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BIOSYSTEMATIC STUDIES ON *ORNITHOGALUM UMBELLATUM* L. S. L.

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ABSTRACT

592 plants from 185 different populations of *Ornithogalum umbellatum* L. s. 1. were studied cytologically and morphologically. Five cytotypes were found to occur: diploids (2n = 18), triploids (2n = 27), tetraploids (2n = 36), pentaploids (2n = 45) and hexaploids (2n = 54). The results of a numerical analysis was that two clusters consisting of triploids and penta-/hexaploids respectively could be distinguished. There is a clear case for giving the penta-/hexaploids specific rather than infraspscific rank and we recognize the triploid *Ornithogalum umbellatum* L. s. str. as being distinct from the penta-/hexaploid *Ornithogalum diver gens* Bor. The tetraploids belong partly to the triploid group and partly to the penta-/hexaploid group. The relationship between the di- and triploid plants deserves further study. The relationship between the characters of the various cytotypes is illustrated in a series of scatter diagrams.

I. INTRODUCTION

Ornithogalum umbellatum L. is a wide ranging and variable taxon with populations that show different combinations of morphological characters. These variants have been variously described as distinct species (TENURE, 1829; BOREATJ, 1849; GODRON, 1854), subspecies (ASCHERSON and GRAEBNER, 1905-1907; ROXTY, 1910), varieties (FIORI and PAOIETTI, 1896-1898) or as having no taxonomic value (TORNADORE and GARBARI, 1979). Of the variants *O. divergens* Bor. in particular seems to be specifically distinct (FErNBRUN, 1941; GADELLA, 1972a and b; GUTNOCHET and HE VHMORTN,

1978; GADELLA and VAN RAAMSDONK, 1979), but NEVES (1952) regards hexaploid forms of *O. umbellatum* and *0. divergem* as indistinguishable and treats them as subspecies of *0. umbellatum.*

Another taxon, *0. paterfamilias* Godr., differs from *0. divergem* in that the former produces bulbils with leaves, whereas the latter forms leafless bulbils under the outer tunic of the bulb. Also, according to NEVES (1956), the only plant of *0. paterfamilias* that had been eytologically studied turned out to be diploid (2n = 18), whereas *0. divergens* is hexaploid (2n = 54). In spite of these facts *0. paterfamilias* is treated as a subspecies by NEVES (1956), who in this respect follows ASCHERSON and GRAEBNER (1. c), ROXJY (1910) and BONNIER (1934). The value and reliability of the characters used to separate these variants are not always clear because in some cases the taxonomic decisions are based on the study of leaves and flowers and in other cases exclusively on the subterranean parts such as bulbs and bulbils.

NEVES (1952, 1956) carried out extensive cytological investigations using Portuguese material, which he supplemented with plants obtained from botanical gardens, the provenance of which was not known exactly. Many authors have demonstrated the occurrence of di-, tri-, tetra-, penta-, hexa-, octo-, deka and dodekaploid plants in various parts of Europe and also aneuploids at most levels of polyploidy: CHIAPPINI, 1968, 1972; CULLEN and RATTER, 1967; CZAPIK, 1961, 1965, 1966, 1968, 1972; GADELLA, 1970, 1972a and *b*, 1976; GADELLA and KLIPHUIS, 1963; GADELLA and VAN RAAMSDONK, 1979 ; GARBARI and TORNADOKE, 1972 ; LUNGEANU, 1971, 1972; MARCHI, 1971; MARKOV A *et al.*, 1972, 1974; NAKÀJIMA, 1936; NEVES, 1952, 1956, 1973; POLYA, 1949; SATO, 1942; SEN, 1973; SPRUMONT, 1928; SUSNIK and LOVKA, 1973; TORNADORE and GARBARI, 1979.

In most of these studies the cytological evidence is not made conclusive for taxonomy. It seems desirable that there should be a taxonomic evaluation of these variants and cytotypes as well as a study of the relationship of *O. umbellatum* s. 1. to other taxa *(O. tenuifolium* Guss., *O. kochii*

Pari, and *O. gussonei* Ten.). When specimens of *0. umbellatum* are growing on poor soils confusion with *0. gussonei* is possible (CZAPIK, 1965). Crossing experiments carried out by CZAPIK (1972) demonstrated that *0. gussonei* is closely related to *0. umbellatum.* Diploid *0. umbellatum* and *0. gussonei* are able to cross and to produce (sterile) hybrids. Fiori (1923-1925) placed *0. gussonei* among the varieties of *0. umbellatum: 0. umbellatum* L. var. *tenuifolium* (Guss.) Fiori, but CULLEN and RATTER (1967) and TORNADOKE and GARBARI (1979) regard *0. umbellatum* and *0. gussonei* as specifically distinct. They are of the opinion that *0. kochii* is a synonym of *0. tenuifolium* (GARBARI and TORNADORE, 1970). Later, however, they regard *O. tenuifolium* as a synonym of *0. gussonei* (TORNADOKE and GARBARI, 1979). In *0. gussonei,* however, cytological differences were demonstrated: 2n = 14 (+ 1-4B) in material from Italy (TORNADORE and GARBARI, 1979) and 2n = 18 (or 2n = 36) from Hungary, Bulgaria and Poland (POLYA, 1949; MARKO VA *et al.,* 1972; CZAPEK, 1972, respectively). These data contributed to the taxonomic confusion regarding the position of *0. gussonei.*

Cytotaxonomic studies should not be restricted to the collective species *O. umbellatum,* but should be extended to some related taxa if we are to arrive at a better understanding of this intricate species complex of the section *Heliocharmos.*

This paper deals with the first results of combined cytological and morphological studies. The first author determined the chromosome numbers of 592 plants from 185 different populations, and worked out the scatter diagrams (fig. 7-31), the second author carried out biomathematic studies of 251 plants in 1977. Most population samples were collected in the Netherlands, but were supplemented by a limited number of plants from south, west and central Europe.

II. MATERIAL, AND METHODS

With the exception of 5 plants all plants (587) were collected in wild populations. A complete list of all plants studied, indicating voucher numbers and chromosome num-

bers, is given in the appendix. Most plants were collected in the Netherlands, where *0. umbellatUm* has been protected by law since 1973. We started collecting in 1965 but were very careful to take only a few bulbils from each population so as not to endanger the species.

All plants mentioned in the appendix are still being grown in pots in the Botanical Garden Sandwijck, de BiIt, State University of Utrecht. For that reason it was only in some cases that voucher specimens (including the bulbs) were deposited in the herbarium. Every autumn the bulbs were repoted and all newly formed bubils were removed. The characters of the bulbs were studied in the autumn only.

In all cases root-tips were used for the study of metaphase plates. They were fixed in Karpecheenko's fixative, embedded in paraffin-wax, sectioned at 15 micron and stained according to Heidenhain's haematoxylin method.

In 1977 we measured fourteen characters of the bulbs and aerial parts of 251 plants of the collection: 128 triploids from 54 localities in Belgium, Denmark, W. Germany and (mainly) the Netherlands; 6 tetraploids from 3 localities in France and the Netherlands; 55 pentaploids from 19 localities in Austria, France, the Netherlands; 60 hexaploids from 19 localities in Denmark, France, the Netherlands and Portugal.

We tried to relate chromosome numbers of the population samples studied to a classification based on general morphological characters and followed the strategy of pattern recognition. For this purpose we used the program system BIOPAT, designed by HOGEWEG and HESPER (1972). This system was developed to facilitate cluster analysis of the population samples, which was described by a vector of (in this case 14) characters each of which had certain values. An agglomerative clustering was performed of which the clustering criterion was minimalisation of the increase in the mean sum of squares to be formed (WARD, 1963). The keywords of the method developed by HOGEWEG and HESPER are: normalisation of the imput-matrix per character (minimum value set to zero, maximum value to one), calculation of the dissimilarities of the plants by the mean character

distance, agglomerative clustering using the criterion «Ward's average» and finally calculation of the optimum number of clusters by a criterion which was also developed by HOGEWEG and HESPER. For the description of these keywords the reader is referred to HOGEWEG (1976a); Further information about numerical taxonomy is given in HOGEWEG (1976b) and SNEATH and SOKAL (1973).

The following characters were studied (differences between two plants which are less than the value mentioned in brackets are meaningless) :

—Ratio bulb height (:) bulb width (indicated as bulb-ratio).
—Bulb width (per mm).
—Number of bulbils.
—The shape of the bulbils (0 means nearly round; 1 means longer than broad, bulb-shape ellipsoid and tapering towards the apex).
— Width of the leaves (per 0.5 mm).
— Length of the leaves (per 0.5 cm).
— Beginning of the flowering period (indicated in April dates, May 1st becomes «April» 31 and June 1st «April» 62).
—The duration of the flowering period (per day).
—The number of flowers per inflorescence.
—The length of the lowermost pedicel (per mm).
— The angle between the lowermost pedicel and the main axis (peduncle) of the inflorescence (per 5 degrees, measured at the end of the flowering period).
— The ratio number of flowers per plant (:) number of fruits (mentioned as fruit ratio).
— Numbers of seeds per fruit.
— Chromosome number.

Another character, the number of veins per leaf, was not used for the cluster analysis because it was measured only in a limited number of plants.

Besides the results of the 1977 investigation there are observations for the years 1976, 1978 and 1979, relating not only to the number of flowers per inflorescence but also to the beginning of the flowering period and the seed-set. These data were treated in the same way as those studied in 1977.

In a number of (modern) Floras the length of the bracts and the ratio of the length of the pedicel to the bract is regarded as taxonomically important. Therefore some observations on these characters were carried out in 1978 and 1979 on 32 Dutch triploids, 9 tetraploid Dutch plants and 23 French and Dutch hexaploids.

We made scatter diagrams in order to study the correlation of at least three characters (one cytological and two morphological).

III. RESULTS

III-A. Some notes on the distribution of Ornithogalum umbellatum L. s. I.

IH-A-I *The Netherlands*

O. umbellatum is a species with a wide range of habitats. In the Netherlands it usually occurs in well drained moist clayey, sandy-clayey or loamy soils, rich in humus and nitrogen, both in shaded woodlands and among shrubs and in open habitats, in grassy places and on waste land. Occasionally the species is to be found on arable land. Most Dutch populations occur on dry southerly exposed slopes of dikes and on river foreland as well as in the woodland zone of older inner dunes that tend to be leached out and poorer in lime than the more exposed sea dunes (see figure 1, general distribution in the Netherlands).

The hexaploid plants can be considered as «stinse plants», see JANSEN and VAN DEE PLOEG (1977). They prefer a special kind of man-made habitat in which many geophytes, flowering early in spring, occur together. These stinse-plants are restricted to old country estates (Dutch: «Stinsen» or «States») and related habitats such as old ramparts, chuchyards and old farmyards. In these habitats the soil is moist, fertile

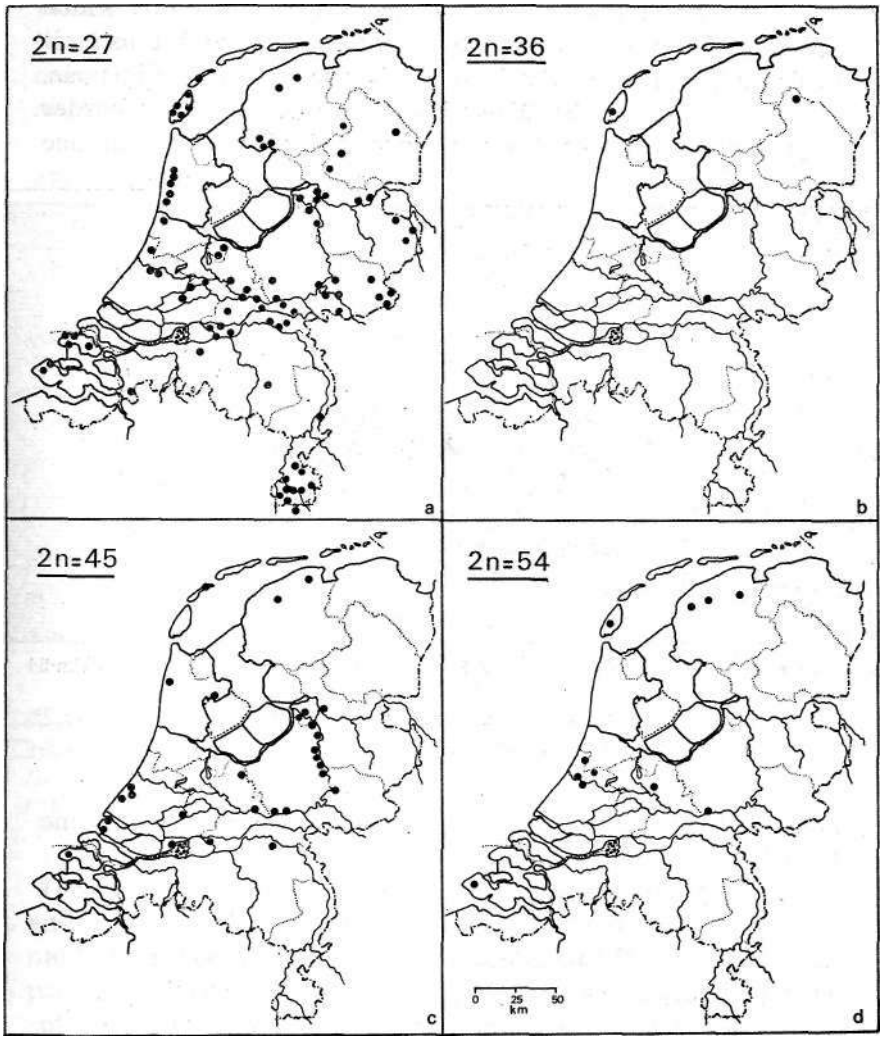


Fig. 1. — The distribution of tri-, tetra,- penta- and hexaploids of *Ornithogalum umbellatum* L. s. 1. in the Netherlands.

and well drained, comparable with so-called «Klebwälder» (see WALTHER and ELLENBERG, 1963).

The triploids and pentaploids may occur in the same kind of habitat, but these cytotypes more often prefer river foreland and woodland on the inner dunes. Hexaploids on

the other hand are confined to the «stinse» habitat, which has been built up by human activities. For that reason it is doubtful whether the hexaploids are in fact indigenous. In one population near Wageningen (see fig. 2) at the border of a southerly exposed steep slope and river foreland one

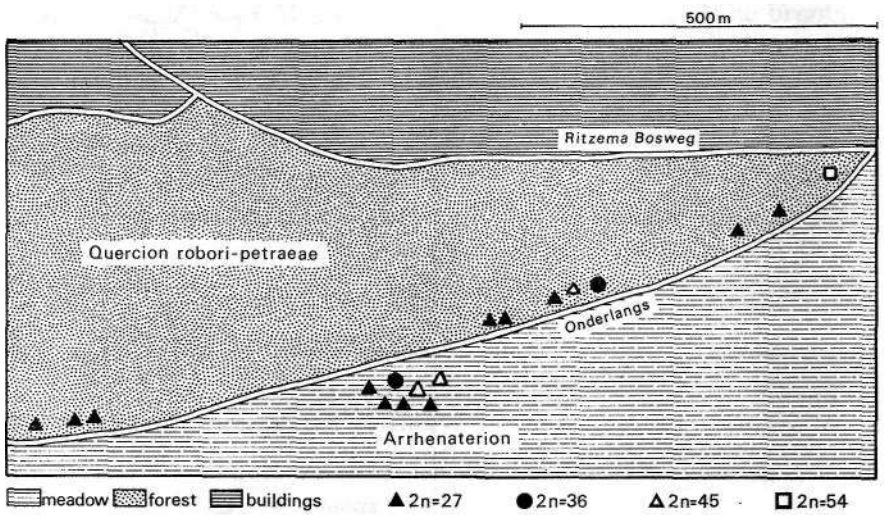


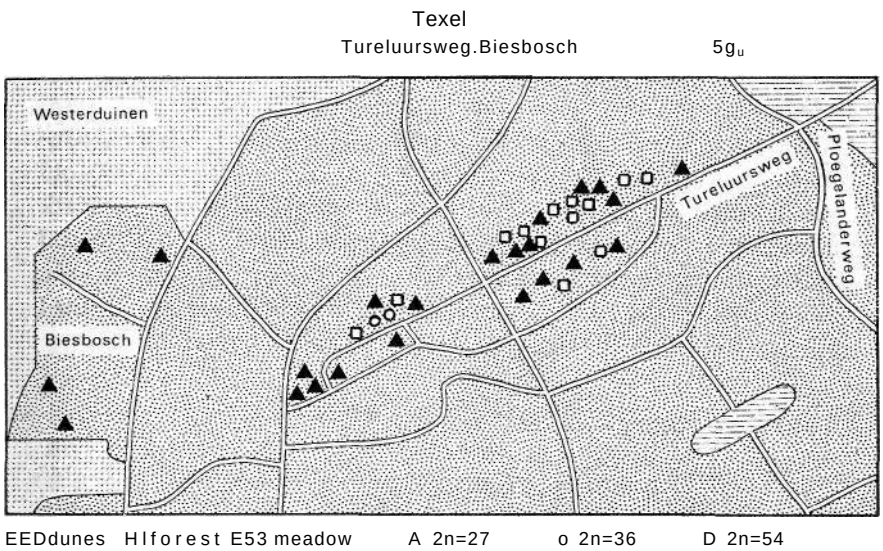
Fig. 2.—The population of *O. umbellatum* near "Wageningen, the Netherlands. Distribution of tri-, tetra-, penta- and hexaploids.

hexaploid plant was found in close proximity to some triploids.

In another population near de Koog (Island of Texel) hexaploids and triploids grow intermingled (see figure 3), and one part of the population is mixed with some tetraploids. This population was in all probability introduced by man. The plants occur in a plantation of Oak and Sycamore into which bulb growers had introduced bulbs of *Galanthus nivalis* for commercial purposes. These bulbs had been dug up in their natural habitat (near Clermont Ferrand and Tours; MANTJE, in lit.) with soil particles attached and it seems probable that some bulbs or bulbils of *O. umbellatum* were thus introduced into the island of Texel. In this habitat, which was also built up by human activities, was found the first and sofar the only occurrence of diploid *Ranunculus*

ficaria in the Netherlands. This race ranges far South of the Dutch borders, which supports the hypothesis that not only these plants but also the hexaploids of Texel are allochthonous.

The triploids and pentaploids, preferring river foreland and the inner dune-zone, are less rare in the Netherlands



Pig. 3.—The population of *O. umbellatum* near de Koog, Island of Texel. Distribution of tri-, tetra- and hexaploids.

and show the following distribution in VAN SOEST'S (1929) phytogeophical districts in the Netherlands: both pentaploids and triploids are regularly found in the so-called «Dune» district and in the «Fluviatile» district. They are rather rare in the «Wadden» district, the «Haf» district the «Drents» district and «Subcentreurope» district and are usually confined in these districts to old country estates, castles, old ramparts and farmyards, i. e. to man-made habitats. In the authors' opinion only the tri- and pentaploids are indigenous to the Netherlands. Usually triploids and pentaploids grow in separate localities; mixed populations were found only very occasionally.

Tetraploids are rare and seem to be confined to the populations of Texel (figure 3), Wageningen (figure 2) and Eeide (figure 1, showing the distribution of all Dutch plants investigated). These tetraploids will be discussed further under the heading «scatter diagrams».

Triploids and sometimes also pentaploids prefer the following plant communities (see WESTHOFF and DEN HELD, 1969) : *Anthrisco-Fraxinetum* (Ash-wood, rich in *Anthriscus sylvestris,* especially in the zone of old inner dunes) and the *Violo odoratae-Ulmetum* (forests with *Ulmus* X *hollandica* and *Ulmus carpinifolia,* often together with *Viola odorata* and *Veronica sublobata),* not only in the zone of old inner dunes, but also near river dunes, e. g. near ZaIk along the river IJssel. Pentaploids prefer river foreland and dikes in the plant community *Arrhenateretum elatioris,* which occurs on well manured grasslands that are grazed or mowed at least twice a year.

M-A-II ***Other European countries***

Relatively few populations have been studied outside the Netherlands. Diploids were found in France (dept. Ht. Alpes, dept. Savoie) in subalpine meadows (1500-1700m). Other investigators encountered diploids in Portugal (NEVES, 1952), Italy (TORNADORE and GARBARI, 1979) and Poland (CZAPIK, 1965). The Polish diploids very often grow in cornfields but are also found in meadows.

Triploids were found in Scotland, Denmark, Belgium and W. Germany. They were also reported from Poland (CZAPIK, 1965), Portugal (NEVES, 1952, 1973) and England (CZAPIK, 1968). In general they seem to have a more northerly distribution than the higher polyploids. TORNADORE and GARBARI (1979) could not demonstrate the occurrence of triploids in Italy. Triploids were often found in meadows and cornfields in Poland (CZAPIK, 1965).

Tetraploids were encountered in Greece by CULLEN and RATTER (1967), in Bulgaria by MARKOVA *et al.* (1972, 1974) and by CZAPIK (1965) in Poland. TORNADORE and GARBARI (1979) found a huge polyploid series, ranging from diploids

to dodekaploids (2n = 18 to 2n = 108) in Italy, but it is remarkable that there were no tetraploids in this euploid series. We counted 2n = 36 in French material, collected in Dept. Var, near la Môle (S. France). CZAPIK (1965) found tetraploids in meadows and cornfields. The data are too scanty to permit any conclusion to be drawn with regard to the distribution of tetraploids.

Pentaploids (often also aneuploids at this level of polyploidy) were found to occur in Turkey (CULLEN and RATTER, 1967), in Italy (TORNADORE and GARBARI, 1979) and in Bulgaria (MARKOVA *et al.*, 1972, 1974), but they were not reported from Poland by CZAPIK (1965). We encountered this cytotype in Austria and France.

Hexaploids (often published under the name *O. divergens* Bor.) were collected in Italy (TORNADORE and GARBARI, 1979), in England (CZAPIK, 1968), in Bulgaria (MARKOVA *et al.*, 1972), in France and Portugal (NEVES, 1956) and by the present authors in material collected in Denmark, in various localities in the South of France, in Italy, Portugal, and Yugoslavia. In the South of France hexaploids are common in vineyards and peach orchards. The general impression is that hexaploids have a more southerly distribution. The only find of this cytotype in Denmark may be an escape; the plant in question grew in a sandy meadow along with thrift *(Armería maritima)* near the Danish coast, fairly close to the village of Ebeltoft. The Dutch hexaploids may also be regarded as introductions from southern Europe, since they are confined to man-made habitats.

III-B. CIusteranalysis

The most important result of the cluster-analysis is that it enables us to divide the 251 studied plants into two clearly distinct groups. The first cluster (A) consists of all triploid plants and three tetraploids (from Eelde in the Netherlands). The other tetraploid plants (from Wageningen, the Netherlands and Col du Canadel, dept. Var, France) could be grouped with the penta- and hexaploid plants in cluster B. All other plants of the collection which were not

used for the cluster-analysis could be assigned without difficulty to either cluster A or B. The mean and standard deviation for each characters is given in table 1.

TABLE 1

Statistical data resulting from the cluster analysis. AU measurements were made on plants grown in pots in the year 1977. For further explanation see text.

Character	Cluster A		Cluster B	
	Mean	S. D.	Mean	S D.
Bulb ratio	1.25	0.08	0.84	0.08
Width of bulb (mm)	19.94	1.76	28.30	4.29
Number of bulbils	6.74	1,30	47.30	23.66
Width of leaves (mm)	0.41	0.05	0.60	0.11
Length of leaves (cm)	17.93	1,81	23,43	2.91
Number of leaves per bulb	21.05	4.71	11.77	3.54
Beginning of the flowering period	44.88	2.86	29.09	5.34
Duration of flowering period (days)	15.31	2.62	23,30	3.85
Number of flowers per inflorescence	7.71	1,61	15.47	3.39
Number of ripe fruits per flower	0.05	0.08	0.04	0.10
Number of seeds per fruit	1.34	0.65	2.25	3.81
Length of lowermost pedicel (cm)	4.17	0.86	5.75	**1.17**
Angle between lowermost fruiting pedicel and peduncle of inflorescence	69.92	6.19	93.44	11.78
Number of plants measured	131		120	

Of the subterranean characters of the plants the most reliable are the shape and the number of bulbils. The plants belonging to cluster A have a few, more or less ellipsoid bulbils, tapering towards the apex, whereas those of cluster B have many more or less round bulbils (which originate under the outer tunic of the bulb, see Plate I).

In cluster A the bulbs are narrower than they are long (resulting in a bulb-ratio > 1), in cluster B the reverse is the ease (resulting in a bulb-ratio < 1). Since the characters of the bulb are not always used for taxonomic purposes (or at least the taxonomic significance of the relation between aerial parts and subterranean parts) one can conclude that

the bulb characters are highly reliable indicators of the cluster to which each plant belongs. In spite of the fact that the tetraploid plants from Texel were analysed after the year in which the cluster-analysis was carried out, the plants undoubtedly belong to cluster A, as do the plants from Eelde.

The diploids have a different way of forming bulbils. Instead of producing a number of bulbils, the bulb divides into two or four parts of equal size, each of which forms a mature plant the following year. The main bulb of the triploid plants, on the other hand, remains clearly distinct from the somewhat smaller bulbils which are leaf-bearing in their first year of life, giving rise to «tufts» of plants (see Pl. I). In the penta- and hexaploids (including the tetraploids from Col du Canadel and from Wageningen) the very small bulbils do not form leaves in their first year of life.

It was not always possible to assign the plants to one of the two clusters when only one of the characters of the aerial parts of the plants was used. In combination, however, the aerial parts too provide reliable characters for the separation of the groups. The plants of cluster A form more, narrower and shorter leaves, fewer flowers, shorter pedicels and smaller angles between the pedicel and the peduncle than the plants of cluster B. See table 1 for further details.

Within cluster B it is possible to distinguish four subgroups, viz B-1 with most of the Dutch pentaploids, B-2 with most Dutch hexaploids, B-3 With the French hexaploids and, finally, B-4 with three tetraploids and a number of hexaploids from the island of Texel. The French plants differ from the Dutch hexaploids mainly by their greater angle between the lowermost fruiting pedicel and the peduncle (they have reflexed pedicels after anthesis). With regard to the width of the leaves and the number of flowers the three tetraploids and the hexaploids from Texel show intermediate values between the other plants of cluster B and those of cluster A.

In 1979 some of the characters of the diploids were tested, which had been used previously for the cluster-analysis. On the basis of the width of the leaves, their length and number, and the number of flowers, these plants seem to be intermediate between those of cluster A and B. The

TABLE 2

Statistical data of some characters measured in diploid plants.

Character	Mean	S. D.
Width of the leaves (mm)	6.00	1.15
Length of the leaves (cm)	19.29	3.30
Number of flowers per inflorescence	9.13	3.64
Length of the lowermost pedicel (cm)	5.29	0.39
Angle between the lowermost fruiting pedicel and the peduncle of the inflorescence	42.14	6.99
Ratio length pedicel (:) length bract	1.4O	0.13
Number of plants measured	7	

angle between the lowermost fruiting pedicel and the axis of the inflorescence, however, is extremely small and this character is probably correlated with the shorter length of the scape of the inflorescence and certainly with the mode of propagation of the bulb (see table 2).

As is shown in table 3 the cytotypes do not show significant differences in the number of veins per leaf. The data on the length of the bracts of the lowermost pedicel

TABLE 3

The number of veins per leaf of tri-, tetra-, penta- and hexaploid plants.

Cytotype	Number of plants	Number of veins per leaf	
		Mean	S. D.
2n = 27	29	7.59	0.98
2n = 36	6	7.00	1.41
2n = 45	28	7.50	1.00
2n = 54	17	8.53	1.55

do not differ significantly for the two clusters. The same applies to the ratio of the length of the pedicel to the length of the bract (see table 4).

Not only the genotype, but also environmental factors influence the phenotype. The absolute number of flowers of triploid, penta- and hexaploid plants may alter according to the year, as can be deduced from figures 4 and 5.

TABLE 4

Statistical data on some characters. All measurements were made on plants grown in pots in 1979.

Character	Triploids		Penta/hexaploids	
	Mean	S. D.	Mean	S.D.
Ratio length pedicel (:) length bract	1.56	0.53	1.32	0.31
Length of lowermost bract		1.09	3.95	0.85
Number of plants measured	39		25	

However, the differences between the two clusters remained fairly constant in each of the four years of observation. In view of the fact that the group of tripioids is comparable to Cluster A and the group of penta-/hexaploids to cluster B, we may expect the differences found as a result of the clusteranalysis of 1977 also tö be present in the other three years in which the same cytotypes were studied.

The cumulative percentages of plants the leaves of which become visible above the ground are shown in figure 6, arranged in periods of 10 days.

As a rule the leaves of penta- and hexaploids appear earlier in the autumn than those of tripioids. As a consequence one might except the penta- and hexaploids to flower earlier the following spring. This is in fact precisely what happened, as is shown in the scatter diagram of figure 22.

The beginning of the flowering period in four successive years (1976-1979) was plotted against the number of flowers in the scatter diagrams illustrated in figures 23-30. Although the onset of flowering was different in these years, the tri-

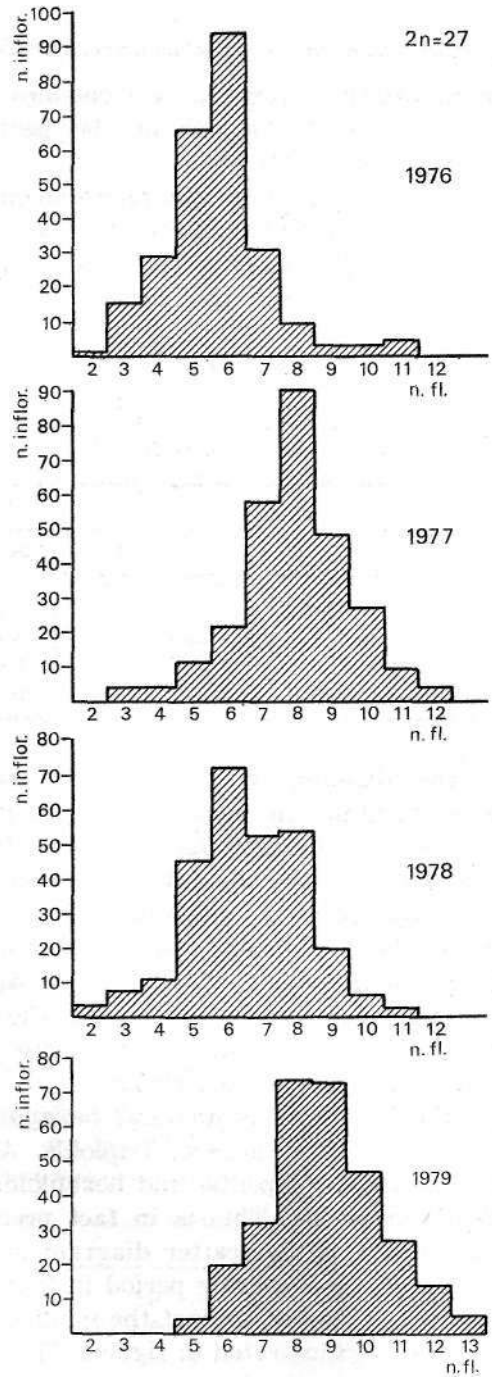


Fig. 4. — Histogram showing the number of flowers per inflorescence in the triploid cytotype in four successive years (1976/1979).

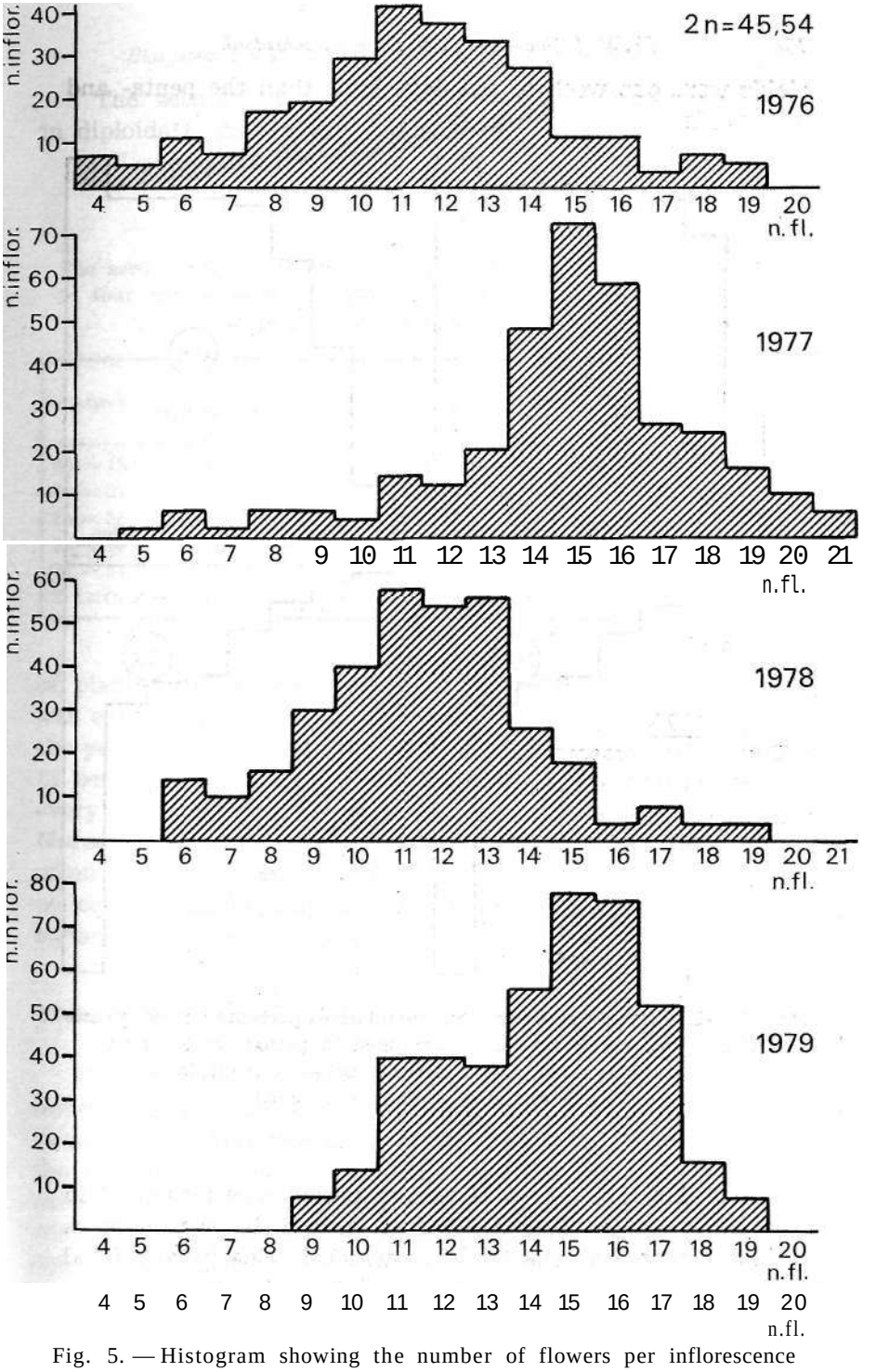


Fig. 5. — Histogram showing the number of flowers per inflorescence in the penta- and hexaploid cytotypes in four successive years (1976/1979).

ploids were one week to ten days later than the penta- and hexaploids.

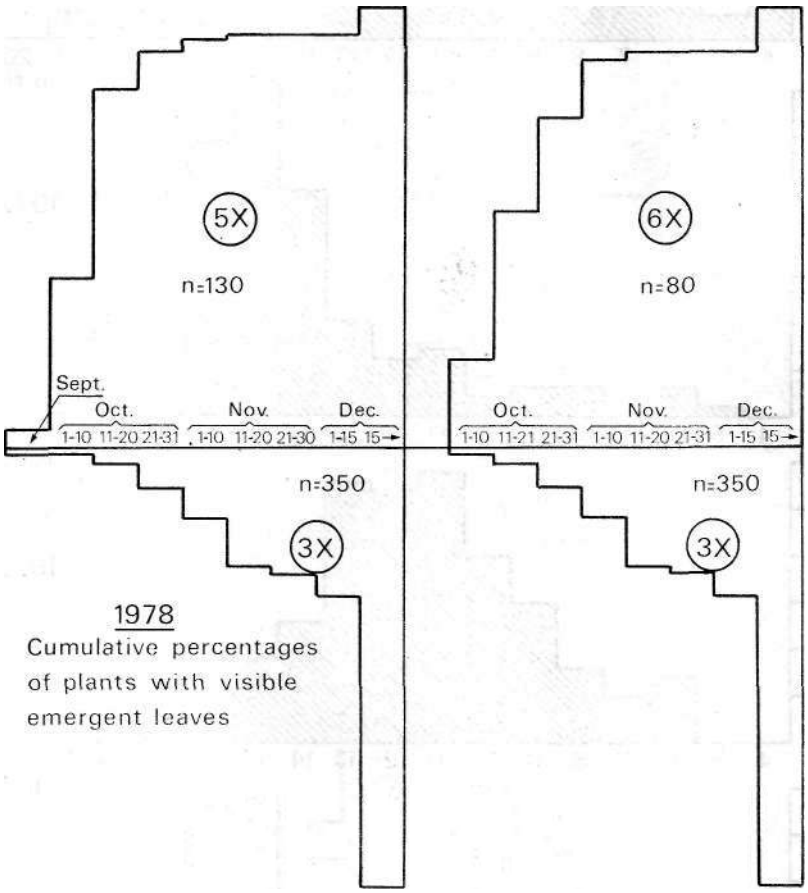


Fig-. 6. — Histogram showing the cumulative percentages of plants with visible emerging leaves, arranged in periods of 10 days (autumn 1978); the difference between triploids and penta-/hexaploids is very clear.

The duration of the flowering period was measured in 1977. The penta- and hexaploids proved to flower some 8 days longer than the triploids, which is clearly correlated with the larger number of flowers of cluster B.

The seed-set is extremely low in all cytotypes (also in diploids!). As we can deduce from table 5, the number

TABLE 5

The seed-set of 325 plants (belonging to five different cytotypes) in four successive years (1976-1979). All plants were cultivated in pots under identical conditions.

Cytotypes	Number of plants studied	Number of plants producing seeds							
		1976		1977		1978		1979	
2n = 18	5					0	0 %	1	20 %
2n = 27	198	8	4 %	52	26.2 %	59	29.7 %	30	15,9 %
2n = 36	5	1	20 %	0	0 %	3	60 %	1	20 %
2n = 45	74	2	2.7%	18	24.3%	15	20:.2%	9	12.1 %
2n = 54	43	2	4.6%	16	37.2 %.	3	6.9 %	3	6.9%
Total	325	13	4.0'%	86	26.5 %	80	24,6 %	43	13.2 %

of plants that produced seeds in the four successive years was extremely low, but a large variation was found between the years. Not only was the seed-set low, so was the number of seeds per plant (see table 1), in view of the fact that every ovary contains about 50 ovules (ca. 17 in each locule). Moreover, plants that produce seeds in a given year, very often lack any ripe seed in the following season. Of the collection studied only one plant produced seeds in four successive years.

M-C. Scatter diagrams

The use of scatter diagrams lets one obtain a visual impression of the correlation between several different characters. In this paper an attempt has been made to show the relationship between cytological and morphological characters. The method has been used to show the morphological variation in different cytotypes, originating from various sources. By the use of different symbols the relationship of at least three characters could be shown.

Correlations between characters of the subterranean parts of the plants (shape of the bulb, number and shape of the bulbils) and other characters (chromosome number; aerial parts of the plants) have been entirely neglected in papers dealing with variation in *O. umbellatum.* For that reason special attention will be given to these aspects. In all scatter diagrams the same symbols have been used for the various cytotypes. For the purpose of showing the relationships of the characters studied the scatter diagrams have been arranged in five groups:

1. The relationships between the characters of the subterranean parts of the plants (figure 7 and 8).
2. The relationships between the characters of the subterranean and aerial parts of the plants (figures 9-12).
3. The relationships between various characters of the aerial parts of the plants (figures 13-21).
4. The relationship between some aspects of periodicity (figure 22).
5. The relationship between some morphological characters and the flowering period (figures 23-31).

The following conclusions can be drawn from the figures:

a. In most figures (8, 9, 10, 11, 14, 17, 18, 20, 21, 24, 25, 30, 31) the group of triploids is very distinct from the group of penta-/hexaploids.
b. In some figures (16, 19, 22, 23, 26, 27, 28, 29) there is no clear demarcation between the group of triploids and the penta-/hexaploids, but it is evident that most plants can be unequivocally assigned to one or other of the two groups.
c. Penta- and hexaploid plants are often indistinguishable. See scatter diagrams of figures: 10, 11, 13, 14, 20, 21, 22.
d. Penta- and hexaploid plants fall roughly into two groups, but there is such an overlap (see figures:

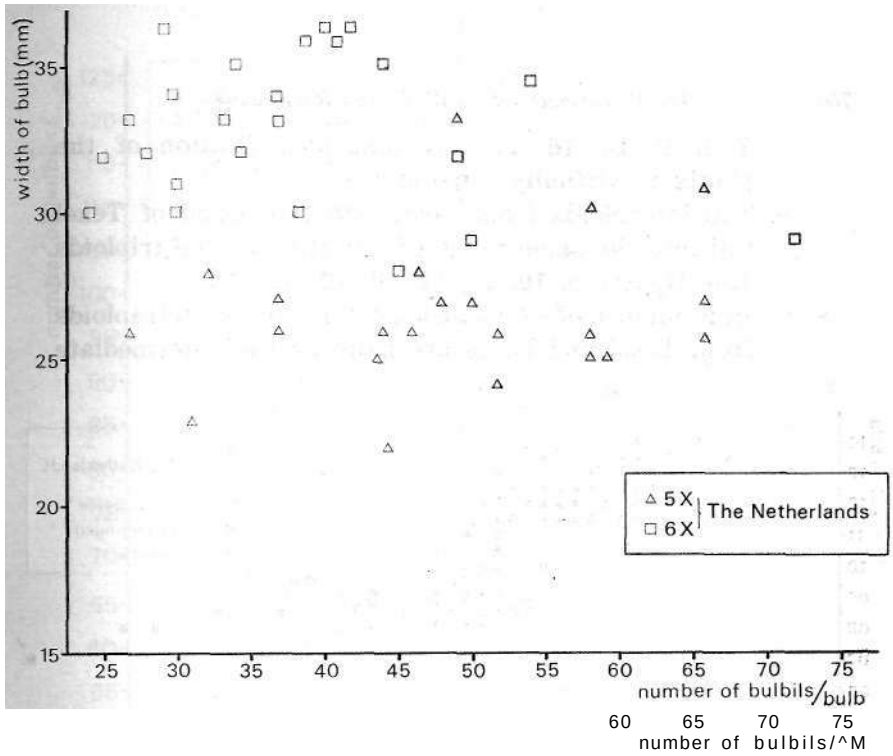


Fig. 7. — Scatter diagram showing the number of bulbils per bulb of penta- and hexaploid plants from the Netherlands, plotted against the length (:) width ratio of the bulb.

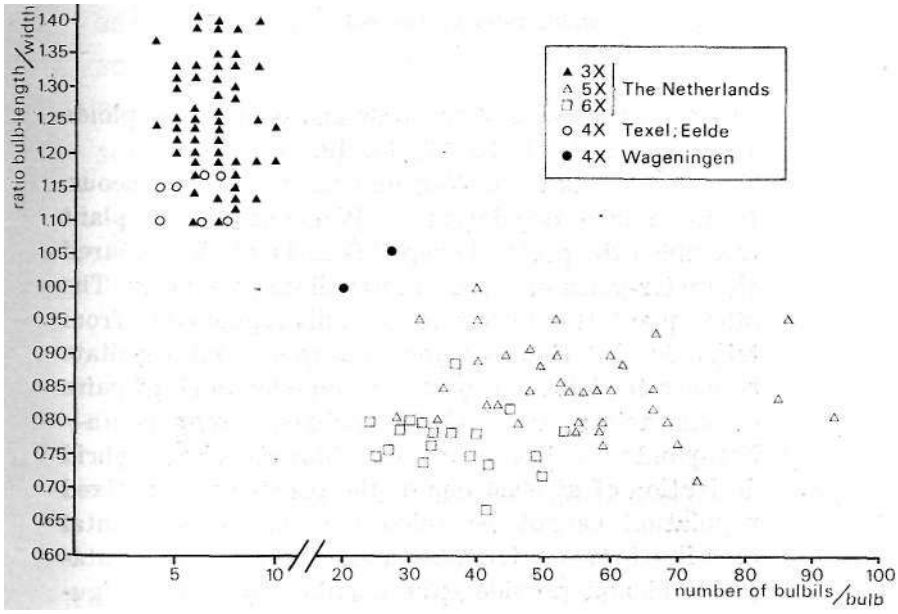


Fig. 8. — Scatter diagram showing the number of bulbils per bulb of tri-, tetra-, penta -and hexaploid plants, plotted against the length (:) width ratio of the bulb.

7, 8, 9, 10, 16, 17, 18) that identification of the plants is virtually impossible.

e. The tetraploids from Eelde and the island of Texel fall into the same range of variation as the triploids (see figures 8, 10, 13, 14, 16, 26, 30, 31).

f. In a number of cases at least some of the tetraploids from Texel and Eelde are more or less intermediate

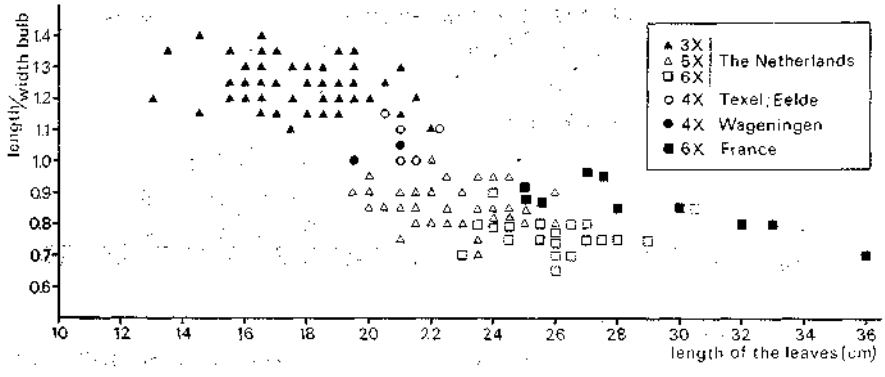


Fig. 10 9. — Scatter diagram showing the length of the leaves of tri-, tetra-, penta- and hexaploid plants, plotted against the length (:) width ratio of the bulb.

between the groups of triploids and penta-/hexaploids (see figures 9, 11, 15, 17, 18, 19, 20).

g. The tetraploids from Wageningen are heterogeneous. In the mixed population of Wageningen one plant resembled the penta-/hexaploids in 11 of 15 compared character-pairs and was intermediate in 4 pairs. The other plant turned out to be indistinguishable from triploids in five scatter diagrams, intermediate between triploids and penta-/hexaploids in eight pairs of characters and indistinguishable from penta-/hexaploids in two pairs of characters. A hybrid derivation of at least one of the plants of this mixed population cannot be ruled out, but experimental crossing between triploids and (presumably) pentaploids should provide experimental proof (see figu-

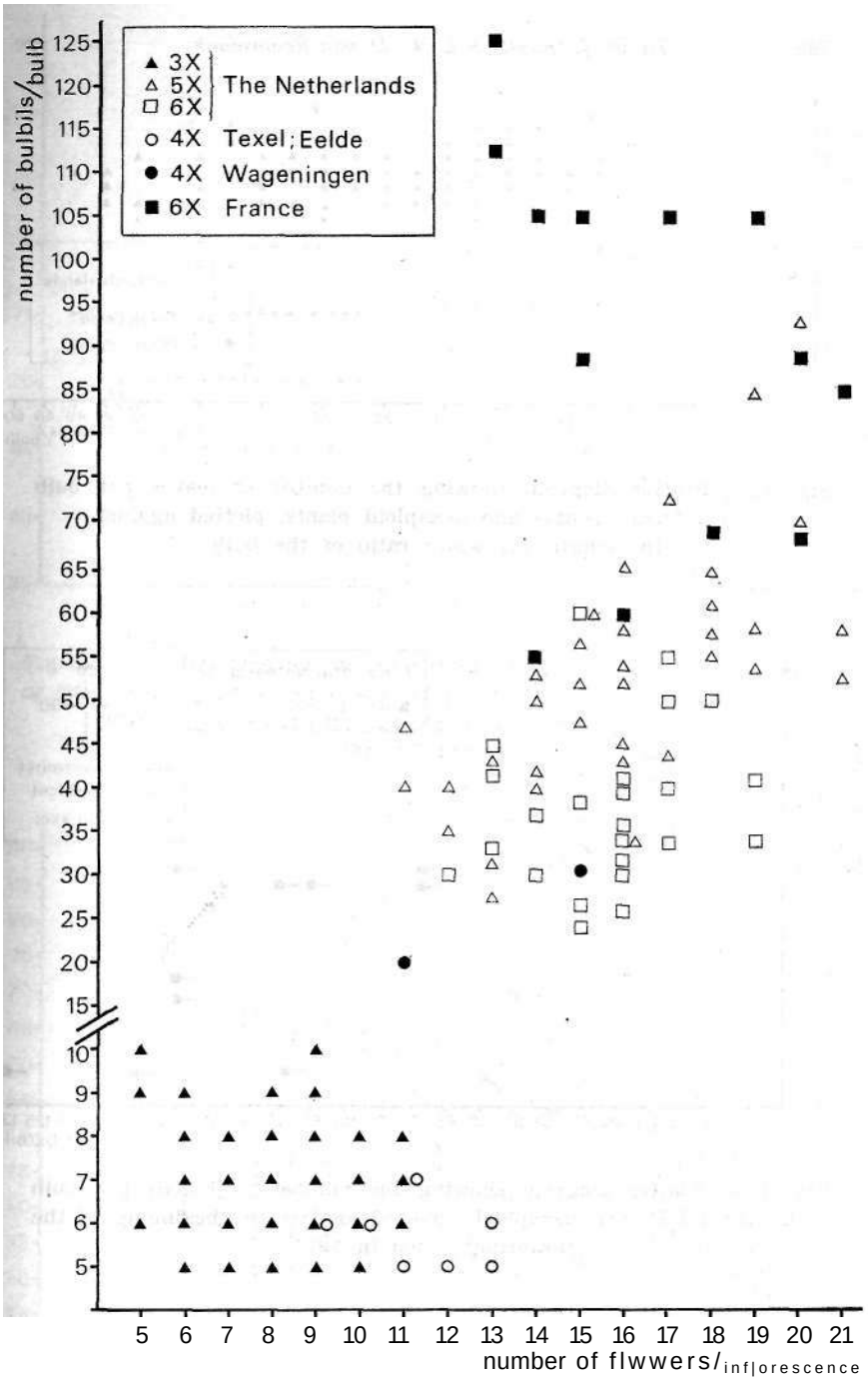


Fig. 10. — Scatter diagram showing the number of bulbils per bulb of tri-, tetra-, penta- and hexaploid plants, plotted against the number of flowers per inflorescence.

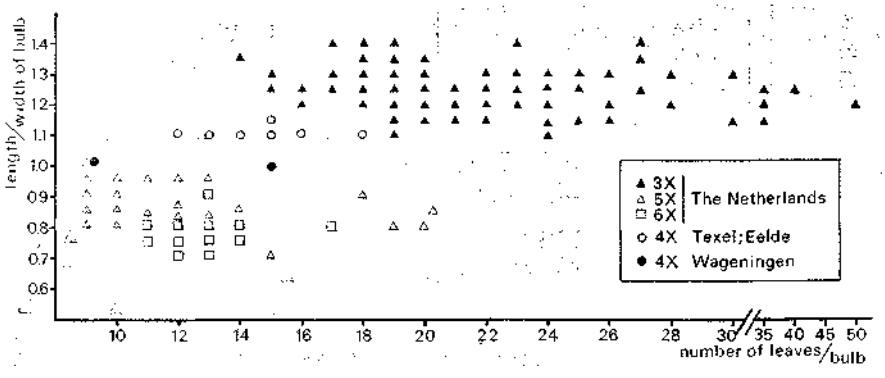


Fig. 11. — Scatter diagram showing the number of leaves per bulb of tri-, tetra-, penta- and hexaploid plants, plotted against the length (:) width ratio of the bulb.

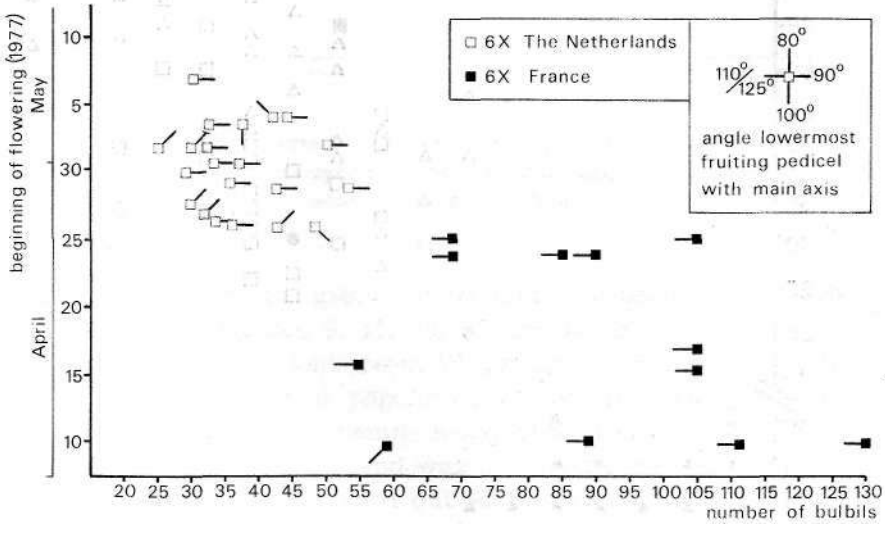
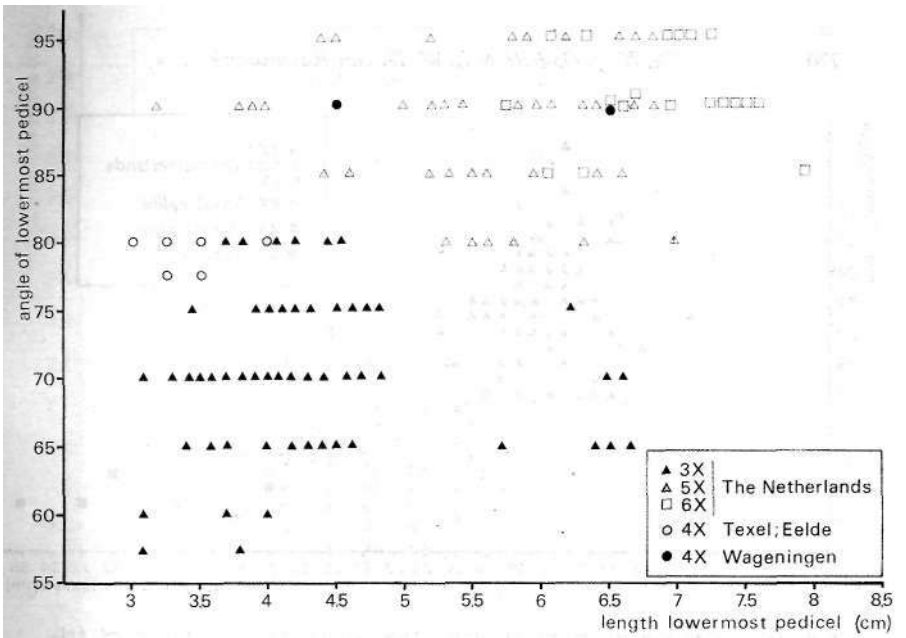


Fig. 12. — Scatter diagram showing the number of bulbils per bulb of Dutch and French hexaploids, plotted against the beginning of the flowering period in 1977.



Pig. 13. — Scatter diagram showing the length of the lowermost pedicel of tri-, tetra-, penta- and hexaploid plants, plotted against the angle between the lowermost fruiting pedicel and the peduncle of the inflorescence.

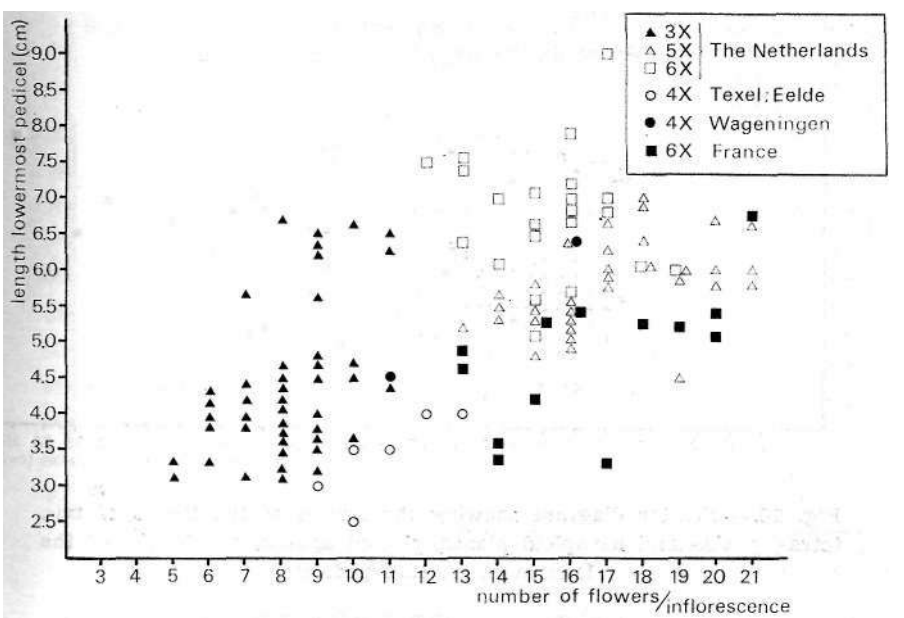


Fig. 14. — Scatter diagram showing the number of flowers per inflorescence of tri-, tetra-, penta- and hexaploid plants, plotted against the length of the lowermost pedicel.

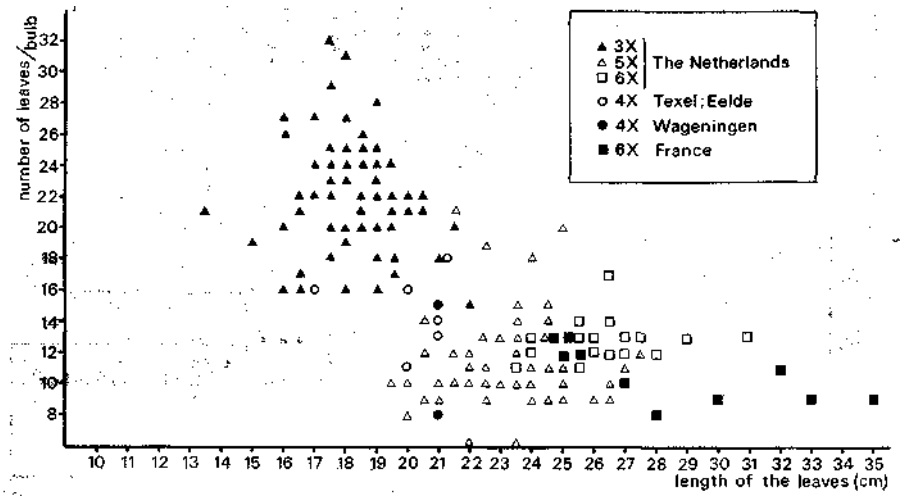


Fig. 15. — Scatter diagram showing the length of the leaves of tri-, tetra-, penta- and hexaploid plants, plotted against the number of leaves per plant.

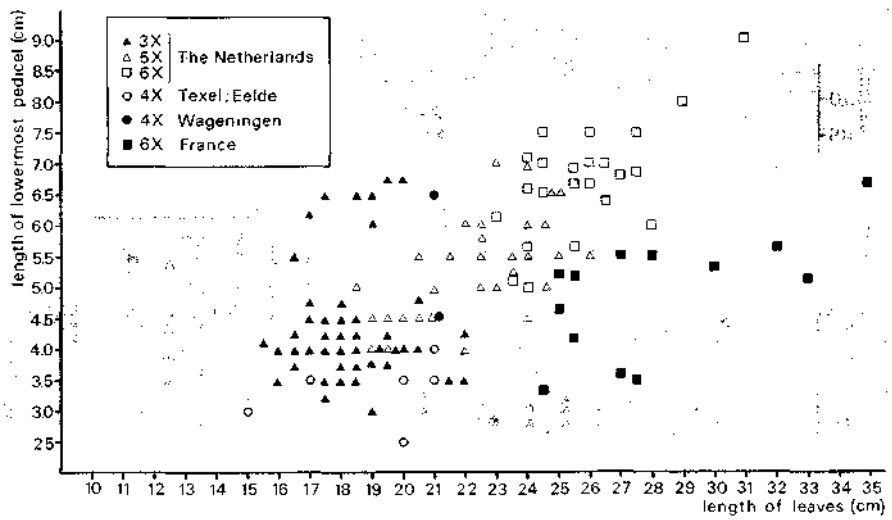


Fig. 16. — Scatter diagram showing the length of the leaves of tri-, tetra-, penta- and hexaploid plants, plotted against the length of the lowermost fruiting pedicel.

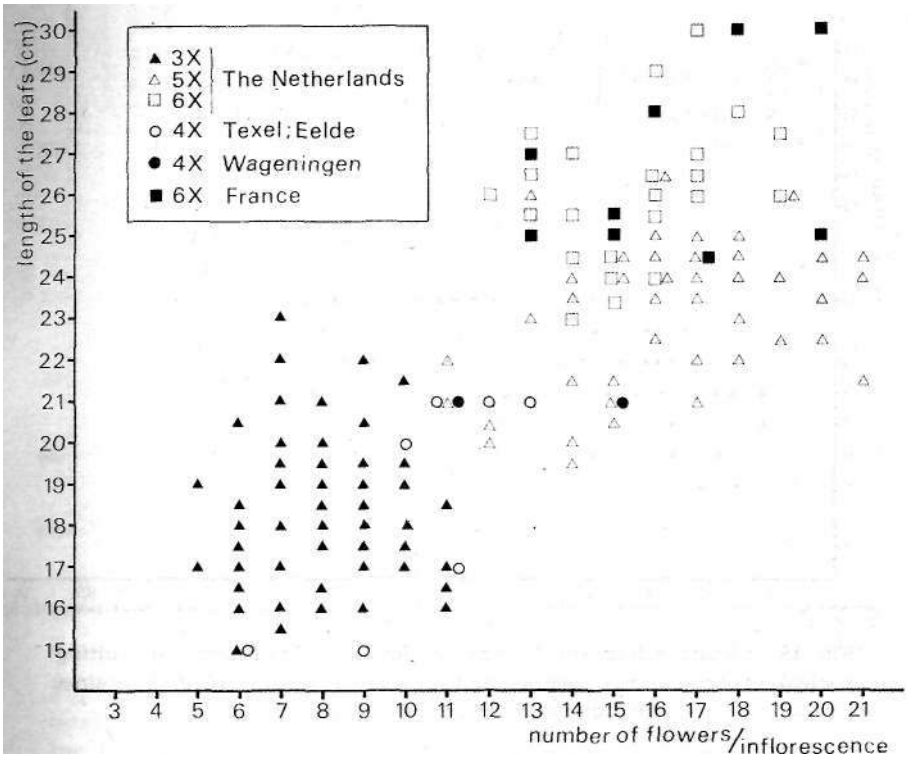


Fig. 17. — Scatter diagram showing the number of flowers per inflorescence of tri-, tetra-, penta- and hexaploid plants, plotted against the length of the leaves.

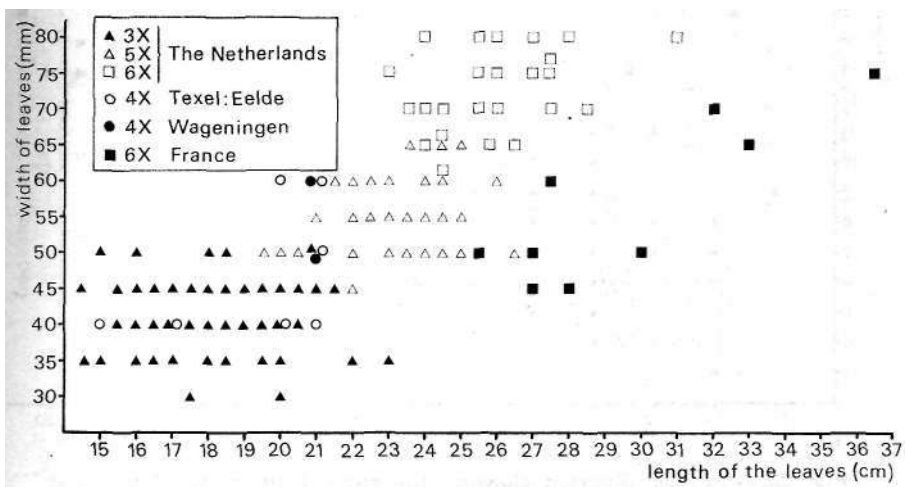


Fig. 18. — Scatter diagram showing the length of the leaves of tri- tetra-, penta- and hexaploid plants, plotted against the width of the leaves.

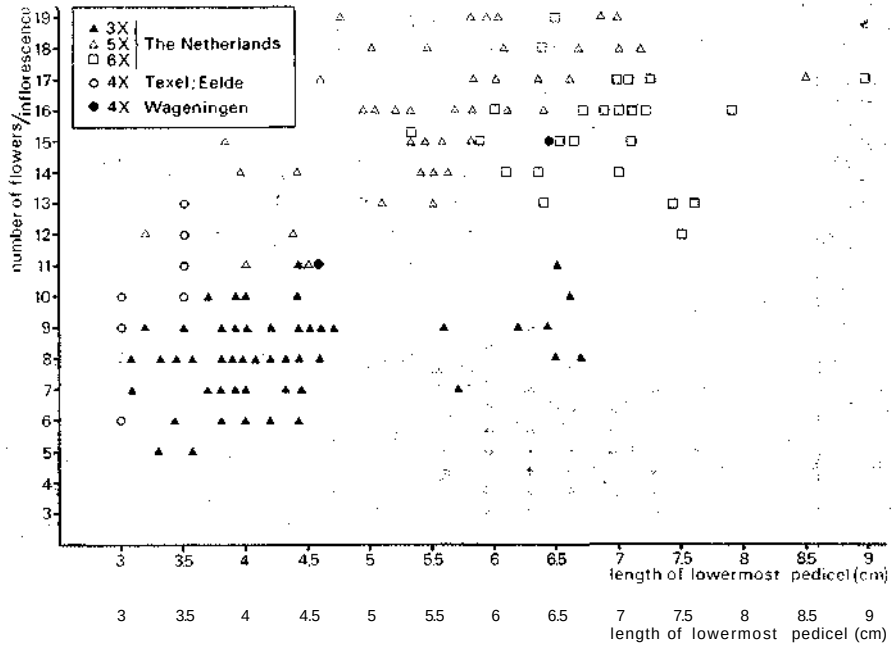


Fig. 19. — Scatter diagram showing the length of the lowermost fruiting pedicel of tri-, tetra-, penta- and hexaploid plants, plotted against the number of flowers inflorescence.

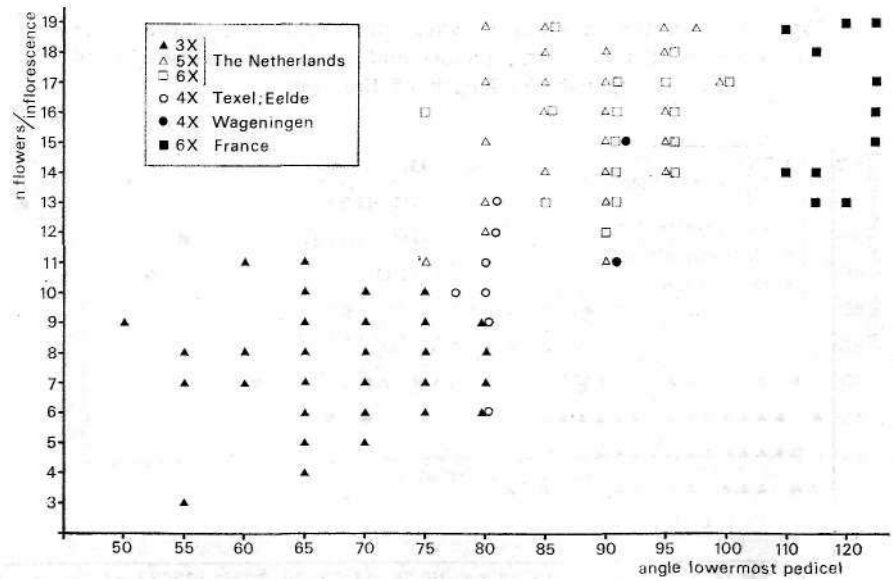


Fig. 20. — Scatter diagram showing the angle between the lowermost fruiting pedicel and the peduncle of the inflorescence in tri-, tetra-, penta- and hexaploid plants, plotted against the number of flowers per inflorescence.

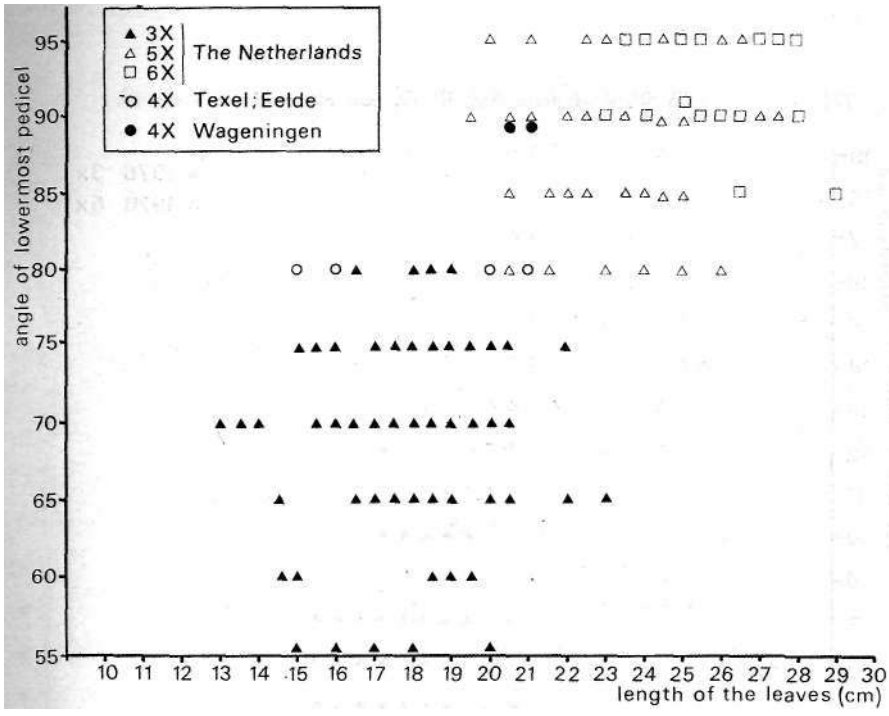


Fig. 21. — Scatter diagram showing the length of the leaves of tri-, tetra-, penta- and hexaploid plants, plotted against the angle between the lowermost fruiting pedicel and the peduncle of the inflorescence.

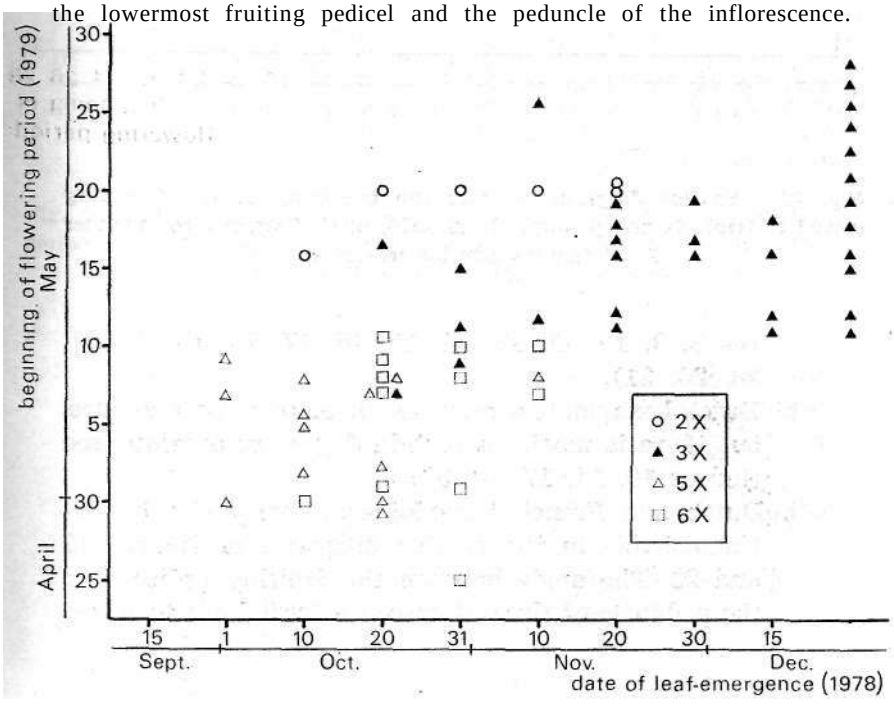


Fig. 22. — Scatter diagram showing the date of leaf emergence (autumn 1978) of tri-, tetra-, penta- and hexaploid plants, plotted against the beginning of the flowering period (1979).

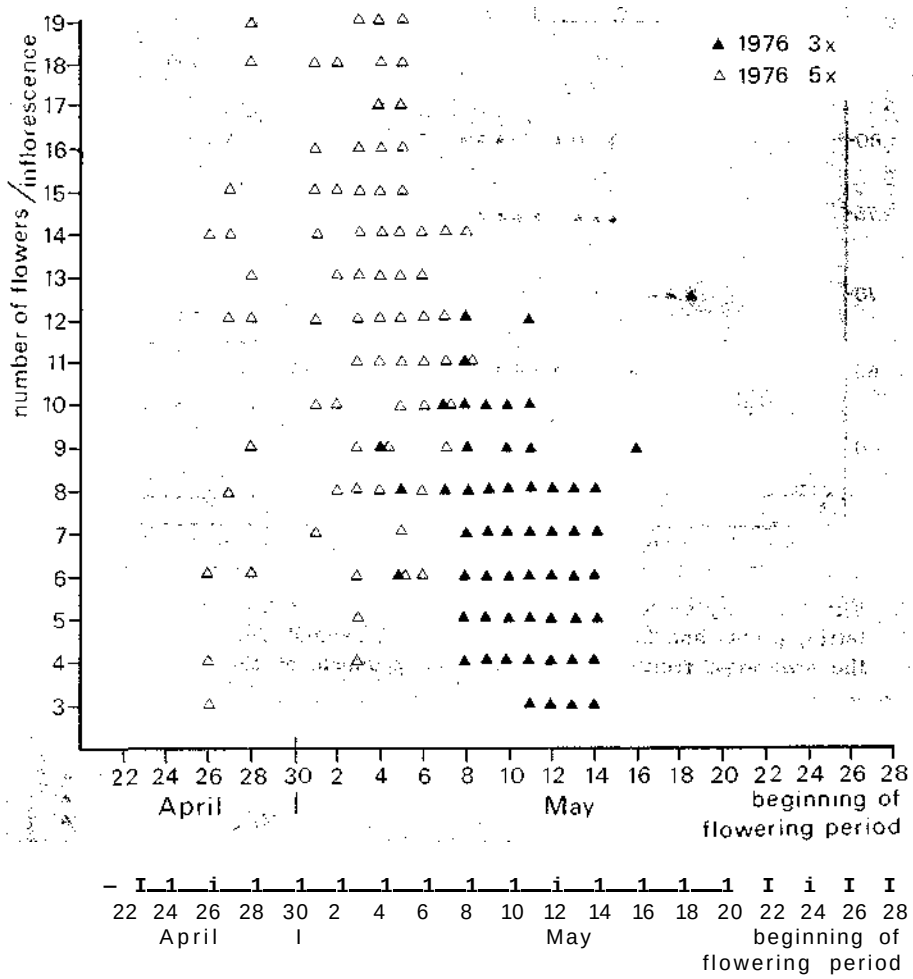


Fig. 23. — Scatter diagram showing the beginning of the flowering period of triploids and pentaploids in 1976, plotted against the number of flowers per inflorescence.

res 8, 9, 10, 11, 13, 14, 15, 16, 17, 18, 19, 20, 21, 26, 30, 31).

h. Dutch hexaploids sometimes differ from French ones, but there is mostly a certain degree of overlap (see figures 10, 14, 17, 18, 30).

i. Dutch and French hexaploids are very clearly distinguishable in the scatter diagrams of figures 12 and 20. The angle between the fruiting pedicel and the peduncle of the inflorescence turned out to be ca.

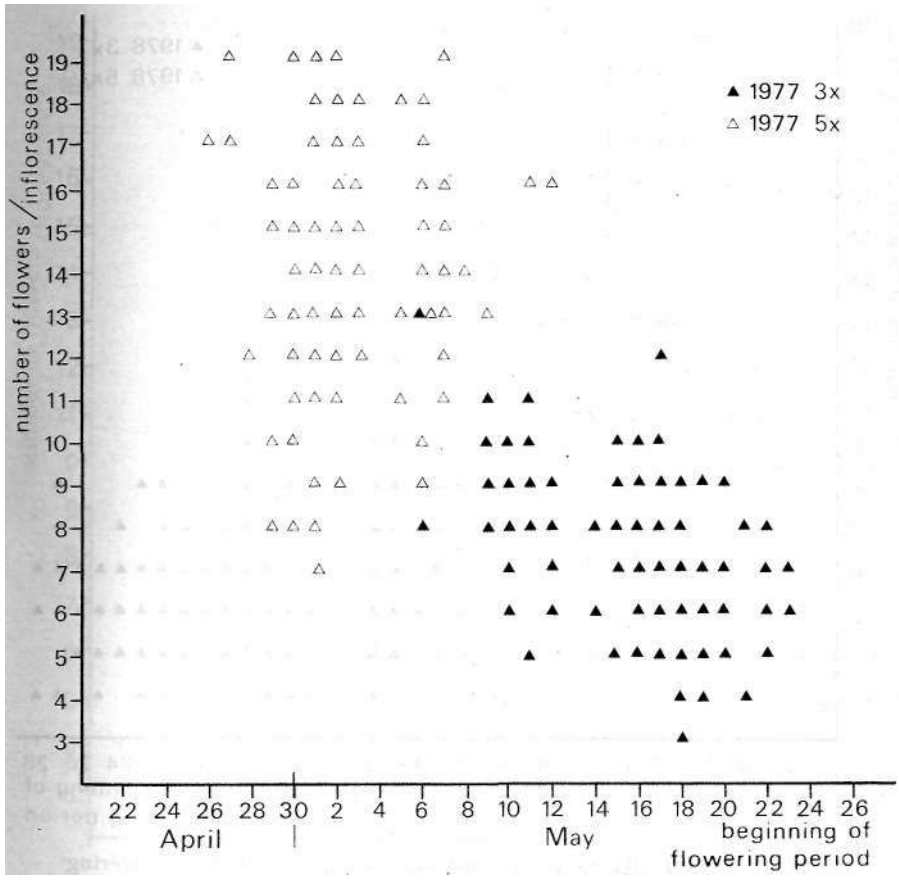


Fig. 24. — Scatter diagram showing the beginning of the flowering period of triploid and pentaploid plants in 1977, plotted against the number of flowers per inflorescence.

120° in two plants from Texel in 1979 (not shown in figure 12). This seems to confirm our supposition that the population from Texel was introduced from France.

j. Some French hexaploids have extremely long leaves (figures 9, 15, 16).

k. The hexaploids from Texel are more or less comparable with the hexaploids from the Dept. Var

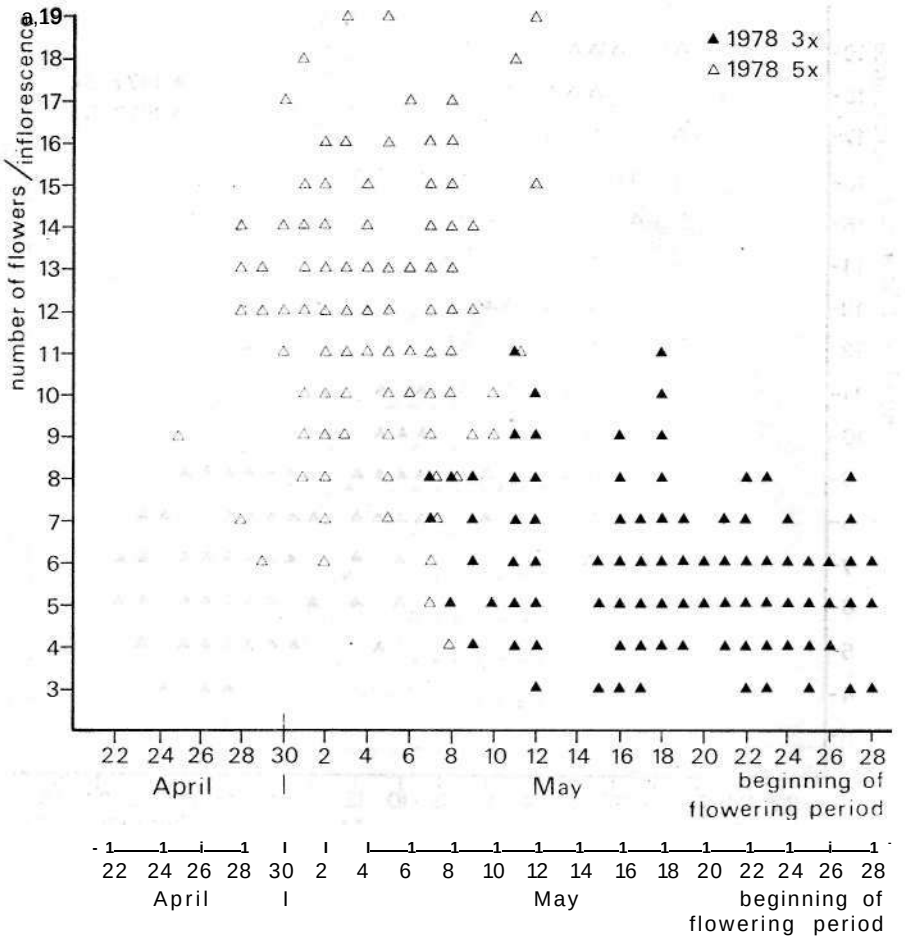


Fig. 25. — Scatter diagram showing the beginning of the flowering period of triploid and pentaploid plants in 1978, plotted against the number of flowers per inflorescence.

(S. France), see fig. 30. They flower rather early in comparison to most other hexaploids from the Netherlands (see fig. 30) but the two Dutch groups are not clearly separable.

1. The triploids are late with regard to both their leaf-emergence in autumn (in spite of a considerable range of dates for leaf-emergence) and their first date of flowering in the following spring. Penta- and hexaploids flower early and their leaves are early visible in the autumn (figure 22).

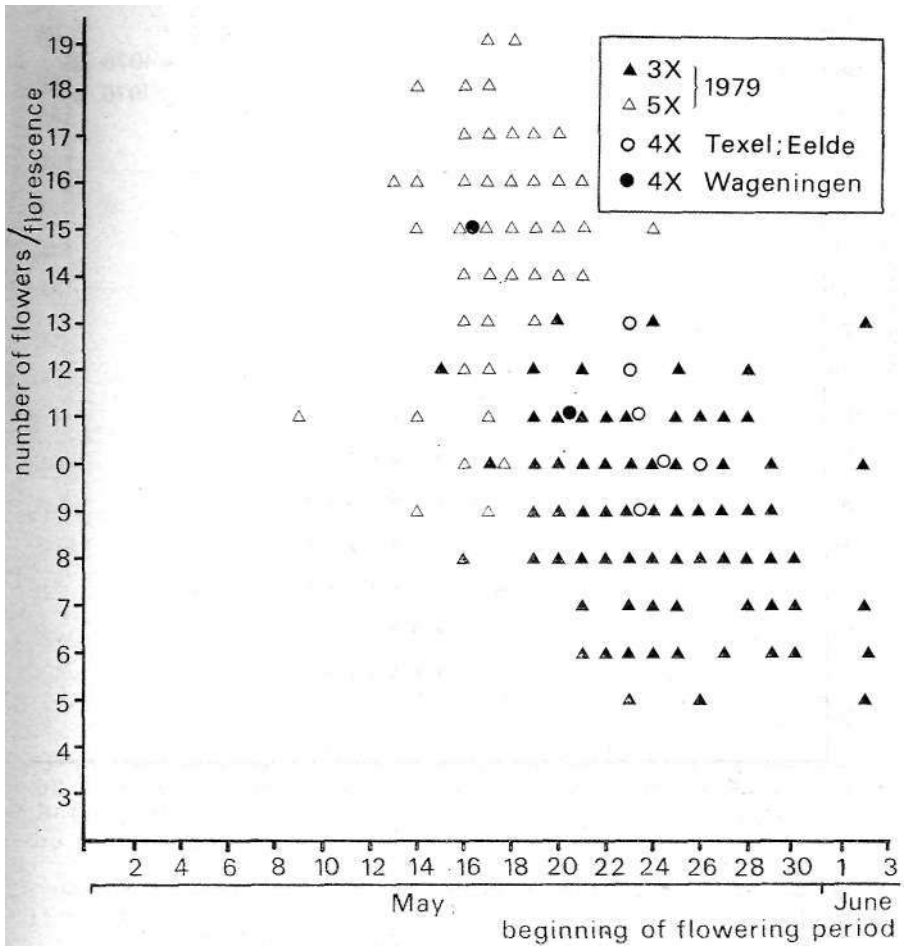
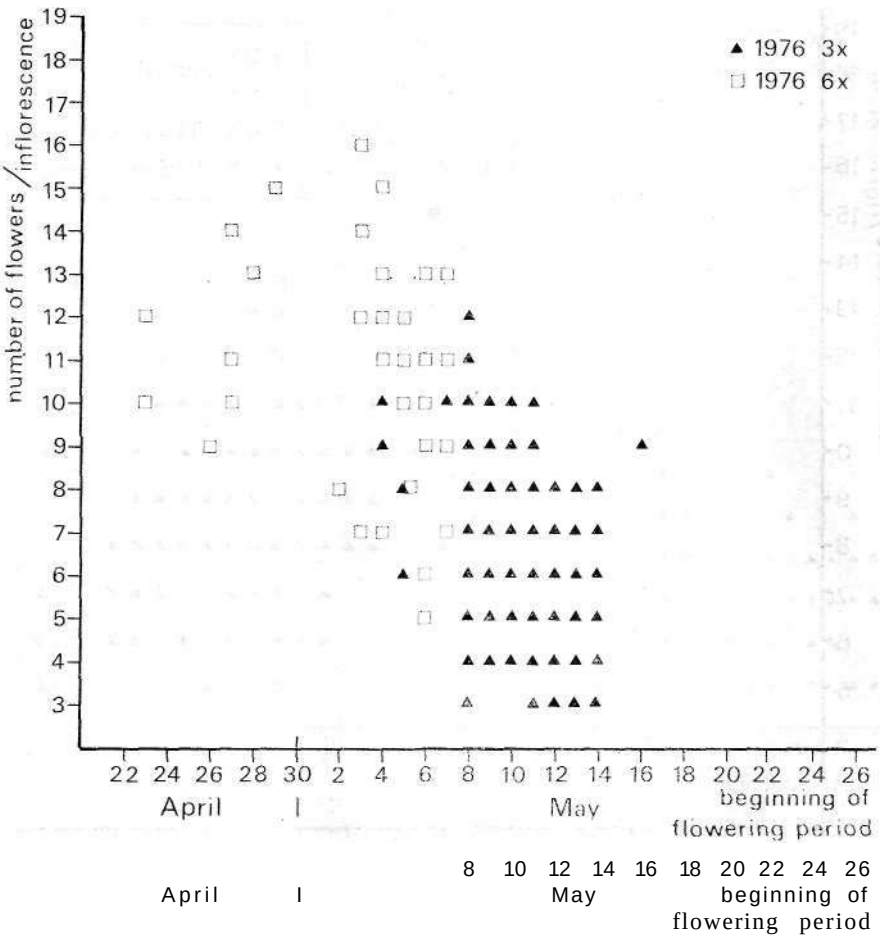


Fig. 26. — Scatter diagram showing the beginning of the flowering period of tri-, tetra- and pentaploid plants in 1979, plotted against the number of flowers per inflorescence.

m. Diploids were not compared, with other cytotypes in the scatter diagrams because they were included in the collection in 1977 and flowered for the first time in the collection in the Botanical Garden in 1978. Most scatter diagrams refer to the situation in 1977.

n. In figures 23-26 the relationship between the beginning of the flowering period and the number of



BHg. 27.— Scatter diagram showing the beginning of the flowered period of triploid and hexaploid plants in 1976, plotted against the number of flowers per inflorescence.

flowers per inflorescence is shown for the triploids and pentaploids during the years 1976-1979. In the years 1976-1978 the groups of triploids and pentaploids were more clearly distinguishable than in 1979. The beginning of the flowering period of the pentaploids in 1979 is comparable to that of the triploids in 1977. Therefore it is inappropriate to compare the beginning of flowering of plants belonging to different cytotypes for different years; care should be taken

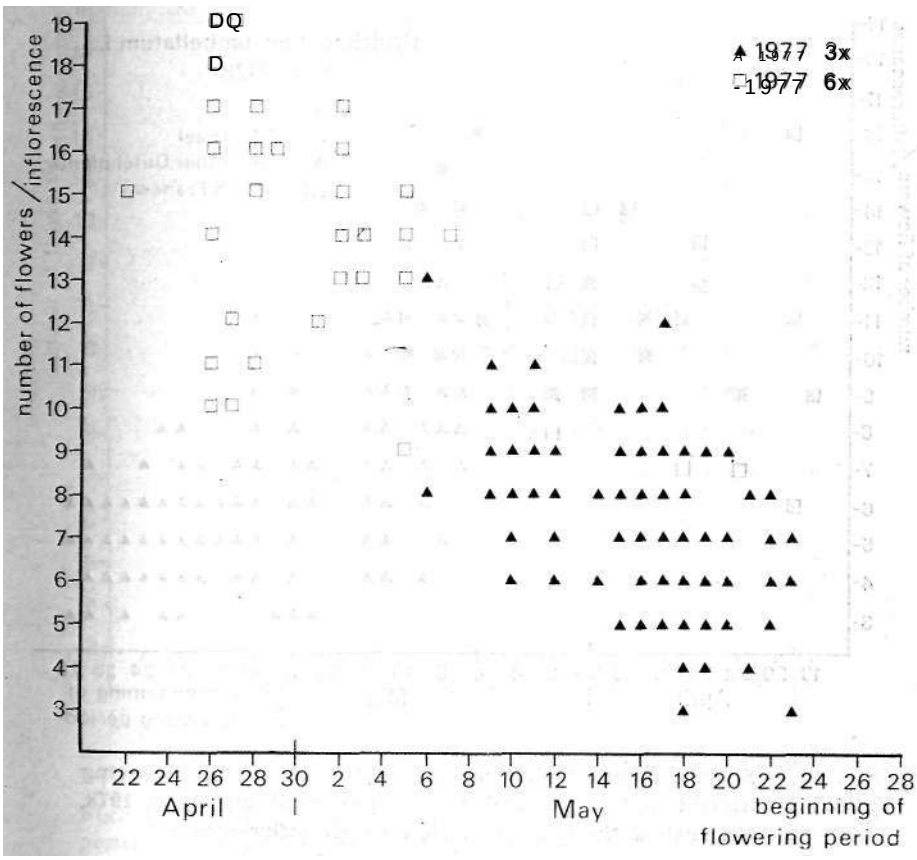


Fig. 28. — Scatter diagram showing the beginning of the flowering period of triploid and hexaploid plants in 1977, plotted against the number of flowers per inflorescence.

that comparisons are made between different cytotypes in the same year,

o. Figures 27-30 demonstrate the same aspects as figures 23-26 but for triploids and hexaploids, as described sub n. Usually there is a difference between these two cytotypes, but here too care should be taken to compare cytotypes in the same year.

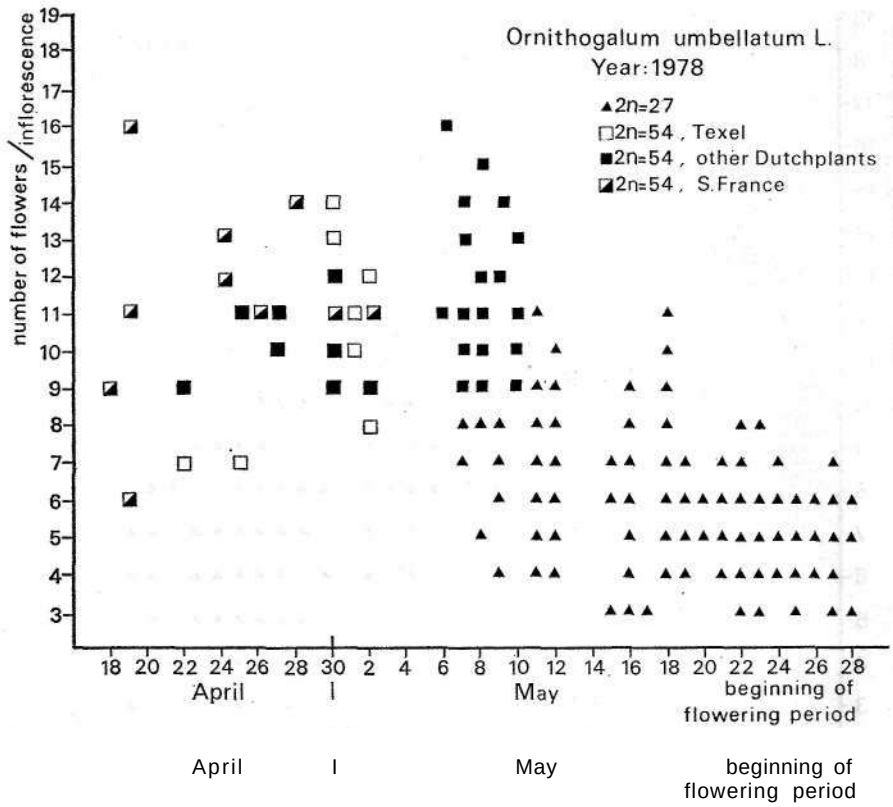
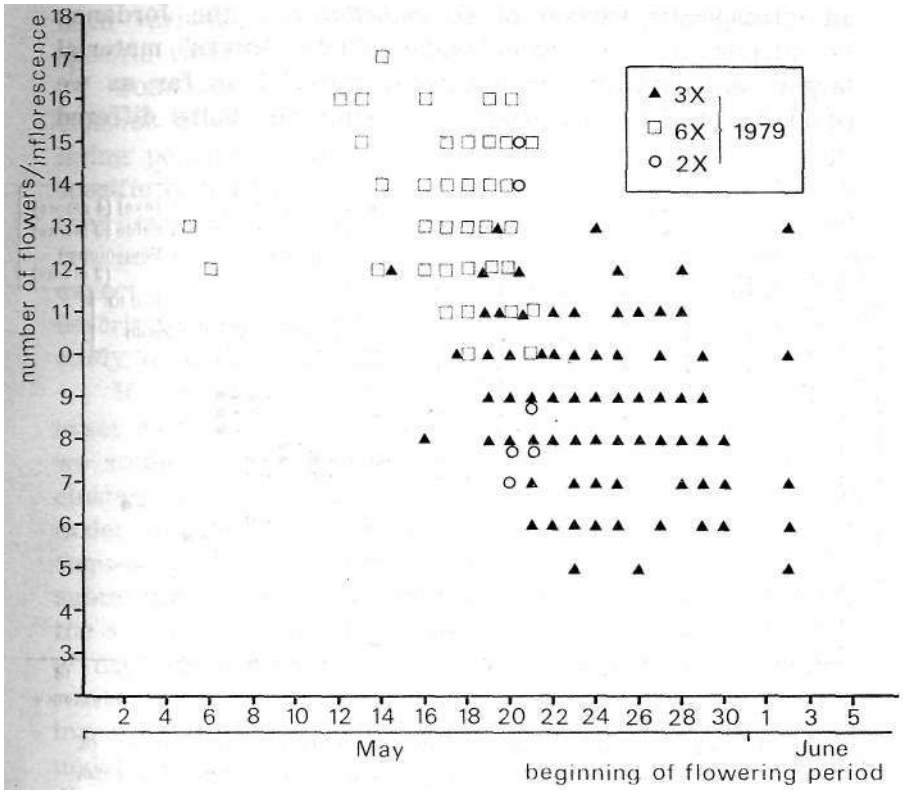


Fig. 29. — Scatter diagram showing the beginning of the flowering period of triploid and Dutch and French hexaploid plants in 1978, plotted against the number of flowers per inflorescence.

IV. DISCUSSION

From the results obtained it is clear that combined cytological and comparative morphological studies revealed constant differences between triploids and penta-/hexaploids. One group of tetraploids clearly belongs to the same group as the triploid plants, a second tetraploid group can be assigned to the penta-/hexaploid group. The two plants from Wageningen (both tetraploid) were somewhat different; both matched the description of the penta-/hexaploid group in most characters, but one of them, being intermediate in a certain number of characters, might be of hybrid derivation.



Pig. 30. — Scatter diagram showing the beginning of the flowering period of diploid, triploid and hexaploid plants in 1979, plotted against the number of flowers per inflorescence.

The diploids are intermediate in some characters, distinct in others or they resemble the penta-/hexaploid group.

Most authors, when treating the variability of the species complex, reach different taxonomic conclusions. Our results seem to indicate that at least the triploid group on the one hand and the penta-/hexaploid group on the other deserve taxonomic recognition. Two groups of plants, however, which make this distinction very difficult, are in the first place the diploid French plants and in the second place the diploid plants from the Botanical Garden of Palermo, studied by NEVES (1956). He considered these diploid plants to be

an intraspecific variant of *0. umbellatum :* the Jordanon (= microspecies) *0. paterfamilias* Godr. NEVES' material largely agrees with our hexaploid material as far as we could deduce from his description. Only the bulbs differed

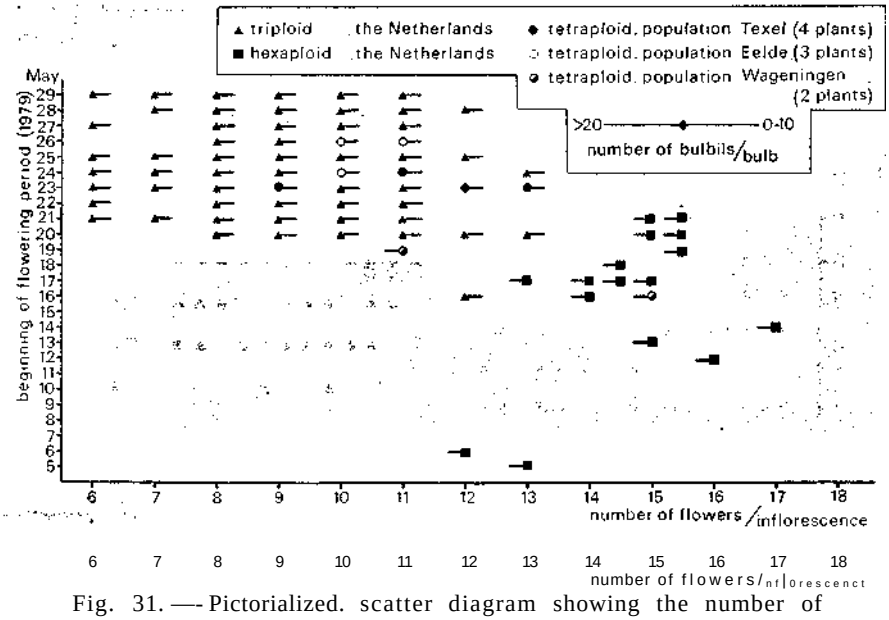


Fig. 31. —- Pictorialized. scatter diagram showing the number of flowers per inflorescence of the tri-, tetra- and hexaploid plants, plotted against the beginning of the flowering period, with an indication of the number of bulbils per bulb.

from our hexaploids in that they produced bulbils with leaves in their first year. His plants differed from our diploid material in having more bulbils and in the pedicels that are reflected after anthesis. The microspecies, *0. paterfamilias* Godron, was treated by BAKER (1873), who studied the type material of GODRON only. The taxon seems to be rare and should be studied in more detail.

According to NEVES (1956) the taxa *O. divergens* Bor. and *0. paterfamilias* Godr. are distinguishable both on karyological and morphological grounds, but NEVES failed to separate *0. divergens* Bor. and *O. paterfamilias* Godr. unambiguously from *O. umbellatum.* For that reason he included

both variants in *0. umbellatum,* treating them as infraspecific variants only.

TORNADORE and GABBARI (1979) arrived at similar conclusions after studying many herbarium sheets and some living populations in Italy, but they did not define infraspecific taxa because of the exterme morphological variation they encountered. Moreover, these authors did not find tri- and tetraploids in Italy, but it seems reasonable to expect that these cytotypes do occur there in view of the descriptions given in their paper, which are based on the study of herbarium material.

If we ignore the aberrant diploid plants of NEVES, the exact wild origin of which could not be traced, the material we studied can be divided into three groups: diploids, and clusters A and B (see tables 1, 2 and the descriptions given under the heading «results»). NEVES-' descriptions of Portuguese cytotypes agree largely with ours as far as the subterranean parts are concerned. He omitted to describe the aerial parts, which is regrettable from the point of view of drawing comparisons. We conclude that at any rate the relation between diploids and triploids requires more detailed investigation. CZAPIK (1986) concluded from her studies that under favourable conditions triploids could give rise to diploids, triploids, tetraploids as well as to aneuploids and that these types would be able to persist in the population by vegetative propagation. She found one mixed diploid/ triploid population in a Polish cornfield and, in addition, among 100 seedlings obtained after open and controlled pollination of a triploid plant, she found the chromosome numbers 18 to 30 and 32 to 36 chromosomes in fixed embryos. From her experiments it becomes clear that at least Polish diploids, triploids and tetraploids may belong to the same cluster. Unfortunately, CZAPIK does not give detailed descriptions of these plants; if she had done so we might have been able to compare our plants with her Polish material. The study of the material from the Island of Texel (see fig. 3) pointed to a very close affinity between tetraploid and triploid plants, or even to the derivation of the tetraploids from the triploids. These factors, however, have still

to be tested experimentally. The relationship of the French diploid plants to the W. European triploids requires further study.

Clearly We need more chromosome counts, supplemented by morphological studies and crossing experiments before we can arrive at a better understanding of the species complex as a whole. We are, however, of the opinion that the penta-/hexaploid group deserves taxonomic recognition and propose to separate these groups from the group to which the triploids are assigned. There is doubt about the taxonomic rank of the two groups. We are of the opinion that specific rank seems most appropriate in view of the extreme scarcity of transitional forms. Our conclusion is: the triploid cytotype (with some rare tetraploids) belongs to the species *O. umbellatum* L. and the penta-/hexaploid cytotypes (with some rare tetraploids) should be assigned to *O. divergens* Bor. The description of *O. divergens* should be based on the data given in table 1. The description of *O. divergens* in various Floras should be modified accordingly. The position of the diploids deserves further study.

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APPENDIX

List of cytologically studied plants of Ornithogalum umbellatum L. s. 1.

2n = 18.

France. Plampinet-Nevaehe (dept. Ht. Alpes, 1500 mtr), 21598A-E; between Valloire and the Col du Galabier (dept. Savoie, 1700 mtr), 21599, 21600.

Ornithogalum umbellatum L. s. str.

2n = 27.

Belgium. Sint Maartensvoeren, 19018-19020.

Denmark. Mens Klint, 13018, 13019; Stege, island of Man, 13020; Tvedkirke, 19217.

Great Britain. Perthshire, Scotland, 13377A-E.

Netherlands, province of Friesland. Dronrijp, 14744-14746; Mirdum, 14738-14740; between Mirdum and Balk, 14735-14737; Nijemirdum, 14732-14734, Oosterwolde, 14717-14719; Oudkerk, 14753-14755; Vlieland, Oude eendekooi, 17487, 17488.

Netherlands, province of Drenthe. Borger, 14764-14769; Diever, 14471-14473; Kolderveen, 14729-14731.

Netherlands, province of Overijssel. Exact provenance unknown, 21068; Denenkamp, 9253; Genne, Huis den Doom, 20990-20993; Genneger Buitenland, 11884, 11886, 11888, 11892; Genne Overwaters, 20994-20996; Hazelbekke near Vasse, 12631; between Holten and Streukel, 11882; Ommen, 14773-14775; Rheeze, 14770-14772; between Weerselo and Deurningen, 12976; Zalk, 11863; de Zande, 14720-14722; de Zijtkolk near Hasselt, 11869, 11870.

Netherlands, province of Gelderland. Ammerzoden, 20871-20873; Appeltern, 14835, 14836; Batenburg, 15921-15923; Bekendelle near Winterswijk, 12665; Bergharen, 15929, 15930; Brakel, 20883-20885; Bronkhorst, 15834, 15835; Deil, 20868-20870; Doesburg, 15868-15870; Echteld, 15939, 15940; Ellecom, 12663; 's Heerenberg, 15797-15802; Hemmen, 12673-12675; Hernen, 15931-15933; Hoog Keppel, 15861, 15862, 15865-15867; Hummelo, 15846-15848; Kotte, 15813, 15814; Loevestijn I, 20877-20879; Loevestijn III, 20886-20888; Meulunteren, 9246; South of Nederhemert, I, 20874-20876; South of Nederhemert, II, 20889-20891; Ratum, 15820, 15821; Ruurlo, 15822-15826; Slijk, 12678-12680; Veessen, 20975-20981; Wagenin-

gen, 17516-17519, 17522-17525, 18965-18967, 18969, 18971, 18972, 18975-18977.

Netherlands, province of Utrecht. Amerongen, castle, 15787-15789; De Bilt, Sandwijck, 13258; Leersum, castle Broekhuizen, 18978, 18979; Linschoten, 15884, 15885; between Oudewater and Haastrecht, 15882, 15883; Wijk bij Duurstede, 15778-15780:

Netherlands, province of Noord-Holland. Bergen, 9203, 9204, 14506, 14507; between Bergen and Wimmenum, 14518, 14519; between Egmond-binnen and Castricum, 14537, 14538; 't Gooi, 18587; Hilversum, Corvers Bos, 14828, 14829; Santpoort, 14554-14556; Schoorl, 9198-9201; Texel, Alloo, 9234-9236, 16000; Texel, Biesbosch, 9237-9239, 20915-20918, 20947, 20-948; Texel, north of Californie, 20903-20908; Texel, Cocksdorp, 20183; Texel, Heidehof, 20913, 20914; Texel, Hoge Berg, 20939, 20940; Texel, de Koog, 9231-9233; Texel, de Krim, 20945, 20946; Texel, Loodsmansduin, 20935, 20936; Texel, Oost, 12628, 12629; Texel, north of Pelikaanweg, 20895-20902; Texel, south of Pelikaanweg, 20909-20912; Texel, Schansweg, 20937, 20938, 20941, 20942; Texel, Skillepaadje, 20931, 20932; Texel, Tureluursweg, 20923-20928, 20943, 20944, 21048-21053, 21056-21060; Texel, vuurtoren, 9240-9242, 20933, 20934; Texel, de Waal, 20182; Texel, Westerslag, 20919, 20920; Vogelenzang, 14389, 14390; between Wimmenum and Egmond, 14520, 14521.

Netherlands, province of Zuid-Holland. Alphen aan de Rijn, 11169-11171; Duinrell 16250, 16251; Leiden, Rhijnhof, 16248, 16249; Valkenburg, 16253-16255.

Netherlands, province of Zeeland. Domburg, 15732, 15733; between Domburg and Westhove, 15730, 15731; Haamstede, 15684-15687, 15695-15698; Renesse, 15699-15702; Schuddebeurs, 15670-15672; Westenschouwen, 15718, 15719.

Netherlands, province of Noord-Brabant. 's Gravenmoer, 15967, 15968; Hoogerheide, 15740, 15741; Nuenen, 13339-13346.

Netherlands, province of Limburg. Beesel, 15894-15896; Cadier en Keer, 19017; Geleen, 16207, 16208; between Houthem and Valkenburg, 15952, 15953; along the Juliana canal near the mouth of the river Geul, 14441-14445; Kunderberg (200mtr), 16227, 19015, 19016; Oirsbeek, 16209, 16210; Oud-Valkenburg, 15790, 15791, 21590; Savelsbos 15692; Ubachsberg (200 mtr), 15956, 15957; Valkenburg, 19494; Voerendaal, 19493; Wijnandsrade, 21592, 21593.

West-Germany. **Lintorf, 14855, 14856.**

2n = 36.

Netherlands, province of Drenthe. Eelde, 14761-14763.

Netherlands, province of Noord-Holland. Texel, Tureluursweg, **20921, 20922.**

Ornithogalum divergens Bor.

2n = 36.

France. Col du Canadel, near la Môle (dept. Var), 16023-16025.
Netherlands, province of Gelderland. Wageningen, 18968, 18974.

2n = 45.

Austria. Kompelstein, 10736.
France. Toulon-Hyères (dept. Var), 17536.
Netherlands, province of Friesland. Dokkum, 14756-14758; Marssum, 14750, 14751; Oost-vlieland 17489, 17490.
Netherlands, province of Overijssel. Deventer, de Worp, 20961-20964; south of Haerst, 20987-20989; north of Welsum, 20973, 20974; south of Nerven, 20982-20986; Wilsum, 20997-20999; a forest in the foreland near Zalk, 21000-21002; de Zande, 14723-14728.
Netherlands, province of Gelderland. Bronkhorst, 15832, 15833; Loevestijn II, 20880-20882; Renkum, 15220-15222; Terwolde, 20965-20968; north of Terwolde, 20969-20972; Voorst, 20955-20960; Wageningen, 17520, 17521, 18970, 18973,
Netherlands, province of Utrecht. Amerongen, 15781-15786; along the Eem, opposite to the Grote Melm, 15871-15881.
Netherlands, province of Noord-Holland. Groet, 14479, 14480; Hoorn, 17483, 17484.
Netherlands, province of Zuid-Holland. Den Haag, 16230-16233; Duinrell, 16238-16247, 16252; along the Merwede, 12925, 14208-14222; Oostvoorne, 14820-14825; Schoonhoven, 17471-17474 Wassenaar-Zuidwrjk, 16234-16237.
Netherlands, province of Zeeland. Haamstede, 15688, 15689.
Netherlands, province of Noord-Brabant. Ravenstein, 15917-15920.

2n = 54.

Denmark. Ebeltoft, 13169, 13170.
France. Cap Camarat (dept. Var), 16026; Col Blanche near Draguignan (dept. Var, 200 mtr), 16027, 16028; Fayence (dept. Var, 400mtr), 16011, 16012; Frejus (dept. Var), 16015, 16016; Grimaud (dept. Var), 16019, 16020; Montélimar (dept. Drôme), 17558; la Motte (dept. Var), 16013, 16014, 16017, 16018; between la Motte and le Muy (dept. Var), 16029-16032; Ramatuelle (dept. Var, 200 mtr), 21621-21625.
Italy. Galzignano, 17540,
Netherlands, province of Friesland. Franeker, 14741-14743; Marssum, 14747-14749, 14752; Veenwouden, 14759, 14760.
Netherlands, province of Gelderland. Wageningen, 14785, 14786.
Netherlands, province of Utrecht. De Bilt, 15958, 15959.

Netherlands, province of Noord-Holland. Texel, Oudeschild, 20929, 20930; Texel, north of Pelikaanweg, 21061; Texel, Tureluursweg, 19051-19060.

Netherlands, province of Zuid-Holland. Leiden, Rhijnhof, 16228, 16229; Leidse Hout, 14351-14353; Rijpwetering, 12980, 12981, 12983-12986; Sassenheim, 10681-10685.

Netherlands, province of Zeeland. Walcheren, 11235-11239.

Portugal. Lisboa, 15960.

Yugoslavia. Exact provenance unknown, 19001, 19002.

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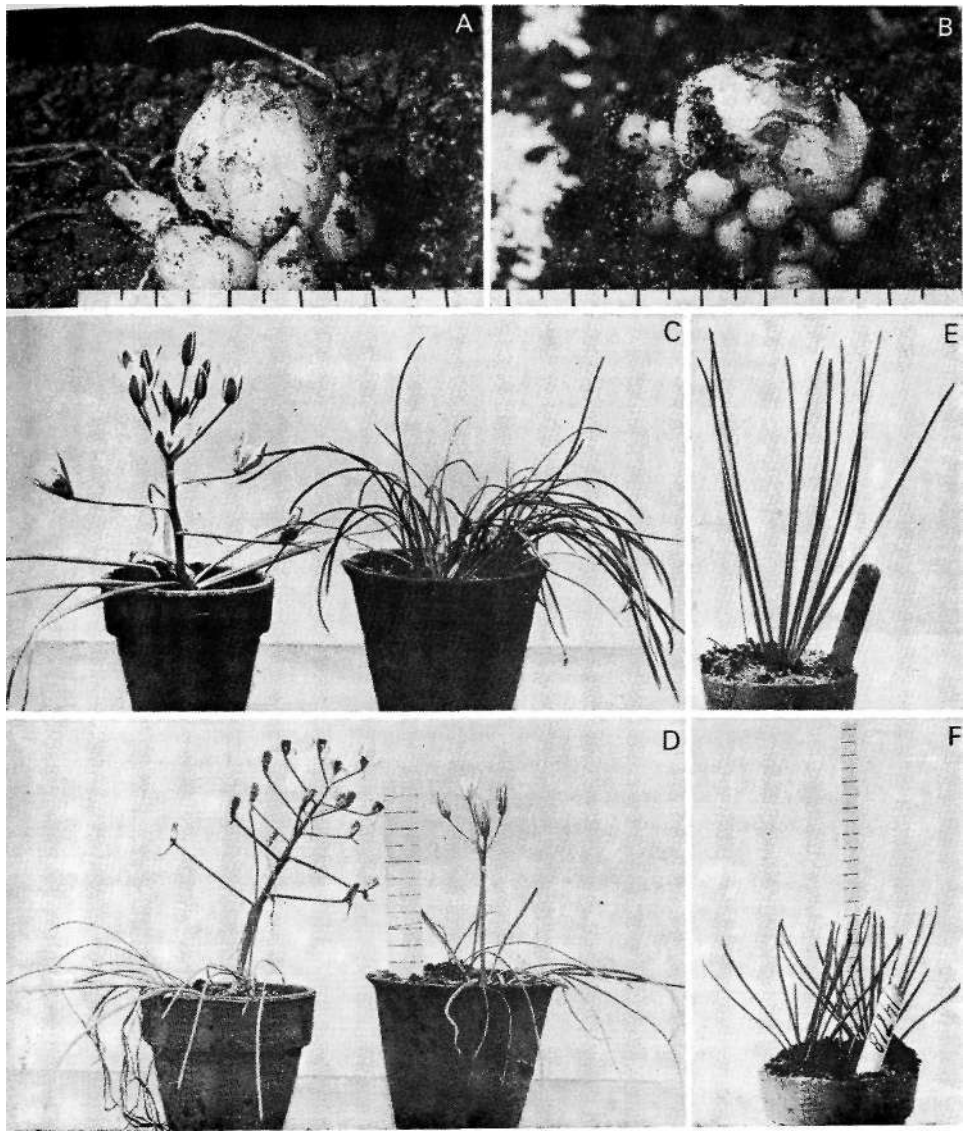

Photographs showing: a. a bulb with bulbils of a triploid plant; b. a bulb with bulbils of a pentaploid plant; c. a triploid and hexaploid plant (May 6th 1971); d. the same plants as illustrated in fig. c (May 19th 1971); e. leaves of a hexaploid plants; f. leaves of a triploid plant.

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COMPARATIVE ECOLOGICAL STUDY OF THE CHROMOSOME RACES IN CERTAIN ROOT PARASITIC PLANTS OF THE SOUTHEASTERN UNITED STATES OF AMERICA *

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SUMMARY

Whichever hemiparasites or hosts are annual, they reassociate with each other randomly every year in temporary unpredictable environments. Some annual hemiparasites have no specific selection of hosts because they might have *to* be automatically, annually preadapted to important segments of new biotic environments. In contrast, autotrophic vigor and its ability of the hemiparasitic plants, particularly annual hemiparasites, could be essential importance maintaining adaptation of individuals, chromosome races, or species to specific habitats. Some species can mature, flower, and set seeds without host contact. The annual hemiparasitic plants have more different chromosome races and more variable karyotypes than the perennial ones do. The chromosome races of all of the four species of the perennial hemiparasites seem to have the common basic chromosome number of X = 6, and to always parasitize woody perennial host species. Their host ranges are mostly narrow.

* This paper is dedicated to Professor ABÍLIO FERNANDES, Botanical Institute of Coimbra University. Because of his contributions in the botany world, this honor could not be awarded to a person more deserving. This article represents field and laboratory work leading to our series entitled «Karyomorphological comparisons in the parasitic species of the Scrophulariaceae» [see *Brittonia* 30: 345-354 (1978)].

INTRODUCTION

AT least twenty-nine species of root hemiparasitic plants representing 18 genera and five families are known in the southeastern United States of America (MUSSEUMAN and MANN, 1978). The hemiparasitic habits were first described for these species, beginning with the original paper by PENNELL (1935) and continuing into the late 1960's and 1970's. Two main characteristics are common to all hemiparasitic plants: (1) the presence of the haustorium, the connective structure between parasite and host as a heterotrophic component; (2) the presence of functional chlorophyll as an autotrophic component.

Anatomical studies of the haustorium development in the hemiparasitic plants of the southeastern United States have been well done by MUSSELMAN and DICKISON (1975). They said, «Early stages in the development of the haustorium are exogenous. Initial periclinal divisions in the epidermis or outer cortex are followed by hypertrophy of cortical parenchyma. These events are followed by development of the vascular core from the pericycle, attachment of haustorium to the host by a specialized layer of cementing cells or root hairs, and penetration of the host by dissolution of host cells». Ecological views of, root parasitism in *Castillejo, coccínea* (L.) Spreng, and *Pedicularis canadensis* L. have been well documented by MALCOLM (1966) and PiEHl, (1963) respectively with special reference to the ,parasite's life history, their host ranges, the connections they make with hosts, and the effects of parasitism on them and their hosts. Damage excess to host plants often arises in discussion of parasitism. Some green root parasites are harmful agricultural, silvicultural and forest plantation pathogens; in the southeastern United States of America, *Striga asiática* (L.) Kuntze introduced from Africa does seriously damage to corn production *(MALCOLM,* 1966), and *Seymeria cassioides* (J. F. Gmelin) Blake does heavily damage and kill three- to four-year-old plants of *Pinus elliottii* Engelm. in plantations (MANN, GRELEN and WillAMSON, 1969). However, most of the other native hemiparasitic species cannot show satis-

factory, distinctively damage to their hosts until it is found what those hemiparasites take from their hosts, hut various problems have been investigated indirectly (PIEHL, 1963; MALCOLM, 1966; KUIJT, 1969; MUSSELMAN and MANN, 1978). A pot culture test with 19 experimental, young, commercial tree species as hosts including several that do not occur in the distribution ranges of the hemiparasites and 19 hemiparasitic species was carefully made by MUSSELMAN and MANN (1978). According to this experiment, all the six species of *Agalinis* used showed to have the broadest host range of any root parasite in the southeastern United States of America. In some cases over 2000 haustoria were found by one parasitic plant on a single one-year-old host in six months. Then, they concluded that the pathogenic potential of *Agalinis* was great.

Hemiparasitic plants grow as annuals, biennials, perennials, woody shrubs, vines, and trees in various habitats from tropical rain forests to arctic and alpine tundra fields. The functional nature of every host-parasite relationship must be related to ecological, biotic and abiotic environmental parameters, among which life history, chromosome race, breeding system, host characteristics, nutritional relations, social relations, seed dispersal mechanism, distribution and physical habitat, climatic relations, and edaphic relations are important. On the other hand, many of the hemiparasitic plants of the world appear to be characterized by descending aneuploidy. When present in the aneuploid series, the individual chromosomes of the complement have a distinct effect on cellular processes and developmental rhythm of the individual plant or individual species. These effects are detected in the altered morphology, anatomy, physiology, life history, eco-geographical distribution pattern, and habitat selection. In *Claytonia* extensive aneuploid complex is inversely proportional to remarkably narrow range of morphological and ecological characteristics (ROTHWELL and KUMP, 1965; LEWIS, OLIVER and SUDA, 1967). Thus, experiments are conducted to determine whether or not aneuploid races of the hemiparasitic species in the southeastern

United States of America confer tolerance to certain range of ecological or environmental conditions.

MATEBIALS AND METHODS

The materials are listed with identifying voucher specimen numbers in Table I. The voucher specimens are deposited in the Herbarium of Old Dominion University (ODU).

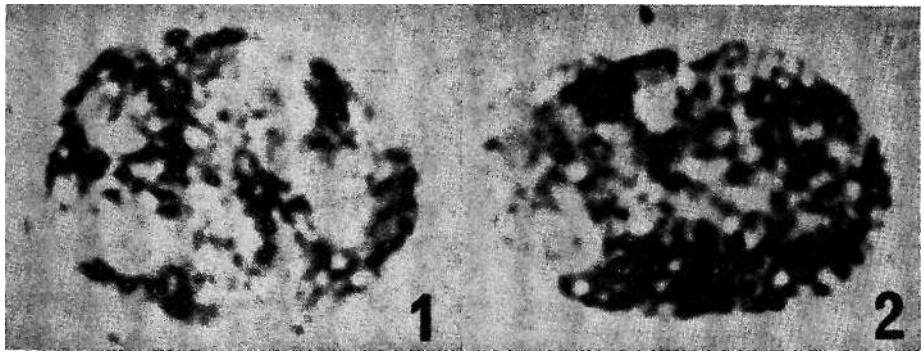
Root-tips were utilized for the study of chromosome races. The root-tips were treated with 0.002 mol. 8-Hydroxyquinoline for two hours at 18° C. They were fixed in 45 % acetic acid at 4^0C for 15 minutes, then hydrolyzed in 2:1 mixture of 1N-hydrochloric acid and 45% acetic acid at 60° C for seven seconds, and then stained and squashed in 1 % aceto-orcein. Ten cells from each individual plant were investigated to get certain chromosome number. Nuclei at interphase in somatic cells were classified according to TANAKA'S (1971) classification of resting nuclei.

Nomenclature of each hemiparasitic plant and host plant studied followed that of RADFORD, AHLES and BELL (1968), and life history, distribution, and habitat of each species followed those studied by MUSSELMAN and MANN (1978) and KONDO (unpublished).

RESULTS AND DISCUSSION

Karyomorphology of one species of *Krameria* in the Krameriaceae, one species of *Ximenia* in the Olacaceae, one species of *Comandra* in the Santalaceae, and nine species of *Agalinis,* two species of *Aureolaria,* one species of *Buchnera,* one species of *Macranthera,* one species of *Schwalbea,* two species of *Seymeria,* one species of *Striga,* and one species of *Castilleja* all in the Scrophulariaceae was investigated in this study (Table I). The karyotype characteristics were based on the data of all measurements of somatic chromosomes at midmetaphase (much of the data collected is not reported due to page limitations).

The interphase nuclei of all clones of the species were of the simple chromocentric type (e. *g.*, Figs. 1-2), which is characterized by light staining over the nucleus with some darkly stained heteropycnotic bodies termed chromocenters according to TANAKA (1971). Among the members of the Scrophulariaceae the hemiparasitic species containing the nuclei of the simple chromocentric type seem to be phylo-



Figs. 1-2.— (X 4000). «Simple chromocentric type» interphase nuclei of: 1. *Agalinis obtusifolia.* 2. *Buchnera americana.*

genetically placed in the advanced group of the family (KONDO, MUSSELMAN and MANN, 1978). Thus, much wider, present results explain that majority of the hemiparasitic plants of the southeastern United States contains the common karyomorphological type of nuclei, simple chromocentric type, although those species can be taxonomically, phylogenetically considered to be divided into four families and three orders; the Krameriaceae belongs to the order Polygalales, both the Olacaceae and the Santalaceae belong to the order Santalales, and the Scrophulariaceae belongs to the order Scrophulariales (CRONQUIST, 1968). The two orders, Polygalales and Santalales, are classified into the subclass Rosidae, while the order Scrophulariales is classified into the subclass Asteridae according to the CRONQUIST'S system (1968).

TABLE I

Comparisons of life history, host species, distribution, and habitats among the chromosome races of certain root parasitic plants studied

Species and source	Life history	Chromosome race (2n)	Host species	Distribution and habitat
Krameriaceae *Krameria lanceolata* Torr. Musselman s. n.	perennial	2n = 12	*Diospyros virginiana* *Opuntia* sp. *Pinus palustris* Miller *Quercus laevis* Walt. *e.t.c.*	Southern Georgia to central Florida; open, sunny, deep sand ridges to sandy flatwood
Olacaceae *Ximenia americana* L. No. 4821	shrubby perennial	2n = 24 *	any neighbouring plant	Central to southern Florida; sand pine scrub
Santalaceae *Comandra umbellata* (L.) Nuttall Kondo 249	perennial	2n = **26**	any neighbouring plant (over 200 species are known)	Virginia, North Carolina, Tennessee to central United States; open, sunny, dry areas, prairies, roadsides
Scrophulariaceae *Agalinis aphyîlla* (Nutt.) Raf. Rich 218, 226, Musselman and Harris 5002	annual	2n = 26	any neighbouring plant	Very rare plant; Atlantic and Gulf Coastal Plains from North Carolina to Louisiana; recently burned areas in pine savannas
Agalinis fasciculata (Ell.) Raf. Musselman and Harris 4992	annual	2n = 28	broad selection of hosts	Weedy plant; Virginia, North Carolina to Texas, most common on the Coastal Plain
Agalinis linifolia (Nutt.) Britt.	perennial	2n = 28	*Taxodium distichum* (L.) Richard	from North Carolina to Mississippi;

Agalinis linifolia (Nutt.) Britt. Rich 230	perennial	2n = 28	*Taxoäium distichum* (L.) Richard	Atlantic and Gulf Coastal Plains from North Carolina to Mississippi; margins of cypress swamp, shallow-ditches in pine savannas
Agalinis purpurea (L.) Penn. Rich 203	annual	2n = 28	no particular selection of hosts (often grow without any host)	One of the most common species of the genus; throughout the southeastern United States; roadsides or other disturbed areas
Agalinis setacea (J. F. Gmelin) Raf. Rich 247, 312, Musselman and Harris 4990	annual	2n = 28	no particular selection of hosts (often grow without any host)	Atlantic Coastal Plain; roadsides or disturbed areas
Agalinis tenella Penn. Rich 214	annual	2n = 28 *	broad selection of hosts	Atlantic Coastal Plain; roadsides or disturbed areas in dry pine-lands
Agalinis obtusifolia Raf. j Rich 219	annual	2n = 30 *	no definite hosts present	Atlantic Coastal Plain; pine savannas and disturbed habitats
Agalinis tenuifolia (Vahl) Raf. Rich 202, Musselman and Harris 4979	annual	2n = 28 *	various hardwood species and pines	Throughout eastern North America; clay roadsides, wooded slopes
Agalinis virgata Raf. Rich 213, 214	annual	2n = 28	no particular selection of hosts	Rare species; Atlantic Coastal Plain, widely scattered localities; pine savannas

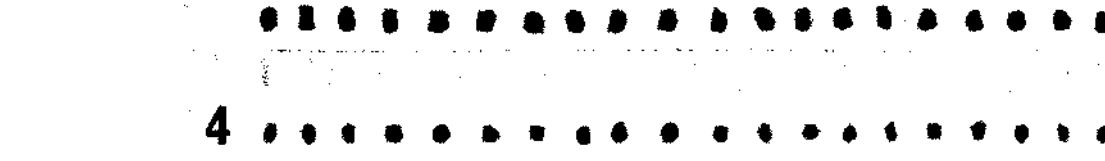
* The chromosome numbers recorded here for the first time.

TABLE I *(Continuation)*

Species and source	Life history	Chromosome race (2n)	Host species	Distribution and habitat
Aureolaria flava (L.) Farwell • r:; Rieh 112.	perennial	2n = 24	various species of *Quercus*	Most widespread species of the genus; Virginia, central Florida to. eastern Louisiana; margin of oak stands, open, dry disturbed áreas
Aureolaria pedicularia (L.) Raf Musselman 4869, Musselman and Harris 4974, 4986	annual or biennial	2n = 28	*Quercus velutina* Lam.	Common species; throughout the eastern United States; open, dry, sand hills or sandy roadsides, man-made disturbed areas, deciduous woodlands
Budinera americana L. Musselman 4693, 5018	biennial	2n = 4 t *	no specific selection of hosts (often grow without any host)	Throughout the southern to centrai United States; moist, sunny, sandy soils, meadow, stream margins, in savannas and piedmont areas
Macranthera flammea (Bartr.) Penn. Rich 209, Musselman and Harris 4998	biennial	2n = 26	*Nyssa sylvatica* Marshall	Endemic to Gulf Coastal Plain from northwestern Florida to southeas-tern Louisiana; open, sunny, *Nyssa* swamp margins
Schwalbea americana L. Musselman 4868	perennial	2n = 36	various tree species	The rarest root parasitic species; each one population in Virginia, Tennessee, and New Jersey, Beau-fort, Charleston, and Horry Cos. of South Carolina, Coastal Plain of North Carolina, Georgia, Ala-bama, Mississippi, and Louisiana; open, sunny, barren, pine savannas or pine woodlands

				open, summy, barren, pine savannas or pine woodlands
Seymeria cassioides (J. F. Gmelin) Blake Muaselman s. *n.*	annual	2n = 26	*Pinus elliottii* Engelm., and other commercial species of *Pinus* (heavily damage to host, commercial trees in plantations)	Throughout the southeastern United States; open, sandhills, upland swamp margins and roadsides in savannas
Seymeria pectinata Pursh Musselman *s. n.,* Musselman and Harris 5016	annual	2n= 26	*Pinus ellittii* Engelm., *Pinus palustris* Miller, *Pinus serótina* Michaux, *Pinus taeda* L, *Populus heterophyïla* L., *Nyssa sylvatica* Marsh., *Carya illinoensis* (Wang.) K. Koch, *e.t.c.*	Narrow populations in North Carolina and South Carolina, Atlantic and Gulf Coastal Plains of Georgia, Florida, Alabama, and Mississippi; *Quercus laevis* scrubs or other oak woods in savannas
Striga asiática (L.) Kuntze Musselman s. *n.*	annual	2n = 24	*Zea mays* L. and other various crops and grasses in the Poaceae and the Cyperaceae	Scattered localities; Coastal Plains in the southeastern United States; cultivated fields and neighbouring-disturbed areas (introduced root parasitic plant)
Castileja coccínea (L.) Spreng. Musselman 4864, 4867	annual or biennial	2n = 46	various vascular species (no particular selection of hosts)	The southeastern United States; meadows, roadsides, and woodland margins

Karyotypes, including chromosome numbers, of five species were newly reported here: 2n = 24 = 24 m for *Ximenia americana* L., 2n=28=24 m + 2 m^{sat} + 2 sm for *Agalinis tenella* Penn., 2n = 30 = 27 m + lmsat + 2 sm for *A. obtusifolia* Raf. (Fig. 3), 2n = 28 = 24 m + 2 msat + 2 sm for *A. tenuifolia* (Vahl) Raf., and 2n = 40 = 40 m for *Buchnera americana* L. (Fig. 4) ; and those of 16 species verified the



Figs. 3-4.— (X 290,0). Karyotypes at mldmetaphase in: 3. *Agalinis obtusifolia,* 2n = 30 = 27 m + 1 msat + 2 sm. 4. *Buchnera americana,* 2n = 4O = 40 m. The symbols for the karyotype descriptions are as follows: m = metacentric chromosome with arm ratio $(= \frac{\text{long arm}}{\text{short arm}})$ of 1.0 to 1.7; sm = submetacentric chromosome with arm ratio of 1.8 to 3.0; st = subtelocentric chromosome with arm ratio of 3.1 to 7.0; t = telocentric chromosome with arm ratio of 7.1 or more; and sat = satellite.

previous reports in the literature: 2n = 12 = 12m for *Krameria lanceolata* Torr. (RAVEN, 1975), 2n = 26 for *Comandra umbellata* (L.) Nuttall (KONDO, 1972), 2n = 26 = 18m + 5 sm + 2 smNOR + 1 $_s$t for *Agalinis aphylla* (Nuttall) Raf., 2n = 28 = 24 m + 2 msat + 2 sm for *A. fasciculata* (Ell.) Raf., 2n = 28 = 27m + 1sm for *A.linifolia* (Nuttall) Britton,

2n = 28 = 24 m + 4 sm for *A. purpurea* (L.) Pennell, 2n = 28 = 20 m + 1 msat + 7_{sm} for *A. setacea* (J. F. Gmelin) Raf., 2n = 28 = 21 m + 7 sm for *A. virgata* Raf., 2n == 24 = 1 m + 1_{m}NOR + 8 sm + 14 st for *Aureolaria flava* (L.) Farwell, 2n=28=5 m +12 sm +1 smsat + 10 st for *A. pedicularia* (L.) Raf., 2n=46=38 m + 7 sm + 1 st for *Castilleja coccínea* (L.) Sprengel, 2n = 26 = 4 m + 5 sm + 17 st for *Macranthera flammea* (Bartram) Renn., 2n — 36 = 32 m + 4 sm for *Schwalbea americana* L., 2n = 26 = 2 m + 1 msat + 14 sm + 9 st for *Seymeria cassioides* (J. F. Gmelin) Bláke, 2n = 26 = 1 m + 4 sm + 2 smsat + 19 st for *S. pectinata* Pursh (KONDO, MUSSELMAN and MANN, 1978), and 2n = 24 for *Striga asiática* (L.) Kuntze (KONDO, 1973). Karyotypes of *Comandra umbellata* and *Striga asiática* could not be determined because of too small chromosomes. Thus, the twenty-one hemiparasitic species studied appeared to be characterized by descending aneuploidy with the chromosome numbers ranging from 2n = 12 to 2n = 46. The karyotypes given consisted of their own graded series of chromosomes which were different in size range, and they showed particularly karyotypie heteromorphology and homologous chromosomes being heteromorphic. Further karyomorphological comparisons and cytotaxonomic detections in relation to the recognized classification of those hemiparasitic genera followed mostly KONDO, MUSSELMAN and MANN (1978).

Concerning life history, the chromosome races of the hemiparasitic species with the somatic chromosome numbers of 2n = 26 and 2n = 28 were mostly annual (a few were biennial as well as annual, depending on where they were growing) and the others with other various chromosome numbers were perennial (Table I).

Among the species of *Agalinis* with 2n = 28, only a perennial species, *A. linifolia,* had the most host-selective haustoria parasitizing *Taxodiunt distichum* (L.) Richard in nature, and had the highest number of metacentric chromosomes indicating the highest karyotypic symmetry. Thus, the highest karyotypic symmetry in *Agalinis* might be correlated with specific selection of host and perennial life history. In contrast, the data given in the two species of *Aureolaria*

indicated just the opposite ; *A. flava* with 2n = 24 and perennial habit had higher karyotypic asymmetry and wider host range within the genus *Quercus* than. *A. pedicularia* with 2n = 28 and annual or biennial habit did (Table I).

The list of host species parasitized by the 21 hemiparasitic species in Table I has been made by growing single hosts in pots with root parasites (MUSSELMAN and MANN, 1978) and by identifying the host plants attacked by haustoria during some of our field works. It was considered that fourteen hemiparasitic species, 2/3 of the species studied, tolerated non-specific hosts and attacked amost any native woody and herbaceous plants and even a few of them could mature, flower, and set seeds without host contact. In contrast, the other seven species, 1/3 of the species studied, adapted highly to select and attack specific host plants as follows : *Agalinis linifolia* parasitized *Taxodium distichum, Aureolaria flava* parasitized various *Quercus* species, *A. pedicularia* parasitized *Quercus velutina* Lam., *Macranthera flammea* parasitized *Nyssa sylvatica* Marshall, *Seymeria cassioides* parasitized various *Pinus* species, *S. pectinata* parasitized various species of *Carya, Nyssa, Pinus,* and *Populus,* and *Striga asiática* parasitized *Zea mays* L. and other crops and various grasses in the Poaceae and the Cyperaceae (Table I). However, it could not be detected that whether those hemiparasitic plants got advantage for growth or life cycle as they formed haustoria on host roots, since they also formed haustoria on such nutritionally inert substances as gravels, pebbles, and piece of dead wood. Although the former group of the hemiparasitic species attacked to several different hosts at some stages of their life cycle, the ability to parasitize to a wide range of hosts did not mean that the members would derive equal nutritional benefit from all grafted partners (ATSATT and STRONG, 1970). In addition, duration of haustorium function of the perennial hemiparasitic species was not investigated well, but it might be depended on duration of the host organs. The chromosome races of all of the four species of the perennial hemiparasites studied, which seemed to contain the common basic

chromosome number of X = 6, always parasitized woody perennial species.

Figure 5 shows the distribution map of the 21 hemiparasitic species studied. The distribution and habitats of the hemiparasites could be depended on those of host species,

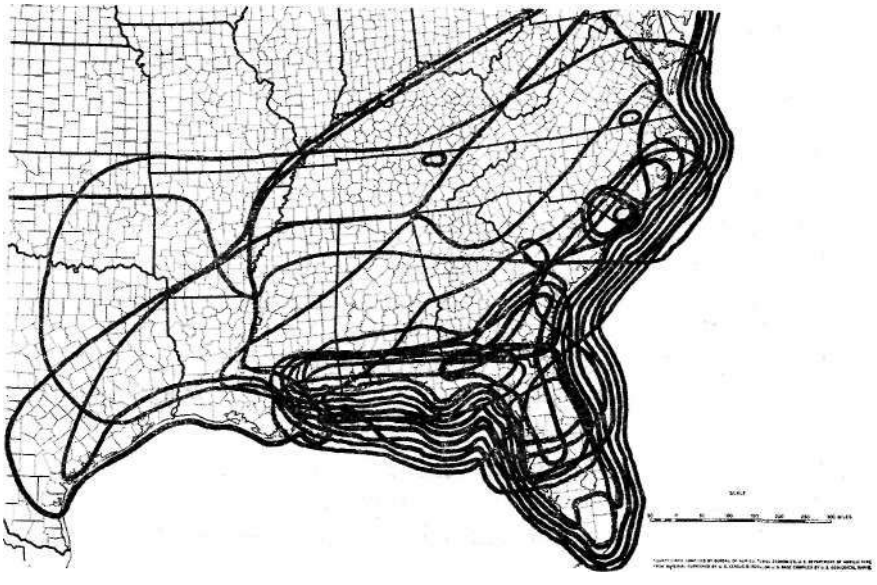


Fig. 5 — Map showing concentrated regions of distribution of certain hemiparasitic species in the Coastal Plains of the southeastern United States.

excepting *Schwalbea americana* had a narrow population size, distribution, and habitats that were not caused by its preference for a specialized host since it grew with various woody species under the laboratory experiment made by MUSSELMAN and MANN (1978). In addition, autotrophic vigour and its ability of the hemiparasitic plants could be of essential importance maintaining the adaptation of individuals, chromosome races, or species to specific habitats. The twenty-one hemiparasitic species were most abundant in sunny, nutrient-poor, acid sites in Coastal savannas of the southeastern United States, and at least ten species were well adapted to disturbed sites and roadsides. However, *Striga asiática*

with 2n = 24 could also get exceptionally into nutrient-rich, agricultural corn fields as a weedy root-parasite. The annual species of *Agalinis* studied, which formed one of the most vigorous autotrophic individuals in their populations, adapted well to grow along with artificially disturbed, wet areas, such as recently burned areas, bog margins, and margins of shallow ditch margins along roadsides. Although the distribution and habitats of *Seymeria* with the chromosome races of 2n = 26 were strongly associated with those of various species of *Pinus,* they seemed to rather like artificial disturbed habitats of pine plantations than natural habitats of pine scrub forests. *Comandra umbellata* had the widest distribution throughout North Carolina, Virginia, to central inland regions of the United States, the broadest range of over 200 host species, and wider range of habitats (PIEHL, 1965). Dispersal of this species could be accomplished by birds and other small animals since its fruits were edible; particularly, birds could be suspected of being involved in the long range distribution of this species.

As a conclusion, some ecological factors involving aneuploid chromosome races in hemiparasitic species could be somewhat attributed to chromosome number and karyotype.

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NOTE ON *ZENIA* (CAESALPINIACEAE) AND ITS POLLENMORPHOLOGY

by

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SUMMARY

Floral morphology and pollenmorphology of *Zenia insignis* Chun have been studied. Pollen is studied for the first time, both LM and SEM techniques are used. Relationships are briefly discussed.

INTRODUCTION

THE monotypic genus *Zenia* was described by CHUN (1946).

Zenia insignis Chun was then reported from 4 localities in the Chinese provinces Kwangtung and Kwangsi. Since then no new collections have been added until recently when the present authors during revisionary work in Paris (Herb, P) under the genus *Cassia* found four collections from Vietnam. We have analysed the floral characters and particularly the pollen. The analysis points out this most recently described taxon is unique within the Caesalpiniaceae.

MATERIAL

All the new material which we have studied was collected in the Tonkin province of North Vietnam:

Chevalier 37986 (coll. Glutron) : Thuyen-Quang, Trung Mon forest reserve (P). *Chevalier* 37987 (coll. Fleury) : Thuyen-Quang, Hui La forest reserve (AAU, p) ; *Chevalier* 40234: Thanh Thuy, Ha Giong territory (P), Poilane 25752, between Lai Chau Muong Toung (AAU, P). Furthermore the

paratype material from China in Herb. A has been chequed (Ko 55656 from Kwangsi).

MORPHOLOGY

From the label of *Poilane* 25752 it is said to be a 20 m high tree with a circumference of c. 2 m.

Following floral characters can be added to the diagnoses, also taken from the label of *Poilane* 25752, the only flowering specimen from Tonkin: Sepals deep granate, petals dark red. Fragrance agreeable but faible. We have compared the flowers of the Tonkinese material with the diagnoses based on Chinese material. The sepals are imbricate with a quincuncial aestivation. This was also found in the type material. The petals, on the other side, offers problems which are less easily solved. CHUN in his diagram shows an upper median petal being exterior in the same way as the vexillum of the Fabaceae, the two upper lateral ones are interior, each enclosing 2 stamens in the bud. The two lower petals are partly interior. We can confirm the position of the upper vexillum-like petal, but we think that also the petals are placed in a quincuncial arrangement, antidromous to that of the sepals. The final solution of this must await the collection of fresh material as it is strictly limited how many flowers can be dissected from the scarse herbarium material available.

The stamens are 4 in number in our material. CHUN reports also 4 and occasionally 5 as occurring. This number has not been observed by us.

POLLEN

As there are no published data on the pollen of *Zenia* the second author has taken up a detailed study including LM (light microscopy), as well as SEM (scanning electron microscopy). The methods used for LM is the same as in LARSEN (1975). The measurements of the size of P (polar axis) and E (equatorial axis) are based on c. 50 pollen grains. For SEM both non-acetolysed and acetolysed pollen grains have been studied. In the first case pollen was sprinkled

on specimen-holders covered with a dry film of acetone-glue (MullEr 1973) and coated under a vacuum with gold. The acetolysed grains were suspensed in distilled water and a drop left to dry on specimen-holders prepared as above. Sections of pollen were studied by lM and SEM after Müller (1. a). The terminology is in accordance with LARSEN (1. c.) except for some alterations after the resolutions admitted at the palynological meeting in Paris 1975.

The pollen grains are single, isopolar, radially symmetrical, prolate-spheroidal (P/E = 1.03-1.11) to spheroidal (P/E = 1) rarely oblate-spheroidal (P/E = 0.89-0.96), equatorial outline ± circular, tricolporate. Size : P (25-) 26-29 um, E (25-) 26-28 (-30) um. Apocolpia (4-) 5 (-6) um. Ectoapertures colpate, fairly long, 20-25 um, 3-5 um wide at equator. Apices acute, slightly invaginated. The colpus membrane finely granulated. Endoapertures apparently subrectangular, 3-5 um long, as broad as the largest width of the ectoapertures but from the inner surface enlarged mainly along the equatorial axis to 8-10 um breadth (Plate II, fig. 2).

Exine 2-2.5 um. thick at the centre of mesocolpia, thicker at the poles, 2.5-3 um, decreasing in thickness towards the apertures. Sexine thicker than nexine, consisting of a perforated tectum and collumellae. From the section Plate II, fig. 3 it is seen that at centre of mesocolpia the tectum-layer is slightly thinner than the columellate-layer; at the marginal area of mesocolpia. Plate I, fig. 4 and Plate II, fig. 4 the sexine is decreasing in thickness and the tectum is almost without perforations, the columellate layer is becoming thinner towards the margins. Neither nexine nor columella are present at the enlargement of the endoapertures, Plate II, fig. 1.

In view of these new data we have tried to speculate on the relationships of *Zenia.* In the vegetative parts the species has some resemblance with some *Cassia* species. A closer study of its flowers, however, does not give much support for any close affinities, particularly the aestivation is unique and peculiar. In the vegetative it should be noted that the leaflets are not opposite in *Zenia* as in Asian *Cassia.* Few recent studies exist on the palynology of *Cassia;* we have

compared our results with those of SRIVASTAVA (1957). The resemblances are so general that they cannot be regarded important. Our conclusions is that *Zenia* may belong to the Cassieae of Caesalpiniaceae but more evidences as e. g. cytology, wood-anatomy, and seed-morphology are necessary for a final decision of where to place it.

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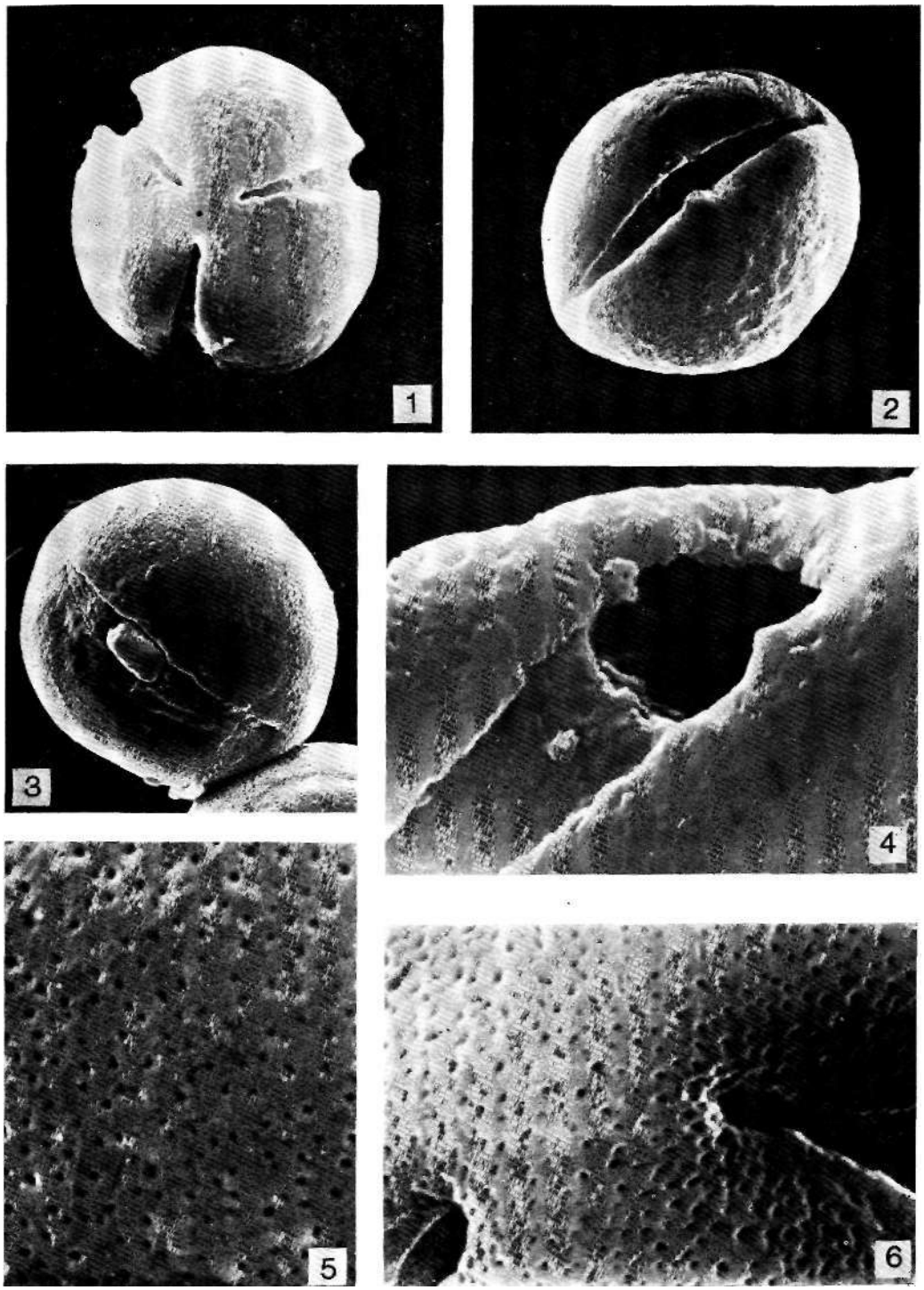

SEM micrographs of acetolysed pollen grains (except Fig. 3); 1. polar view; 2. equatorial view; 3. non-acetolysed pollen grain, equatorial view; Pig. 4-5. details of tectum surfaces; 4. marginal area of mesocolpium, X 900; 5. mesocolpium, X 8650; 6. polar area, X 9000. Fig. 1-3 X 1800.

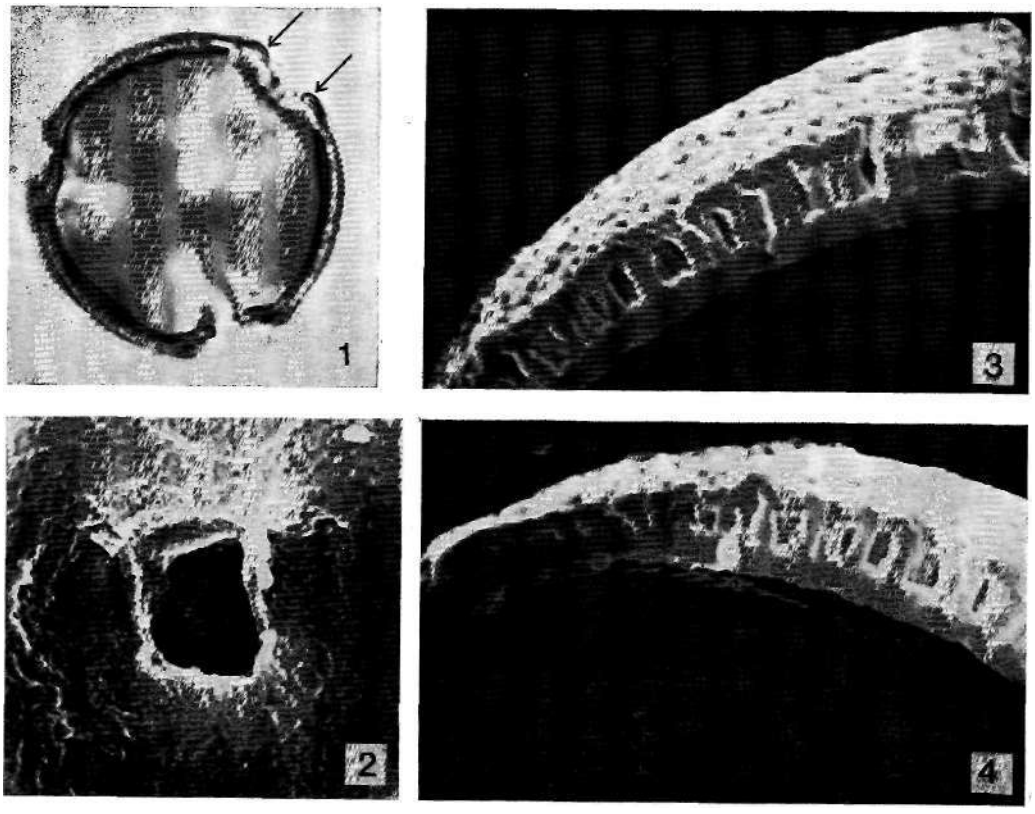

Fig. 1. LM micrograph polar view, arrow showing sexine at enlargement of endoaperture, X 1450; Fig. 2-4. SEM micrographs of acetolysed pollen grains; 2. inner surface of endoaperture, X 4350; 3 & 4. section of exine at mesocolpium; 3. center area, X 8550; 4. marginal area, X 8550.

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TRlTICuM-AEGILOPS CROSS-INCOMPATIBILITY SYSTEM BASED ON THE POLAR-NUCLEI ACTIVATION HYPOTHESIS

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SUMMARY

There was found a close relationship between seed incompatibility of 69 intergeneric hybridizations, 11 *Aegilops* species crossed with 8 *Triticum* species, and activation index of the polar nuclei (AI, %) calculated from a standpoint of the PNA hypothesis. That is, the crosses with AI less than 10 or 20%, 20-80%, 80-110%, and 110-250% were generally observed to be abortive or barely successful, successful, abortive or hardly successful, and completely abortive, respectively (Fig. 1). A few unexpected cases should be re-examined in further crossing experiments in detail.

CROSS-incompatibility in higher plants is generally caused by several sexual barriers in pre- and postfertilization of male and female gametes. In wide crosses one of the important barriers was found to be imperfect development of the endosperm, resulting in deformed or abortive seeds. Based on the histology of hybrid seeds and interspecific crossing data in *Avena,* NISHIYAMA and YABUNO (1978, 1979) interpreted the principal mechanism of seed abortion in terms of the polar-nuclei activation (PNA) hypothesis. The outline of the hypothesis is briefly represented as follows: It was first assumed that early development of the embryo and endosperm was mainly ascribed to the activation of the

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egg and polar nuclei by the male nuclei in double fertilization. The activation degree of the *egg* nucleus and the two polar nuclei was indicated by activation index (AI, %) calculated from the formula $\frac{AV}{RV}$ (%) and $\frac{AV}{2RV}$ (%), respectively. AV (activating value) and RV (response value) show intensity of activating action of the male nucleus, and of reaction of the female nucleus, respectively. The same species or variety indicates AV = RV, and AI of the egg and polar nuclei is always 100 % and 50 %, respectively under which seeds develop normally. However, in interspecific crosses AI may vary widely, because AV or RV seems to be peculiar to each species, being certainly controlled by a gene or genes. Distorted AI can cause abnormal development of the embryo and endosperm. The greater the deviation in the plus or minus direction from the standard 50% the greater the abnormality to over- or inhibited growth of the developing seed. In histological studies of abortive crosses, and successful artificial culture of excised embryos from them, e. g. *Triticum aestivum* X *Aegilops squarrosa* with AI = 10 % (RILEY and CHAPMAN 1960), it was also known that the growing endosperm was more sensitive to the unusual activating action than was the developing embryo, probably associated with triple fusion of the primary endosperm nucleus. Thus, there was found a close relationship between AI of the polar nuclei and abortion of hybrid seeds. The hypothesis was very well applied to interspecific cross-incompatibility in *Triticum* and in *Aegilops* (NISHIYAMA 1979a, b).

The present paper further deals with that activating values separately adopted in interspecific hybridizations in *Triticum* and in *Aegilops* are still valid in intergeneric crosses between them.

MATERIALS AND METHODS

The present author owed *Aegilops-Triticum* crossing data, using 11 *Aegilops* and 8 *Triticum* species to many earlier workers (AASE 1930, BOCHEV and KOSTOVA 1973, BLEIER 1930, DOSBA and CAUDERON 1972, KAGAWA 1928, 1929,

KATAYAMA 1933, KrHARA 1929, 1937, KIHARA and LILIENFELD 1932, LILIENFELD and KiHARA 1934, MATSUMOTO and TABUSHI 1956, PERCIVAL 1930, RILEY and CHAPMAN 1960, SEARS 1941, TANAKA 1959). However, it regrets to say that there are only a few or no crossing data showing details of development and germination of hybrid seeds which are necessary for discussing the present problem. The species used, their genome types, refered to KIHARA and TANAKA (1970), and activating values (or response values) are listed as follows:

Aegilops	Genome	AV	***Triticum***	Genome	AV
Ae. squarrosa L.	D	0.6	*T. monococcum* L.	A	0.9
Ae. uniaristata Vis.	M^u	0.7	*T. boeoticum* Boiss.	A	1.0
Ae. umbellulata Zhuk.	C^u	0.9	*T. timopheevi* Zhuk.	AG	1.5
Ae. comosa Sibth. et Sm.	M	1.1	*T. dicoccoides* Kön.	AB	1.7
Ae. caudata L.	C	1.4-(1.7)	*T. dicoccum* Schrank.	AB	1.7
Ae. speltoides Tausch.	S	1.5	*T. durum* Desf.	AB	1.75
Ae. ventricosa Tausch.	DM'	1.6	*T. spelta* L.	ABD	2.9
Ae. ovata L.	$C^UM°$	1.65	*T. aestivum* L.	ABD	3.0
Ae. triaristata Willd, 4x	C^UM^t	2.1	*(T. vulgare* Host.)		
Ae. cylindrica Host.	CD	2.2			
Ae. triuncialis L.	C^u C	2.2			

In the genus *Triticum* the activating value of 1 was arbitrarily adopted for *T. boeoticum* and the derived values 0.9-3.0 were assigned to the other species by the degree of abnormality of seed development in certain representative crosses. The AV = 1 of *T. boeoticum* was further employed as a standard for estimation of a relative activating value 0.6 for *Ae. squarosa* from which 0.7-2.2 were derived for the other *Aegilops species* (NISHIYAMA 1979a, b).

RESULTS AND DISCUSSION

Previous articles dealt with interspecific cross-incompatibility systems in *Triticum,* and *Aegilops* in view of the PNA hypothesis (NISHIYAMA and YABUNO 1978, 1979, NISHIYAMA 1979a, b). A similar inspection has been made

on intergeneric hybridization between them. In Tables 1 and 2 are listed crossing data of 11 *Aegilops* species crossed with 8 *Triticum* species, refered to many authors. Most of the crosses were made to get F_1 hybrids for cytological investigations without details of hybrid-seed development. Then, the successful crosses are simply indicated with a mark + or percentage seed germination if available. Activation indices (%) are also given to all cross-combinations. They are greatly ranging from 10% to 250%. The data are again summarized in a graphic illustration, especially showing the frequency distribution of the activation indices of the 69 crosses (Fig. 1). The failure of hybrid crosses is marked with an asterisk. Owing to a few or no experimental data it is difficult to discuss detailed seed development in connection with the activation index. However, the seeds from *Aegilops-Triticum* crosses are compared with those from interspecific crosses of *Aegilops* or *Triticum*, and they might be classified into four kinds of seed development, designated W-, N-, Ps- and E-type. W-type seeds develop weakly and are sometimes wrinkled slightly. They show poor or no germination. N-type seeds indicate normal or nearly normal development and high germination. In the E-type the seed content begins to degenerate at the early stage of embryogenesis, and results in shrivelled, empty seed-coat at maturity in an extreme case. Ps-type seeds are just intermediate between N- and E-type. being partially shrivelled and germinable or not. It is remarkable that the W-, N-, Ps- and E-type appear to correspond to the activation-index groups, less than 10 or 20%, 20-80%, 80-110% and 110-250 %, respectively as shown in Fig. 1. The boundary between two types will be more clearly shown when sufficient crossing data become available. On the boundary there were often found intermediate types, though widely variable, which seem to be much attributed to environmental conditions or modified factors if present.

In the N-type, however, three crosses, *Ae. triaristata* crossed with *T. boeoticum* (♂), *T. durum* (?) and *T. aestivum* (♀),were found to be unsuccessful, but only 6-14 seeds from each cross were used for a single germination test.

TABLE 1

Reciprocal crosses between *Aegilops* and *Triticum* species, I

"^^v. *Triticum* AV *Aegilops*^_^ RV ^ \	*monococcun* 0.9			*boeoticum* 1.0			*timopheevi* 1.5			*dicoccoides* 1.7		
	AI i)	Germ. [2]J	**Auth.** [3])	AI	Germ.	**Auth.**	AI	Germ.	Auth.	AI	Germ.	Auth.
squarrosa				83.3	0	Ka'33					—	
0.6				30.0	62	Ka'33 ([4])				17.6	+	T'59
uniaristata				—	—	—						
0.7				35.0	+	S'41						
umbéllulata				—	—	—	—	—				
0.9				45.0	+	S'41	30.0	+	MT'56			
cowosa	—	—	:—	45.5	50	Ka'33	68.2	61	LK'34			
1.1	61.1	+	S'41	55.0	+	S'41						
caudata				35.7	+	K'37	36.8	18	LK'34			
1.4-(1.7)												
speZíoídes	30.0	+	KL'32				50.0	61	LK'34			
1.5												
ventñcosa	28.1	+ •	P'30	31.3	+	KL'32	46.9	13	LK'34	53.1	+	P'30
1,8												
ouaía	27.3	+ •	B'30	30.3	+	KL'32	45.5	83	Ka'33	51.5	71	Ka'33
1.65							55.0	53	Ka'33	48.5	+	K'29
triaristata			[c]	23.8	0	Ka'33	105.0	0	Ka'33			
2.1				105.0	0	Ka'33	34.1	78	LK'34			
ei/Z¿ndr¿ea				22.7	+	KL'32				38.6	+	P'30
2.2							73.3	0	Ka'33			
triMwcíaíis							34.1	+	K'29	38.6	+	K'29
2.2												

1) Activation index (%), 2) Seed germination (+or<%), 3) Author, 4) Lower line indicates the reciprocal cross.

Abbreviation: A AASE, B BLEIER, BK BOCHEV and KOSTOVA, DC DOSHA and CAUDERON, K KIHAEA, Ka KATAYAMA, Kg KAGAWA, KL KIHARA and LILIENFELD, LK LILIENFELD and KIHARA, MT MATSUMOTO and TABOSHT, P PERCIVAL, S SEARS, T TANAKA.

TABLE 2

Reciprocal crosses between *Aegilops* and *THttcum* species, II (continued)

^^•v. *Triticum* ^ \ ^ AV / RV ^ \	*dicoccam* 1.7			*duram* 1.75			*spelt a* 2.9			*aesiivum* 3.0		
	AI	Germ.	Auth.	AI	Germ.	Auth.	AI	Germ.	Auth.	AI	Germ.	Auth.
squarrosa				145.8	0	Ka'33				250.0	0	Ka'33
0.6	**17.6**	+	T'59	17.1	23	Ka'33				10.0	0	Ka'33
comosa				79.5	45	Ka'33				136.3	0	Ka'33
1.1:												
caudata				62.5	23	Ka'33				107.1	22	Ka'33
1.4-(1.7)												
speiíoide's										100.0	0	Ka'33
1.5												
ventricosa				54,7	23	Ka'33				93.8	0	Ka'33
1.6				45.7	83	Ka'33				26,7	50	DC'72
ovata	51.5	50	BK'73	. 53.0	91	BK'73	**87.9**	• +	P'30	90.9	56	BK'73
1.65				**47.1**	+	K'29				27.5	+	P'30
triímsíaía				41.7	75	Ka'33				71.4	40	Ka'33
2.1				60.0	0	Ka'33				35.0	0	Ka'33
oyUndrica	38.6	+	Kg'29	39.8	+	B'30	65.9	+	B'30	68,2	+	Kg'28
2.2							37.9	+	A'30	36.7		Kg'28
triuncialis	38.8	+	K'29	39.8	77	BK'73	65.9	+	K'29	68.2	82	BK'73
2.2							37.9	+	K'29	36.7	+	K'29

Explanation of the abbreviation and symbol is shown in Table 1.

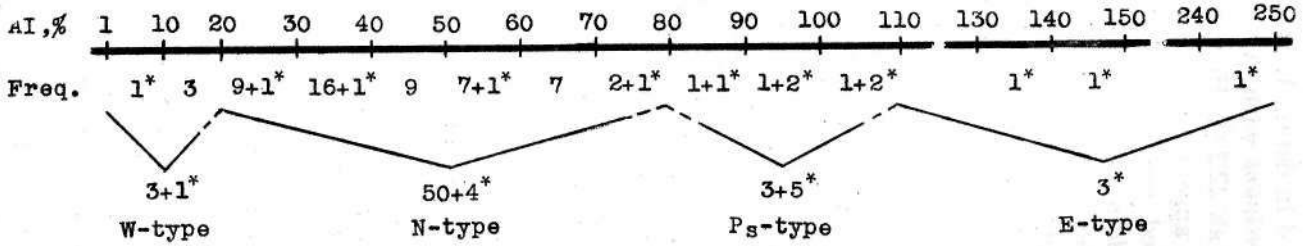

Fig. 1. — Putative cross-incompatibility system in *Aegilops-Triticum* crosses, especially showing the activation index series with frequency distribution of the 69 crosses.
* Abortive cross.

They should be re-examined in detail. It is difficult to expect that *Ae. caudata* X *T. aestivum* with AI — 107.1 % showed seed germination as high as 22.2%. However, certain modifying factors, if present, appear to be associated with the production of nearly filled seeds. On the other hand, if AV (or RV) of *Ae. caudata* is adjusted to be 1.7 instead of 1.4 or that of *T. aestivum* goes down from 3 to 2.7 the cross might give moderately good seeds. Anyhow the nature of these exceptional crosses becomes fairly known in an extensive crossing experiment, and a comparative study of the physiology and histology of developing seeds from related interspecific crosses.

In conclusion, the polar-nuclei activation index is well applicable for establishing an *Aegilops-Triticum* cross-incompatibility system. Only AV = 0.6 of *Ae. squarrosa* was estimated in comparison with AV = 1 of *T. boeoticum,* and they were independently used as a standard activating value for the other species in each genus. It is also noteworthy that the AV (or RV) series of *Triticum,* and *Aegilops* are still available in the intergeneric crosses between them. The fact suggests that the activating values had mainly differentiated quantitatively in the evolutional pathway of both closely related genera.

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ACTION DE LA TERBUTRINE (HERBICIDE) SUR LA CELLULE VÉGÉTALE —I

ÉTUDE CYTOLOGIQUE DES EFFETS PRODUITS SUR DEUX ALGUES VERTES : *RHIZOCLONIUM HIEROGLYPHICUM* (KÜTZ.) STOCKM. ET *TETRAEDRON MINIMUM(A.* BRAUN) HANSGIRG.*

par

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ABSTRACT

Some species of Algae harvested on rice field of the banks of Mondego river were isolated and cultured in a climatized chamber. Among the referred algae a filamentous Chlorophyta *(Rhizoclonium hieroglyphicum)* as well as an unicellular one *(Tetraedron minimum)* were selected to study the alterations produced by terbutryn (2-tert. butylamino-4-ethylamino-6-methylthio-s-triazine). The drug was used in concentrations of 0.025, 0.05, 0.1 and 0.5 mg/1 for treatment of juvenile cultures (2-3 weeks) for periods of time from few hours up to several days, under different light conditions. Periodically, samples of these cultures were removed for electron microscopy study. The most evident effect of terbutryn on both algae species is the depletion of starch from chloroplasts, whether this polysaccharide is pyrenoid-associated or not. This effect is not observed whenever the algae supplied with an exogenous hydrocarbon source (2% glucose). Moreover a variable degree of inhibition of dictyosome activity and, particularly in *Tetraedron minimum,* the swelling of chloroplast-thylacoids were observed. The intensity of these effects rises up in parallel with the duration of the treatment and herbicide concentration. These results are compared with the ones obtained by others after treatments by similar drugs.

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DfTRODUCTION

DEPUIS quelques ans un des Auteurs a consacré une partie de son travail à la recherche des altérations de la structure des cellules des plantes supérieurs induites par l'application de drogues, dont les effets, au point de vue physiologique, sont très variés. C'est ainsi qu'on a déjà étudié l'action de quelques substances douées de propriétés mitoclasiques, radiomimétiques et antibiotiques (MESQUITA, 1966, 1967a e b, 1968, 1970, 1971). Cependant, dans ce domaine de la Cytologie Végétale, un des aspects les plus importants au point de vue pratique, disons même économique, est sans doute la recherche des altérations cellulaires produites par les herbicides. En effet la connaissance de ces modifications, en ensemble avec les données physiologiques concernant leur mécanisme d'action, peuvent être utiles dans le choix de l'herbicide et des conditions plus convenables de son application. Alors, en profitant du fait que le seconde Auteur travaille depuis longtemps à la taxonomie des Algues, nous avons pris la résolution d'étendre les études rapportées ci-dessus à ce groupe, en choisissant, pour cela, quelques espèces infestantes des rizières du Mondego.

Dans nos expériences, nous avons employé la terbutrine, puisque, d'après ce que nous connaissons, il n'y a aucun travail concernant les éventuels altérations produites par cet herbicide dans la célule végétale.

MATÉRIEL ET MÉTHODES

La récolte des algues a été efectuée dans les rizières du Mondego (localisées d'un et l'autre côté de la route Coimbra-Figueira da Foz). Après leur identification, quelques unes ont été isolées et cultivées dans des milieux appropriés, selon la méthode décrite ailleurs (MESQUITA & M. FÁTIMA SANTOS, 1976). Parmi ces algues on a choisi, pour les études expérimentales, deux espèces d'algues vertes *(Chlorophyta),* une filamenteuse *(rhizoclonium hieroglyphicum)* et l'autre unicelulaire *(Tetraedron minimum).*

Pour faire les traitements des algues on a essayé d'abord de les repiquer pour le milieu de culture additionné de la terbutrine[1] .

Cependant, étant donné l'effet inhibiteur de la drogue sur de développement des algues, on n'arrive pas à obtenir du matériel en quantité suffisante, c'est à dire susceptible d'être manipulé pour l'étude au M. E.

Alors, on a utilisé la méthode suivante: *a.* Répiquage pour des boîtes à Petri contenant le milieu de culture solidifié avec l'agar (1,2%). 6. Contrôle, au M. O., du vieillissement des cultures faisant attention, essentiellement, aux dimensions des cellules, au développement de l'appareil vacuolaire et à la quantité d'amidon. De cette façon, on a constaté que les cultures âgées de 2-3 semaines étaient satisfaisantes pour les essais prétendus, *c.* Traitement des cultures seleccionées par des solutions aqueuses de terbutrine qui étaient versées sur la gélose, de manière que les algues restaient couvertes par une fine nappe d'eau.

L'herbicide a été utilisé aux concentrations de 0.025, 0.05, 0.1 et 0.5 mg/1 et la durée du traitement a varié de 6 h à 20 jours.

Le développement des témoins et des cultures traitées s'est effectué dans une chambre de culture à la température de 16-18° C, soit à l'obscurité, soit avec une photopériode de 10h lumière sur 24 h (intensité lumineuse de 850 lux à peu près).

Pour l'observation au M. E., le matériel a été fixé au glut/OsO4 et inclu dans l'epon (pour plus détails voir les «Résultats»). Les coupes, faites au ultramicrotome Ultratome III (LKB), ont été contrastées par l'acétate d'uranyle et le citrate de plomb (REYNOLDS, 1963) et étudiées dans le microscope Siemens Elmiskop 101.

[1] Nous tenons à remercier à la Ciba-Geigy Portugaise qui nous a offert un échantillon de cet herbicide. Il s'agit d'une poudre blanche, hidrosoluble, dont la composition chimique est le 2-tert.butylamino-4-ethylamino-6-methylthio-s-triazine, et qui, d'après les renseignements de cette Firme, est commercée au Portugal avec le nom de Igran-80. (8% de terbutrine).

RÉSULTATS

Pour facilitier la description nous allons traiter séparemment les deux espèces étudiées et après nous en discutirons les résultats:

1. **Rhizoclonium hieroglyphicum** (Kütz.) Stockm. (Cladophorales).

Il s'agit de filaments simples, à structure partiellement cénocitique et souvent fixés par une cellule rhizoïdal différenciée. Les «cellules», ou bien les «apocytes», à paroi relativement mince, ont 10-35 um en largeur, la longueur étant 2 à 5 fois plus grande. Après coloration au carmin-acétique, on voit nettement que le nombre des noyaux, dans chaque apocyte, varie de 2 à 8, étant le nombre deux le plus fréquent dans les filaments jeunes.

Le chloroplaste (chromatophore) réticulaire, à mailles plus au moins serrées, contient plusieurs pyrénoïdes, normalement à deux calottes d'amidon. Particulièrement dans les filaments vieux on distingue encore, à la région axial des apocytes, de nombreux vacuoles, parfois fortement serrées les unes contre les autres.

En ce qui concerne l'étude au M. E., il faut remarquer que nous avons trouvé de grandes difficultés techniques à réussir une conservation acceptable de l'ultrastructure de cette espèce. Après quelques essais, pendant lesquels nous avons fait varier la concentration du glutaraldéhyde (1, 2.5, et 5 %), le tampon utilisé comme véhicule du fixateur (tampon phosphate et tampon PIPES selon SALEMA & BRANDÃO, 1973) et leur molarité (0.005M, et 0.1M), nous avons constaté que les meilleurs résultats s'obtiennent avec le glutaraldéhyde à 1 % dans le tampon phosphate 0.005M, pH 6.8 ou le tampon Na-PIPES 0.1% pH 7 (Pl. I, figs. 1 et 2). La fixation, la post-fixation au tétroxyde d'osmium, ainsi que toutes les autres étapes de la technique, ont été faites comme habituellement (MESQUITA & FÁTIMA SANTOS, 1976).

Les altérations que nous avons observé dans le matériel soumis à l'action de la terbutrine ne sont pas très specta-

culaires. Elles concernent essentiellement la disparition progressive de l'amidon, soit celui qui forme les calottes des pyrénoïdes, soit l'amidon extrapyrénoïdal (Pl. I, fig. 3; Pl. II, fig. 2), et une inhibition, plus au moins accentuée de l'activité des dictyosomes. En effet, tandis que dans le témoin ces organites produisent normalement de nombreuses vésicules, ce qui donne aux régions cytoplasmiques correspondantes un aspect caractéristique (Pl. III, figs. 1 et 2), dans les algues traitées, la production de vésicules golgiennes est nettement réduite, parfois presque nulle (Pl. III, fig. 3). Il faut encore remarquer que, chez cette espèce, les images de corrélation entre le reticulum endoplasmique et les corps de Golgi, au moyen de vésicules de transition, sont extrêmement fréquentes (Pl. III, figs. 1 et 2). Souvent tous les profils de dictyosomes visibles dans une cellule montrent cette corrélation.

L'étude comparative du matériel traité par la terbutrine aux plusieurs concentrations (0.025, 0.05, 0.1 et 0.5mg/l), pendant des périodes de temps variables (½, 1/4, *1*, 2, 4, 15 et 20 jours), a montré que l'intensité des effets décrits ci-dessus est en rapport direct avec la concentration de la drogue et la durée du traitement. C'est ainsi que, à la concentration de 0.025 mg/1, les effets sont négligeables, même après des traitements assez longs (2-4 jours) (Pl. II, fig. 1). Par contre, un traitement de 15-20 jours par une solution à 0.05 mg/1 montre une efficacité identique à celle d'un traitement de 4 jours avec une concentration double (0.1 mg/1) (Pl. I, fig. 2; Pl. n, fig. 2).

La présence d'une source hydrocarbonée exogène (glucose) pendant le traitement annule les altérations qu'on vient de décrire. L'observation de nombreuses coupes nous a laissé l'impression que les traitements assez longs, aux concentrations plus élevées (0.1, 0.5 mg/1), peuvent déterminer aussi, au niveau de l'appareil photosynthétique, un épaississement des grana, parfois suivi de leur désarticulation par rapport aux membranes intergranaires, et une prolifération des plastoglobuli (Pl. III, figs. 1 et 2). Mais, étant donné que sous cet aspect les témoins montrent des caractéristiques assez

variables, nous ne sommes pas sûres qu'il s'agit là d'un effet de l'herbicide.

2. **Tetraedron minimum** (A. Braun) Hansgirg (Chlorococcales).

Il s'agit d'une petite algue unicellulaire, normalement tétraédrique sans épines ou apophises, les bords des cellules étant concaves. Le nombre de chloroplastes est variable et les dimensions de nos échantillons varient de 5-15 um.

Par rapport au *Rhizoclonium,* cette espèce est beaucoup moins sensible aux conditions de fixation. Ses caractéristiques ultrastructurales s'accordent d'une façon générale, avec la description de PICKETT-HEAPS (1972) pour l'espèce *T. bitridens.* La comparaison du témoin avec les cellules traitées a montré que, chez cette espèce, les altérations plus remarquables produites par la terbutrine se localisent au niveau du chloroplaste. En effet, en dehors de la disparition de l'amidon et une apparente inhibition de l'activité golgienne, constatées également chez *Rhizoclonium,* il y a aussi une tuméfaction bien évidente des locules des thylacoïdes. Ceux-ci assument alors la forme de saccules tuméfiés, plus ou moins allongés, ou bien de vésicules aux dimensions variées (PL *TV,* fig. 2). Par suite de ces altérations, les chloroplastes, et d'une façon générale les cellules elles-mêmes, prennent un aspect caractéristique, ce qui permet les distinguer très facilement des cellules non traitées (Pl. *TV,* figs. 1 et 2).

De même que pour le *Rhizoclonium,* ces effets s'intensifient avec la concentration de l'herbicide et le temps de l'action de la drogue. Alors, sauf une faible raréfaction de l'amidon extrapyrenoïdal, la concentration de 0.05 mg/1 ne produit aucune altération significative, même après une expérience de 96 h. Des résultats comparables s'obtiennent avec un traitement de 48 h par une solution doublement concentrée (0.1 mg/1). Néanmoins, lorsqu'on prolonge la durée du traitement avec cette solution (par ex., 96h) ou s'élève la concentration de l'herbicide (0.5mg/1), les altérations deviennent beaucoup plus nettes: l'amidon extrapyrenoïdal disparait complètement et celui des pyre-

noïdes reste réduit à des calottes très minces (Pl. IV, fig. 2) ; la tuméfaction des thylacoïdes s'accentue considérablement, ce qui donne aux chloroplastes l'aspect rapporté ci-dessus (Pl. *TV,* fig. 2). Nous avons constaté aussi que lorsqu'on additionne de la glucose (2%) à la solution de terbutrine, ces altérations ne se manifestent plus: l'amidon apparaît en grande quantité (à la fin de 96 h de traitement il est encore plus abondant que dans le témoin) et il ne s'aperçoit point de dilatation des locules chloroplastidales (Pl. V). De même, si les expériences sont faites à l'obscurité (par ex. 0.1 mg/I-96 h), en dehors de la dégradation normale de l'amidon, il ne s'observent pas d'autres altérations significatives.

DISCUSSION

D'après ce qu'on connaît dans la littérature, c'est la première fois qu'on envisage le problème des altérations ultrastructurales produites spécifiquement par la terbutrine (2-tert.butylamino-4-ethylamino-6-methylthio-s-triazine) sur la cellule végétale [1]. On peut dire même que les travaux de nature cytologique concernant des substances similaires, c'est-à-dire les dérivés des triazines, sont aussi rares. Par contre, sont déjà assez nombreuses les études faites avec le bût d'éclaircir leur mode d'action. Depuis Ia décade de 60, plusieurs travaux ont montré que, effectivement, quelques dérivés de la triazine (aminotriazines symétriques) peuvent être très utiles dans le contrôle des plantes infestantes de plusieurs cultures. Normalement, pour assurer un contrôle efficace, il suffit d'employer des doses relativement baisses, bien que des concentrations beaucoup plus élevées puissent être utilisées dans une stérilisation préalable des sols pas encore cultivés.

[1] D'ailleurs, les seuls données que nous connaissons sur la terbutrine sont ceux de NEWBOLD (1975). Dans cet article de nature technique-agricole, l'auteur présente un résumé des résultats connus, concernant l'efficacité de nombreux herbicides (terbutrine comprise) dans le contrôle des algues et d'autres plantes aquatiques.

Alors, quelques auteurs ont essayé d'éclaircir le mécanisme d'action de ces herbicides. C'est ainsi que MORELAND *& col.* (1959), en travaillant avec des plantules d'orge *(Hordeum vulgare)* traitées par la simazine (2-chloro-4, 6-bis-ethylamino-s-triazine), arrivent à la conclusion de que l'action létal de cette drogue doit résulter d'une inhibition du processus photosynthétique. En plus, ils ont demonstré que l'activité photochimique des chloroplastes isolés de cette plante est très sensible à la simazine, même lorsque celle-ci est très peu concentrée; alors, dans le mécanisme complexe de la photosynthèse, la photolise de l'eau doit être le point exact d'action de l'herbicide (MORELAND *& col.,* 1959).

Beaucoup d'autres aminotriazines symétriques sont douées de cette propriété et on peut dire que les herbicides de ce type, la terbutrine comprise, bloquent la photosynthèse avec plus au moins d'efficacité (HILTON & *col.,* 1963). Par contre, on ne connaît pas beaucoup sur les éventuelles modifications de Fultrastructure cellulaire produites par ces drogues. Néanmoins, ASHTON *& col.* (1963) ont décrit les altérations de l'ultrastructure des chloroplastes de *Phaseolus vulgaris* induites par l'atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), de la manière suivante: disparition de l'amidon; désorganisation de l'arrangement granaire, par suite de la destruction des «fret-membranes»; tuméfaction des locules des grana suivie de leur destruction et du collapsus de l'enveloppe plastidale. Étant donné que ces altérations ne s'observent pas à obscurité, les Auteurs pensent qu'elles sont produites par quelques substances toxiques provenant de l'interaction de l'atrazine avec la lumière, en présence de la chlorophyle (ASHTON *& col.,* 1963).

Plus tard la même équipe (ASHTON *& col.,* 1966) fait une étude semblable, cette fois-ci utilisant dans leurs expériences une algue verte unicellulaire, c'est-à-dire, *Chlorella vulgaris.* Au niveau de la structure cellulaire, ils ont constaté, tout simplement, la disparition de l'amidon et une faible inhibition de l'activité des corps de Golgi, qui ne s'observent pas en présence de la glucose exogène.

Quant à nos résultats, et considérant, d'une part, la similitude chimique entre la terbutrine et l'atrazine, et d'autre

part, la parenté phylogénétique de *Tetraedron* et *Chlorella*, il faut remarquer le suivant: en ce qui concerne le métabolisme de l'amidon, nos résultats s'accordent avec ceux obtenus par ASHTON *& col.* (1966) chez *Chlorella* traitée par l'atrazine; quant aux effets sur ultrastructure du chloroplaste et, au contraire de l'apparente ineficacité de l'atrazine sur *Chlorella* (ASHTON & *col.,* 1966), la terbutrine agit sur *Tetraedron minimum* d'une façon très semblable à celle de l'atrazine sur *Phaseolus vulgaris* (ASHTON *& col.,* 1963). Comme ces effets ne se manifestent pas à l'obscurité, on peut peut-être admettre, en accord avec l'hypothèse de ASHTON *& col.* (1963), que les altérations de l'ultrastructure des chloroplastes de *Tetraedron* ne sont pas déterminées par la terbutrine elle-même, mais plutôt par des substances toxiques secondaires résultantes de l'interaction de la drogue avec la lumière.

Quoi qu'il en soit et au contraire de ce qu'il paraît être suggéré par ASHTON *& col.* (1966), il ne semble pas y avoir une corrélation significative entre la parenté ou l'habitat des plantes et les altérations de l'appareil photosynthétique produites par ce type d'herbicides, car, sous cet aspect, *Tetraedron* s'éloigne de *Chlorella* et il s'approche de *Phaseolus.*

Il s'agit plutôt d'une question génétique, c'est-à-dire, indépendemment de leur habitat ou du fait des espèces être ou non phylogénétiquement proches, il y aura des génotypes sensibles et d'autres résistants.

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EXPLICATION DES PLANCHES

1. Méthode de préparation du matériel: toutes les microphotographies électroniques concernent du matériel fixé au glut./OsO inclu dans l'Epon et contrasté par l'acétate d'uranyle et le citrate de plomb. (Pour les détails de la technique voir les Résultats et les légendes des figures).
2. Abréviations: a, amidon; ch, chloroplaste; d, dictyosome; m, mitochondrie; n, noyau; nu, nucléole; p, paroi cellulaire; py, pyrenoïdes; re, reticulum endoplasmique; v, vacuole.

PLANCHE I

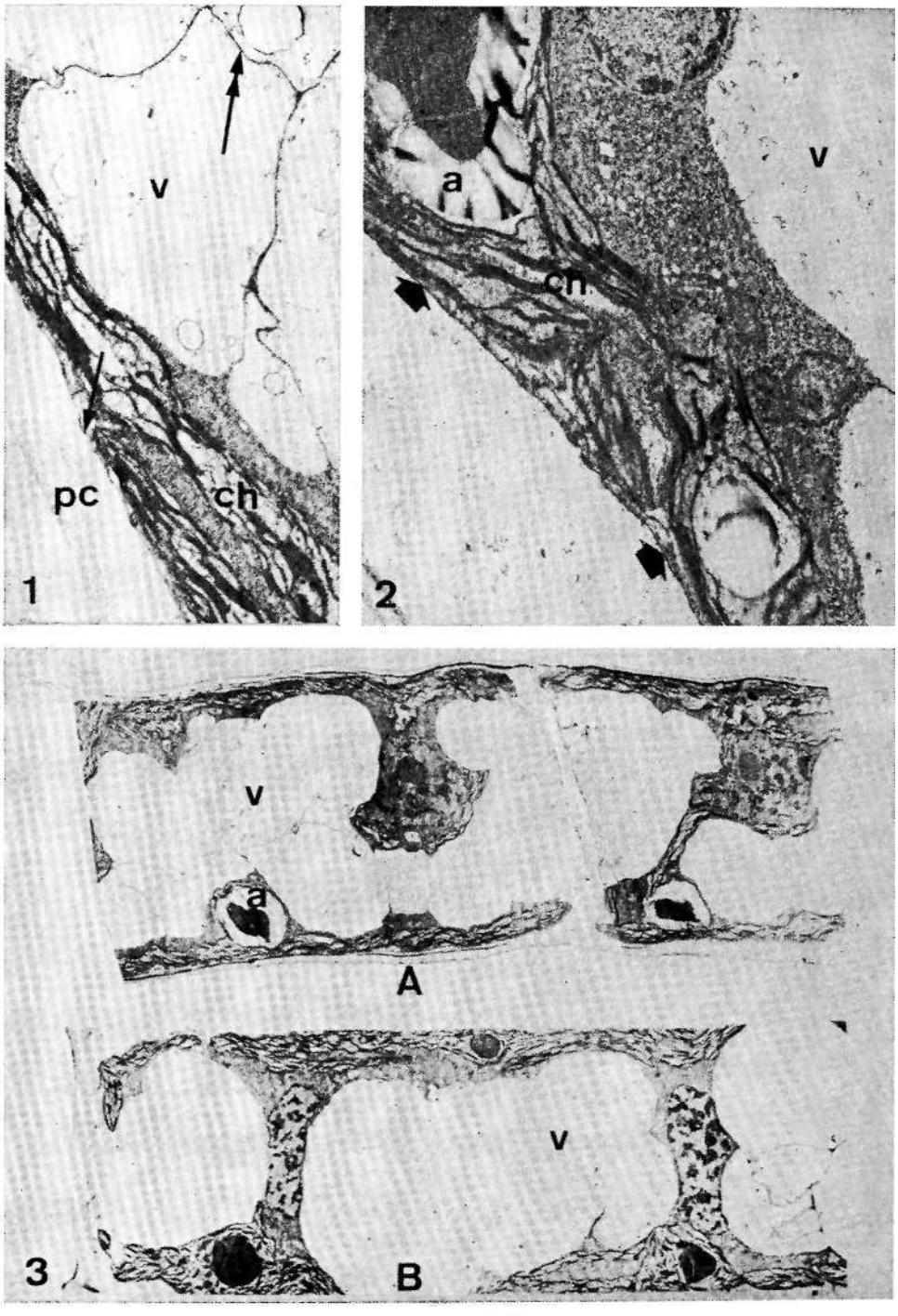
— *Rhisoclonium hieroglyphicum.* Fixation dans le glutaraldéhyde à l % dans le tampon Pipes (0.1 %, pH 7). Remarquer l'absence de Plasmolyse (flèche simple) et la bonne conservation du tonoplaste (flèche double). X 10.300.

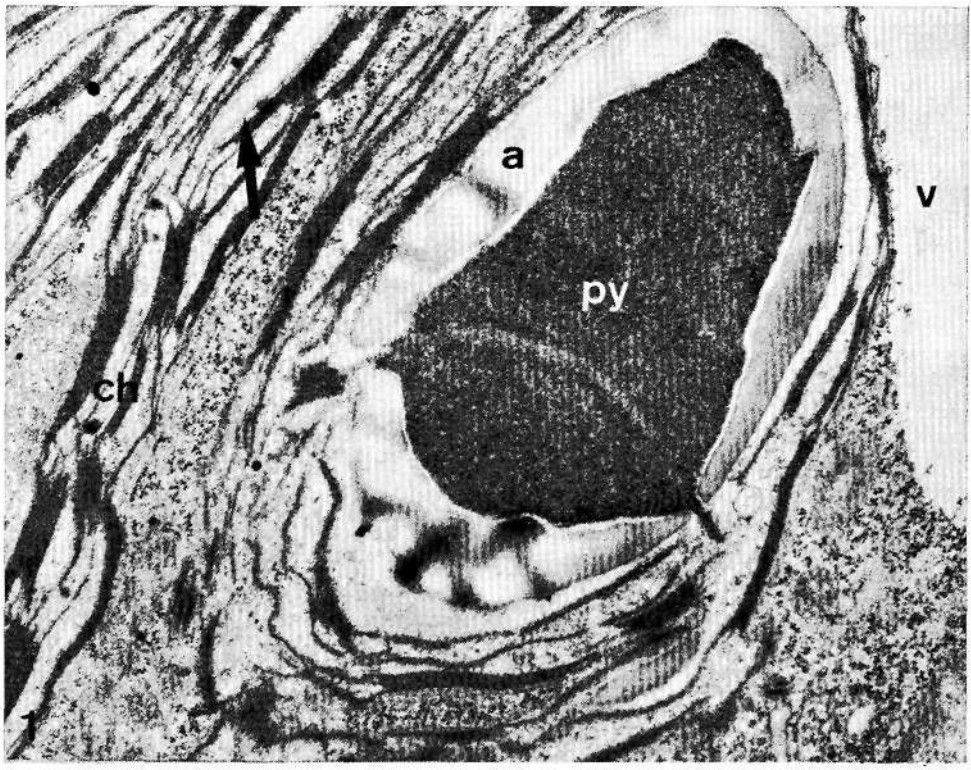
— Idem. Fixation dans le glutaraldéhyde à 2.5% dans le tampon phosphate (0.025M, pH 6.8). On voit un détachement généralisé du plasmalemme (flèches). X 12.400.

— Idem.

A. Algue non traitée (témoin).

B. Algue traitée par la terbutrine (0.1 mg/1, 96 h). Par rapport au témoin, on remarque l'absence d'amidon autour des pirénoïdes.

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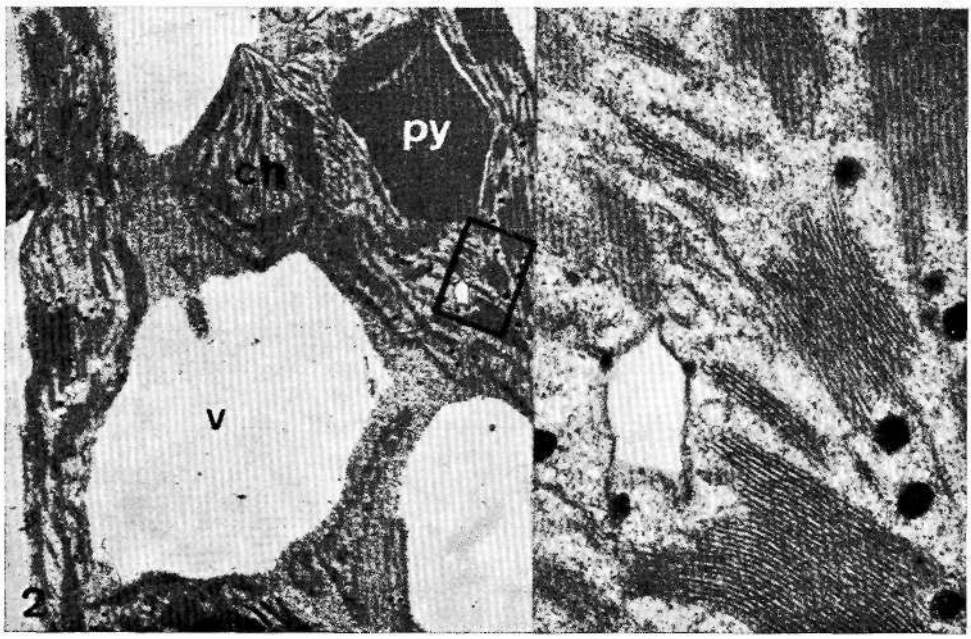
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PLANCHE II

Pig. 1. — R*hisoclonmm hieroglyphicum.* Traitement par la terbutrine (0,.025 mg/l-48h).
Les calottes d'amidon autour du pyrénoïde sont encore bien développées, ainsi que l'amidon extrapyrénoidal (flèche). X 28.000.

Fig. 2. — Idem. (Terbutrine à 0.05mg/I, 20 jours). L'amidon est complètement disparu.
Apparement, il semble y avoir une augmentation du nombre das plastoglobuli et un épaississement des grana (voir le détail à droite). X 8.200. «Inset»: X 42.000.

PLANCHE III

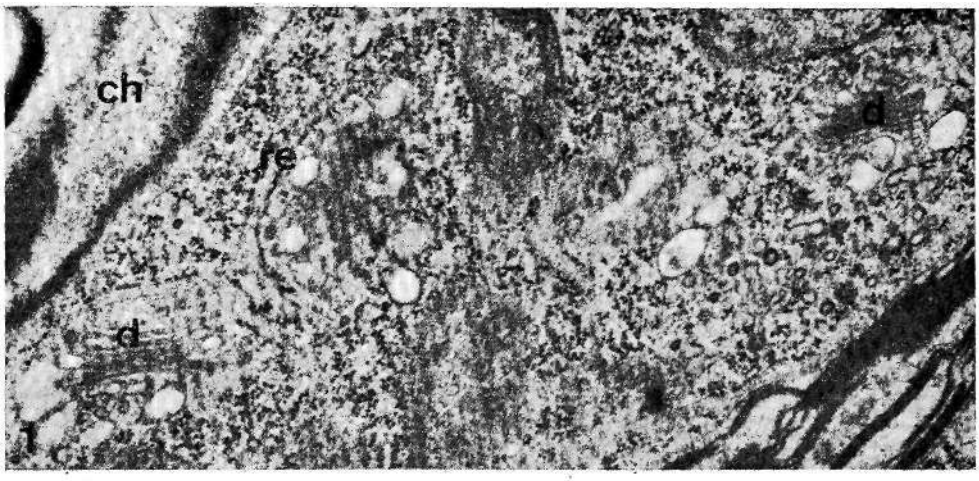
Fig. 1. — *Rhizoclonium hieroglyphicum* (témoin). Les dictyosomes apparaissent systématiquement associés à des profils du reticulum endoplasmique et produisent de nombreuses vésicules. X 36.800.

Fig-. 2. — Idem. Dans cette microphotographie, on voit le complexe R. E. dictyosome dans une maille du réseau chloroplastidal. X 36.800.

Fig. 3. — Idem. Algue traitée par la terbutrine à 0.05 mg/1 pendant 20 jours. Remarquer l'absence de vésicules au voisinage du dictyosome, traduisant l'inactivité de cet organite. X 24.800.

Fig- 4. — *Tetraedron minimum.* Aspect général de l'ultrastructure. On voit le noyau en rapport avec deux dictyosomes, des chloroplastes, des mitochondries, des vacuoles et du reticulum endoplasmique. X 16.500>.

Fig. 5. — Idem. Détail faisant ressortir l'association noyau-dictyosome: l'enveloppe nucléaire burgeonne, et les petites vésicules qui en résultant (flèche) se fusionnent pour donner des saccules golgiens à la face proximale du dictyosome. X 33.100.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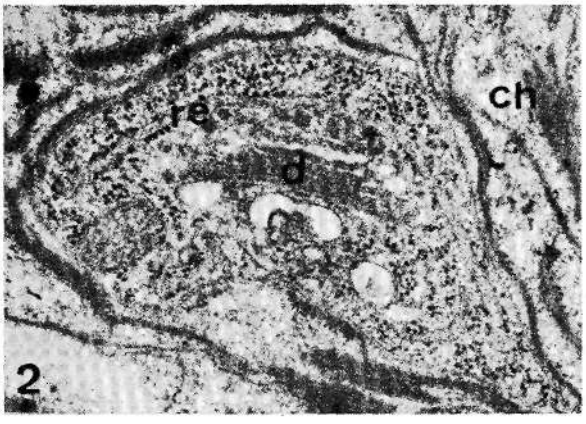
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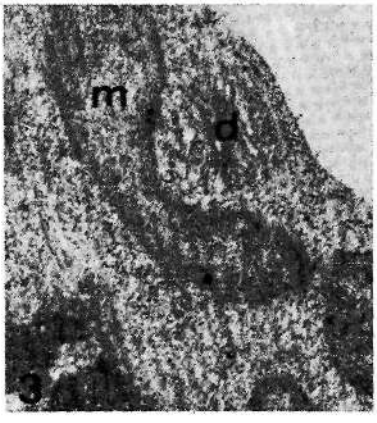
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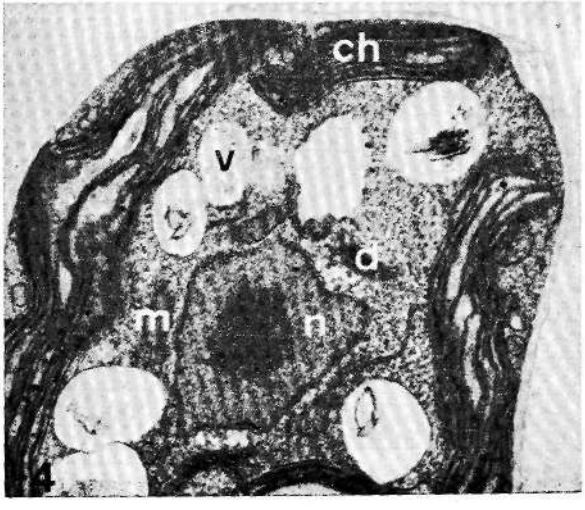
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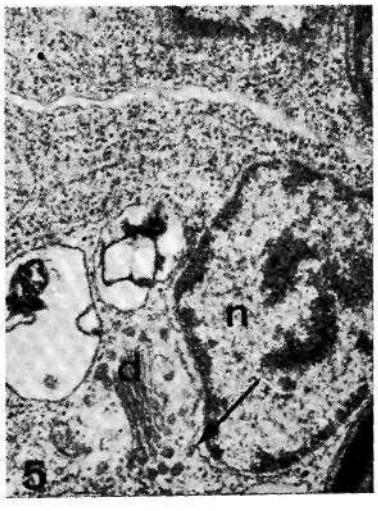
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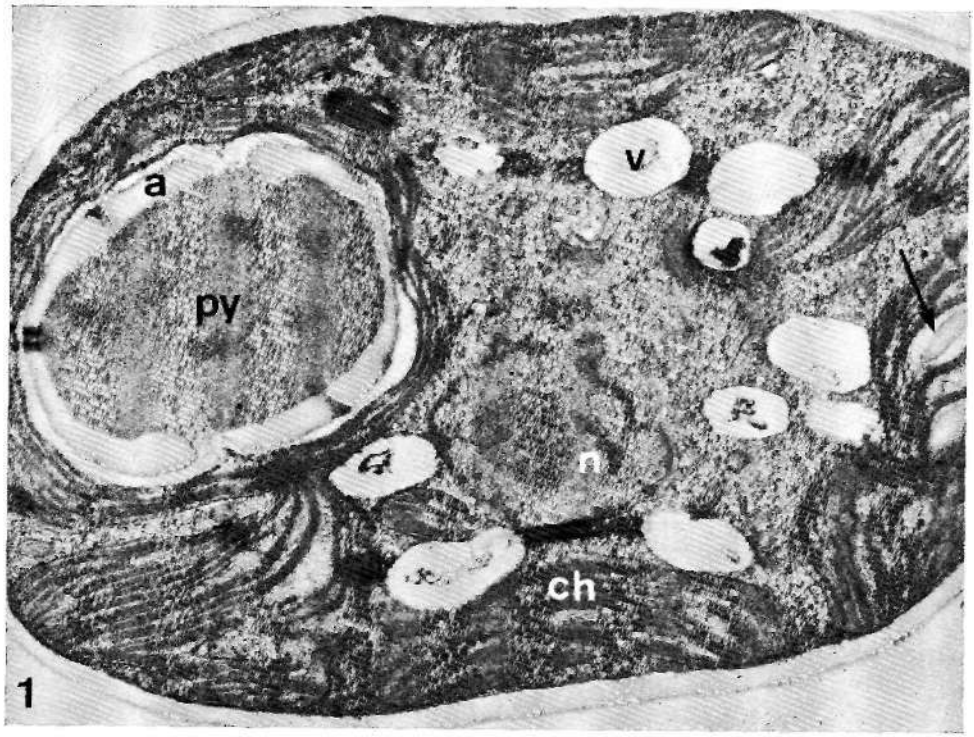
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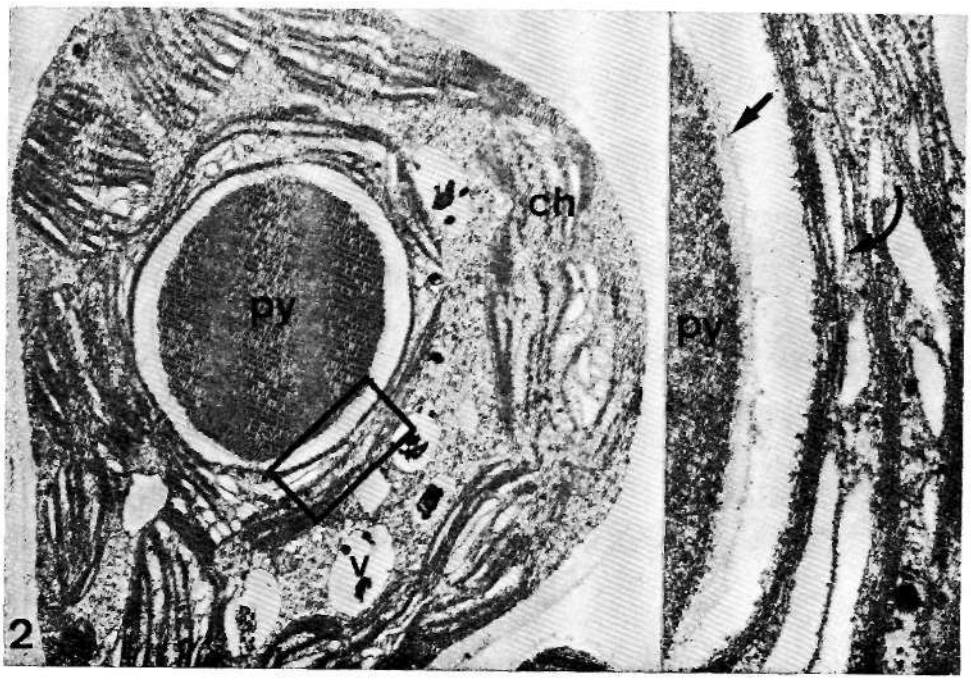
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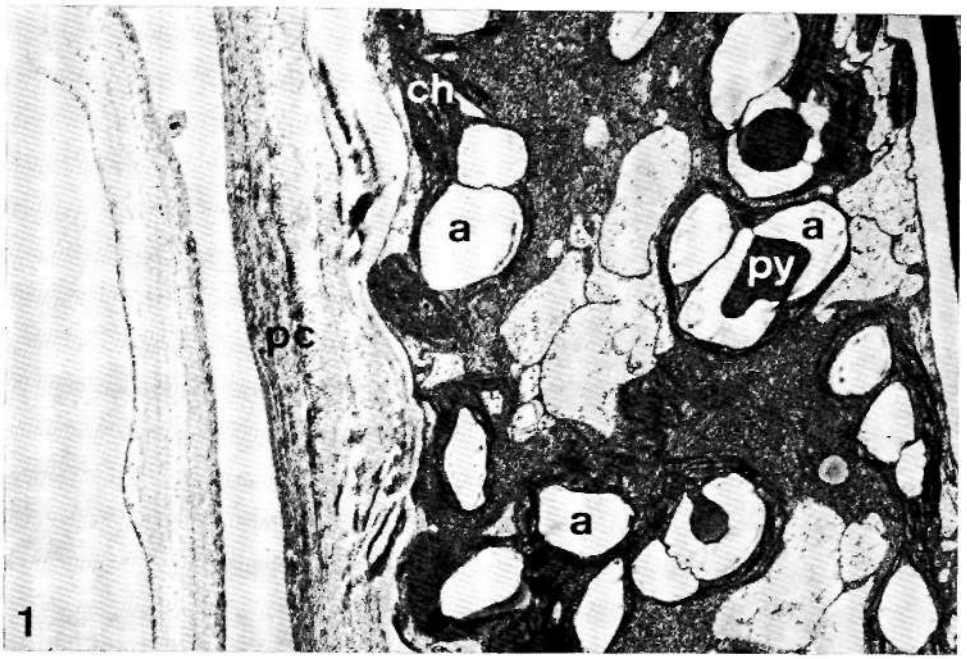
PLANCHE IV

Fig. 1. — *Tetraedron minimum.* Ultrastructure d'une cellule non traitée (témoin). En dehors des caractéristiques mentionées dans la légende de la fig. 4 — Pl. III, on voit aussi deux calottes d'amidon autour.du pyrénoïde et des grains d'amidon extrapyrénoidal (flèche). X 16.000.

Fig. 2, — Idem. Cellule traitée par la terbutrine à 0.5 mg/1 pendant 96 heures. Par rapport au témoin (fig. 1), il ressort l'absence presque totale d'amidon et l'aspect vésiculeux de l'appareil photosynthètique des chloroplastes. Ces caractéristiques se voient plus nettement dans l'«inset» à droite: les calottes d'amidon sont réduites à des lames très minces (flèche droite) et les vésicules chloroplastidales, plus ou moins applaties, proviennent de la tuméfaction des thylacoïdes (flèche courbe). X 14.500. «Inset»: X 60.000.

PLANCHE V

Rhisoclonium hieroglyphicum. Traitement effectué pendant 96 heures par une solution aqueuse de terbutrine (0.5mg/l) additionnée de glucose *(2%).* Il ressort la grande abondance d'amidon. X 6.200.
Tetraedron minimum. Cellule traitée dans les conditions mentionnées ci-dessus. Tandis que l'amidon est abondant, on ne voit aucun signe des altérations ultrastructurales du chloroplaste rapportées dans le texte. (Comparer avec la fig. 2— Pl. IV). X 14.500.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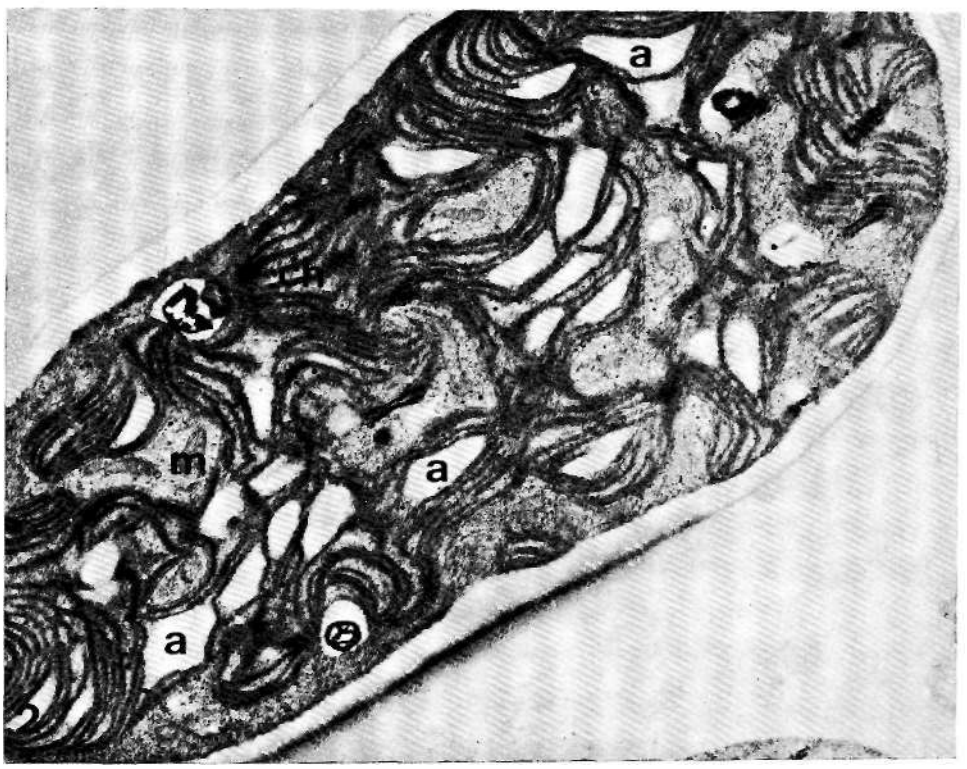
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Bol. Soc. Brot., Sér. 2, 53(2): 839-854 (1981).

ACTION DE LA TERBUTRINE (HERBICIDE) SUR LA CELLULE VÉGÉTALE — II

ÉTUDE DES EFFETS PRODUITS SUR LE RIZ *(ORYZA SATIVA L.)*

par

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ABSTRACT

The effects produced by terbutryn (an herbicide) on rice plants *(Orysa sativa* L.) are studied.

In a climatized chamber, plants were developed on vermiculite, saturated with Hoagland solution, either alone or with different concentrations of terbutryn added (0.1, 1, 10 and 100 mg/1). The growth of the plants was throughout S weeks and was determined by weekly mesurement of 70-100 samples from each culture. At same time, samples of leaf blades were fixed (glut/$Os0_4$) and routinely processed for electron microscopy. The most prominent results from this study are: 1. Only the highest concentration (100mgr/l) is clearly growth-inhibitory from the begining and more so from the 5th week on. On the contrary, all the other concentrations (particularly that 10 mg/1) stimulate somewhat the growth of the plants. 2. Some alterations in the chloroplasts of the mesophyl cells were observed. These alterations are mostly due to the progressive disappearance of the fret-membranes leading to the more or less disordered distribution of grana. Likely the effect on the growth, these alterations are only evident in plants treated with the highest concentration used (100 mg/1) and from 5th week on. These results, show that this grass *(Orysa sativa)* is very resistant to the action of the terbutryn, comparison with some infestuous algae of the same rice fields (MESQuitA & FÁTIMA SANTOS, 1981).

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INTRODUCTION

DANS un travail antérieur (MESQUITA & FÁTIMA SANTOS, 1981) nous avons analise, pour la première fois, les altérations ultrastructurales produites par la terbutrine (herbicide) sur quelques algues assez fréquentes dans les rizières du Mondego.

Alors, nous avons pensé qu'il serait intéressant d'étendre cette étude à l'espèce cultivée, c'est-à-dire, le riz *{Oryza sativa* L.), et comparer les éventuels effets produits par la drogue sur cette graminée avec ceux que nous avions déjà constaté dans les algues infestantes.

Ce sont les résultats obtenus que nous présentons maintenant dans cet article.

MATÉRIEL ET MÉTHODES

Des graines d´*Oryza sativa*, préalablement stérilisées à l'hypoclorite de calcium, ont été mis à germer dans des pots pleins de vermiculite, qui ont été périodiquement arrosés, soit avec du milieu de Hoagland (témoin) soit avec ce milieu additionné de terbutrine aux concentrations de 0.1, 1, 10 et 100 mg/1.

Après la germination des graines (une centaine dans chaque pot), le développement des plantules a eu lieu dans les conditions suivantes: température, 16-18° C; humidité, 60-65%; intensité lumineuse, 850 lux; photopériode de 10 h lumière sur 24 h.

La croissance des plantes a été appréciée, pendant deux mois, par la variation de leur taille moyenne. Pour cela, chaque semaine on a mesuré 70-100 exemplaires, tant du témoin que des plantes traitées, et les moyennes des valeurs obtenues ont été comparées statistiquement par le test de Student. Pour l'étude cytologique au M. E., des échantillons du tissu foliaire (mésophyle) ont été prélevés sur des plantes âgées de 3, 6 et 8 semaines, et tout de suite plongés dans le glutaraldéhyde (2%, tampon phosphate 0,05M, pH 6,8). Après une fixation de deux heures, le matériel a été rincé dans le tampon et post-fixe par le tétroxyde d'osmium (1 %,

tampon phosphate 0.05M, pH 6,8) pendant 1,30 h. à la température de la pièce.

La deshydratation à l'alcool et l'inclusion dans l'Epon ont été faites selon la technique habituelle. Les coupes ultrafines, ramassées sur des grilles sans membrane, ont été soumises à une double «coloration» par l'acétate d'uranyle et le citrate de plomb (REYNOLDS, 1963 ; VENABLE & GOGGESHALL, 1965) et étudiées dans un microscope électronique Siemens Elmiskop 101.

RÉSULTATS ET DISCUSSION

Au bout d'une semaine, tant les graines témoin que celles traitées dans les conditions rapportées ci-dessus (voir Matériel et Méthodes) ont germé dans un pourcentage de 87-95%. Alors, la terbutrine ne semble avoir aucun effet inhibiteur sur la germination, ce qui, d'une façon générale, est d'accord avec les données concernant les triazines (HILTON & *col*, 1963).

Pendant deux mois, le développement des plantules, d'après leur taille, a été analisée chaque semaine, étant que les premières mesures ont été faites sur des individus âgés de 15 jours (70-100 exemplaires dans chaque pot) (fig. 2). Alors, les courbes que nous avons obtenu avec les moyennes ont été rassemblées dans une seul graphique (fig. 1). On y voit que la terbutrine, aux concentrations de 0.1, 1 et 10%, n'inhibe pas le développement du riz, mais, bien au contraire, elle l'intensifie un peu, particulièrement à la concentration de 10 mg/1 (4-6 semaines de traitement). Par contre, la courbe correspondante à un traitement avec une solution dix fois plus concentrée (100 mg/1) montre un effet inhibiteur de la croissance, ce qui devient très évident à partir de la 5.^{ème}e semaine (fig. 1).

En ce qui concerne l'ultrastructure des plastes des tissus foliaires, nos observations réjoignent, d'une façon générale, celles de MIYAKÉ & MAEDA (1976a et &). En effet, dans ces tissus, en dehors de l'épiderme, ils ressortent les types de plastes suivants: 1. Plastes des tubes criblés qui sont normalement sphériques, à 1-15 um de diamètre, et contiennent

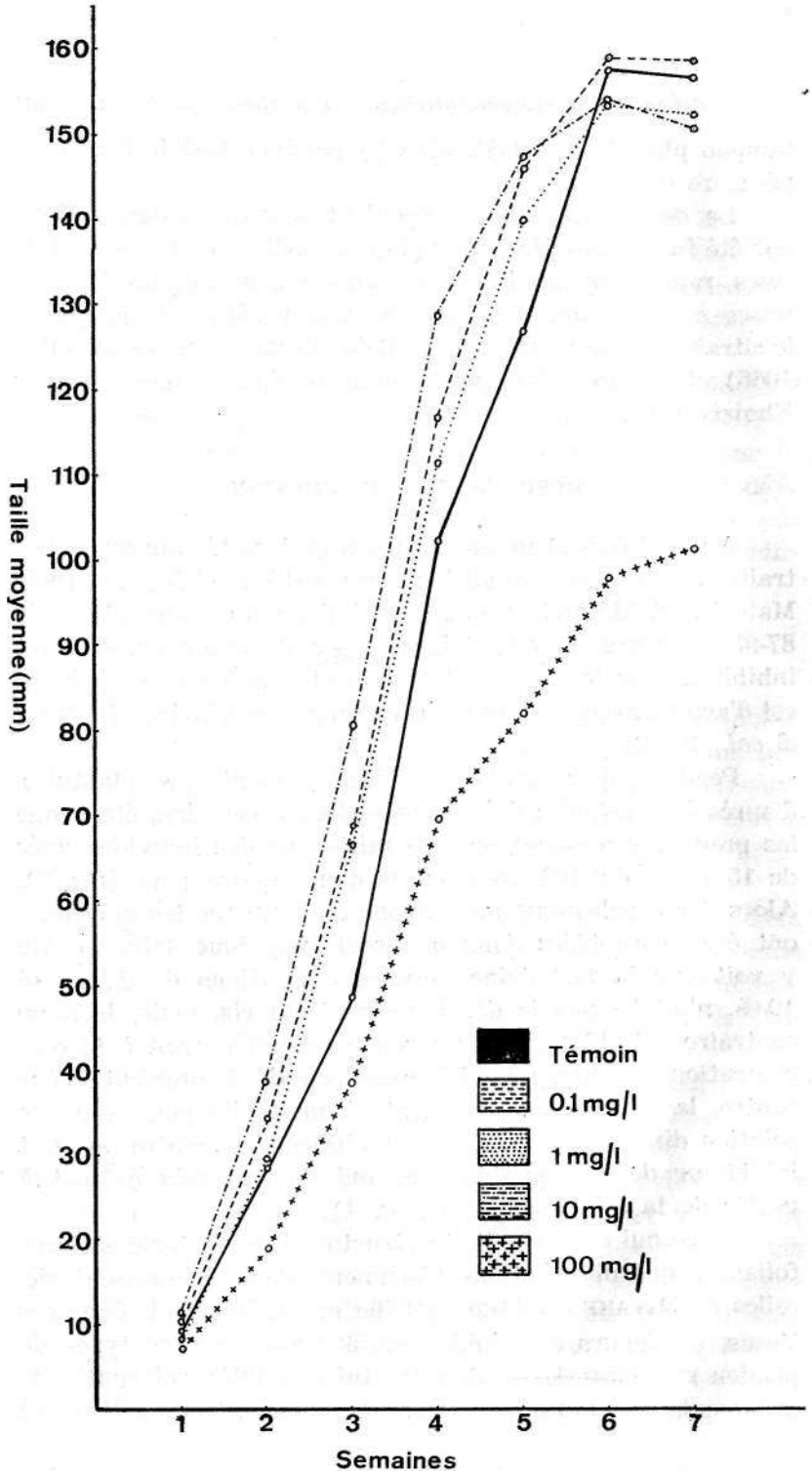

Fig. 1.—Variation de la taille moyenne (en milimètres) des plantules de riz cultivées, pendant sept semaines, soit dans le milieu de Hoagland (témoin), soit dans ce milieu aditioné de terbutrine aux concentrations de 0.1, 1, 10, l00mg/1.

toujours plusieurs inclusions, amorphes ou paracrystalines, probablement de nature protéinique (Pl. I, figs, *a* et c). 2. Plastes des cellules annexes, à morphologie et dimensions semblables à celles des plastes des tubes criblés, mais à structure plus simple: leur stroma est homogène et on n'y voit pratiquement pas des membranes, de l'amidon ou d'autres inclusions (Pl. I, fig. 6). 3. Plastes des cellules du parenchyme phloèmique. Ces plastes, en règle, sont plus petits

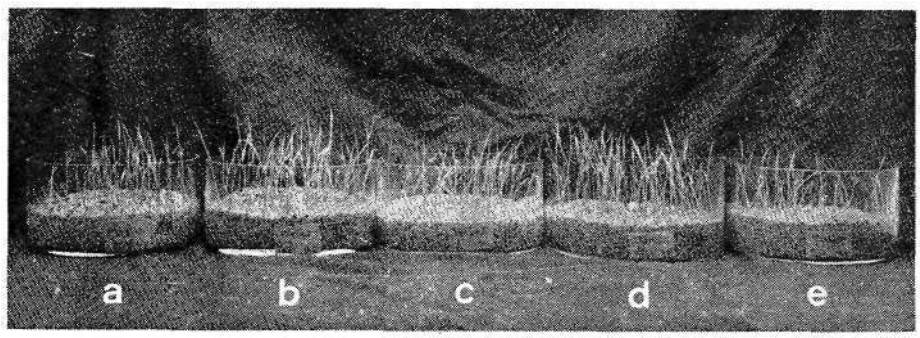

Fig. 2. — Développement des plantilles de riz dans le milieu de Hoagland additionné ou non de terbutrine. *a.* Témoin, *b-e.* Plantules soumises à l'action de la terbutrine aux concentrations, respectivement, de 0.1 mg/1 *(b),* lmg/1 *(c),* 10 mg/1 *-(d),* et 100 mg/1 *(e).* Cultures âgées de six semaines. Remarquer la taille des plantules dans les pots *(d)* et *(e)* par rapport au témoin (Voir le texte).

que ceux décrits ci-dessus, mais, par contre, ils montrent plus fréquement des lamelles ou des invaginations digitiformes en rapport avec la membrane interne de l'enveloppe plastidale; de même, ils ne contiennent point de l'amidon (Pl. I, fig. *d), 4.* Plastes des cellules parenchymateuses du mésophylle et du manchon fasciculaire. Dans les feuilles adultes, ces plastes montrent une structure qu'on peut considérer typique des plantes supérieures, c'est-à-dire, avec un appareil photosynthétique à zones granaires et intergranaires bien différenciées. Cependant, on doit signaler que les grana, normalement peu épais, se présentent constitués par des thylacoïdes à locules très réduits et bien serrés les uns contre les autres. Tout cela donne parfois aux grana l'aspect de

«bandes sombres» qui ressortent du stroma, mais dont la structure n'est pas toujours facilement décelable aux grossissements courants. Par contre, les ribosomes et les zones à DNA sont particulièrement évidents dans ces chloroplastes (Pl. II, fig. *b* et Pl. IV, fig. 2). D'accord avec les observations de MIYAKÉ & MAEDA (1976a), nous avons aussi constaté que les chloroplastes des feuilles adultes ne contiennent pas des grains d'amidon. 5. En dehors des types plastidales qu'on vient de décrire, nous avons observé, soit dans les cellules du manchon fasciculaire, soit dans celles du mésophylle, un autre type de plaste, à structure bien différente, et qui n'a pas été signalé par MIYAKÉ & MAEDA (1976a, *b).* Il s'agit de plastes assez volumineux, polymorphes et dont la structure se caractérise par la présence de nombreuses vésicules dispersées dans le stroma (Pl. III). Apparément, ces vésicules proviennent de la tuméfaction précoce, soit des thylacoïdes incipients, soit des digitations produites par la membrane interne de l'enveloppe plastidale (Pl. II, fig. *b).* Ils ont alors l'allure de certains plastes mutés (SALEMA & ABREU, 1972a et *b),* ou bien ils ressemblent les chloroplastes de plantes soumises à l'action de quelques antibiotiques (SIGNOL, 1961, 1962; KIRK & JUNIPER, 1963: MESQUITA, 1971) ou herbicides (BREZEANU & *col.,* 1976). Ces plastes, que nous avons aussi observé dans des feuilles de *Zea mays* (MESQUITA, 1979), se trouvent souvent à côté de chloroplastes normales dans la même cellule. Probablement, nous sommes alors en présence de «mixed-cells» (Pl. III, fig. *c)* (SALEMA & ABREU, 1972a et b), bien que leur fréquence ne soit pas suffisament élevée pour déterminer des modifications du phénotype (couleur des feuilles). Étant donné que ces plastes aberrants apparaissent, tant dans les témoins que chez les plantes traitées, ils ne représentent sûrement pas des altérations provoquées par la terbutrine.

Néanmoins, les plantules traitées pendant six semaines par une solution de cet herbicide à la concentration de 100 mg/1 montrent déjà, par rapport aux témoins, quelques altérations au niveau de l'ultrastructure chloroplastidale. Ces altérations, n'étant pas spectaculaires, se traduisent essentiellement par la désorganisation progressive des lamelles

intergranaires (Pl. IV, fig. *c;* Pl. V). Par suite de cette destruction, les grana deviennent desordonés dans le stroma (Pl. V, *a* et 6), autrement dit, l'appareil photosynthétique perd son organisation spaciale typique qui s'observe dans les chloroplastes des plantes non-traitées (Pl. TV, fig. *a).*

D'une façon générale, les composés similaires de la terbutrine, c'est-à-dire, les triazines et leurs dérivés, bloquent la photosynthèse par suite de leur action inhibitrice sur la réaction d'Hill (MORELAND *& col.,* 1959; HILTON *& col.,* 1963). Alors, il n'est pas étonnant que les chloroplastes soient les organites les plus sensibles à l'action de ce type de drogues. Cependant, l'intensité des altérations produites dépend, probablement, de plusieurs facteurs variables selon les espèces étudiées.

En realité, d'après HILTON & *col.* (1963), la plupart des herbicides sont fort et rapidement metabolises, pouvant leur toxicité sélective s'expliquer par des différences entre les espèces traitées, en ce qui concerne leur capacité de réaliser cette métabolisation. En plus, ces variations interspécifiques de susceptibilité seront aussi dépendantes de caractéristiques morphologiques, physiologiques et biochimiques, lesquelles, à un moment donné, déterminent la concentration du matériel toxic à l'endroit où il va agir (HILTON *& col.,* 1963).

Il se peut que soit là l'explication des différences de sensibilité et, par conséquence, du degré d'altération chloroplastidale, signalée chez des espèces traitées, soit avec des herbicides analogues de la terbutrine (ASHTON *& col.,* 1963), soit avec la terbutrine elle-même (MESQUITA *&* FÁTIMA SANTOS, 1981). En effet, nous avons constaté, par exemple, qu'un traitement de 96 h par la terbutrine, à la concentration de 0.1 mg/1, produit déjà des altérations remarquables dans les chloroplastes de quelques espèces d'Algues (MESQUITA *&* FÁTIMA SANTOS, 1979), tandis que chez le riz, comme nous venons de le signaler, la même concentration, ou même d'autres beaucoup plus élevées (1 et 10 mg/1) n'altèrent point l'ultrastructure cellulaire, même après 6-8 semaines d'action.

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EXPLICATION DES FIGURES

Toutes les figures concernent des tissus foliaires du riz *(Orysa sativa* L.).

Fixation: Glutaraldéhyde/tétroxyde d'osmium.
Contraste: Acétate d'uranyle/citrate de plomb.

Abréviations: Chl, chloroplaste; esp., espace intercellulaire; g, granum; gl, globule lipidique; m, mitochondrie, mb, «microbody»; n, noyau; pc, paroi cellulaire; pl, plaste; re, reticulum endoplasmique; v, vacuole.

PLANCHE I

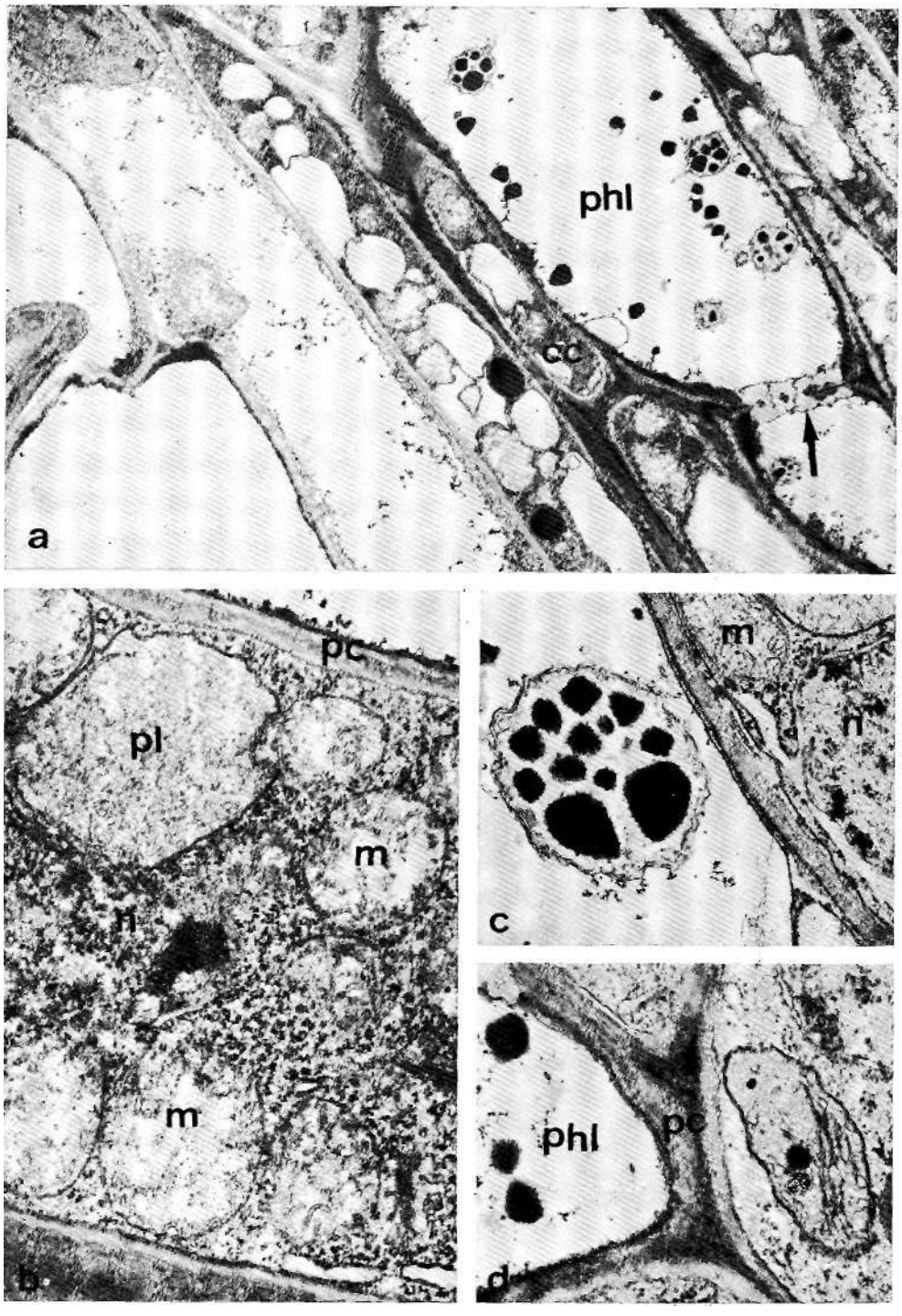
Différents aspects de l'utrastructure des cellules de la région fasciculaire des feuilles du témoin (plantes non-traitées).

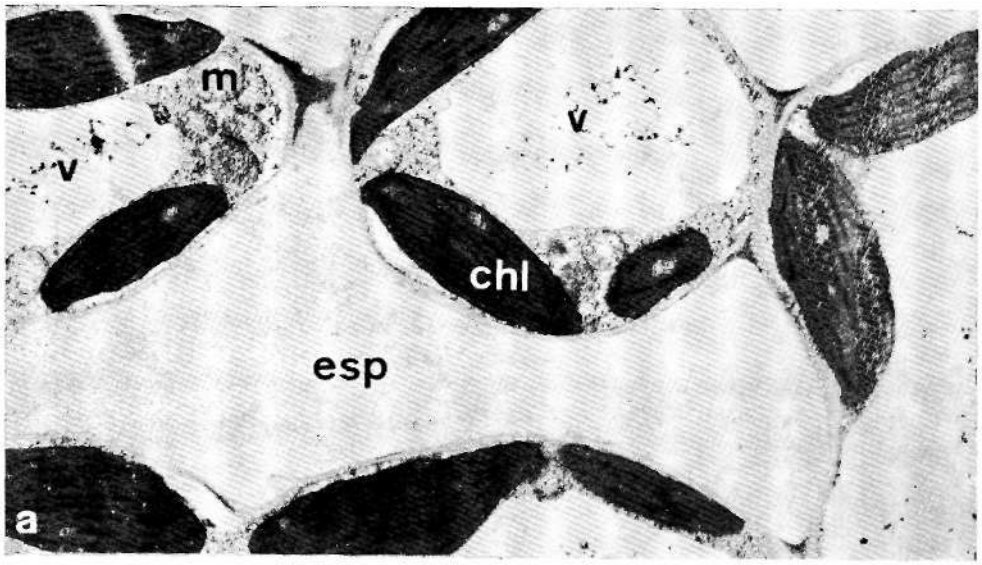
Fig. *a*. — Coupe longitudinale montrant, parmi d'autres cellules, deux éléments d'un tube criblé du phloème (phi) séparés par un crible (flèche) et des cellules annexes (c c). X 12.400.

Pig. 6. — Détail d'une cellule annexe. En dehors du cytoplasme dense à polyribosomes très nombreux, on remarques des mitochondries (m), uñe partie du noyau (n) et un plaste (pl). Celui-ci montre une structure très simple sans éléments figurés (mebmanes ou inclusions) dans le stroma homogène. X 33.000.

Fig. c. — On voit un plaste typique du phloème à nombreuses inclusions denses. X 33.000.

Fig. *d*. — Remarquer la structure d'un plaste d'une cellule de parenchyme phloèmique (à droite). X 33.000 (Voir le texte et comparer avec les figs, b et c).

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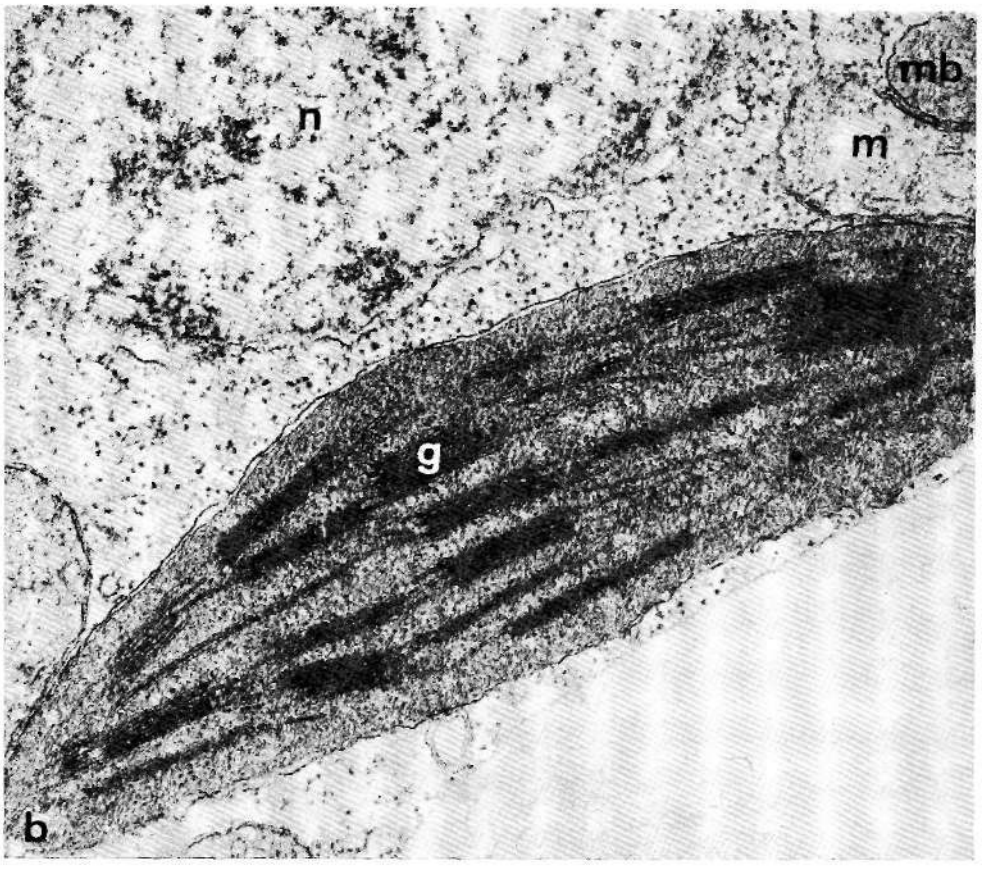
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PLANCHE II

Ultrastructure des cellules du mésophylle des plantules-témoin âgées de six semaines.

Fig. *a*, — Aspect générale du tissu. Il ressort le grand développement des vacuoles et des espaces intercellulaires. X 8.200.

Fig. *b*.—Vue partielle d'une cellule du parenchyme chlorophyllien. Remarquer l'organisation typique de l'appareil photosynthétique du chloroplasté à zones granaires (g) et intergranaires bien différenciées. x 48.000.

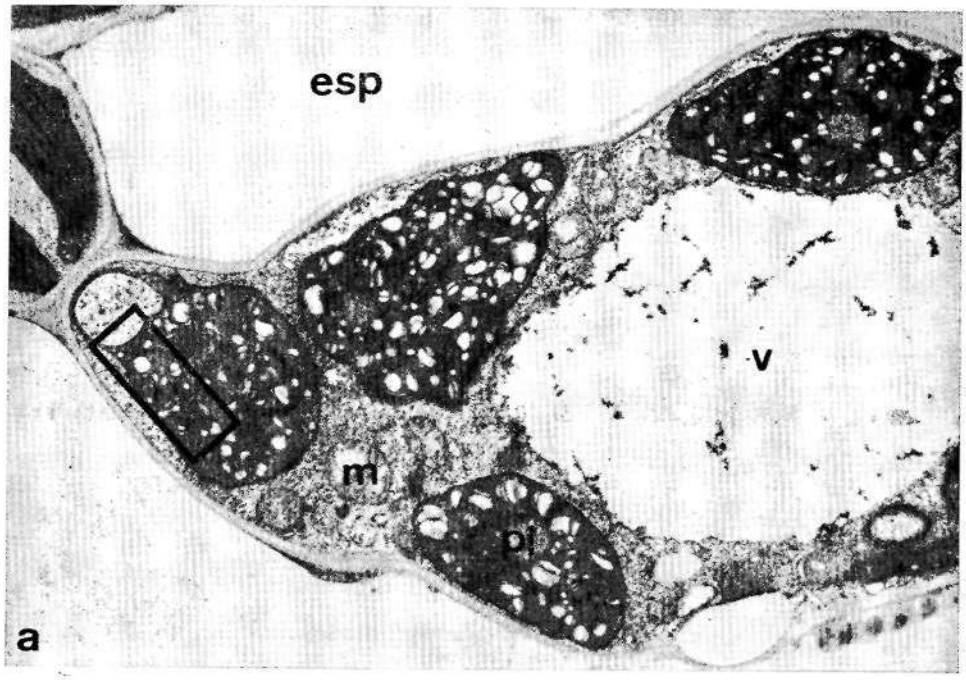
PLANCHE III

Aspects ultrastructurales des cellules du mésophylle des plantules du riz-témoin (continuation).

Fig. *a*. — Dans cette cellule on voit quatre profils de plastes aberrants. X 10.300.

Fig. *b*. — Détail de la fig. *a*. Remarquer que les vésicules plastidales semblent résulter de la dilatation des thylacoïdes de grana incipients (flèches). X 50.000.

Fig. c. — Vue partielle d'une «mixed-cell» montrant un chloroplaste normal (1) à côté d'un autre aberrant (2). X 20.700.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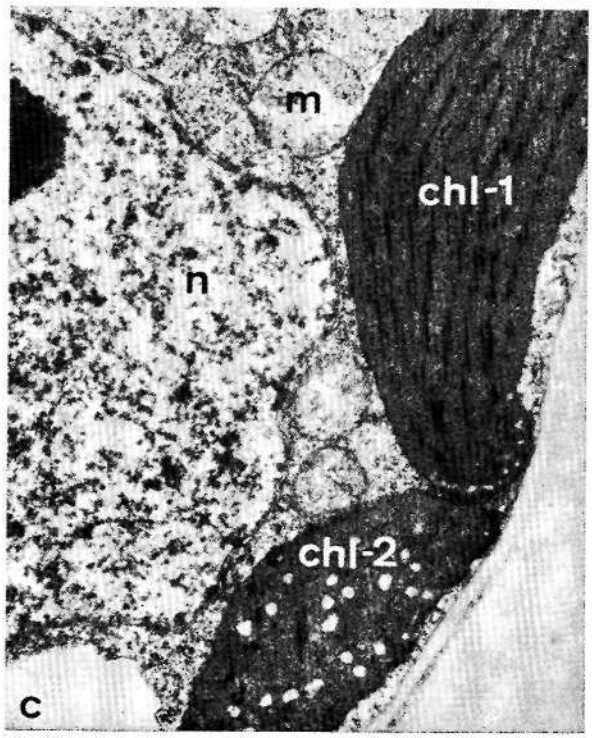
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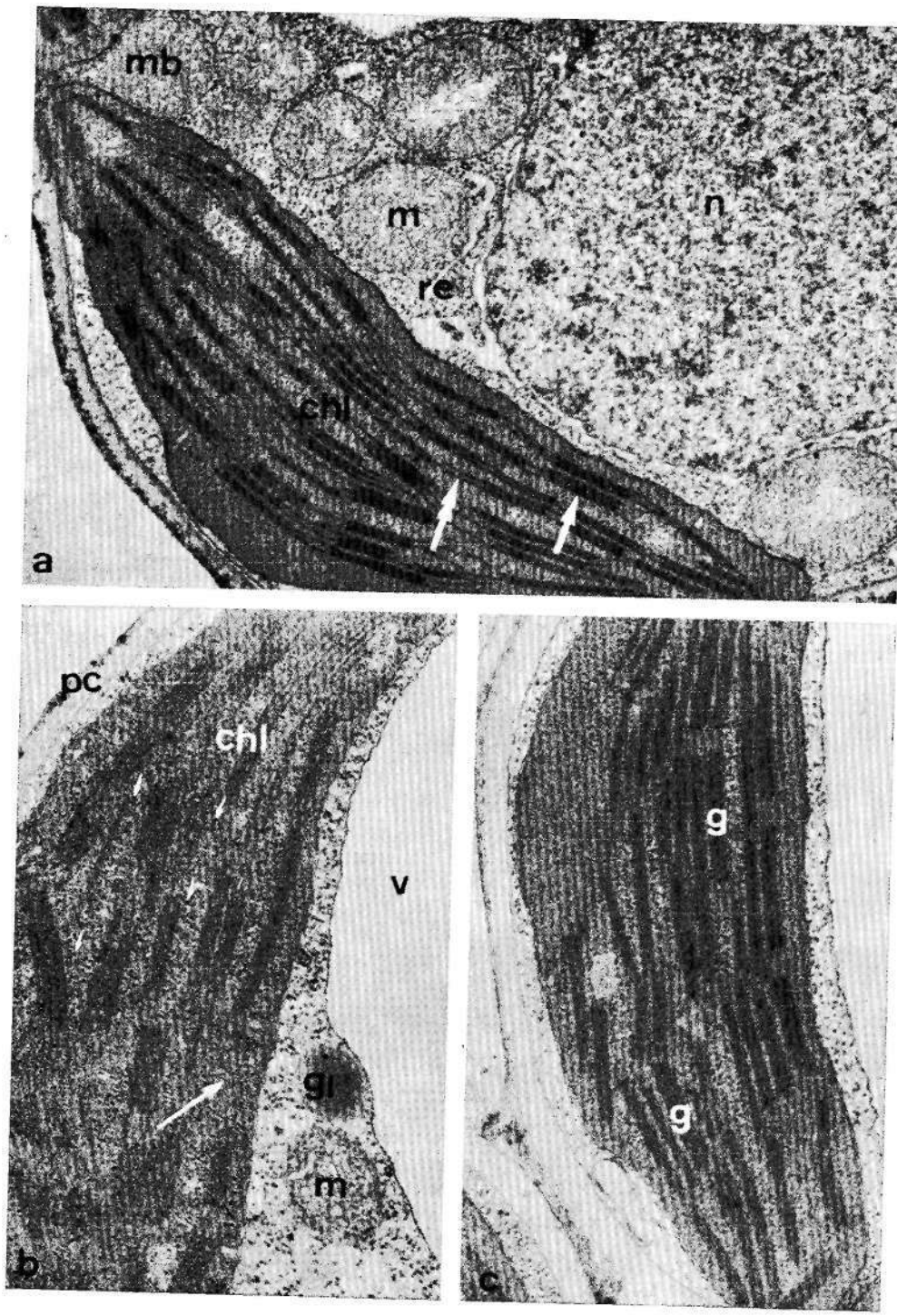
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PLANCHE IV

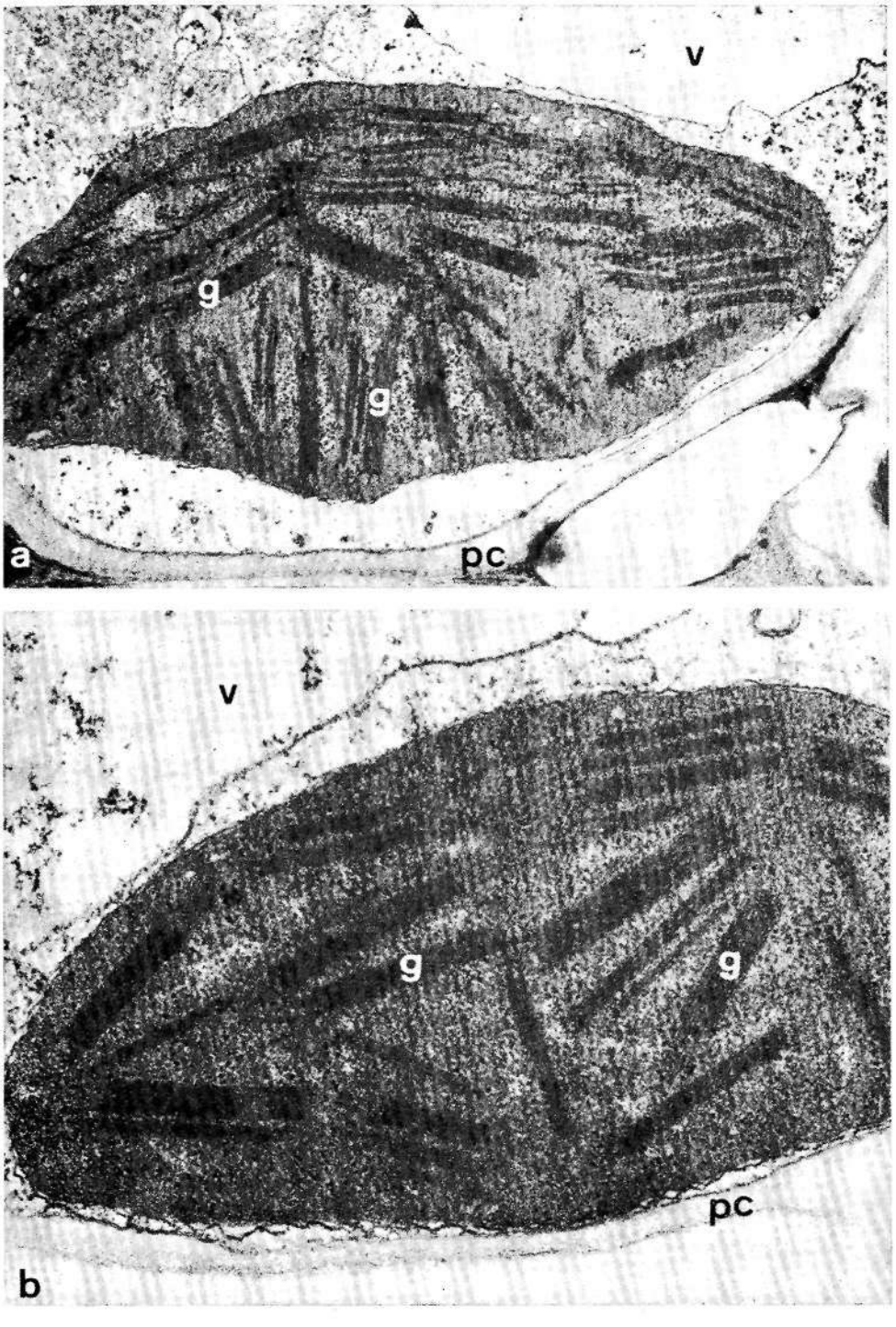
Fig. *a*. — Témoin. Ultrastructure du protoplasme pariétal d'une cellule du mésophylle. On voit presque tous les organites cellulaires: noyau (n), mitochondries (m), «microbody» (mb), chloroplaste (chl) et reticulum endoplasmique (re). Celui-ci est en continuité avec l'enveloppe nucléaire. L'appareil photosynthétique du chloroplaste montre une structure typique, c'est-à-dire, bien différenciée en des zones granaires (flèche simple) et intergranaires (flèche double). X 27.000.

Fig. *b*.—Traitement par la terbutrine (l mg/1-8 semaines). L'ultrastructure du chloroplaste est semblable à celle des plantes non traitées (comparer avec la fig. *a* et la fig. *b*, Pl. II). Les ribosomes chloroplastidales (petites flèches) sont bien évidents, ainsi que les lamelles stromatiques (grandes flèches). X 33.000.

Fig. c. — Idem (l00mg/1-6 semaines). Remarquer la disparition presque totale des lamelles stromatiques du chloroplaste. Néanmoins, les grana (g) mantiennent encore leur position relative (comparer avec les figs, o et *b).* X 41.000.

PLANCHE V

Figs, a et 6. — Traitement par la terbutrine (100 mg/l 7-8 semainés). Par rapport au chloroplaste de la fig. *c*, Pl. IV, et en dehors d'une certaine augmentation du volume de l'órganite, on doit remarquer la désordre de la distribution des grana (g), c'est-à-dire, la destruction de «l'architecture» typique de l'appareil photosynthétique (comparer avec la fig. *b*, Pl. II et la fig. a, Pl. IV).
Fig. *a:* X 33.000.
Fig. *b:* X 54.000.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FLORA CANARIA: NOTAS TAXONOMICO-COROLOGICAS— I

por

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RESUMEN

Se amplia el área eorológica de cuatro raros endemismos canarios: *Descurainia gonzalezii* Svent., *Micromeria glomerata* P. Pérez, *Sanguisorba moquiniana* (Webb et Berth.) Nordb. y *Tolpis glabrescens* Kammer. Se redescubre la existencia de *Onopordum carduelium* Bolle en Gran Canaria. Se hacen consideraciones acerca de la taxonomía y ecología de las especies tratadas.

ABSTRACT

In this paper data is presented that amplifies the chorological area of 4 rare Canary endemic plants: *Descurainia gonzalezii* Svent., *Micromeria glomerata* P. Pérez, *Sanguisorba moquiniana* (Webb et Berth.) Nordb. and *Totpis glabrescens* Kämmer. Also notification of the rediscovery of *Onopordum carduelium* Bolle on Cran Canaria is given. Comments are made on the taxonomy and ecology of the above mentioned species.

E N relación con los trabajos que el Departamento de Botánica tiene emprendidos en las Islas Canarias, hemos efectuado abundantes herborizaciones y recorridos por las mismas, durante los cuales han aparecido datos que, por su interés para el mejor conocimiento taxonómico, corológico y ecológico de la rica flora del Archipiélago, creemos oportuno dar a conocer. En esta ocasión se tratan cinco especies bastante raras. Una considerada ya como probablemente extinguida es redescubierta. Junto a ella, las otras

cuatro están fuertemente amenazadas por ese peligro inminente que acecha a un elevado porcentaje de la flora canaria: su extinción. Esperamos que su mejor conocimiento nos concience y comprometa a todos a sua conservación.

Descurainia gonzalezii Svent., JSoZ. *Inst. Nac. Inv. Agron.* 28: 17 (1953).

En 1977, en una excursión realizada a los montes de Granadilla, entrando por la pista forestal del Lomo Blanco (Vilaflor), en el Sur de Tenerife a unos 1600 m de alt., descubrimos la existencia de *Descurainia gonzalezii,* que crece con profusión sobre los derrubios de ambos lados de la pista a la que, en ocasiones, invade.

Considerada como bastante rara en la localidad clásica por su descubridor, SVENTENIUS, esta especie no habla sido encontrada fuera del Circo de Las Cañadas, en donde se le tiene como una verdadera rareza. En contra por tanto, de lo que es casi una norma para los endemismos canarios, esta planta ha debido tener en épocas recientes una notable difusión, que nos permite ampliar en la actualidad su dominio natural. No obstante, debe considerarse la posibilidad de que la planta fuese desde siempre abundante y que haya sido lo extraviado que resultaba el acceso a la zona, hasta la reciente construcción de la pista forestal, el motivo de no haberla encontrado en ella antes.

EXSiCCATA : Inmediaciones del Campamento Madre del Agua (Granadilla, Vilaflor), Tenerife, 30.IV.1979, P. *L. Pérez* y *C. Hernández* (TFC 6443; 6499); Pista forestal del Lomo Blanco, 1600m, Vilaflor, Tenerife, 13.V.1979, Ibid. (TFC 8028, 8029, 8030, 8031 y 8032).

Comentario taxonómico y ecológico

En el aspecto morfológico no hemos notado diferencias apreciables con los ejemplares de Las Cañadas, hecha la excepción de algunas pequeneces observadas en los ejemplares de mayor talla, que pueden alcanzar hasta 1 m de alto X 1-1,5 m de diám. En estos casos los escapos inflores-

cendales, que en los ejemplares menores suelen ser simples o ramificados solamente una o dos veces, pueden ramificarse hasta tres y cuatro veces y las dimensiones de las piezas florales pueden exceder ligeramente las medidas ordinarias.

Respecto a la ecología que la planta presenta en la zona, en una segunda visita realizada en mayo de 1979, hicimos las siguientes apreciaciones. Las inmediaciones donde crece la planta se hallan habitadas por un pinar ralo, cuyos claros ocupa un escobonal de *Chamaeoytisus proliferus* evidentemente mermado en favor de las repoblaciones de *Pinus canariensis.* La inclusión de la vegetación potencial en el dominio de la *Cytiso-Pinetea* Rivas-Goday et Esteve (1965) in ESTEVE (1969) no ofrece duda. Sin más pretensión que la de tener una visión de la composición florística de las comunidades en las que, en mayor o menor grado participa nuestra especie, se adjunta la siguiente tabla. De su análisis se deduce el carácter serial asi como las claras apetencias por suelos removidos y algo nitrófilos que presenta la planta.

Para completar una somera aproximación del panorama vegetal que nos ocupa, habría que añadir las marcadas disyunciones ecológicas y florísticas que suponen los barrancos que surcan la zona, en los que llama poderosamente la atención el elevado contingente de especies consideradas habitualmente como exclusivas de Las Cañadas, propias de cotas por lo general superiores a los 2000 m. Entre ellas, además de la *Descurainia gonzálezii,* anotamos *Echium wildpretii, Pterocephalus lasiospermus, Silene nocteólens, Nepeta teydea, Erysimum scoparium, Carlina xeranthemoides, Scrophularia glabrata, Pimpinella cumbrae* y otras que, pese a reconocérseles una mayor plasticidad ecológica, son consideradas también en general como endemismos de Las Cañadas: *Adenocarpus viscosus, Spartocytisus supranubius, Andryala pinnatifida* var. *teydea, Polycarpaea tenuis,* etc. A la vista de ello, estos barrancos deben considerarse como importantes vectores de dispersión, que permiten la fuga a niveles inferiores de plantas que alcanzan su óptimo en las cumbres de la isla.

En el caso concreto de *Descurainia gonzálezii,* la abundancia y enorme vitalidad con que crece entre los 1600 y

TABLA I

	1	2	3	4	5
	79/34	79/35	79/36	79/37	79/38
	160O	1600	1600	1625	1600
	SW	SW	S	S	S
	50	25	25	9	9
	5	5	IO	20	
	95	80	70	80	70
	8	13	14	8	7
Características de la clase *Cytiso-Pinetea* y unidades subsecuentes					
Pinus canariensis Chr. Sm. ex DC.	3.2	1.1	+		
Chamaeeytisus proliferus (L. f.) Link	4.4	1.1	1.1	+	
Argyranthemum adauetum (Link) Humphr. ssp. adauetum	+	3,3	1.1	1.1	2.2
	11	1.1	+	1.1	1.1
Cistus symphytifolius Lam. . . .	—	1.1	2.2		
Diferenciales de *8partocytision-mubigeni* y unidades subsiguientes					
Spartocytisus supranubius (L. f.) W. et B.			2,2		
Adenocarpus viseosus (Willd.) W. et B.	1.1	2,2	1,1		
Tolpis webbii Seh Bip. ex W. et B.	+	1.1	1.1	1.1	
Andryala pinnatifida Ait. var. teydea Webb.	1.1	1.1	+	1.1	1.1
Carlina xeranthemoides L. f. . . .	—	+	2.2		
Descurainia gonzalezii Svent. . . .	—	2.2	2.2	2.3	3.3
Compañeras accidentales					
	+	2.2	1.1	2,2	1.1
Avena occidentalis Dur.	—	+	2.2	1,1	+
	—	2.2	1.1		
Hirschfeldia incana (L.) Lagr.-Foss.				2.2	1.1

Loe. — 1. Parcela exponente de la climax (pinar/escobonal denso); 2. Claros de la formación anterior, sitios pedregosos; 3, Ibid.: malpais lávico subreciente; 4 / 5 . Borde y derrubios de la pista forestal.

1800 m, frente a su relativa rareza en Las Cañadas, nos hace pensar en la hipótesis inversa, es decir, más que una planta de las cumbres que tenga aquí su límite inferior, pudiera tratarse de una especie que teniendo su óptimo en estas cotas, alcanzara ocasionalmente Las Cañadas. Sin embargo no debe olvidarse la posibilidad de que la gran dispersión que presenta en esta localidad sea secundaria. La planta pudo haber llegado vía los barrancos o cualquier otro vector y al encontrarse con terrenos removidos, fruto de la construcción de pistas y repoblaciones forestales, que parecen favorecer su desarrollo, los colonizara, originándose un nuevo foco de expansión de la especie.

Micromeria gíomerata P. Pérez, *Vieraea,* 3 (1-2) : 77 (1974).

Este endemismo, que puede contarse entre los más raros de la isla de Tenerife, solamente conocido de su localidad clásica (Roque de Enmedio-Taganana), lo hemos encontrado en dos nuevas localidades situadas también en las inmediaciones de lo que los geólogos llaman «El arco de Taganana», sobre materiales traquifonolíticos : Dique del Roque Marrubial y al pié del Roque del Fraile, sobre Afur (Leg. E. BARQUÍN, 1978).

EXSICCATA : Dique del Roque Marrubial (Anaga-Tenerife), 21.IV.1979, P. *L. Pérez* (TFC 8313) ; Roque del Fraile (Afur--Anaga), VIII.1979, Ibid. (TFC 8314).

A pesar de ampliar su área de distribución, las características morfológicas y ecológicas de la planta, no difieren sensiblemente de las ya conocidas (P. L. PÉREZ, 1978).

Sanguisorba moquiniana (Webb et Berth.) Nordb., *Opera Bot.* 11 (2): 72 (1966).

Bencomia moquiniana Webb et Berth., *Phyt. Canar.* 2: 11; Tab. 39 (1846).

Marcetella moquiniana (Webb et Berth.) Svent., BoZ. *Inst. Nac. Invest. Agron.,* 18: 11 (1948).

La disparidad de criterios existente entre los distintos autores que se han ocupado del estudio taxonómico de esta especie, ha dado como resultado el que se haya situado en tres géneros diferentes. En 1846 WEBB et BERTHELOT, sus descubridores, la dan a conocer por primera vez bajo el nombre de *Bencomia moquiniana,* fundamentando la descripción solamente en plantas masculinas, e ignorando la flor femenina y fruto, por no haber encontrado pies de este sexo. Un siglo después, en 1948, SVENTENIUS, al descubrir en el Monte de Los Silos en Tenerife unas plantas femeninas, basándose fundamentalmente en el fruto samaroideo, claramente alado, que posee la especie, revisa el criterio de WEBB et BERTHELOT y describe un género nuevo, *Marcetella,* pasando la planta a denominarse *M. moquiniana.*

Finalmente, G. NORDBORG en 1966, publica un documentado trabajo acerca de la delimitación y subdivisión de los géneros *Sanguisorba* L., *Sarcopoterium* Spach y *Bencomia* Webb et Berth. En él considera insuficientes los caracteres apuntados por SVENTENIUS para independizar con rango de género a *Marcetella* Svent. de *Sanguisorba* L., con lo cual se llega a la tercera y discutida denominación de *S. moquiniana.*

Corológicamente *S. moquiniana* se consideraba hasta hace muy poco como un endemismo de Tenerife. Recientemente, sin embargo, ha sido encontrada en el Barranco de Guayadeque, Gran Canaria (KuNKEL, 1973) y en una localidad de la isla de La Gomera (M. FERNÁNDEZ, inéd.).

En Tenerife, dentro de su relativa rareza, se creía limitada a los profundos barrancos del SW de la isla, desde la Ladera de Güímar hasta el macizo de Teno, desde donde se extiende por el Norte hasta la Ladera de Tigaiga (BURCHARD, 1929), por lo general entre los 200 y 900 m de altitud. No había sido encontrada en la parte oriental de la isla, circunstancia que ya llamó la atención a BURCHARD en 1929. Nosotros encontramos en 1973 una reducida población en la ladera izquierda del Bco. del Batán (Anaga) entre los 250 y 400 m de altitud, pasando así a engrosar la lista de paleoendemismos que presentan una distribución disjunta entre los

sectores oriental y occidental de la isla, geologicamente afines *y* mucho más antiguo.

EXSICCATA : Bco. del Batán, 350m, Anaga — Tenerife, 11.VI.1979, *P. L. Pérez* (TFC 4125) ; Ibid., 250 m (TFC 8141) ; Ibid., 300 m (TFMC 302); Ibid., 400 m (TFC 8140).

Comentario ecológico

La mayor parte de los ejemplares de esta localidad se encuentran refugiados en acantilados inaccesibles, relictos de una vegetación más exhuberante del pasado, rica en endemismos, en su mayoría asimilables a los matorrales mesofíticos de transición entre el piso basal xerofítico y el montano húmedo. Entre ellos destacamos por su elevada presencia y significado: *Apollonias barbujana, Pistacia atlántica, Olea europea* ssp. *cerasiformis, Lavatera phoenicea, Convolvulus floridus, Sideroxylon marmulano, Maytenus canarienses, Jasminum oäoratissimum, Globularia salicina,* etc., especies, que en su mayoría solamente podemos ver hoy en los andenes termófilos de estos acantilados, que en los sitios de mayor verticalidad y pobreza en suelo se hallan poblados por especies características de la Al. *Soncho--Aeonion* SUNDING (1972) : *Sonchus congestus, Aeonium canariense* y *Davallia canariense* fundamentalmente.

Tolpis glabrescens Kämmer, *Bot. Jahrb. Syst.,* 97 (1) : 155 (1976).

Esta especie descrita por KAMMER en 1976, la habíamos observado nosotros por primera vez en marzo de 1972, en los escarpes del promontorio que con el nombre de Chinobre se destaca por encima del fayal-brezal, a unos 900 m de altitud en la vertiente Norte de las cumbres de la península de Anaga.

Plantas de esta especie las mantuvimos en cultivo por un período de más de tres años, durante los cuales se llevó a cabo su estudio, estando a punto[1] de ser dada a

[1] La especie se iba a dar a conocer con el nombre de *T. wildpretii* en *Vieraea* 6 (1), publicado en julio de 1976.

conocer en 1976 cuando, por sorpresa, apareció la publicación de KÄMMER. Una vez más sufrimos la descortesía de algunos investigadores extranjeros poco comunicativos, que parecen ignorar la existencia de botánicos en las islas.

El haber estudiado con detalle la especie y el hallazgo de una nueva localidad para la misma en las inmediaciones del Roque Anambro en la cordillera de Anaga, nos motiva ahora a transcribir algunas de las observaciones que habíamos hecho en aquella ocasión.

Descripción

Caméfito de hasta 25 cm de alt., más o menos ramificado en la base, cespitoso; raíz crasa, sinuado-tortuosa, escamosa y apenas laticífera, látex acuoso. TaZZo anodino, ca. 0,5-1 cm de diám., con las cicatrices de las hojas marcadas y cubierto por los residuos de las hojas y pecíolos viejos; dividido hacia el ápice en varias rósulas de 5-15 cm diám. *Hojas* dispuestas en roseta, parecidas a las de *Hypochoeris oligocephála,* carnosas, coriáceas, glabrescentes, las jóvenes verde--alegre, las más viejas amarillentas o castaño-oscuro, brillantes, bastante desiguales, generalmente de 3-12 cm 1. X 1--1,5 cm a., espatulado-lanceoladas, atenuadas en un corto pecíolo, en el ápice ligeramente mucronadas; margen remoto e irregularmente sinuado dentado, subpinnado-partidas, algunas veces brevemente aladas en la base, con el nervio principal prominente en el envés. *Panícula* erecta, de 10-20 cm de alt., glabra, estriada, marcescente, con pocos capítulos (usualmente de 3-6) y apenas 1-3 hojas reducidas; *pedúnculos* subarqueados, glabros, muy levemente pruinosos por debajo del capítulo, orlados por 2-5 brácteas lineares y agudas. *Capítulos* de 15-30 (80) flores, de 15-25 mm diám. e involucro de 6-7 mm de 1., formado por 15-25 brácteas, linear-lanceoladas, agudas, subpruinosas, margen hialino, las exteriores arqueado-patentes, las interiores erectas, algunas veces imperceptiblemente sublanosas en el ápice. *Receptáculo alveolado* de 4-5 mm de diám. Lígulas amarillas, de 12-15 mm de 1. X 2-4 mm de a.; tubo la mitad más corto que el limbo, erizado de pequeños pelos, amarillo-tenue; limbo ovado-

cuneado, penta-dentado, dientes deltoides, más raramente bilabiado, de color amarillo, a menudo listado de oscuro en el envés. *Estambres* la mitad más cortos que la lígula; estilo exerto por encima de las anteras, terminado en un estigma de lacinias subiguales, pequeñas y divaricadas. *Aquenios* de 2 mm de 1. X 0,5 mm de a., estrechos, subpentágonos, atenuados en la base, con un solo surco entre los ángulos, glabros, oscuros, ornamentados por pequeñas arrugas transversales. *Vilano* blanquecino formado por 5-7 setas grandes, mezcladas con otras más pequeñas y escamiformes.

Florece desde finales de primavera hasta finales de verano.

T. glabrescens se trata, al parecer, de un endemismo local conocido solamente de algunos puntos aislados en las crestas de la cordillera central del Macizo de Anaga, entre los 700 y 900m de altitud. Se ha citado con interrogación (KUNKEL, 1977) para el macizo de Guayedra en Gran Canaria. No podemos confirmar esta cita por ahora, pues estudiado material de *Tolpis* procedente del Bco. Oscuro (Tamadaba) •— TFC 8142—, próximo a aquella localidad y muy semejante al iconografiado en la lámina que se incluye en *Cuad. Bot. Canar.*, 28: 55 (1977), al confrontarlo con el de Tenerife, difiere notoriamente por la mayor laxitud de las rosetas foliares y el mayor tamaño de los escapos florales, al igual que por las características ecológicas reinantes en los respectivos habitats de origen. No obstante ,teniendo en cuenta el polimorfismo que caracteriza a los *Tolpis* en Canarias es necesario el estudio comparativo de más material, antes de descartar definitivamente tal relación.

EXSIOCATA : Chinobre, Anaga, 900 m, 5.VIII.1974, *P. L. Pérez* (TFC 4967; duplic. in MA y MAF) ; Ibid., VIII. 1976, *P. L. Pérez* (TFMC 280, 281 y 282); Anambro, Anaga, VIII. 1976, Ibid. (TFMC 283).

Comentario taxonómico y ecológico

Por el porte, conformación de. las rosetas, aspecto, consistencia y coloración de las hojas e incluso remotamente

por la ecología, *a priori, T. glábrescens* parece guardar mayor afinidad con *Hypochoeris oligocephála,* que con cualquiera de las especies descritas del género *Tolpis* en el Archipelago. Sin embargo esta primera impresión se desvanece al examinar la morfología de las brácteas del involucro, el receptáculo sin escamas y las peculiaridades de la flor y del fruto.

Asimismo, ciertas formas empequeñecidas de *T. proustii* en la isla de El Hierro, que crece en las partes más expuestas de los riscos sobre Sabinosa, en situaciones ecológicas muy similares a las existentes en las cumbres de Anaga (influencia casi constante de la niebla y del viento), recuerdan en principio a *T. glábrescens,* similitud que se deshace al observar la variabilidad poblacional de la primera especie.

Pensamos que estas relaciones aparentes parecen deberse más bien a ciertas convergencias ecológicas, que a una mayor proximidad filogenética.

En la isla de Tenerife, las dificultades para su separación dentro del género, convergen en *T. crassiuscula,* de la cual sin embargo, a nuestro juicio es fácil separar por las diferencias observadas, que se recogen en el cuadro siguiente:

	T. crassiuscula Svent.	*T. glábrescens* Kämmer
Hojas Fig. 1)	simiado-lóbuladas; hacia el peciolo albo - tomentosas; las mayores de hasta 20 X 5 cm; ± flácidas.	sinuado-dentadas; totalmente glabras; las mayores de 12 X 3 cm; coriáceas.
Panícula	albo-tomentosa en la base; netamente pruinosa en el ápice.	glabra
Capítulos	multif loros (150-200 fl.); 2-3,5 cm de diám.	paucifloros [15-40 (80) fl.] ; 1,5-2,5 cm de diám.
Receptáculos	5-7 mm de diám.	4-5 mm de diám.
Involucro	30-40 brácteas.	15-25 brácteas.
Brácteas	7-8 mm; lanosas en el ápice.	6-7 mm imperceptiblemente lanosas en el ápice.
Florece	Mayo-Junio.	Junio-Septiembre.

TABLA n

N^0 de orden . .	1	2	3			5
Altitud m s.n.m. .	150	150	100	175		**900**
Area m^2	2	5	1	2		1
Exposición . . .	N	NE	NW	N		N
Inclinación % . .	95	90	90	100		90
Cob, vegetación %	20	25	15	25		25
N° de especies . .	IX	11	7	7		7
Características asociación (Al. *Soncho--Aeonion)*					*Ibid.*	
Sonchus radicatus Ait.	1.1	—	1.1	**2.2**	Tolpis glabrescens Kämmer . . .	2.3
Aeoniuna tabulaeforme (Haw.) Webb et Berth.	1.1	1.1			Aeonium cuneatum Webb et Berth.	1.1
Diferenciales locales						
Tolpis crassiuscula Svent. . .	1.1	1.1	2,2	**1.1**		
Hypochoeris oligocephala (Svent et Bramw.) Lack.		1.1	1.1	**2.3**		
Monanthes gilensis.(Praeger) Svent	1.1			**1.1**		
Características de AL, Or., y Cl *(Soncho--Aenion, Soncho-Aeonietalia* y *Aeonio--Greenovietea)*					*Ibid.*	
Braehypodium arbuscula Knoche . . .	1.1	1.1		**1.1**	Selaginella denticulata (L.) Link	3.4
Monanthes laxiflora (DC.) Bolle . . -	1.1			**1.1**	Blechnun spicant (L.) Roth. .	1.1
Vieraea laevigata (Brouss. ex **WiUd.)** Webb.	1.1	1.1				
Lobularia intermedia Webb et Berth.		1.1				
Lotus glaucus Ait.		1.1				
Reichardia ligulata (Vent.) Aschers. . .	1.1					
Sideritis argosphacelus (Webb et Berth.) Clos.		1.1				
Transgresivas Al. *Frankenio-Astyda-•mion* y Al. *Kleinio-Euphorbion*					Transgresivas Al., Or. y Cl. *(Fayo-Ericion, Andryalo-Ericetalia, Pruno-Lauretea)*	
Frankenia ericifolia Chr. Sm. ex DC.			+			
Euphorbia aphylla Brouss.		2,1			Erica scoparia L. ssp. platycodon (W. et B.) Hans, et Kun.	1.1
Argyranthemum coronopifolium (WiUd.) Humphr.	1.1		1.1		Viola cf. maderensis Lowe	1.1
Descurainia rnillefolia (Jacq.) Webb et Berth.	1.1				Hypericum grandifolium Choisy . .	1.1
					Ranunculus cortusaefolius WiUd. . .	
Accidentales						
Micromeria varia Bentham ssp. varia		1.1	1.1			
Psoralea bituminosa L.	1.1	+	1.1	+		

Loc. — 1, 3 y 4: El Fraide (Teno); 2, Los Andenes (Teno). 5, 6 y 7: Chinobre (Anaga).

Las apetencias ecológicas de ambas especies, muestran asimismo diferencias manifiestas. *T. crassiuscula* se cría en las fisuras de los diques y coladas basálticas, no demasiado secas, del piso basal de la isla. *T. glabrescens*, por el contrario, es marcadamente higrófila desarrollándose en uno de los sectores más húmedos del archipiélago, sometido a los efectos de una alta condensación nebulosa y al goteo casi permanente que la misma trae consigo.

En la Tabla II, se suma a las ya comentadas diferencias morfológicas y ecológicas, otras de índole sociológico, que ponen de manifiesto la composición florística de los inventarios realizados en las áreaos clásicas de las dos especies.

Onopordum carduelium Bolle, *Bonplandia* 7: 297 (1859).

Onopordon carduelinum auct. pl.

Descubierto por BOLLE y dado a conocer por él en 1859, este raro endemismo no había sido vuelto a encontrar posteriormente, a pesar de la anotación de su autor: «...in adscensu de la Cumbre, Cazadores et Cuevas blancas versus, abunde».

En Agosto de 1974, durante las herborizaciones que efectuábamos en la isla de Gran Canaria, en una visita realizada en compañía de A. SANTOS a las inmediaciones del Roque o Risco Grande de Tentiniguada (Valsequillo), observamos hacia los 1250 m de altitud, en la ladera SE al pié de dicho Risco, un cardo que por el porte y la morfología de sus tallos alados sospechamos pudiera tratarse de la especie bolleana. Al encontrarse el material completamente agostado en esa ocasión, no pudimos confirmar nuestras sospechas hasta mayo de 1979, fecha en que visité la localidad y herboricé dos plantas a punto de florecer, que transplantadas al jardín del Dpto. de Botánica en La Laguna, florecieron y fructificaron. En junio del mismo año, en una segunda visita a la isla, herboricé material en flor y en él basamos la descripción que se incluye a continuación.

Descripción

Hemicriptófito con raíz potente, axonomorfa, tuberosa. *Hojas* basales dispuestas en una amplia roseta, oblongo-lanceoladas, atenuadas en la base, semiamplexicaules, subsésiles,

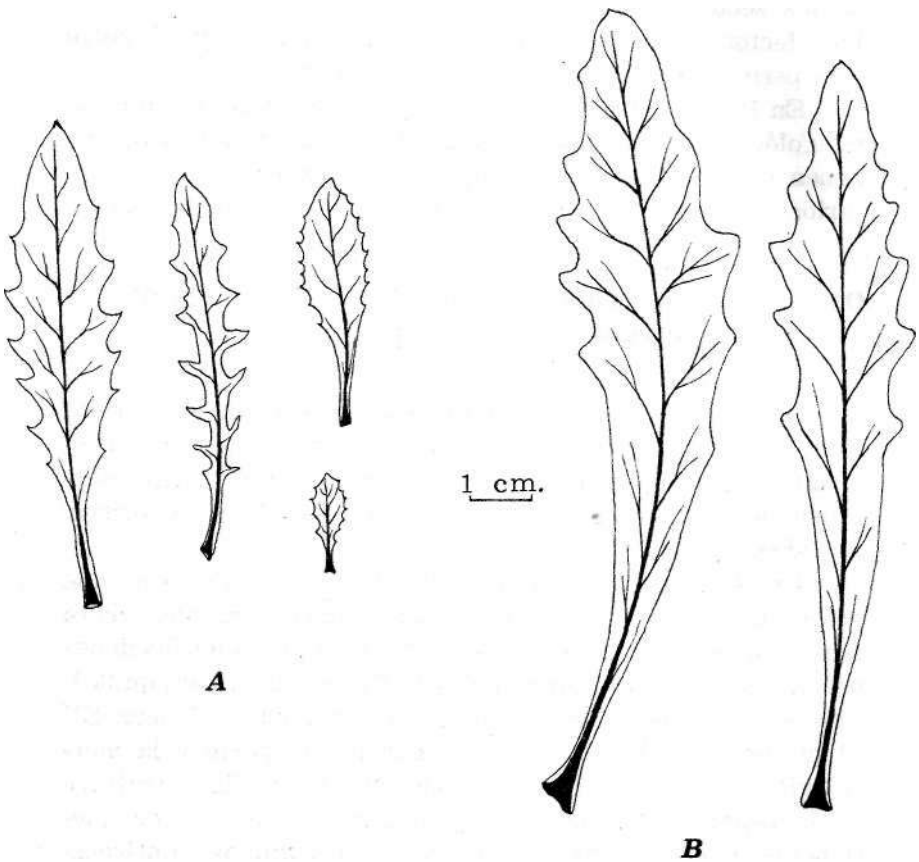

Fig. 1. — Comparación de la morfología foliar de: A. *Tolpis glábrescens* Kämmer y B. *Tolpis crasiuscula* Svent.

pinnatipartidas, de lóbulos recortados, subtriangulares y espiniscentes, con espinas amarillas marginales, muy punzantes; aracnoideo-tomentosas en ambas caras, blancas en el envés y más oscuras en la haz; usualmente de 30-35 X 10-12 cm, las mayores de hasta 50 X 15 cm, marcescentes. Las cauli-

nares más pequeñas, decurrentes, transformándose en alas que recorren el tallo. *Tallos floríferos* erectos de 0,5-1,8 m de alto; netamente mayores que las hojas basales, recorridas por alas irregularmente recortadas y provistas de espinas amarillas, horizontales, de 2-10 mm de long. Rama principal coronada por una cabezuela que es, por lo general, la primera en florecer; laterales arqueado-erectas, igualmente alado espinosas, también monocéfalas, pudiendo ocasionalmente la más superior superar en longitud a la rama principal. *Capítulos* de 3-5 cm de diám., subesféricos, truncados en la base. *Brácteas* involúcrales glabras, purpurescentes en el tercio superior y terminadas en una espina rígida, de color pajizo; las internas linear-lanceoladas y las externas lanceoladas y reflexas; decrecientes de dentro a fuera. *Receptáculo* profundamente alveolado; alveolos cuadrangulares o pentagonales, delimitados por una membrana escarioso recortada con un marcado apículo en los vértices. *Flósculos* de 30-35 mm de long. ; tubo de 2-2,5 mm de long., ensanchado y esparcidamente glandular en Ia parte superior; lóbulos linear-subulados, rosáceo-purpúreos, glandulosos en los márgenes y superficie externa; glándulas puntiformes, brillantes. *Estambres* insertos en el ensanchamiento superior del tubo; *filamentos* cortos, de 3-4 mm, glabros, hialinos ; *anteras* de 10-11 mm de long, excediendo ligeramente (0,5 mm) a los lóbulos de la corola; glabras; apéndice terminal subulado, purpurescente, de 3-3,5 mm de long. *Estilo* netamente exerto, de 30-40 mm de long., blanco hialino, glabro. *Estigma* de ca. 5 mm, purpurescente al igual que los apéndices de las anteras; lacinias linear-obtusas, adnatas. *Aquenios* jóvenes lisos, brillantes, subcilíndricos ; maduros tetrágonos o pentágonos, con 4-5 costillas bien marcadas; 4-5 mm de long. X 2-3 mm de a., truncados en el ápice y atenuados en la base, estriado-rugulados longitudinalmente, cubiertos de un tomento muy fino, aracnoide-blanquecino, al menos en las $^{3/4}$ partes inferiores de su longitud ; pardos, más oscuros en el tercio superior. Vilano de aproximadamente doble long, que el aquenio; setas rígidas, desiguales, soldadas entre sí en la base, divergentes, finamente plumosas, blanco--amarillentas.

Florece: Junio-Julio; *fructifica* Julio-Agosto.

Typus. «*Onopordum carduelium* Bolle; Loc: Al pié del Risco Grande en Tentiniguada, Valsequillo, Gran Canaria, 1300m —Loc. cl.—; 2.VI.1979, P. L. Pérez in Herb. Fac. de Ciencias (Biológicas) TFC 8143 (1-4) ex Herb. *P. L. Pérez* (n° 21) ». *Neotypus.*

Tipificación. Se ignora la suerte que corrió el material de BOLLE en el que este basó su descripción y que constituiría el *Typus* de esta especie. Su búsqueda en los herbarios B, FI, K, MO, P-CO, S y W en los que existía la remota posibilidad de hallarse algún duplicado del material original, ha resultado infructuosa. Del herbario de Berlin-Dahlem se nos comunicó que posiblemente el material estudiado por BOLLE desapareció en la catástrofe sufrida durante la Cegunda Guerra Mundial. En vista de ello, se ha creído conveniente elegir *Neotypus* al ejemplar que, desglosado en 4 pliegos, se conserva en la actualidad en el Herbario del Dpto. de Botánica de la Fac. de Ciencias (Biológicas) — TFC 1843 (1-4) de la Universidad de La Laguna.

Comentario taxonómico y ecológico

El género *Onopordum,* L. está representado en las Islas Canarias por dos especies: *0. carãuelium* Bolle y *O. nogalesii* Svent., endemismos de las Islas de Gran Canaria y Fuerteventura respectivamente. La existencia, supuesta por BOLLE (1859 Z. c.) de otra especie en la Isla de La Gomera no ha podido confirmarse y el material originario procedente de las semillas por él enviadas a los jardines de Berlín y Florencia, parece haber desaparecido según se nos comunicó de estos Centros.

De acuerdo con nuestras observaciones y las que se desprenden de la descripción hecha por SVENTENIUS (1960) de *O. nogalesii,* las dos especies presentan diferencias manifiestas,, tanto en el porte como en los detalles taxonómicos de las brácteas del capítulo, flor y fruto. En *O. carãuelium*

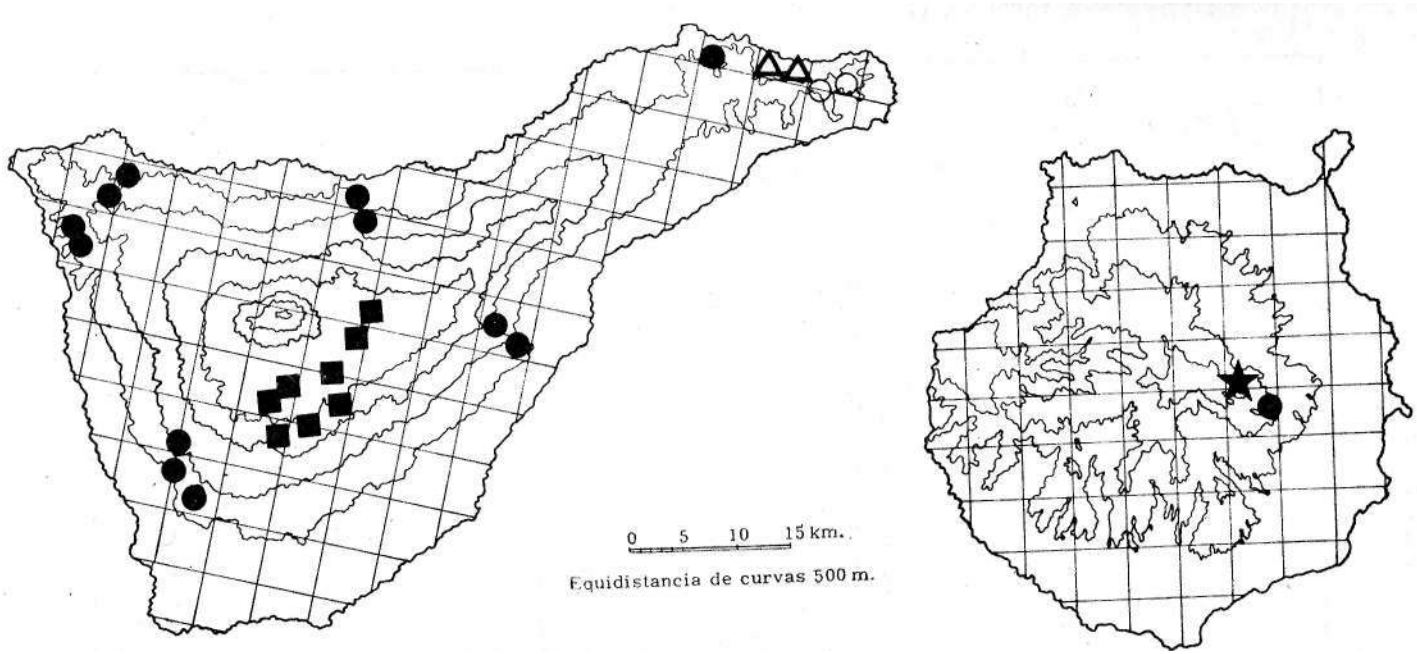

Fig. 2. - Distribución conocida de las 5 especies estudiadas

■ Descurainia gonzalezii Svent.

● Sanguisorba moquiniana (W. & B.)Nordb.

△ Micromeria glomerata P. Pérez

O Tolpis glabrescens Kämmer

Onopordum carduelium Bolle

«INVENTARIO»

	79/25
	1250
	100
	SE
	45
	70
N° de especies	17
Características de la, Al. *Micromerio-Oytision congesti* o unidades subsecuentes.	
Teliae mierophylla (DC.) Gibbs et Dingw. (= Cytisus congestus Ball.)	3.3
Argyranthemum adauctum (Link) Humphr. ssp. canariense (Sch. Bip.) Humphr.	2.2
Erysimum scoparium (Brouss. ex WiUd.) Wettst.	1.1
Sideritis dasygnaphala (Webb) Clos	+
	2.1
Características de orden y clase: *Oytiso-Pinetalia* y *Cytiso-Pinetea.*	
Chamaecytisus proliferas (L. f.) Link (= Cytisus proliferas L. f.)	1.1
Micromeria benthami Webb et Berth.	1.1
Diferenciales o transgresivas de otras comunidades.	
Euphorbia obtusifolia Poir.	11
	11
	1.1
	1.1
(rapí colas)	2.2
	1.1
Echium callithyrsum Webb ex Bolle	1.1
(accidentales)	1.1
Asphodelus aestivus Brot.	1.1
Micromeria varia Benth.	1.1

los tallos floríferos son más gráciles ;y mayores, al menos de 1,5-2 veces la longitud de las hojas basales. Las ramas laterales son más alargadas y monocéfalas. Las espinas apicales de las brácteas involúcrales son menores que en *O. nogálesii.* Los flósculos son mayores (30-35 mm) asi como el apéndice terminal de las anteras (3-3,5 mm). El estigma tiene las lacinias adnatas y en los aquenios estudiados faltan las estrías transversales entre las costillas, tan típicas en *O. nogálesii.*

O. carduelium crece, en esta localidad, sobre una extensión aproximada de 5000m^2, en la ladera inclinada que se extiende al pié del Roque Grande, sobre el Ricón de Tentiniguada. Aquí contamos alrededor de 150 ejemplares que viven en las fisuras de las rocas, entre los bloques de piedra desprendidos del Roque y en los pequeños terraplenes de la ladera, hincando sus potentes raices napiformes hasta por debajo de los 0,5 m de profundidad.

La vegetación del paisaje está caracterizada por un matorral, en ocasiones casi cerrado de *Teline micropkylla,* que en determinados sitios debido, en parte, a la fuerte inclinación del terreno se ve aclarado, siendo en estos calveros donde se localizan un mayor número de ejemplares de *Onopordum.*

El inventario adjunto, realizado sobre un área de 100 m^2 nos da una idea bastante fiel de la composición florística del conjunto de esta localidad, asimilable al dominio de la Al. *Micromerio-Cytision congesti* Esteve —• 1969 —(.= *Cytision canariensis* Sunding —1972 — p. p.).

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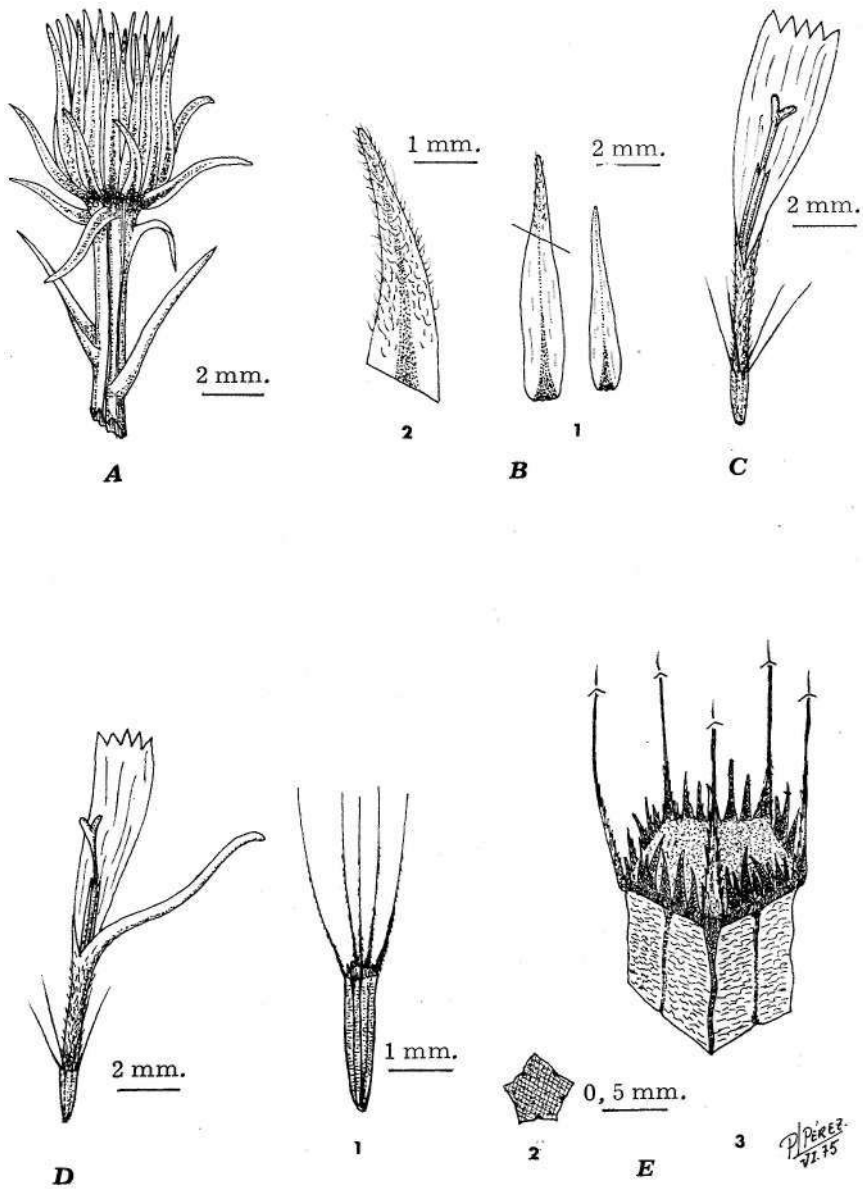
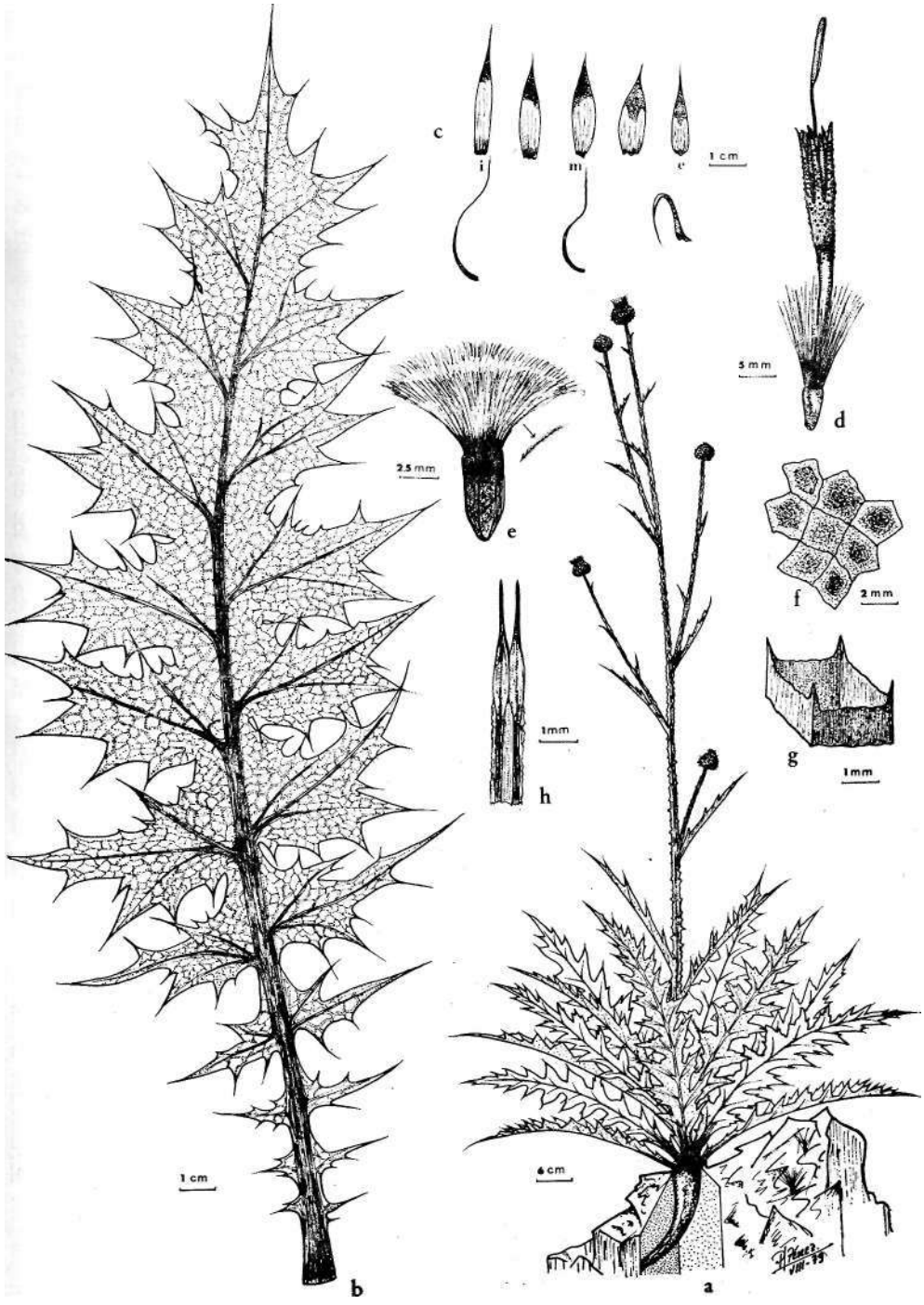
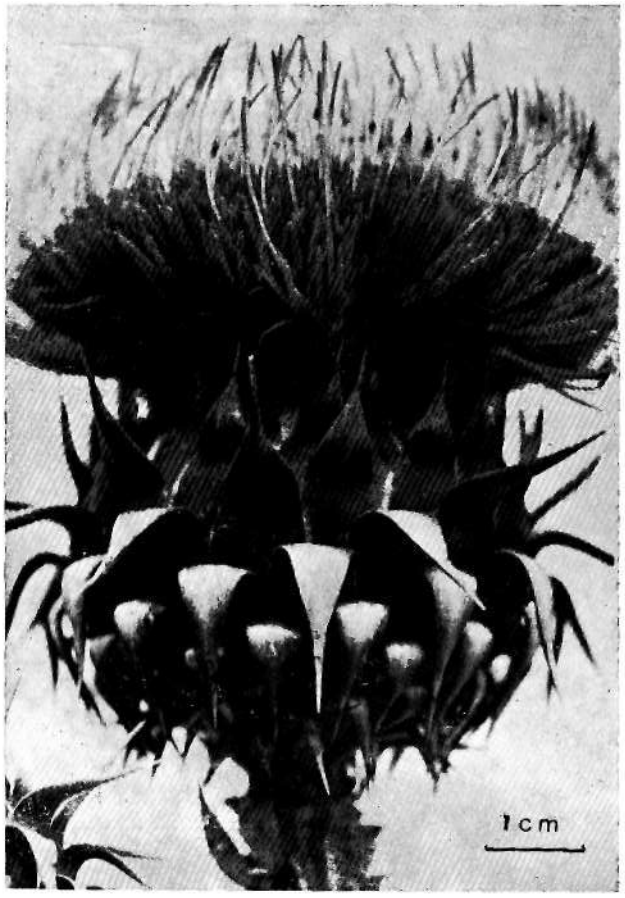
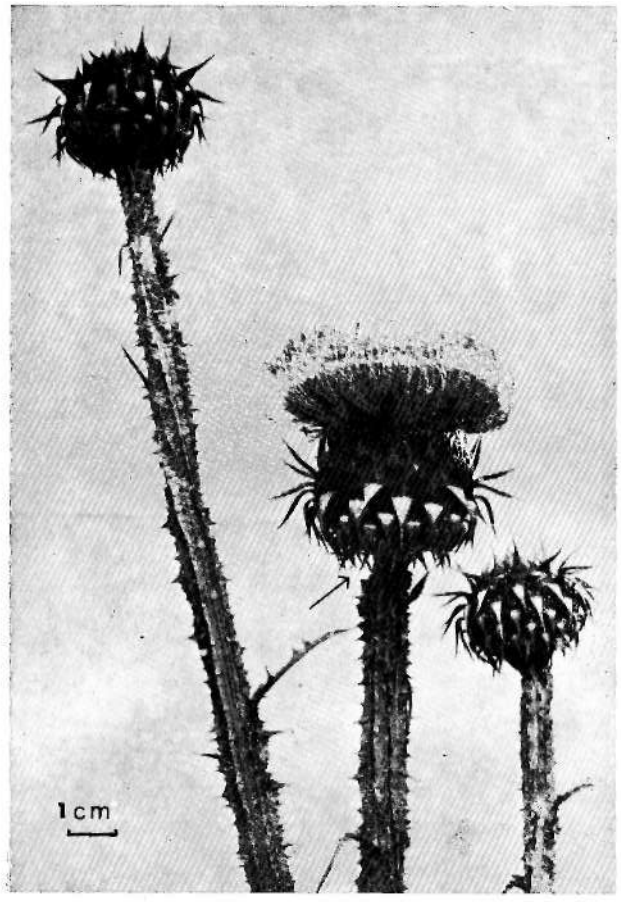


Tolpis glabrescens Kämmer. Detalles del capítulo, flor y fruto: A. Involucro. B. Brácteas: 1. enteras; 2. ápice de una de ellas. C. Lígula. D. Curioso aspecto teratológico de una lígula bilabiada. E. Aquenio: 1. completo; 2. sección transversal; 3. detalle de la inserción de las setas — muy aumentado.

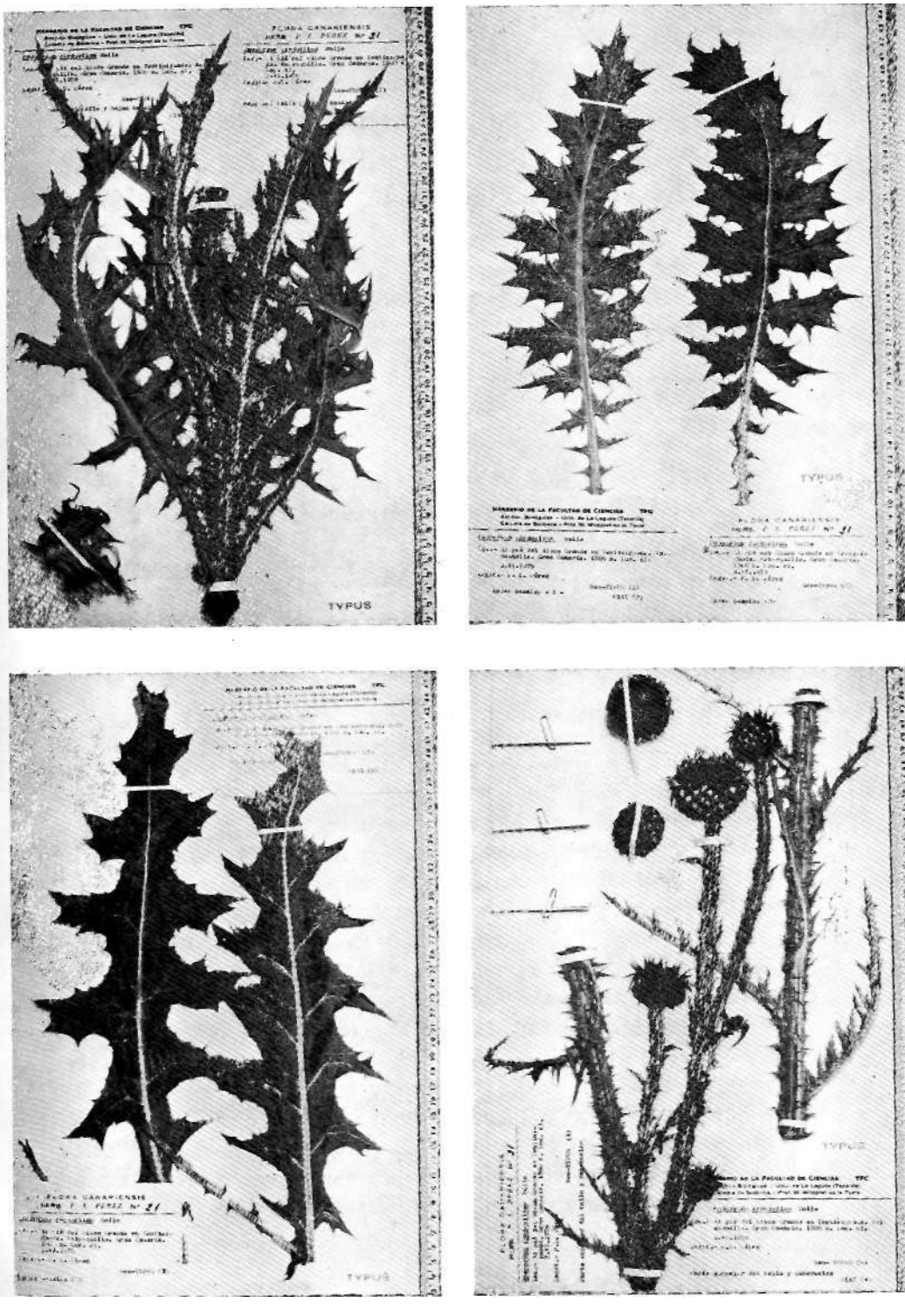


Onopordum carduelium Bolle, a. Aspecto general, b. Detalle de una hoja basal (vista por el envés), c. Brácteas del involucro: i = internas; m = medias; e = externas. Arriba vista dorsal; abajo vista de perfil, d. Flósculo. e. Aquenio. f. Detalle de los alvéolos del receptáculo. *g.* Detalle de un alvéolo — muy aumentado, h. Detalle del apéndice terminal de las anteras.





Onopordum carduelium Bolle. A. Detalle de un capítulo. B. Aspecto de los capítulos y parte superior de los tallos floríferos. Nótese (flecha) el efecto de las mordeduras de las hormigas (*Camponotus rufoglaucus feae* Emery).



Onopordum carduelium Bolle; NEOTYPUS in Herb. TFC.

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ESTUDIO PRELIMINAR SOBRE LA DISTRIBUCIÓN DE *ASPLENIUM ADIANTUM-NIGRUM SENSU* LATO EN GALICIA

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KESUMEN

Se inicia el estudio de la distribución de *Asplenium adiantum-nigrum,* sensu lato, en Galicia y se confirma la presencia de *A. cuneifolium* Viv. en su flora.

SUMMArY

This is the beginning of a study of the distribution of *Asplenium adiantum-nigrum,* sensu lato, in Galicia and it confirms the presence of *A. cuneifolium* Viv. in this flora (NW Spain).

OFRECEMOS el resultado de nuestras herborizaciones durante 1979 y la revisión efectuada de los herbarios MA, MAC, Herb^{0} Merino de la Facultad de Ciencias de Santiago, lamentando profundamente el aspecto negativo que reveló la consulta de SANT.

En principio nos interesamos por la presencia de *Asplenoum cuneifolium* Viv. que cita I. BARRERA (1977) para Moeche (MAC, n° 4782) y Cedeira, ambas en la provincia de Coruña.

Todavía no hemos herborizado, en su totalidad, el área de serpentinas o de rocas metabásicas (PARGA-PONDAL, 1967; DEN TEX, in LÓPEZ LÓPEZ, L, 1978) en el País gallego, pero sí creemos poder indicar la presencia del citado taxon en cinco localidades, pertenecientes a las provincias de Coruña

y Pontevedra. Estando pendiente otras, de la recolección de material en mejores condiciones que nos permitan salir de dudas.

Asplenium onopteris, A. adiantum-nígrum y *A. cuneifolium* están estrechamente relacionados y cada uno de ellos presenta además, una morfología muy variable que hace difícil la determinación de cada uno de los taxones. Recientemente SLEEP, A. *et al.* (1978) ; SCANNEIX, M. J. P. (1978) ; DESCHATRES, R., *et al.* (1978) ; O'MALLEY, D. J. S. (1979) ; PAGE, C. N. & FRANCES M. BENNELL, (1979) y ROBERTS, R. H. (1979) plantean el problema en las Islas Británicas y en Córcega.

Incluyen el estudio morfológico en distintos habitats, estudio del citotipo, tamaño de las esporas, así como el porcentaje de viabilidad de las mismas.

Del material de herbario consultado podemos establecer como hipótesis de trabajo que *Aspleniumadiantum-nigrum* L. var. *corunnense* Christ (herb^{0} Merino carpeta n° 10, n° 42 y MA n° 850) que cita el P. MERINO (1909), corresponderá a *A. cuneifólium,* por lo menos alguna de las frondes presentes en ambos pliegos. De igual modo el MA n° 842 de Begoña-Vizcaya — revisado por RIVAS MARTINEZ en 1963, como tal — e el MA n° 843 de Durango-Vizcaya y revisado por el mismo autor como *Asplenium adiantum-nigrum* L. deben corresponder a *A. cuneifólium.* El otro pliego MA n° 846 dé Mürguía? (Alava), con la misma acepción, revisado también por el mismo autor como *A. onopteris,* está formado por tres frondes, libres, dos de las cuales corresponden a esa revisión, mientras que la tercera, es muy joven y difícil de precisar su entidad taxonómica.

En las localidades de Merza (Pontevedra), río Ulla--embalse de Portodemouros (línea divisoria entre Coruña y Pontevedra), Melide, A Caleira, río Mera-Somozas, todas en la provincia de Coruña, hemos podido comprobar que conviven las tres especies.

Podemos concluir, en este estudio preliminar, que *Asplenium cuneifólium* Viv. forma parte de la flora de Galicia [JALAS, J. & J. SUOMINEN (1972); LAÍNZ, M. (1973)]. En ulteriores trabajos esperamos llegar a conocer algo más

sobre la biología del grupo, estudiando, por una parte las poblaciones en las que viven conjuntamente y por otra el comportamiento de *A. adiantum-nigrum* L. y *A. conopteris* L. fuera de esa área.

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ESTUDIOS CARIOHISTOLOGICOS EN EL GENERO *TRIFOLIUM*

por

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RESUMEN

En esta comunicación se presentan los datos correspondientes al estudio de la relación n^{os} cromosómicos/n^0 sacos polínicos de nuevas especies de *Trifolium.*

Se confirman los resultados obtenidos anteriormente (ANGULO & FiGUERAS, 1979) tanto en las especies primitivas de la Sección *Amoria* como en las más avanzadas de la Sección *Eulagopus.*

Asimismo los datos cariotípicos concuerdan plenamente con las tendencias evolutivas del género y confirman la relación evolutiva entre números cromosómicos y sacos polínicos en *Trifolium.*

E N una reciente comunicación sobre números cromosómicos y sacos polínicos en *Trifolium* (ANGULO & FIGUERAS, 1979) habíamos llegado a la conclusión de que, desde el punto de vista evolutivo, existe una estrecha relación entre lá dotación cromosómica de una especie y las características numéricas y estructurales de los microsporangios de las anteras, estableciéndose esta conexión en función de los datos obtenidos en el estudio cariohistológico de plantas de trebol.

Como continuación de este trabajo ampliamos nuestras investigaciones a nuevas especies de *Trifolium,* primitivas y más evolucionadas, algunas incluidas en la Sección *Amoria* (Subsec. *Falcatula, Micranthemum* y *Euamoria)* com números cromosómicos 2n = 16 y otras, más avanzadas, con 2n = 14 que pertenecen a la Sección *Eulagopus* (Subsec. *Probatostomá).*

MATERIAL, Y MÉTODOS

Las nuevas especies cuyos sacos polínicos hemos estudiado son las siguientes: *T. ornithopoãioiães* (L.) Sm., *T. suffocatum* L., *T. nigrescens* Viv., *T. isthmocarpum* Brot., *T. thallii* Vill., *T. leucanthum* Bieb., *T. smyrnaeum* Boiss., *T. striatum* L., *T. pratense* L., *T. stellatum* L., *T. incarnatum* L. y *T. arvense* L.

Las semillas fueron suministradas por la Sección de Ecofisiología del Instituto de Edafología y Agrobiología del C. S. I. C.

Para los cortes histológicos se siguió la técnica de RANDOLPH (1935) modificada por la de LACADENA & VAZQUEZ (1971) y para las preparaciones citológicas la de TJIO & LEVAN (1950) completada con un tratamiento de hidrólisis. La nomenclatura de las Secciones, Subsecciones y especies es la de VICIOSO (1951-52).

OBSERVACIONES

Realizamos esta segunda tanda experimental con objeto de confirmar nuestros postulados anteriores sobre relación evolutiva n^{os} cromosómicos/n^0 sacos polínicos, utilizando nuevas especies, incluidas en las Secciones *Amoria* y *Eulagopus.*

Dentro de la Sección *Amoria* los números cromosómicos de cinco de las especies utilizadas, habían sido establecidos anteriormente. BLEIER (1925) en un trabajo clásico sobre *Trifolium,* indica únicamente el número haploide n = 8 para *T. thallii,* número que hemos comprobado obteniendo 2n — 16 en mitosis, como se observa en el idiograma que presentamos. RODRIGUES (1953), BREWBAKER (1955) y LASSEN (1956) atribuyen a *T. nigrescens* el número diploide 2n = 16. AHUJA (1955) encuentra también 2n = 16 en *T. isthmocarpum* y KLIPHUIS (1962) estabelece este mismo número para *T. ornithopoãioiães.* El número diploide de *T. suffocatum* 2n = 16 fué establecido por nosotros en 1973 (GONZALEZ--BERNALDEZ *et al.).* Estas determinaciones cromosómicas realizadas por distintos autores coinciden entre sí y sirven

de apoyo a nuestro criterio de que las especies más primitivas poseen los números básicos más altos, x — 8 dentro del género *Trifolium.*

Hemos verificado, mediante cortes histológicos, que cuatro especies de complemento diploide 2n = 16 *(T. nigrescens, T. isthmocarpum* y *T. thálii* de la Subsec. *Euamoria* y *T. suffocatum* Subsec. *Micranthemum)* presentan también dos sacos polínicos lo que viene a confirmar este carácter como factor asociado a los grupos filogenéticamente más primitivos. Unicamente *T. ornithopodioides* (Subsec. *Falcatula)* muestra características peculiares en cuanto a número de sacos polínicos ya que además de dos aparecen a veces tres y hasta cuatro (Láminas I y II).

La Sección *Eulagopus* presentaba marcado interés desde el punto de vista evolutivo ya que dentro de la misma se manifestán distintas tendencias; un grupo menos evolucionado de espécies con diferentes números cromosómicos y dos sacos polínicos que, en su aspecto filogenético, podemos considerar como formas intermedias o de transición de mayor flexibilidad evolutiva, y otro grupo más avanzado, de menor flexibilidad, donde aparecen formas de 10 cromosomas y cuatro s. p. (ANGULO & FIGUERAS, 1979).

Sin embargo nos faltaba información más amplia sobre el grupo de especies de 14 cromosomas, número más común dentro de esta Sección, razón por la cual incluimos en este trabajo cinco taxones *(T. incarnatum, T. stellatum, T. arvense, T. pratense* y *T. striatum)* estudiados previamente por nosotros en su aspecto cariológico, habiendo establecido sus cariotipos (ANGULO *et al.* 1971, 1972 y 1973) como base para el conocimiento de las tendencias evolutivas del género. También incluimos *T. suffocatum* 2n = 13 de la Sección *Amorta* (loc. cit.).

De las cinco especies de complemento diploide 2n = 14 incluidas en la Sección *Eulagopus* (Subsec. *Probatostoma)* hemos podido observar que las cuatro primeras presentan anteras con cuatro sacos polínicos, mientras que en *T. striatum* solamente aparecen dos s. p. como carácter proprio de esta especie la cual y siguiendo el criterio del trabajo

anterior (ANGULO & FIGUERAS, 1979) podemos considerar como forma intermedia o de transición (Láminas III y IV).

Estas nuevas aportaciones vienen a confirmar una relación significativa en el orden evolutivo entre números cromosómicos y sacos polínicos.

Al mismo tiempo hemos estudiado también dicha relación a nivel cariotípico, mediante el estudio comparativo entre cariotipos de especies primitivas y mas avanzadas.

Incluimos en el cuadro 1, además de los datos actuales, ejemplos representativos tomados de trabajos anteriores (ANGULO *et al.* 1968-79). De la observación del mismo se deducen las siguientes consideraciones generales:

Los cariotipos de las especies primitivas (Sección *Amorta)* se caracterizan por su estabilidad, son regulares y uniformes (simétricos) y presentan escasa variabilidad estructural en sus tipos cromosómicos. Sus n^{os} básicos, x = 8 son los más altos del género que nosotros conozcamos y poseen anteras con dos sacos polínicos como carácter asociado a su escaso nivel de especialización. Los cariotipos de las especies filogenéticamente más avanzadas (Sección *EuIagopus)* son más irregulares (asimétricos) y muestran un alto grado de polimorfismo al estar formados por diferentes tipos de cromosomas. En esta Sección están representados todos los números básicos del género, y gran parte de las especies presentan anteras con cuatro sacos polínicos, a excepción de *T. smyrnaeum* con número variable de sacos polínicos y de cromosomas, pudiendo representar el eslabón de enlace entre los caracteres primitivos de la Sec. *Amoria* y los más avanzados de la Sec. *Eulagopus.*

En la Sección *Calycomorphum* también se manifiesta el carácter de cuatro sacos polínicos propio de las especies más avanzadas en la escala evolutiva.

Si atendemos a los tipos cromosómicos que constituyen el cariotipo de las cuatro especies primitivas incluidas, encontramos que predominan los cromosomas de dimensiones medias, de tipo submediano, seguidos de los metacéntricos cuyos tamaños relativos son sensiblemente similares.

Las diferencias de longitud cromosómica entre los ocho pares de mayores dimensiones son apenas significativas ya

CUADRO 1

		N.o s. p.	N.o croms.	Tipos de cromosomas	Características del cariotipo
	Subs. *Micranthemum*				
	T. glomeratum	2	2n = 16	3 pares V; 4 L; 1 SAT	Estabilidad cariotípica. Estructuras similares y uniformidad cromosómica propia de cariotipos simétricos de especies primitivas.
	T. suffocatum	2	2n = 16	3 » V; 4 L; 1 SAT	
	Subs. *Buamoria*				
	T. retusum	2	2n = 16	2 » V; 4 L; i J; 1 SAT	Cariotipo algo menos uniforme por modificación en un par de cromosomas lo que parece significar un pequeño avance en el orden evolutivo.
	T. thallii	2	2n = 16	2 » V; 4 L; 1 J; 1 SAT	
	Subs. *Stenostoma*				
	T. leucanthum	4	2n = 16	2 » V; 3 L; 2 J; 1 SAT	Estruturas semejantes y diversidad de tipos cromosómicos que sitúan estas especies en un escalón más avanzado de la escala evolutiva.
	T. smymaeum	2, 3, 4	2n=14-16	2 » V; 3 L; 2 J; I- SAT	
	T. squarrosum	2	2n = 16	2 V; 2 L; 1 J; 2 dos sonst. 1 SAT	Polimorfismo cromosómico más acusado. Esp. en periodo de transición, más evolucionada en constitución cromosómica que en n^{oB} sacos polínicos.
	Subs. *Probatostoma*				
	T. striatum	2	2n = 14	1 » V; 4 L; 1 J; 1 SAT	Cariotipo con polimorfismo menos acusado y predominio de cromosomas submedianas.
	T.Àncanatum	4	2n = 14	2 » V; 2 L; 2 J; 1 SAT	Cariotipo más común entre las especies de 14 cromosomas con variabilidad de tipos cromosómicos (encontrado en cuatro espacies).
	T. stéllatum	4	2n = 14	2. » V; 2 L; 2 J; 1 SAT	
	T. arvense	4	2n = 14	2 » V; 3 L; i J; 1 SAT	Polimorfismo cromosómico con diferencias estructurales marcadas.
	T. pratense	4	2n = 14	3 » V; 2 L; 1 J; 1 SAT	idem idem
	T. cherleri	4	2n = 10i	1 » V; 1 L; 2 J; 1 SAT	Alto grado de polimorfismo cromosómico (especies muy evolucionadas).
	T. hirtum	4	2n = 10.	1 » V; 1 L; 2 J; 1 SAT	idem idem
	T. scabrum	4	2n = 10	1 » V; 1 L; 2 J; 1 SAT	idem idem
	I Ecotipos españoles				
	T. subterraneum				
	BA-5 ssp. *brachycalicinum*	4	2n = 16	2 » V; 2 L; 2 J; 1 dos 30nst. 1 SAT	Polimorfismo cromosómico con diferencias estructurales muy marcadas.
	CC-2 ssp. *brachycalicinum*	4	2n = 16	2 » V; 2 L; 2 J; 1 dos sonst. 1 SAT	idem idem
	var. *Taïlarock* ssp. *subt.*	4	2n = 16	2 » V; 2 L; 2 J; 1 dos sonst. 1 SAT	idem idem
	T. israeliticum	4	2n = 12	— — — 1 L; 4 J; 1 SAT	Alto grado de polimorfismo cromosómico.

que, teniendo en cuenta los valores medios de los mismos, equivalen al 0,6, valores que corresponden al 1,7, en los cincuenta y seis pares de cromosomas restantes.

La observación de los cariotipos de las tres especies avanzadas de la Sección *Eúlagopus* (Subsec. *Stenostoma)* 2n == 16, muestran diferencias notables de dimensiones y nuevas estructuras, como son cromosomas subterminales y submedianos con constricciones secundarias, manifestando el polimorfismo cromosómico de estas especies.

Lo mismo ocurre con las ocho especies pertenecientes a la Subsec. *Probatostoma,* 2n = 14 y 2n — 10, en las cuales aparecen cromosomas de tipo subterminal y son considerables las diferencias de longitud cromosómica, tanto entre los dieciseis pares grandes como entre los ochenta y cuatro restantes de tipo medio.

En la Sección *Càlycomorphum,* la más avanzada del género, donde figuran números cromosómicos 2n = 16 y 2n = 12, es notorio el polimorfismo cromosómico en sus especies, ya que aparecen cromosomas subterminales y de dos constricciones siendo muy considerables las diferencias de longitud cromosómica sobre todo en los pares medianos.

En el cuadro n° 2 se recogen estos datos mediante la comparación entre cariotipos de especies primitivas y más avanzadas.

DISCUSIÓN

El estudio de nuevos grupos de especies primitivas y más avanzadas viene a confirmar los resultados obtenidos anteriormente y muestra que, desde el punto de vista evolutivo, existe una relativa relación entre n^{os} cromosómicos/ /n° sacos polínicos.

Hemos comprobado con nuevos elementos de juicio que las especies primitivas de 16 cromosomas poseen 2 sacos polínicos y son formas estables dentro de las tendencias evolutivas del género.

Unicamente *T. ornithopodioides* presenta menor estabilidad en cuanto a número de sacos polínicos, ya que,

CUADRO 2

Secciones	N.o esp.	N.o cromosómico	N.o total cromosomas	Tipos de cromosomas					Diferencias de longitudes cromosómicas entre especies primitivas y avanzadas			
				Met.	Subm.	Subt.	SAT.	2 const.	Pares grandes	Valores medios (*)	Pares medianos	Valores medios
AMORIA												
Esp. primitivas	4	2n = 16	**64**	20	32	4	8	—	8	0,6	56	1,7
EULAGOPUS (Subs. Stenostoma)							«					
Esp. avanzadas	3	2 n = 16	48	12	16	10	6	4	6	1,3	42	4,5
Subs. *Probatostoma*												
Esp. avanzadas	8	2n = 14 (5) 2n = 10 (3)	**100**	26	32	26	16	—	16	2,8	84	5,1
CALYCOMORPHUM												
2 Esp. + 2 ssp. (muy avanzadas)	4	2n = 16 (3) 2n = 12 (1)	60	12	14	20	8	6	8	1,3	52	6,1

(*) Corresponden a la media de las dimensiones cromosómicas del complemento diploide de cada grupo de especies, cuyos cromosomas se dividen en grandes (dos pares) y medianos (los pares restantes).

además de dos, aparecen también tres y cuatro esporádicamente.

Al mismo tiempo confirmamos que, atendiendo a los criterios de estabilidad y flexibilidad evolutiva de PREVOSTI (1978), y a la amplia información de FERNANDES & QUEIRÓS (1978), la Sección *Eulagopus* (subsec. *Stenostoma)* al presentar 2, 3 y 4 s. p. apuntaría a la inestabilidad evolutiva, y la (Subsec. *Próbatostoma)* quedaría constituida por tres grupos de especies con distinto grado de evolución, resaltando en primer lugar el formado por las especies de 14 cromosomas y cuatro sacos polínicos como el grupo más numeroso, homogéneo y estable de la Sección, al que podemos considerar como zona de menor flexibilidad en relación con los cambios evolutivos. A este grupo seguiría uno más restringido compuesto por especies de 10 cromosomas y cuatro sacos polínicos, también estable. Y finalmente (ANGULO & FIGUERAS, 1979) un tercer grupo correspondiente a especies de transición, de mayor flexibilidad evolutiva con diversos números eromosómicos y dos sacos polínicos y que pueden constituir el eslabón de enlace con las especies más primitivas.

Estas características concurren en el caso de *T. striatum* com 2n = 14 y 2 sacos polínicos, carácter asociado a las especies más primitivas en la escala evolutiva, por lo que puede considerarse como una forma de transición en la cual coexisten un mayor grado de evolución cromosómica y el carácter primitivo de sus sacos polínicos.

Por otra parte los datos cariotípicos presentan una estrecha concordancia con las tendencias evolutivas del género *Trifolium* y confirman los postulados mantenidos sobre relación n^{os} cromosómicos/n^0 sacos polínicos, poniendo de relieve que las especies primitivas poseen 2n = 16 cromosomas, cariotipos simétricos, y dos sacos polínicos, mientras que las más avanzadas varían en número cromosómico, poseen cariotipos asimétricos y cuatro sacos polínicos.

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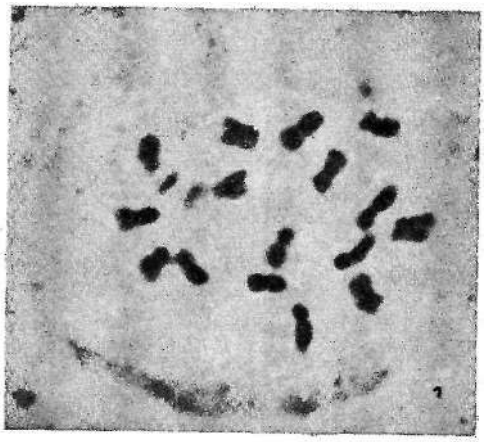

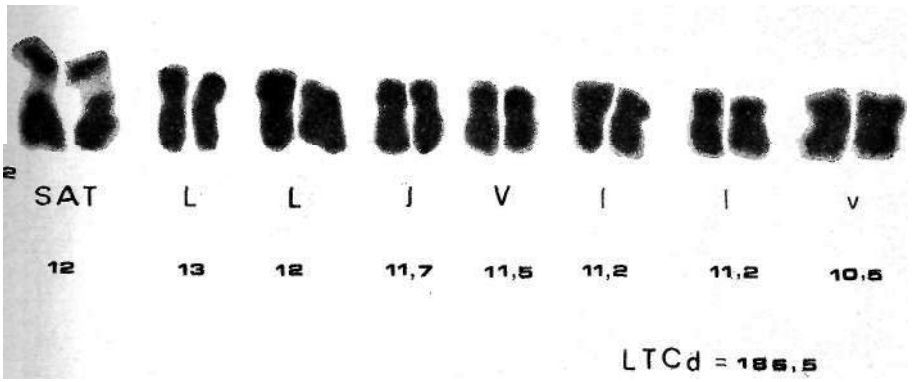

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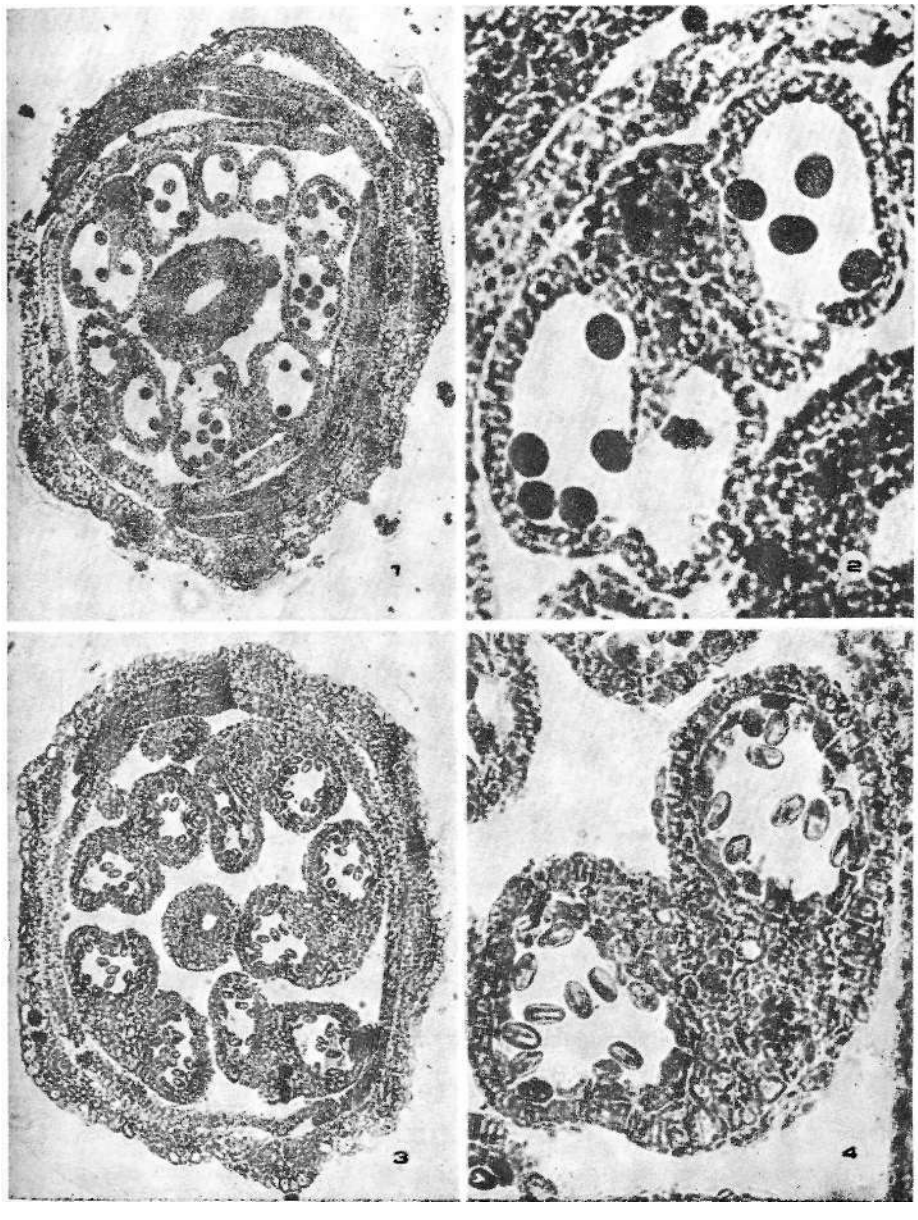
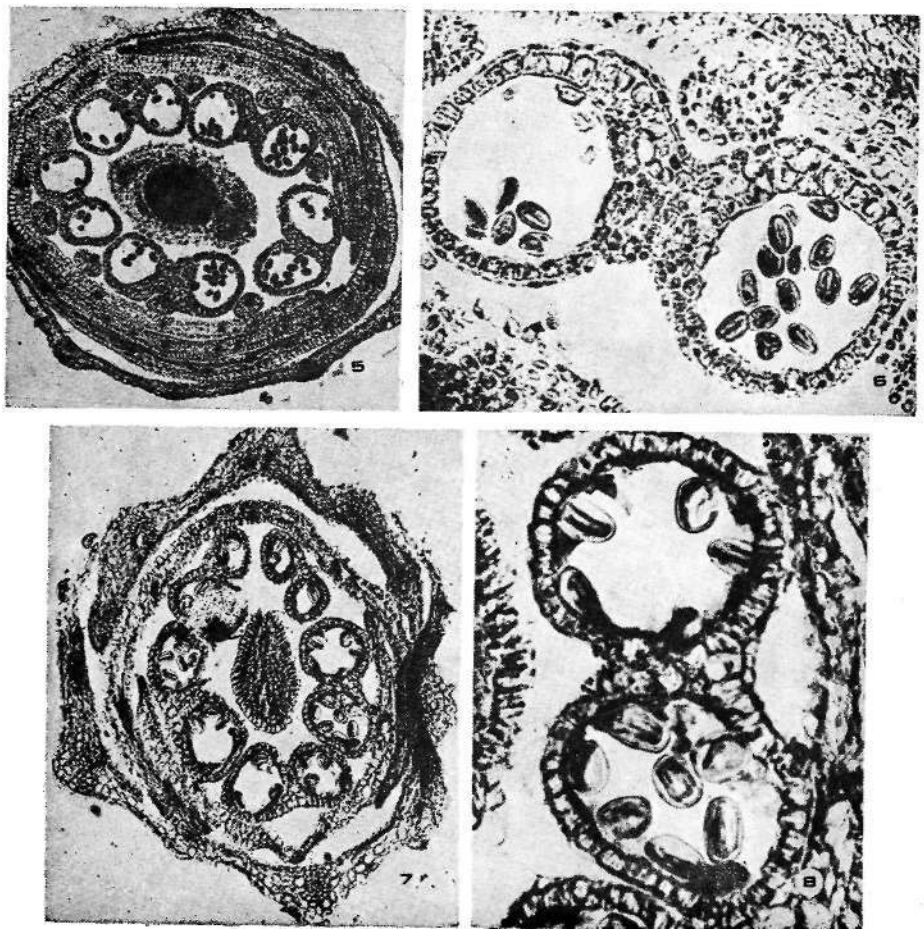
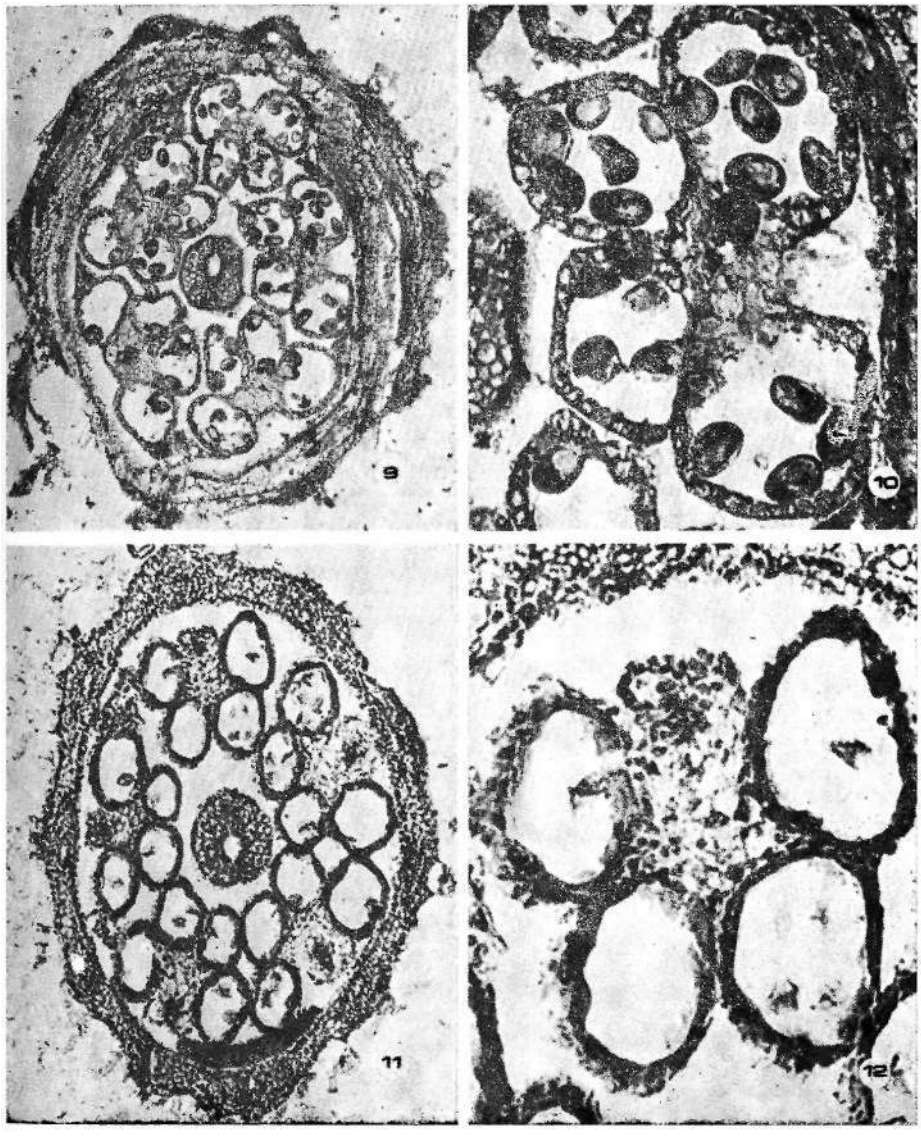
Fig. 1—Metafase X 4096. Fig. 2 — Características del cariotipo; tipos cromosómicos y longitud media de los ocho pares de homólogos (Metafase X 6144).



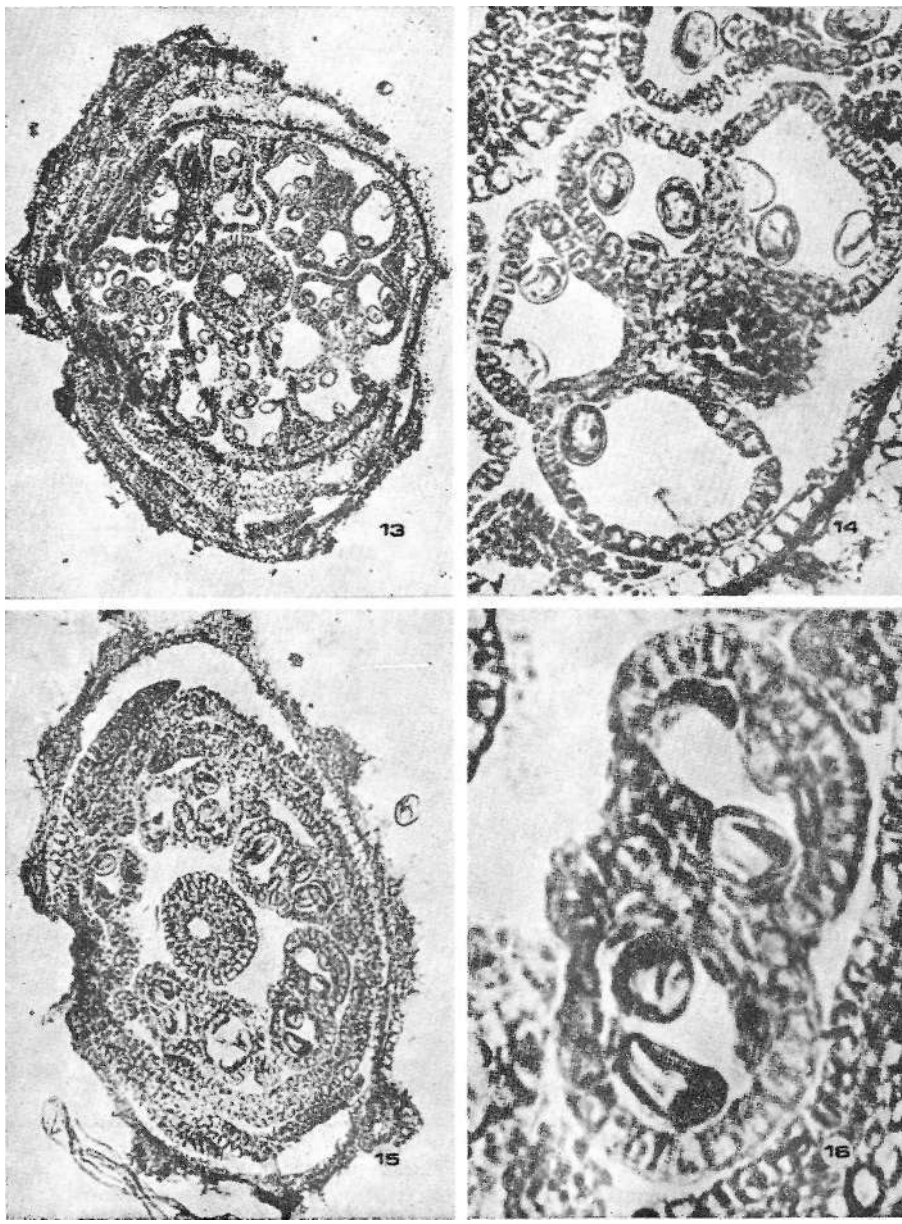
Especies de *Trifolium* de la Sección Amorío (2n = 16 y dos sacos polínicos). Pigs. 1 y 2. T. *ornithopodioides* (Subsección *Falcatula); Figs.* 3 y 4. T. *nigrescens* (Subsscción *Euamoria).*



Especies de *Trifolium* de la Sección Amoria (Subsection *Euamoria).* (2n = 16 y dos sacos polínicos); Figs. 5 y 6. T. *isthmocarpum;* Figs. 7 y 8. T. *thallii.*



Especies de *Trifolium* de la Sección *Eulagopus* (Subsección *Probatostoma)* (2n = 14 y cuatro sacos polínicos); Figs. 9 y 10. *T. incarnatum;* Figs. 11 y 12. *T. stellatum.*



Especies de *Trifolium* de la Sección *Eulagopus* (Subsección *Probatostoma). F*igs. 13 y 14. *T. pratense* (2n = 14 y cuatro sacos polínicos); Fig. 15. *T. striatum* (2n = 14 y dos sacos polínicos).

Bol. Soc. Brot., Sér. 2, 33(2): 887-906 (1981).

CONTRIBUTION A L'ETUDE CYTOTAXINOMIQUE DU GENRE *CAMPANULA L.* EN AFRIQUE DU NORD ET CENTRALE

JULIETTE CONTANDRIOPOULOS

RÉSUME

Une étude cytotaxinomique du genre *Campanula* en Afrique a été réalisée à partir de 14 taxons d'Afrique du Nord et centrale.

Des nombres chromosomiques nouveaux ont été déterminés pour les taxons suivants: *C. bordesiana* Maire ssp. *bordesiana,* n = 40-42; *C. filicaulis* Dur. var. *filicaulis* et var. *pseudoradicosa* Lit. et Maire, n = 12; *G. mairei* Pau var. *anremerica* Lit. et Maire, var. *atlántica* (Jah. et Maire) Maire et var. *flahaultiana* Emb., n = 17; *G. mollis* L., S = 12; *G. mollis* L. var. *rifana* Emb., 2n = 46, 48, 50, 52; *G. numidica* **Dur.,** n = 12; *G. rapunculus* L. var. *hirta* Ten. forma *verruculosa* (Hoffgg. et Link) Maire, n = 10.

Des phénomènes de dysploïdie semblent se produire chez *C. filicaulis* et chez *C. mollis.* Des phénomènes de Polyploidie accompagnés d'aneuploïdie ont été observés chez *G. bordesiana* ssp. *bordesiana* et chez *G. mollis* var. *rifana.* Les parentés morphologiques de *G. bordesiana* ssp. *bordesiana* ont été établies et la signification évolutive du caractère particulier de ses capsules à 5 loges discuté.

ABSTRACT

A cytotaxinomical study of the genus *Campanula* in Africa from 14 north and central Afriea taxa has been effected.

New chromosome numbers have been found for following taxa: *C. bordesiana* Maire ssp. *bordesiana,* n = 40-42; *G. filicaulis* Dur. **var.**

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filicaulis and var. *pseudoradicosa* Lit. & Maire, n = 12; *C. mairei* Pau var. *anremerica* Lit. & Maire var. *atlántica* (Jah. & Maire) Maire and var. *flahaultiana* Emb., n = 17; *C. mollis* L., n = 12; C. *mollis* L. var. *rifana* Emb., 2n=46, 48, 50, 52; *C. numidica* Dur., n=12; C *rapunculus* L. var. *hirta* Ten. forma *verruculosa* (Hoffgg. & Link) Maire, n = 10.

Dysploidy phenomena with C. *filicaulis* and *C. mollis,* polyploidy and aneuploidy in *C. bordesiana* ssp. *bordesiana* and *C. mollis* var. *rifana* have been observed.

Morphological affinités of C. *boräesiana* ssp. *bordesiana* are established and evolutive significance concerning particular characters of quinquelocular capsule is discuted.

DANS le cadre des recherches cytotaxinomiques que nous effectuons depuis plusieurs années sur les *Campanulaceae* méditerranéennes et qui ont porté jusqu'ici sur des espèces du bassin méditerranéen oriental (cf. J. CONTANDRIOPOULOS 1964, 1966, 1970a, 19706, 1971, 1972a, 19726, 1973, 1976), nous abordons, dans ce travail, l'étude cytotaxinomique du genre *Campanula* en Afrique du nord et centrale.

Bien que ce genre ne soit représenté en Afrique que par un petit nombre d'espèces (25 environ), il n'en constitue pas moins un groupe très complexe en raison de sa grande diversification infraspécifique et de la multitude de petites formes, le plus souvent bien fixées, qui apparaissent. QUEZEL (1953) a élaboré une monographie des *Campanuïaceae* en Afrique du Nord et exposé quelques-uns des problèmes soulevés dans la famille. Cependant il n'existe aucune étude cytotaxinomique d'ensemble portant sur la famille et sur le genre *Campanula* en particulier. Seuls des dénombrements chromosomiques épars concernent quelques campanules d'Afrique du nord ou orientale. Citons: *C. mairei* Pau (QUEZEL 1953), *C. saxifragoides* Doumergues, *C. guinocheti* Quezel (QUEZEL 1957), *C. filicaulis* Dur. (QUEZEL 1957, HUMPRHIES & al. 1978), *C. jurjurensis* Chabert (PODLECH 1965), *C. dichotoma* L., *C. edulis* Forsskäl et *C. keniensis* Thulin (THULIN 1975).

MATÉRIEL ET MÉTHODE

Le matériel qui a servi de base aux présentes recherches a été récolté par nous en Algérie du nord et au Sahara

algérien au cours d'une mission de botanique subventionnée par le C. N. R. S. en 1978. Les plantes du Maroc nous ont été procurées par Mme CAUWET, Maître-Assistante Dr au Collège Scientifique Universitaire de Perpignan (1972) et par le Professeur C. FAVARGER de l'Université de Neuchâtel (1976).

Les fixations ont toutes été effectuées directement sur le terrain à l'alcool acétique ($4/_1$) avec mordançage au carmin acétique-acétate ferrique, La coloration a été faite au carmin acétique-acétate ferrique selon la méthode des écrasements.

Les échantillons fixés ont été conservés à une température de -15^9. Les exsiccatas témoins se trouvent dans notre herbier déposé à l'Université de Provence (Marseille).

Toutes les figures ont été dessinées à l'aide du tube à dessin du Photomicroscope III de ZEISS (Oculaire 12,5, Objectif 100 X).

OBSERVATIONS EX DISCUSSIONS

C'est donc l'étude cytotaxinomique de 14 taxons appartenant au genre *Campanula* que nous avons entreprise. Les dénombrements chromosomiques sont consignés dans le Tableau N° 1 sur lequel figure aussi l'origine du matériel étudié. Les figures correspondantes se trouvent sur la Planche **I.**

Campanula bordesiana Maire ssp. bordesiana

Nous avons récolté au Hoggar dans l'Oued Tarouda vers 2000 m *C. bordesiana* ssp. *bordesiana* endémique de ce massif où elle n'est signalée que dans quelques localités: Ideies, 1500 m (MAIRE 1929), Oued Tarouda (MAIRE 1929) et nous-même), Oued Tamada (QUEZEL 1953), Tihantakert 2080-2100 m (MAIRE 1929) ainsi qu'au Teffedest dans l'Oued Ahor (QUEZEL 1953).

C. bordesiana est représenté au Tibesti par le ssp. *tibestica* Quezel.

Remarquable par sa rareté, *G. bordesiana* l'est aussi par son nombre chromosomique hautement polyploïde difficilement explicable : n = 40-42. Pl. I, fig. 1.

Pour MAIRE (1929) puis pour QUEZEL (1953) *G. bordesiana* appartiendrait à une lignée mésogéenne orientale des campanules de la section *Medium* à capsules quinquéloeulaires. Or la comparaison morphologique de cette espèce avec les campanules de la Méditerranée orientale rattachées à ce groupe ne nous permet pas de confirmer cette hypothèse. Les caractères morphologiques ne concordent pas et le nombre chromosomique de base est complètement différent puisqu'il est de n = 17 dans la presque totalité des espèces étudiées, n = 15 a été dénombré chez une seule espèce. Enfin aucun cas de polyploïdie n'a été détecté chez les campanules de la *section Medium* à capsules quinquéloculaires (CONTANDRIOPOULOS loc. PHITOS (1965), FEDOROV et KOVANDA (1976) in Flora Europaea, DAMBOLDT et PHITOS (1978) in Flora of Turkey, vol. VI).

Par contre il existe dans les montagnes de l'Afrique orientale une espèce vivace très polymorphe qui semble avoir différencié de massifs en massifs une série de micromorphes réunis les uns aux autres par des formes de transition. Ii s'agit du groupe de *G. edulis* Forsskäl qui habite les fissures des rochers et les pelouses rocailleuses entre 600 et 3000 m dans les montagnes est africaines du Soudan, de l'Ethiopie, de la Somalie, de l'Uganda, du Kenya, de la Tanzanie ainsi qu'au Yemen. Cf. Figure 1.

THULIN (1975) qui a étudié *G. edulis* rattache à cette espèce tous les taxons qui ont été décrits sur les montagnes de l'Afrique orientale et du Yemen sous les noms de *G. esculenta* A. Rich., *C. quartitiana* A. Rich., *C. rigidipïla* A. Rich., *G. sarmentosa* A. Rich., *G. schimperi* Vatke, *C. schimperi* Vatke var. *quartitiana* (A. Rieh.) Vatke, *C. schimperi* Vatke var. *rigidipïla* (A. Rich.) Vatke, *G. schimperi* Vatke var. *sarmentosa* (A. Rich.) Vatke, *G. rigidipïla* A. Rich. var. *quartitiana* (A. Rich.) Engl., *C. rigidipïla* A. Rich. var. *esculenta* (A. Rich.) Di Capua et var. *sarmentosa* (A. Rieh.) Engl.

En comparant les exsiccatas contenus dans les herbiers du Muséum National d'Histoire Naturelle de Paris, de Montpellier (herbier général, herbiers Maire et Sauvage), de Marseille (herbier Quézel), il apparait un lien incontes-

TABLEAU I

Campanula d'Afrique du Nord et du Hoggar

CAMPANULA	Témoin	n	2n	Lieu de récoite
C. bordesiana Maire ssp. *bordesiana*	78.571	40		Algérie: Sahara au Hoggar: Oued Tarouda, 1700 m sables húmidas de la berge à proximité de la route
C. dichotoma L. ssp. *dichotoma* .	78.608		24	Algérie: Roknia, talus humides en bordure de la route, 180 m
	78,613	12	24	Algérie: Rocher de Constantine, fossé bordant la route au pied de la falaise
	78.625		24	Algérie: Algérois, gorges de Ben Amrane
C. dichotoma L. ssp. *afra* (Cav.) Maire	70.07		24	Maroc: Entre Tanger et Larache sur le bord de la route
	78.615	14	28	Algérie: Rocher de Constantine à proximité de la piscine
G. filicaulis Dur. var. *filicaulis* . . .	72,31		24	Maroc: Haut Atlas central, Tizi-n-Fedhrat, 2000 m
C. filicaulis Dur. var. *pseudoradiocosa*	76.03		24	Maroc: Haut Atlas siliceux, Toubkal près du refuge Nelter, 3200 m
	76.05	12	24	Maroc: Haut Atlas calcaire, Djebel Ahrabout, 2200 m
C. mairei Pau var. *anremerica* Lit. et Maire.	76.06		34	Maroc: Haut Atlas siliceux, Touhkal, dans des xérophytes épineux au-dessus du refuge Nelter
C. mairei Pau var. *atlántica* (Jah. et Maire) Maire (=C. *herminii* Hoff m.	72.38	17	*	Maroc: Haut Atlas central, Mont Aioui, 2000 m
C. mairei Pau var. *flahaultiana* Emb.	76.04		34	Maroc: Djebel Ayachi, pentes nord, 2950 m
	78.623		24	Algérie: Algérois: gorges de Ben Amrane
C. mollis L. var. *rifana* Emb. et Maire	76.01	25,26	46,48 50,52	Maroc: Rif, bords de la route dans une callitraie à 400 m dans une gorge au-dessus da Talembose
	76.02	24	48	Maroc: Rif, au Mont Kraa vers 2100 m
	78.614	12	24	Algérie: rocher de Constantine, falaise qui surplombe la route vers la piscine
C. rapunculus L. var *hirta* Ten. . . .	78.610	10		Algérie: Constantinois, maquis à Roknia
	78.620	10	20	Algérie: Constantinois, près de Roknia à 100 m
	72.08	10	20	Maroc: Entre Rabat et Larache
	72.02	10		Maroc: Tanger
	72.65		34	Maroc: Moyen Atlas, forêt de Jaba au Nord d'Ifrane

table entre les taxons du groupe *C. edulis* et *C. bordesiana,* parenté supposée d'ailleurs par THULIN.

G. bordesiana ssp. *bordesiana* se différencie du groupe *C, edulis* par son système radiculaire généralement napiforme, par ses fleurs toujours plus petites et ne dépassant pas 13 mm, par ses feuilles oblongues, lancéolées, subspathulées lâchement hispides alors que chez *G. edulis* il y a de nombreuses racines, des fleurs généralement plus grandes pouvant atteindre jusqu'à 25-30 mm de long. Les feuilles crénelées dentées sont plus longues et souvent plus larges que celles de *G. bordesiana* ssp. *bordesiana.*

QUEZEL a distingué chez *G. bordesiana* deux sous-espèces : les ssp. *bordesiana,* endémique du Hoggar et *tibestica* Quézel, endémique du Tibesti, localisé sur les crêtes de l'Emi Koussi vers 3000 m. Ce dernier taxon, par sa corolle velue sur le sommet des lobes et par son calice dont les dents sont bordées de poils dressés, assure la transition entre l'endémique du Hoggar et *G. edulis.*

Enfin une autre endémique du Tibesti *G. monodiana* Maire (1943) appartiendrait elle aussi au groupe de *G. edulis* dont elle se distingue par sa souche grêle, peu ramifiée comme celle de *C. bordesiana,* sa corolle glabre à lobes aigus, par ses tiges peu anguleuses et enfin par son nanisme, la plante n'excédant pas 4 cm. Isolé sur le Mt Toussidé (3000 m) *C. monodiana* de même que *G. bordesiana* ssp. *bordesiana* du Hoggar forment des taxons bien différenciés et distincts de *C. edulis* (Fig. 1).

Au niveau des capsules, nous avons observé, suivant les taxons, des différences qui selon nous sont d'une certaine importance. En effet, pour les plantes du Hoggar se rapportant au *G. bordesiana* ssp. *bordesiana* nous n'avons trouvé que des capsules quinquéloculaires (QUEZEL signale cependant 3 à 5 stigmates et nous avons noté qu'il y avait parfois concordance entre le nombre de stigmates et celui des loges). Pour le ssp. *tibestica* nous avons remarqué que les capsules triloculaires semblaient plus nombreuses que les capsules quinquéloculaires. Chez *G. monodiana,* les capsules sont triloculaires. Enfim, chez *C. edulis,* pour lequel THULIN

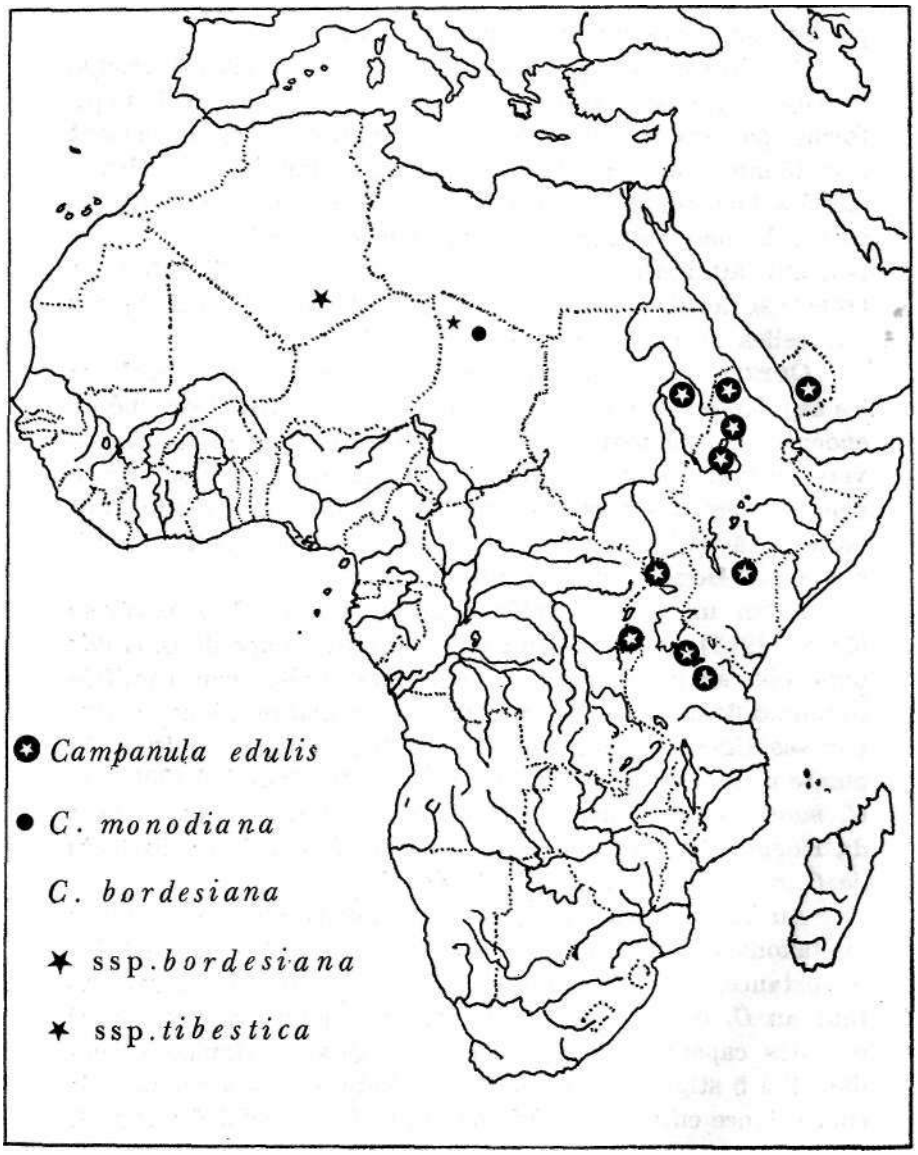
Campanula edulis
C. monodiana
C. bordesiana
ssp. bordesiana
ssp. tibestica

Fig. 1.

signale également 3-5 loges dans les capsules, nous avons vu toutes les formes de passage de 3 à 5.

— des capsules triloculaires dont une loge est plus grande que les autres suivant la description classique des capsules des campanules de la section *Medium* (LE MAOUT 1846);
— des capsules triloculaires dans la partie centrale, une quatrième loge apparaissant dans la grande loge à partir du milieu d'une cloison qui semble se dédoubler;
— des capsules tétraloculaires à peu près égales;
— des capsules tétraloculaires dans la partie centrale avec une cinquième loge apparaissant vers la partie externe;
— des capsules quinquéloculaires (Fig. 2).

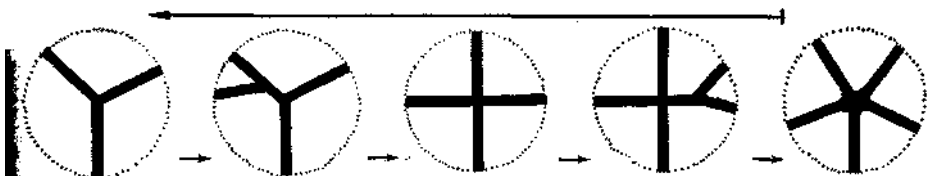

Fig. 2. — Schéma de l'évolution des loges de la capsule.

Enfin, comme pour les loges, nous avons dénombré, dans les échantillons examinés, un nombre variable de stigmates, 3 stigmates, 3 stigmates dont un bifide, 4 stigmates, 4 stigmates dont un bifide et 5 stigmates.

Nous avons déterminé dans la population étudiée de *G. boräesiana* ssp. *bordesiana* (Hoggar: Oued Tarouda), un nombre chromosomique dont le degré de polyploïdie était très élevé avec n = 40-42 alors que pour *C. edulis* THULIN (1975) donne un nombre chromosomique également polyploïde mais à un degré moindre, de n = 28 (2n = 56) pour des plantes d'Ethiopie (Anasella) et de Tanzanie (Nigorongoro). Il se pourrait, compte tenu des parentés morphologiques qui semblent exister entre ces deux taxons, que *C. bordesiana* ssp. *bordesiana* dérive de *C. edulis* par polyploïdie et peut-être aneuploïdie. La méiose de *C. bordesiana* ssp. *bor-*

desiana n'est pas très régulière et en métaphase I des cellules mères de grains de pollens, on peut voir des univalents et des polyvalents (Planche I, figure 1). Ce nombre chromosomique, peut-être 12-ploïde, est difficilement explicable sans informations complémentaires. Il conviendrait d'étendre ces recherches à d'autres populations de *C. bordesiana* ssp. *bordesiana* et au ssp. t*ibestica* ainsi qu'à C *monodiana* afin de préciser l'origine de ce polyploïde dont *G. edulis* constitue peut-être l'un des parents.

Signalons aussi qu'au Tibesti QTJEZEL (1961) a décrit une autre campanule, *C. filicaulis* Dur. var. *tïbestica* espèce méditerranéenne d'Afrique du nord. Nous ne connaissons pas le nombre chromosomique de ce taxon mais nous verrons que pour d'autres variétés du *C. filicaulis* le nombre est n=12 (cf. p. 897). Par son degré de polyploïdie *C. bordesiana* ssp. *bordesiana* représente un taxon plus jeune que *C. edulis,* mais le caractère concernant le nombre de loges des capsules semble, à première vue, contradictoire avec cette idée et pose aussi un problème systématique.

Chez les campanules, se rapportant à la section *Medium,* le nombre de loges des capsules est considéré comme un caractère discriminatoire lorsqu'on tente de déterminer une espèce appartenant à cette section (cela explique d'ailleurs les parentés supposées par MAIRE (1929) et par QTJEZEL (1953) pour la plante du Hoggar avec des espèces de la Méditerranée orientale puisque la plupart des campanules quinquéloculaires habitent ce domaine). Or le fait que chez une même espèce on trouve des capsules quinquéloculaires et triloculaires met en question la valeur systématique du caractère concernant le nombre de loges des capsules et pose un problème évolutif à l'intérieur de ce groupe. Il est généralement admis, chez les Gamopétales que l'évolution est accompagnée d'une contraction des pièces florales. Dans cette éventualité, les campanules à capsules triîoculaires seraient plus évoluées que celles à 5 loges avec lesquelles elles sont apparentées. Si chez *C. edulis,* le nombre de loges dans la capsule est fluctuant et aussi, mais à un degré moindre chez *C. bordesiana* ssp. *tïbestica,* les plantes que nous avons examinées pour le ssp. *bordesiana* possédaient

toutes 5 loges. Il semblerait que dans ce cas, les caractères évolutifs associés au très haut degré de polyploïdie de ce taxon se manifestent au niveau des capsules par le passage de 3 loges à 5 loges suivant le schéma de la Figure 2. Dans cette hypothèse, le caractère des capsules quinquéloculaires de *G. bordesiana* ssp. b*ordesiana* pourrait être interprété comme un exemple d'évolution secondaire qui redonne la morphologie florale du type primitif alors qu'il s'agit, en fait, d'un taxon plus jeune.

Cette hypothèse mérite d'être vérifiée en étudiant conjointement, chez les taxons polymorphes de *G. edulis* et aussi de *C. bordesiana,* les relations susceptibles d'exister entre le degré de polyploïdie et le caractère concernant le nombre de loges des capsules afin de tenter de déterminer les processus évolutifs dans cette lignée de campanules et de rechercher le type primitif à partir duquel ont pris naissance ces différents taxons.

Sur le plan biogéographique, la présence d'une lignée de campanules qui habitent les montagnes de l'Afrique orientale et centrale, du Yemen et aussi le Mont Sinaï avec *G. dulcis* Decne. que Naomi FEINBRUN-DOTHAN (1978) considère comme un élément arabico-saharien est un fait extrêmement intéressant qui illustre les liens floristiques qui existent sur tout le système montagneux de l'Afrique centrale et orientale.

En limite d'aire *G. edulis* a donc donné naissance à des taxons qui se sont individualisés, *G. bordesiana* ssp. *bordesiana* au Hoggar et ssp. *tibestica* au Tibesti (Emi Koussi) et *C. monodiana* au Tibesti (Toussidé), alors que dans le centre de l'aire *C. edulis* apparait comme un taxon très polymorphe.

BRUNEAU DE MIKE et QUEZEL (1961) ont mis en évidence les affinités floristiques qui existent entre ces différentes montagnes. Ils ont montré que des mêmes espèces peuvent se rencontrer sur plusieurs massifs telles que *Commicarpus montanus, Aptosimum pumilum, Blumea gariepina* qui habitent aussi bien en Ethiopie sur le Mt Mara, au Tchad au Tibesti, au Mt. Ennedi et à *YMr,* qu'en Algérie au Hoggar. Dans d'autres cas ce sont des groupes fortement apparentés

qui se sont diversifiés sur l'ensemble du territoire. Citons par exemple *Silène macrosolen* d'Afrique orientale et du Djebel Marra qui est remplacé au Tibesti à l'Emi Kou Koussi par S. *mirei* Chev. et Qzl. et par le *S. toussidona* Qzl. et le *S. guichardi* Qzl. au Toussidé. *Bromus adoenensis* d'Afrique orientale et centrale donne au Tibesti *B. tïbestica* et au Hoggar *B. gammas.*

BRUNEAU DE MIKE et QUEZEL décrivent en Afrique centrale, une flore spéciale aux montagnes situées à la lisière méridionale du Sahara et estiment qu'il a du y avoir des échanges floristiques considérables entre ces sommets. Actuellement «ces montagnes iles» dont l'isolement est absolu conservent des types qui, en raison même de cet isolement, ont évolué sur place permettant la différenciation de montagnes en montagnes des types différents.

C. dichotoma L.

L'aire de distribution de cette annuelle s'étend des Canaries à la Méditerranée occidentale et centrale : en Afrique du nord, au Sud de la péninsule ibérique, en Italie méridionale en Sicile et en Grèce.

Cette espèce est assez diversifiée et comprend 3 sous-espèces: le ssp. *dichotoma* dans la partie orientale de l'aire (Italie méridionale, Sicile, Grèce, Tunisie, Algérie orientale), le ssp. *afra* (Cav.) Maire dans la partie occidentale de l'aire depuis l'Algérie occidentale, le Maroc, le sud de la péninsule ibérique et les Canaries et le ssp. *kremeri* (Boiss. et Reut.) qui habite les massifs côtiers de l'Espagne méridionale, du Maroc, de l'Algérie et de la Tunisie et qui croît donc à des altitudes plus élevées. Chacun de ces trois taxons occupe une aire qui lui est propre.

Nous confirmons, Pl. 1, fig. 2 et 3 — pour les deux taxons étudiés les nombres chromosomiques déterminés par GADELLA (1964) sur des plantes de Palerme pour le ssp. *dichotoma* et par THULIN (1975) pour les ssp. *dichotoma* (Algérie à El Malia) et *afra* (Canaries dans l´ile de Tenerife). THULIN a compté le même nombre pour le ssp. *kremeri* provenant de FOranais, soit 2n = 24.

La diversification morphologique et écologique des différentes sous-espèces du *G. dichotoma* n'a pas été accompagnée de variations dans le nombre chromosomique.

C. erinus

Nous confirmons le nombre chromosomique 2n = 28 (Pl. I, fig. 4) de cette espèce qui, malgré le polymorphisme observé dans son aire de distribution circum-méditerranéenne, reste caryologiquement très stable.

C. filicaulis Dur.

Cette espèce est largement répandue dans toutes les régions montagneuses d'Afrique du nord où elle croît aussi bien sur calcaire que sur silice.

C. filicaulis forme un groupe extrêmement complexe en raison de son très grand polymorphisme qui a permis la distinction de nombreuses variétés qui assurent la transition entre des types extrêmes et que QUEZEL (1953) regroupe en 3 principaux phyllums évolutifs:

«— un rameau oriental individualisé surtout par la diminution de la pilosité, par la linéation des feuilles et la diminution de la taille des fleurs: var. *elata* (Faure et Maire) Qzl. et var. *reboudiana* (Pomel) Maire;

— un rameau occidental dont les caractères distinctifs sont inverses de ceux du rameau oriental: par l'intermédiaire des var. *parielii* Qzl. *intermedia* J. Vindt, *mairei* Qzl. *pseudo-atlantica* Qzl. il s'achève avec le var. *antiatlantica* (Maire, Weiller et Wilczek) QzL;

— un rameau septentrional caractérisé par sa vigueur de végétation et la grande taille de ses fleurs: il a donné naissance, dans le moyen Atlas au var. *gattefossei* (Maire et Weiller) Qzl. et dans le Rif au var. *gomerica* Font Quer;

— Enfin dans toutes les zones montagneuses du Maroc, le var. *pseudoradicosa* Maire paraît répondre à des

conditions stationnelles spéciales liées aux pâturages des montagnes plus ou moins humides tandis que le var. *filicaulis* répandu dans toute l'aire de l'espèce habite en général des pâturages plus ou moins rocailleux et secs. Sous couvert forestier elle individualise un écotype le var. *cedretorum* Lindberg».

Nous avons déterminé pour les var. *filicaulis* et *pseudoradicosa* un nombre chromosomique de 2n — 24 et n = 12 (Pl. I, fig. 5 et 6). Ce nombre est en désaccord avec ceux donnés par QUEZEL (1957) soit 2n = 16 pour le var. *réboudiana* et 2n = 48 pour les var. *filicaulis* et *pseudoradicosa.* Nous pensons que le nombre de 2n = 16 dû var. *reboudiana* est erroné. Quant aux races tétraploïdes des var. *filicaulis* et *pseudoradicosa,* il est indispensable d'étudier un plus grand nombre de populations pour se faire une opinion.

Ce nombre est également en désaccord avec celui donné pour *C. filicaulis* par HUMPHRIES & al. (1978) qui ont déterminé un nombre chromosomique de n — 13 pour des plantes provenant de Tanger (col entre le Cap Malabata et Ceuta, 550 m et du grand Atlas dans les gorges du Haut Dades vers 2300 m). Il se pourrait que des races dysploïdes aient pris naissance à la suite d'irrégularités méiotiques bien que dans notre matériel, nous ayons observé une méiose tout à fait normale avec 12 bivalents accompagnés parfois de chromosomes B (Pl. I, fig. 6).

Dans nos figures de métaphases somatiques, nous avons observé la présence d'une paire de chromosomes plus longs que les autres (cf. Pl. I, fig. 5).

Enfin dans le Tibesti QUEZEL (1961) signale la présence d'un taxon endémique de souche méditerranéenne *C. filicaulis* var. *tïbestica* Quézel, isolé sur l'Emi Koussi. Ce dernier avec d'autres espèces telles que *Verbascum dentifolium, Myrtus nivellei* ou encore *Spergularia tïbestica* (QUEZEL et MONNIER 1958) dont les affinités sont également tournées vers des taxons méditerranéens, soulignent les liens et les échanges floristiques qui ont dû se produire entre les flores mésogéennes et celles des montagnes d'Afrique centrale et l'extension de la flore méditerranéenne dans ces montagnes.

C. mairei Pau

Cette endémique du haut Atlas est extrêmement polymorphe en raison peut-être de son manque de spécificité écologique (on la trouve aussi bien dans les lieux humides que dans les éboulis ou les landes à xérophytes épineux ou encore dans les anfractuosités des rochers. Cependant la formation de colonies isolées a pu favoriser, par dérive génique sans doute, la naissance de petites races génotypiquement bien individualisées qui habitent des niches écologiques permettant leur conservation. Ainsi les variétés *anremerica* Lit. et Maire, *teñera* Emb. et Maire et *flahaultiana* Emb. se localisent uniquement dans les anfractuosités des rochers calcaires du grand Atlas oriental alors que le var. *atlántica* (Jah. et Maire) Maire, le plus répandu se rencontre aussi bien sur sols humides que sur un substrat très sec (QUEZEL 1953).

Les 3 variétés que nous avons étudiées les var. *anremerica, atlántica* et *flahautiana* ont un même nombre chromosomique de 2n = 34 et n = 17 (cf. Planche I, fig. 7, 8 et 9), contrairement au comptage de QUEZEL (1953) qui donne n = 8 pour cette espèce.

C. mollis L.

C. mollis, dont l'aire de répartition géographique est localisée dans le sud et le sud est de l'Espagne et en Afrique du nord sur les montagnes du Rif, et sur le littoral méditerranéen dans la région de Tlemcen — Oran, présente une distribution de type bético-rifain.

Très polymorphe cette espèce comprend plusieurs variétés. Or, si nous avons déterminé un nombre chromosomique diploïde de 2n — 24 (cf. Planche I, fig. 10) sur des plantes d'Algérie se rapportant à la variété *oranensis* Maire, les deux populations du Rif qui appartiennent au var. *rifana* Emb. se sont révélées être tétraploïdes. Les plantes de la population du Mt Kraa donnent un nombre chromosomique stable de n = 24 et 2n = 48 (cf. Planche I, fig. 14). Celles de la gorge de Talembose ont montré un phénomène d'aneu-

ploïdie qui s'est manifesté par des nombres somatiques de 2n = 46, 48, 50, 52 et des nombres méiotiques de n = 25 et 26 pour les plantes examinées (cf. Planche I, fig. 11, 12, 13). Il apparaît donc dans cette population des perturbations chromosomiques provoquant une aneuploïdie ascendante et descendante.

En Espagne PODLECH et DAMBOLDT (1964) donnent pour *C. mollis* un nombre chromosomique de 2n = 26 pour des plantes provenant de la province de Malaga récoltées à Grazalema. S'agit-il d'une race dysploïde? La figure qu'ils donnent montrent des chromosomes somatiques sensiblement de même taille à l'exception d'une paire de chromosomes légèrement plus longs que les autres. Dans le matériel des gorges de Ben Amrane, il apparait 2 paires de chromosomes qui semblent plus longs que les autres (Pl. **I,** fig. **10).**

Campanula numidica Durieu

Cette remarquable endémique étroitement localisée dans la ville de Constantine et à sa périphérie possède le même nombre chromosomique que celui de *G. filicaulis,* soit 2n=24 (cf. Planche I, fig. 15) dont certaines formes rupicoles marocaines semblent lui être nettement apparentées.

Campanula rapunculus L.

C. rapunculus possède une aire de répartition très vaste depuis l'Europe centrale et méridionale jusqu'en U. R. S. S., l'Asie mineure et l'Afrique du nord.

Très polymorphe sur toute l'étendue de son aire, *C. rapunculus* l'est également en Afrique du nord où plusieurs variétés ont été décrites, les var. *rapunculus, grandiflora* F. Q. et *hirta* Ten. C'est à cette dernière que se rattache les échantillons que nous avons étudiés et qui se rapportent à la forme *verruculosa* (Hoffgg. et Link) Maire. Le nombre chromosomique n = 10 de *C. rapunculus* est remarquablement constant, parmi les différentes variétés qui ont été décrites de part et d'autre dans son aire de répartition, il y a souvent des chromosomes B (cf. Planche I, fig. 16).

Campanula trachelium L.

Largement répandue en Europe, en U. R. S. S., en Asie mineure et en Afrique du nord cette espèce présente également un nombre chromosomique constant de 2n = 34 dans toutes les formes qui ont été décrites.

CONCLUSION

Les *Campanula* d'Afrique septentrionale et centrale par leur polymorphisme et les nombres chromosomiques différents qui ont été dénombrés posent d'intéressants problèmes de spéciation qui mériteraient une étude plus approfondie portant sur un nombre important de populations par espèce.

Cette brève étude a déjà mis en évidence des phénomènes de polyploïdie accompagnée d'aneuploïdie chez *G. bordesiana* ssp. *bordesiana,* de dysploïdie et d'aneuploïdie chez *G. mollis,* de dysploïdie et peut-être de polyploïdie chez *G. filicaulis.*

L'étude comparative de *G. bordesiana* ssp. *bordesiana* avec les campanules d'Afrique centrale et orientale a montré son appartenance à une lignée de campanules qui vivent sur les montagnes qui bordent le Sahara méridional et de l'Afrique orientale et qui se seraient différenciées à partir de *C. edulis,* espèce extrêmement polymorphe, à la limite de son aire de répartition. Citons : *C. bordesiana* ssp. *bordesiana* et *tïbestica* et *G. monodiana.*

Le nombre chromosomique de *G. bordesiana* ssp. *bordesiana* (n = 40-42) dont le degré de polyploïdie est très élevé, est difficilement explicable. Les parentés morphologiques entre *G. edulis* et *G. bordesiana* laissent supposer que *G. edulis* est peut-être un des parents (le nombre de chromosomes de cette espèce est n = 28 THULIN 1975).

Enfin nous avons posé le problème de la valeur systématique qu'il faut accorder au caractère du nombre de loges des capsules (3 ou 5) chez les campanules de la section *Medium.* Considéré généralement comme discriminatoire, ce caractère est fluctuant chez *G. edulis* où nous avons pu observer les formes de passage entre 3 et 5. Chez *C. bor-*

desiana ssp. *tibestica* nous avons vu des plantes à capsules triloculaires et d'autres dont les capsules possédaient 5 loges. Chez le ssp. *bordesiana* toutes les plantes examinées avaient des capsules quinquéloculaires. Le caractère «capsule quinquéloculaire» de ce dernier taxon pourrait être interprété comme un caractère évolutif secondaire qui trouverait sa confirmation dans le degré élevé de polyploïdie par rapport à celui de *G. edulis. C. bordesiana* correspondrait donc à un taxon jeune.

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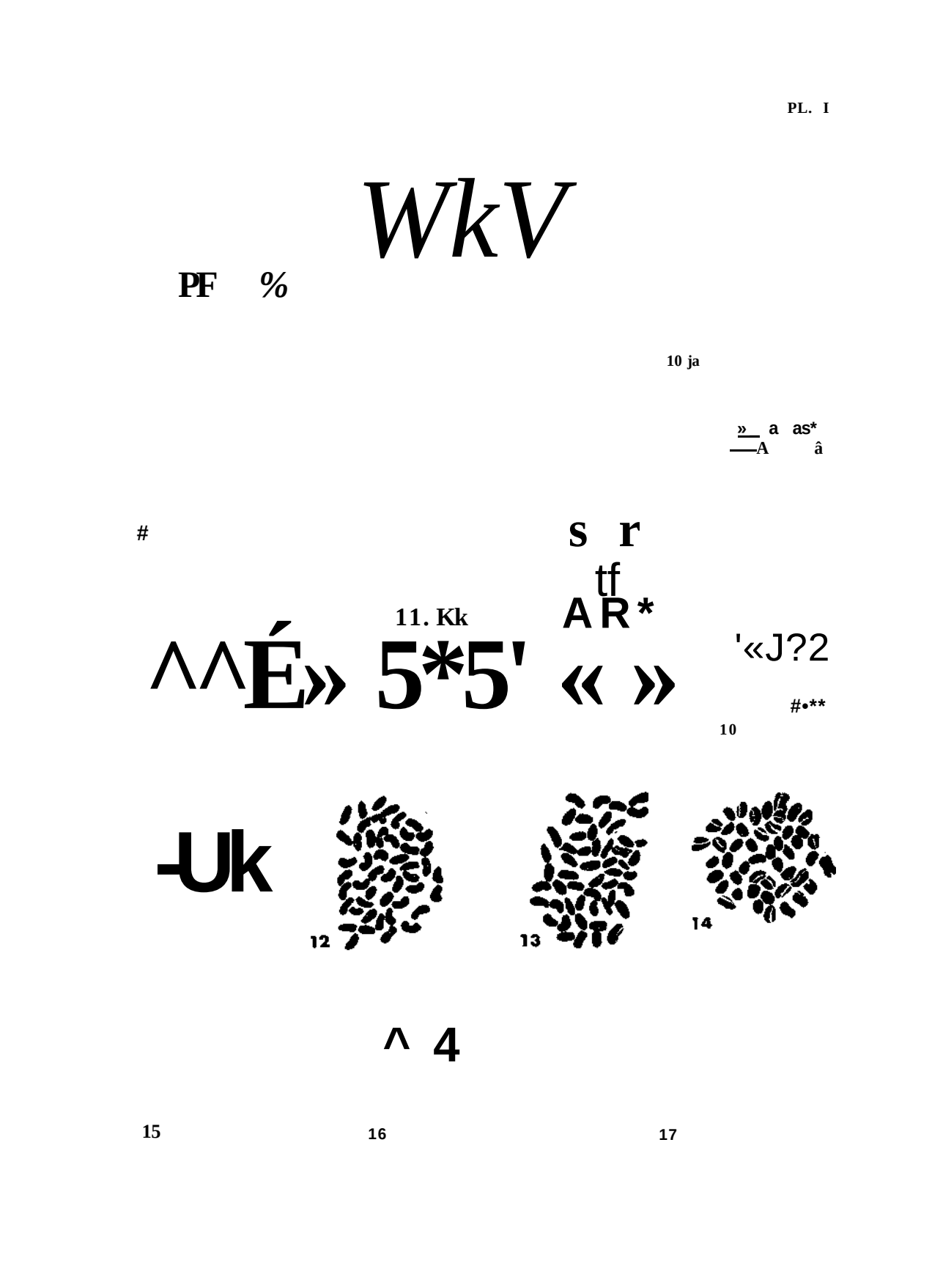
PLANCHE

PLANCHE I

Fig. 1. — *G. bordesiana* ssp. *bordesiana,* métaphase I dans une C. M. P., n = 40.
Fig. 2, — *G. dichotoma* ssp. *dichotoma,* métaphase I dans une C. M. P., n = 12.
Fig. 3. — *C. dichotoma* ssp. *afra,* métaphase somatique dans l'ovaire, 2n = 24.
Fig. 4. — *C. erinus,* métaphase dans une maerospore, n = 14.
Fig. 5. — *C. filicaulis* var. *filicaulis,* métaphase somatique dans l'ovaire, 2n = 24.
Fig. 6. — *G. filicaulis* var. *pseudoradicosa,* métaphase I dans une C. M. P., n = 12 + 1 B.
Fig. 7. — *G. mairei* var. *anremerica,* métaphase somatique dans l'ovaire, 2n = 34.
Fig. 8. — *C. mairei* var. *atlántica,* métaphase pollinique, n = 17.
Fig. 9. — *G. mairei* var. *flahaultiana,* métaphase somatique dans l'ovaire, 2n = 34.
Fig. 10. — *G. mollis,* métaphase somatique dans l'ovaire, 2n = 24.
Fig. 11, 12, 13. — *G. mollis* var. *rifana,* métaphases somatiques dans l'ovaire: dysploïdie, 2n = 46, 48, 52.
Fig. 14. — *G. mollis* var. *rifana,* métaphase somatique dans l'ovaire, 2n = 48.
Fig. 15. — *G. numidica,* métaphase somatique dans l'ovaire, 2n = 24.
Fig. 16. — *G. rapunculus* var. *hirta,* forme *verruculosa,* mitose pollinique, n = 10 + 1 B.
Fig. 17. — *G. tracheliwm,* métaphase somatique dans l'ovaire, 2n = 34.

C. M. P. = Cellule mère de grains de pollens.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CARYOLOGIE DE TAXONS ENDÉMIQUES MAROCAINS DU GENRE *ARTEMISIA* L.

par

Melle A. OUYAHYA et Mme J. VIANO *

RÉSUMÉ

Des nombres chromosomiques nouveaux ont été comptés pour *Artemisia negrii* Ouyahya (2n =7x = 63), *Artemisia mesatïantica* Maire (2n = 18), *Artemisia ifranensis* Did. (n = 9 et 2n = 18). De plus, des nombres chromosomiques ont été confirmés sur de nouvelles populations chez *Artemisia alba* Turr. subsp. *chitachensis* (Coss.) Maire (n = 9) et *Artemisia flahaultii* Emb. et Maire (n = 9 et 2n = 18). Des individus diploïdes à 2n = 18 ont été rencontrés chez *Artemisia atlántica* Coss.. et Dur. var. *maroccana* (Coss.) Maire, où seuls des tétraploïdes avaient été précédemment décelés.

Le nombre de base des Armoises étudiées est toujours égal à 9, ce qui est le cas plus fréquent pour le genre *Artemisia.*

L'existence de plusieurs degrés de ploïdie pour les taxons *Artemisia negrii* Ouyahya et *A. atlántica* Coss. et Dur. var. *maroccana* (Coss.) Maire confirme l'instabilité génétique du genre *Artemisia.*

La. Polyploidie joue certainement un rôle décisif dans l'évolution de ce genre.

SUMMARY

New chromosome numbers are proposed for *Artemisia negrii* Ouyahya (2n = 7x = 63), *Artemisia mesatïantica* Maire (2n = 18), *Artemisia ifranensis* Did. (n = 9) et (2n = 18). Other chromosome: numbers have been confirmed on new populations for *Artemisia alba*

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Turr. subsp. *chitachensis* (Coss.) Maire (n = 9) et *Artemisia flahaúltii* Emb. et Maire (n = 9) et (2n = 18).

Diploids have been found to *Artemisia atlántica* Coss. et Dur. var. *maroccana* (Coss.) Maire where only tetraploid have been proposed before.

The basic number of these species is ever such as n = 9 and it is presently the most frequent number found about the genus *Artemisia.* The authors notice also the presence of some polyploids who confirm the genetic instability among the genus *Artemisia.*

The polyploidy seem to be a decisive element for the differentiation and the evolution of the genus.

INTrODUCTION

Au cours de recherches de Taxinomie expérimentale sur le genre *Artemisia* au Maroc, entreprises au laboratoire de Taxinomie et Ecologie végétales dans le cadre d'un Doctorat de III Cycle, il nous a paru intéressant de réaliser une étude caryologique sur plusieurs taxons non encore étudiés sur ce plan: *A. alba* Turr. subsp. *chitachensis* (Coss.) Maire, *A. atlántica* Coss. et Dur. var. *maroccana* (Coss.) Maire, *A. negrii* Ouyahya, *A. mesatlantica* Maire, *A. flahaultii* Emb. et Maire, *A. ifranensis* Did. Tous ces taxons sont strictement localisés au Maroc.

D'après les données caryologiques contenus dans divers ouvrages (DARLINGTON 1945, LÖVE and LOVE 1961, TISCHLER 1950, MOORE 1967-1974), deux résultats intéressants sont apparus concernant le genre *Artemisia:* d'une part l'existence d'un nombre de base différent selon les espèces étudiées (x = 8 ou x = 9), d'autre part la présence de plusieurs degrés de ploïdie (2x à 1Ox).

MATÉRIEL, ET MÉTHODES

Le matériel étudié provient uniquement des plantes récoltées dans les lieux d'origine. Il s'agit de boutons floraux prélevés sur le terrain même. De plus, à partir d'échantillons cultivés nous avons procédé à des vérifications afin de contrôler le matériel récolté.

Les fixations de boutons floraux et de jeunes racines ont été faites dans un liquide contenant $^{3/4}$ alcool absolu et $^{1/4}$ acide acétique.

Une précoloration à base de carmin acétique a été réalisée quelques jours avant l'écrasement. La technique utilisée est celle des squashes au carmin acétique (BELLING, 1926).

De plus, un prétraitement à lVmonobrornonaphtalène conseillé par DARLINGTON et LA COUR (1969) a été utilisé pour raccourcir les chromosomes de certaines espèces étudiées afin d'en faciliter le dénombrement.

Après avoir effectué toute une série de mesures sur la longueur des fleurons, on a pu déterminer que sur les espèces étudiées, les méioses des cellules-mère de grains de pollens ont lieu pour des fleurons longs de 1 à 1,5 mm.

De même, nous avons constaté par des fixations à diverses heures de la journée, que le maximum de mitoses et de méioses se situe entre 12 h et 14 h.

Les comptages chromosomiques ont été le plus souvent réalisés pour une même espèce sur plusieurs échantillons appartenant à une même population ou provenant de populations différentes.

RESULTATS

Une étude d'ensemble de la structure du noyau quiescent et du noyau en division des différents taxons étudiés, nous **a** permis d'envisager l'utilisation des caractères du noyau quiescent et du noyau en division pour préciser les affinités entre les 6 armoises.

Artemisia alba Turr. subsp. **chitachensis** (Coss.) Maire

Provenance des échantillons étudiés: Moyen Atlas oriental, Jbel Bou Naceur, flanc Nord, 2450 m, sol rocailleux, 22.VII.1978.

Nombre chromosomique: n = 9. Sur des métaphases I méiotiques dans l'étamine en vue polaire, nous avons compté n = 9 (Planche 1, figure 1). De même, sur des diacinèses, ce nombre a été retrouvé (Planche 2, microphoto 1). Des irrégularités méiotiques ont été également observées laissant apparaître un nombre aberrant de chromosomes

(n = 8. 10.11). Le nombre chromosomique de ce taxon a été déterminé pour la première fois par QuEZeL (1957) sur des populations du Haut Atlas au M' Goun. Le nombre de base n = 9 se trouve donc confirmé par nous observations.

Il faut également signaler l'existence d'individus tétraploïdes découverts par TUTIN (1976) chez l'espèce *s. str.* et par CESCA (1972) chez le var. *incanescens* Fiori.

La polyploïdie jouerait donc un rôle dans l'évolution de ce taxon. Sans doute, au cours de nos futures investigations caryologiques, rencontrerons nous des taxons à degré de ploïdie différent.

Aire de répartition: le ssp. *chitachensis* Maire est spécial au Maroc. (Grand Atlas et Moyen Atlas). Mais l'espèce possède une aire de répartition étendue (Péninsule ibérique, France, Belgique, Suisse, Italie, Hongrie, Algérie).

Artemisia atlántica Coss. et Dur. var. maroccana (Coss.) Maire ¡.

Provenance des échantillons étudiés: Haut Atlas central, gorges du Dadès, 1730 m, 4.X.1978.

Nombre chromosomique: 2n = 18. Sur des métaphases somatiques de jeunes pointes de racines, nous avons dénombré 2n = 18 (Planche 1, figure 2; Planche 2, microphoto 2). Le caryotype de ce taxon se compose de 18 chromosomes dont 4 paires plus longues (2,5 u environ) et 5 paires assez courtes (environ 1 u).

Chez ce taxon aussi, il semblerait y avoir des individus polyploïdes. En effet, QUEZEL (1957) sur des populations provenant du M'Korn (Haut Atlas central) a compté n = 18 chez *A. atlántica.* Ce fait pourrait indiquer que le nombre chromosomique varierait selon là distribution géographique du taxon considéré. Cette hypothèse sera sans doute vérifiée lors d'une prochaine mission effectuée dans cette région du Haut-Atlas central.

Aire de répartition: L'espèce se rencontre en Afrique du Nord mais le var. *maroccana* est une endémique marocaine (Grand Atlas et Moyen Atlas).

Artemisia negrii Ouyahya (= *A. mesatlantica* var. *subsimplex* Maire et Humbert)

Provenance des échantillons étudiés:

— Haut Atlas central, à 26 km d'Agoudal, 2790 m d'altitude, 24.XI.1978.
— Haut Atlas, Jbel Afadaï, 1850 m, 25.VII.1978.

Nombre chromosomique: (2n = 7x = 63). Sur des métaphases somatiques de racines, nous avons mis en évidence une forme heptaploïde (Planche 3, microphoto 3). Le caryotype montre des chromosomes courts d'environ 1 u, certains possèdent des constrictions médianes bien visibles. De plus, nous avons noté la présence d'un nombre élevé de nucléoles (3 à 5). L'existence de formes de ce degré de polyploïdie est signalée pour la première fois dans le genre *Artemisia.*

MAIRE et HUMBERT (1926) ont considéré ce taxon corne une variété (var. *subsimplex)* de *A. mesatlantica* Maire. Cependant, il est polyploïde (2n = 7x = 63), tandis que l'armoise de l'Atlas est diploid (2n = 18). Les données caryologiques semblent donc soutenir les données morphologiques et écologiques que nous avons établies précédemment. Ce qui nous a autorisé à élever le var. *subsimplex* au rang spécifique et à le nommer *A. negrii* (OUYAMYA, 1980).

Aire de répartition: cette espèce est une endémique marocaine, localisée dans le Grand-Atlas où elle croît à haute altitude.

Artemisia mesatlantica Maire

Provenance des échantillons étudiés:

— Moyen Atlas oriental, Tmatert, sol rocailleux, 1850 m, 24.Vn.1978.

— Moyen Atlas central, route d'Ifrane à Boulmane, 31.X.1978.

Nombre chromosomique: 2n = 18. La numération chromosomique a été faite sur des métaphases somatiques de jeunes pointes de racines germées à partir de graines récoltées dans le Moyen Atlas central. Sur plusieurs plaques équatoriales, nous avons dénombré 2n = 18 (Planche 1, figure 3; Planche 2, microphoto 4). Les chromosomes sont assez longs de 1 à 2,5 u. avec des constrictions peu nettes.

Ce nombre est nouveau pour l'espèce.

Aire de répartition: Cette espèce est spéciale au Maroc, localisée dans le Grand, le Moyen et l'Anti-Atlas.

Artemisia flahaultii Emb. et Maire

Provenance des échantillons étudiés:

Moyen Atlas oriental : Jbel Bou Naceur, flanc nord, altérites, 2450 m, 17.VIII. 1978.

Nombre chromosomique: n = 9, 2n = 18.

A partir de plaques métaphasiques I de cellules méiotiques, nous avons compté aisément n = 9 (Planche 1, figure 4). Ce nombre a également été rencontré sur des mitoses somatiques dans des pointes radiculaires obtenus après germination (Planche 1, figure 5; Planche 2, microphoto 5).

Nous avons distingué dans le caryotype 4 paires de chromosomes longs à constriction médiane et sous-médiane et 4 paires de chromosomes isobrachiaux deux à deux ainsi qu'une paire de chromosomes courts à constriction médiane. La longueur des chromosomes varie entre 1,5 et 2,5 u.

Ce résultat confirme celui établi par QUEZEL en 1957 sur des populations provenant aussi du Jbel Bou Naceur.

Aire de répartition: Endémique marocaine localisée dans le Moyen-Atlas oriental.

A. ifranensis Did.

Provenance des échantillons étudiés: Moyen Atlas central, Michlifène, 1600 m, 29.VIII.1978.

Nombre chromosomique: n = 9, 2n = 18.

A la métaphase hétérotypique, les chromosomes sont suffisamment bien éloignés les uns des autres pour qu'un comptage tout à fait satisfaisant soit possible (Planche 1, figure 6; Planche 2, microphoto 8). Nous avons observé dans ce matériel de très belles figures de diacinèse (Planche 2, microphoto 7), sur lesquelles nous avons dénombré 9 bivalents. La longueur des chromosomes varie entre 1,5 et 3 u. Le dénombrement des chromosomes a été aussi effectué sur des méristèmes radiculaires. Nous avons compté sans difficulté 2n = 18. A ce stade quelques uns des chromosomes sont déjà clivés (Planche 1, figure 7; Planche 2, microphoto 6).

Le nombre chromosomique de ce taxon est rapporté ici pour la première fois.

Aire de répartition: Cette espèce est pour l'instant spéciale au Maroc (Moyen Atlas central).

CONCLUSIONS

1 — D'après nos résultats, le nombre de base pour les 6 taxons étudiés *[A. alba* Turr. subsp. *chitachensis* (Coss.) Maire, *A. atlántica* Coss. et Dur. var. *maroccana* (Coss-) Maire, *A. negrii* Ouyahya, *A. mesatlantica* Maire, *A. flahaultii* Emb. et Maire, *A. ifranensis* Did.] est toujours égal à 9. Ceci est en accord avec les travaux antérieurs réalisés sur le genre *Artemisia* où la majorité des espèces étudiées possèdent un nombre de base n = 9 et une minorité n = 8.

2 — Le nombre chromosomique a été compté pour la première fois chez *Artemisia negrii* Ouyahya (2n = 7x = 63),

Artemisia mesatlantica Maire (2n = 18), *Artemisia ifranensis* Did. (n = 9 et 2n = 18).

3 — De plus, des nombres chromosomiques ont été confirmés sur de nouvelles populations chez *Artemisia alba* Turr. subsp. *chitachensis* (Coss.) Maire, *Artemisia atlántica* Coss. et Dur. var. *maroccana* (Coss.) Maire, *Artemisia flahaultii* Emb. & Maire.

4 — Parmi les 6 taxons étudiés, nous n'avons rencontré qu'un seul polyploïde : *Artemisia negrii* Ouyahya (2n — 7x = 63). Pourtant, il existe des individus tétraploïdes chez *A. atlántica* Coss. et Dur. var. *maroccana* (Coss.) Maire et *A. alba* Turr. subsp. *chitachensis* (Coss.) Maire. Ceci nous laisse supposer que les espèces utiliseraient la polyploïdisation dans leur évolution. Aussi le fait d'avoir rencontré des individus polyploïdes (2n = 7x = 63) chez une espèce nouvelle *A. negrii* présente un grand intérêt pour la caryologie du genre *Artemisia.* En effet, même si l'individu qui le possédait est unique, son existence révèle bien l'évolution du genre *Artemisia* L.

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PLANCHES

PLANCHE 1

Dessins de la garniture chromosomique des Armoises

Fig. 1.—*A. alba* Turr. subsp. *chitachensis* (Coss.) Maire, n = 9, métaphase I méiotique dans l'étamine.

Fig. 2.—*A. atlântica* Cocc. et Dur. var. *maroccana* (Coss.) Maire, 2n = 18, métaphase somatique dans une racine.

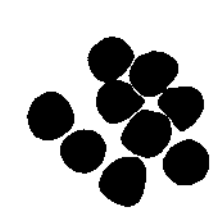
Pig. 3. — *A. mesatlantica* Maire, 2n = 18, métaphase somatique dans une racine.

Fig. 4. — *A. flahaultii* Emb. et Maire, n = 9, métaphase I méiotique dans l'étamine.

Fig. 5. — *A. flahaultii* Emb.et Maire, 2n = 18, métaphase somatique dans une racine.

Fig. 6. — *A. ifranensis* Did., n = 9, métaphase I méiotique dans l'étamine.

Fig. 7. — *A. ifranensis* Did., 2n = 18, métaphase somatique dans une racine.



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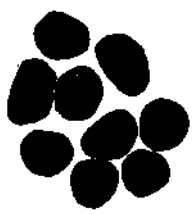

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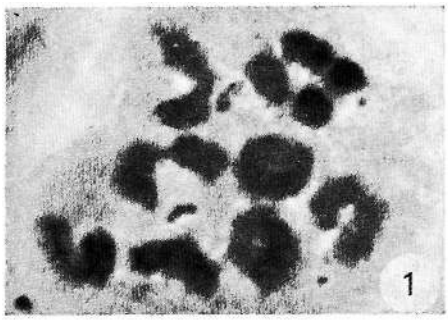
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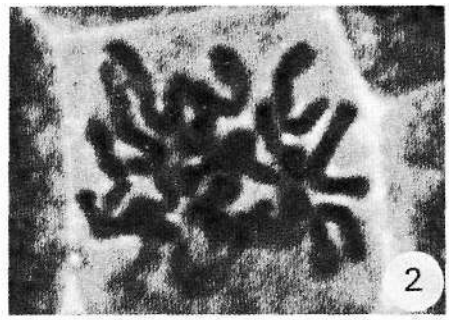
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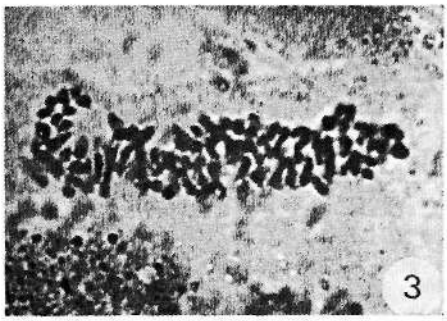
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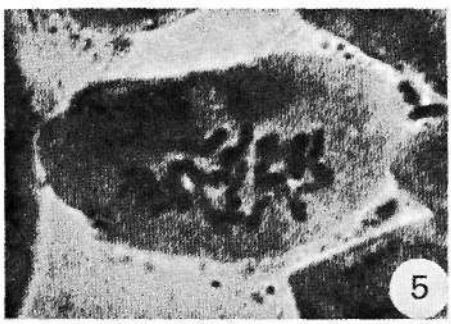
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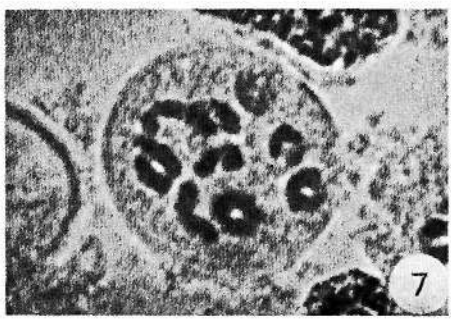
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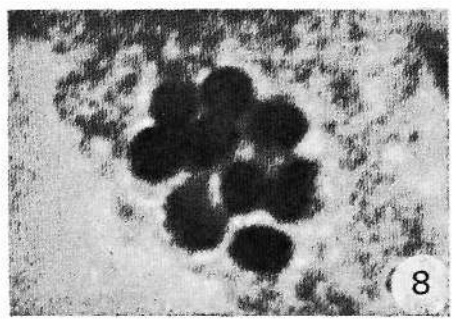
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PLANChE 2

Documents photographiques des Armoises étudiées

Microphoto 1. — *A. alba* Turr. subsp. *chitachensis* (Coss.) Maire, n = 9, diacinèse.

Mierophoto 2. — *A. atlântica* Cocc. et Dur. var. *maroccana* (Coss.) Maire, 2n = 18, métaphase somatique dans une racine.

Microphoto 3. — *A. negrii* Ouyahya, 2n = 63, métaphase somatique dans une racine.

Microphoto 4. — *A. mesatlantica* Maire, 2n = 18, métaphase somatique dans une racine.

Mierophoto 5. — *A. flahaultii* Emb. et Maire, 2n = 18, métaphase somatique dans une racine.

Microphoto 6. — *A. ifranensis* Did., 2n = 18, métaphase somatique dans une racine.

Microphoto 7. — *A. ifranensis* Did., n = 9, diacinèse dans l'étamine.

Microphoto 8. — *A. ifranensis* Did., n = 9, métaphase I méiotique dans l'étamine.

Bol. Soc. Brot, Sér. 2, **58(2):** 921-931 (1981).

IRIS PSEUDOPUMILA TIN. AND IRIS ATTICA BOISS. & HELDR.: TWO VERY SIMILAR KARYOTYPES

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SUMMARY

Some investigations on the karyotypes of *I. pseudopumila* Tin. and *I. attica* Boiss. & Heldr. are carried out. Evidence is shown of the similarity between the karyotypes of the two species and some hypothesis on their distibution area at the end of Miocene age are put forward. Further investigations on the *I, pseudopumila* and *I. attica* morphology and on their hybrids would be necessary in order to bring out real differences existing between the two species.

Iris pseudopumila Tin. is a dwarf Pogoniris characterized by a oneflowered scape, yellow or purple flowers and by perianth-tube shorter than in *Iris pumila* L. and longer than in *Iris chamaeiris* Bertol. (DYKES 1931). RANDOLPH & REICHINGER (1954) as well as SIMONET (1956) have reported that its distribution area is Southern Italy and Sicily. MACCHIA (1969) points out that the area where this species is present includes: Puglia, Basilicata and Sicily. MITRA (1956) and RANDOLPH & MITRA (1959) reported its presence also near Zadar (Yugoslavia).

Closely related to this species is *Iris attica* Boiss. & Heldr., present in Greece (RANDOLPH & REICHINGER 1954; SIMONET 1956). This species was regarded by BAKER (1892) and DYKES (1913) as a variety of *I. pumila* L., probably because of similarities in their morphology. It is, however, a well definited species, characterized by yellow flowers,

long perianth tube and a number of chromosomes different from that of *I. pumila* L. (SIMONET 1932, 1955).

We have investigated karyotypes of different specimens of *I. pseudopumila* from Puglia and Sicily and of two plants of *I. attica* from Greece.

MÄTErIAL AND METHODS

The specimens of *I. pseudopumila* investigated have been gathered in the following areas in Puglia and Sicily (one specimen from each area) : Porto Cesareo (Lecce) ; Gargano, Val Carbonara (Foggia) ; Palagiano (Taranto) ; Martina Franca (Taranto) ; Manduria (Taranto) ; Santeramo in Colle (Bari) ; Cassano delle Murge (Bari) ; Montelepre (Palermo); Lercara Friddi (Palermo); Madonie (Palermo).

The two specimens of I. *attica* are from Cape Sounion near Athens[1]

The karyological investigations were carried out on root tip meristematic cells, by the usual methods of squashing after colchinization with -bromonaphtalene and 8-hydroxy-quinoline and staining with Gomori haematoxyline.

For each area the karyotype was constructed by calculating the r- value (long arm/short arm ratio) and by subdividing the chromosomes into groups according to the r-value (LEVAN *et aï.* 1964). It is quite easy to find out the group the various chromosomes belong to, whereas it is not possible to distinguish, in the group, the various chromosome couples owing to the extremely close resemblance between the various chromosomes, unless they present particular characteristics such as satellites.

The karyotype of Iris pseudopumila Tin.

We have carefully investigated the karyotype of the specimen from Porto Cesáreo and have compared it with

[1] We are grateful to: FRANCINI CORTI E., GARBARI F., NARDI E., RiCCERi C, STEINBERG C, for gathering the specimens and thus contributing to our study.

the karyotypes of the irises from other areas. The chromosome number 2n = 16 agrees with all the data from the literature (SIMONET 1955; MITRA 1956; RANDOLPH & MITRA 1958, 1959; GARBARI *et al.* 1973).

In the karyotype from the Porto Cesáreo specimens the following chromosome groups have been brought out (Fig. 1) :

I Group: Couple 1: chromosomes with centromere in median region, r mean value about 1.06.
II Group: Couples 2, 3, 4, 5. 6, 7, 8: chromosomes with centromere in subterminal region, with r mean value varying roughly, between 5.80 and 8.60, according to the couples considered.

Couple 7 and 8 carry satellites on the short arms of both partners. The satellites consist of a heterochromatic filament with euchromatic thickening at the tip. In one metaphase plate one chromosome in couple 3 showed a very tiny satellite. In another case one satellite on the long arm of one partner in couple 2 was observed (Fig. 1).

Iris pseudopumila from the other areas present karyotypes similar to the one described above. There are differences only as regards satellites (Table I). In fact, specimens from Gargano (Fig. 2), Palagiano, Santeramo in Colle, Madonie, Lercara Friddi, show satellites on both members of couple 7 and 8. The specimen from Montelepre (Fig. 3) carries satellites in couple 8. It is doubtful however that it carries satellites in couple 7 too, as in this latter couple two tiny satellites were observed only once.

In the specimens from Martina Franca and Cassano delle Murge, couple 7 is heteromorphic owing to the presence of one satellite in only one partner. In the Manduria specimens instead couple 7 does not show any satellites.

A tiny satellite on one member of couple 3 was observed also in the specimens from Gargano and Manduria.

The tiny satellite on the long arm of couple 2, observed in the specimen from Porto Cesáreo, was never found in any other specimen.

TABLE I

Number of satellites on chromosomes of couples 2, 3, 7, in *I. pseudopumila* Tin. from the different localities

Localities	Number of satellites couple 2	Number of satellites couple 3	Number of satellites couple 7	Number of satellites couple 8
Porto Cesáreo . . .			2	**2**
Gargano			2	**2**
Cassano Delle Murge			1	**2**
Palagiano			2	**2**
Santeramo in Colle .			2	**2**
Martina Franca . .			1	**2**
Manduria				**2**
Montelepre			**2?**	**2**
Madonie			**2**	**2**
Lercara Friddi . . .			**2**	**2**

The karyotype of Iris attica Boiss. & Heldr.

The chromosome number 2n = 16 agrees with the data reported in the literature (SIMONET 1932; MITRA 1956; RANDOLPH & MITRA 1959).

The following chromosome groups were brought out in the karyotype (Fig. 4) :

I Group: Couple 1: chromosomes with centromere in median region, r mean value about 1.22.

II Group: Couples 2, 3, 4, 5, 6, 7, 8 chromosomes with centromere in subterminal region, with r mean value varying rougly, between 5.59 and 8.06, according to the couples considered.

Couples 2 and 7 turned out to be supplied with satellites. They show a small satellite consisting of a heterochromatic filament with an euchromatic thickening at the tip in both partners of the couple. In one case tiny satellites were observed on one member of couple 1 and 3 respectively.

CONCLUSIONS

The karyotypes described for *I. pseudopumila* Tin. and *L attica* Boiss & Heldr. agree, on the whole, with the data reported in the literature (SiMONET 1932, 1934; MITRA 1956; RANDOLPH & MITRA 1959). The number of satellites, we observed, is different from the number reported by RANDOLPH & MITRA who observed as many as 3-4 satellites-supplied couples. GARBARI *et al.* (1973) too noticed a smaller number of satellites and they also observed secondary constrictions on the short arms of a chromosome couple. In the *I. attica* karyotype too RANDOLPH & MITRA observed a higher number of satellited couples (three) than observed by SiMONET (1932) and ourselves (two). Besides, according to RANDOLPH & MITRA, couple 1 is submetacentric and not metacentric as in our investigations which follow the classification by LEVAN *et al.* (1964). Actually the two arms of couple 1 have not the same length exactly, like those in *I. pseudopumila,* as their ratio is about 1.20.

The karyotypes of *I. pseudopumila* and *I. attica* are therefore characterized by 16 chromosomes — one couple being metacentric and the others subacrocentric — and by number of satellites varying according to the areas or better to the clones investigated. We observed a karyotype with a similarly variable number of satellites also in another dwarf Pogoniris: *Iris chamaeiris* Bertol. s. 1. (MAUGINI & BiNi MALECT 1973; 1974; BiNi MALECT 1976). These karyotypes appear to be characteristic of plants with some chromosome instability which mostly grow by vegetative reproduction and thus preserve any possible chromosome mutation.

The remarkable resemblance between the karyotypes of *Iris pseudopumila* and *Iris attica,* which basically differ only in the slightly different position of the centromere in couple 1, raises the problem of their affinity. Geological events involved Puglia and Aegean peninsula at the end of Miocene (Pontic). At that time Puglia was the most western part of what is called the Aegeic continent (GRIDELLI 1950; PASA 1953 in FRANCINI CORTI 1966). If we take this into account, several hypotheses ' can be formulated on

possible contacts between *I. pseudopumila* and *I. attica.* It can be assumed, as MACCHIA (1969) also suggests, that the two species had a wider distribution area and were sympatric. On the other hand, owing to their remarkable similarity, there might have been one species only at that time. This species later isolated in Greece and in Puglia, by subsequent geological events, migth have diversified and engendered *I. pseudopumila* and *I. attica.*

We believe that further investigations on the distribution areas of these plants and on their morphology and hybrids should be carried out. Evidence might be found of transitions forms between *I. pseudopumila* and *I. attica* and at the same time a helpful contribution might be given to the knowledge on the origin of *I. pumila* L. which SIMONET (1934) regarded as a autotetraploid of *I. attica* and RANDOLPH & MITRA (1959) as an amphidiploid hybrid of *I. pseudopumila* and *I. attica.* To conclude the problem of amphidiploidy and allopoliploidy is related to the differences existing between the two species.

While this paper was still in press, some papers were published on *Iris pseudopymila* and 7ns *attica.* (COLASANTE, RICCI 1979; SAUER 1979; SAUER & LEEP 1979; SAUER & STEGMEIER 1979). Recorded data and conclusions agree, on the whole, with those of our paper.

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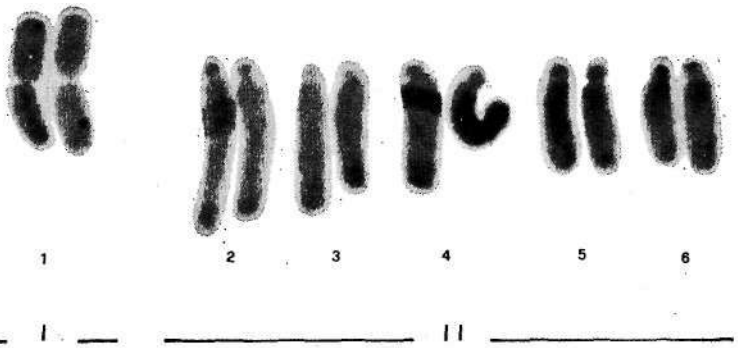
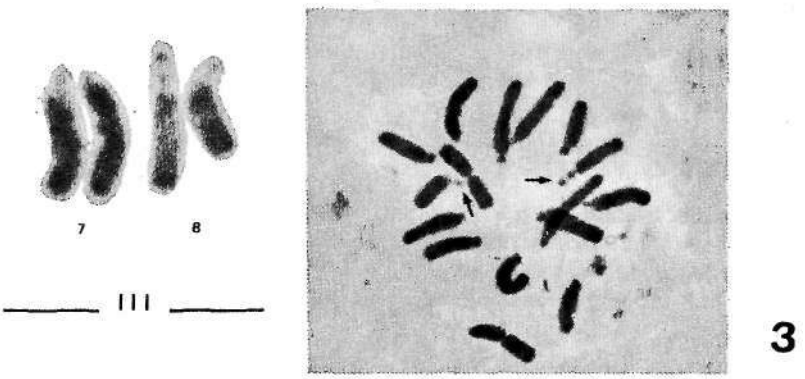
PLATES

PLATE I

-Karyotype (X 2100) and metaphase plate (X 1100) of *I. pseuäopumila* from Porto Cesáreo. Short arrows indicate satellites on couple 7 and 8. Long arrow indicates the satellite on long arm of one partner of couple 2.

-Karyotype (X 2100) and metaphase plate (X 1100) of *I. pseuäopumila* from Gargano. Short arrows like in fig. 1. Long arrow shows the short satellite on one partner of couple 3.





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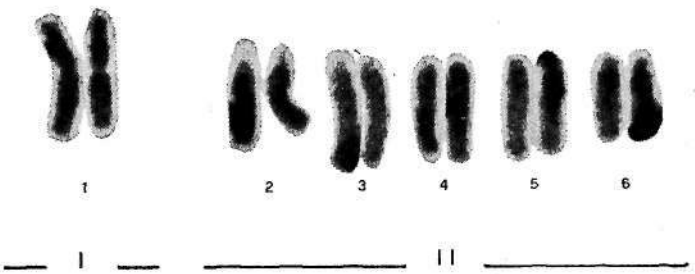

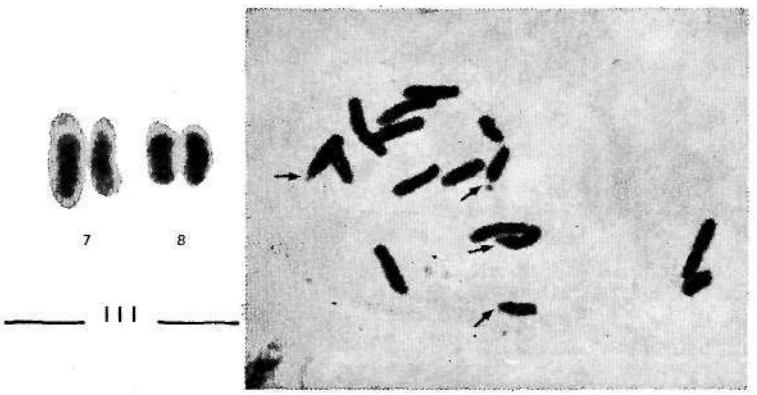

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PLATE II

Fig. S. — Karyotype (X 2100) and metaphase plate (X 1100) of *I. pseudopumila* form Montelepre. Arrows indicate satellites on couple 8.

Pig-. 4. — Karyotype (X 2100) and metaphase plate (X 1100) of *I. attica* from Cape Sounion. Arrows indicate satellites on couple 7 and 8.

Bol. Soc. Brot., Sér. 2, 53 (2): 933-942 (1981).

POTYVIRUSES RECORDED IN PORTUGAL. PURIFICATION, SEROLOGY AND HOST-VIRUS ULTRASTRUCTURAL RELATIONSHIPS (*)

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ABSTRACT

Bean common mosaic virus (BCMV), bean yellow mosaic virus (BYMV), beet mosaic virus (BMV), carnation vein mottle virus (CVMV), onion yellow dwarf virus (OYDV), potato virus Y (PVY), soybean mosaic virus (SBMV) and sugarcane mosaic virus (SCMV) have been recorded.

An improved purification method is described which preserves infectivity and prevents agrégation and fragmentation of the virions.

Serological assays revealed positive although erratic relationships among some of the viruses but never between BCMV and BMV. The several isolates of BYMV, referred to as strains, are serologically related although showing different titers.

The ultrastructural observations permited to include BCMV in sub-division I proposed by EDWAEDSON (1974) for the Potyvirus group; BYMV and BMV in sub-division II and PVY and CVMV in sub-division III although the latter with some restrictions.

The presence of filaments, interpreted as virions, contiguous to the mitochontria in broad bean cells infected with BYMV and in *Datura, metel* cells infected with PVY, suggests a role of the mitochondria in its synthesis.

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THE most common viruses infecting annual crops in Portugal belong to the Potyvirus group. Isolates have been obtained from *Solanaceae, Leguminosae, Iridaceae, Chenopodiaceae, Liliaceae, Caryo-phyllaceae* and *Gramineae.*

Identification and characterization of each virus as well as the study of some aspects of their epidemiology, was undertaken years ago with a view to obtaining the necessary information for achieving a better control of the diseases for which they are responsible. Some results have already been published (ASCENSÃO *et al.*, 1974; BORGES, 1966, 1971, 1976; BORGES & DAVID-FERREIRA, 1968; BORGES *et ca.*, 1968; FERREIRA & BORGES, 1958 ; PEDROSO, 1969 ; SANTOS, 1962 and VASCONCELOS, 1964). These studies are concerned with onion yellow dwarf virus (OYDV), soybean mosaic virus (SBMV), sugarcane mosaic virus (SCMV) and potato virus Y (PVY).

The aim of the present paper is both to report all the Potyviruses recorded in Portugal and to describe some recent results obtained with bean common mosaic virus (BCMV), bean yellow mosaic virus (BYMV), beet mosaic virus (BMV), carnation vein mottle virus (CVMV) and potato virus Y concerning purification, serological aspects and some host-virus ultrastructural relationships.

MATERIALS AND METHODS

Virus isolates. Viruses have been obtained from field plants referred as original hosts and maintained in convenient hosts in glasshouses (Table 1). The propagation species have been also used as assay species for purification, serology and electron microscopy, unless when otherwise indicated.

Virus purification. Two purification methods have been used. One based on Wetter's method (BORGES, 1966) and another devised during the present work. Only the last one will be described here (Table 2). Whenever it was possible the work was done in a cool room at a temperature of 4° C to 7° C. The low speed centrifugations were obtained

in a Martin Christ Junior II and high speed centrifugations in a Spinco-Model L.

Suspensions of the partially purified viruses were negatively stained with sodium phosphotungstate 2%, at pH 7.0 and observed under the electron microscope. The absorption spectra of these suspensions in the range 230 to 325 nm were obtained in a Unicam SP 800 ultraviolet spectrophotometer.

Serology. Antisera for BCMV, BMV, BYMV and PVY were prepared in rabbits by intramuscular injections of purified suspensions of these viruses, emulsified with Freund's incomplete adjuvant. Emulsions were injected twice with a 10-day interval and the rabbits were bled 10 days later for a first evaluation of serum titer, and then twice a month. Serum titers were estimated by precipitin tests in microscope slides (WETTER, 1965).

Electron microscopy of ultrathin sections. Leaf pieces 0.5 X 2 mm were fixed in 5 % glutaraldehyde in 0.08 M NaOH-PIPES buffer, at pH 8.0 (SALEMA & BRANDÃO, 1973) and rinsed three times in the same buffer 0.02 M. They were post-fixed in 2% OsO_4 in the last buffer at pH 6.8. Dehydration was achieved with graded acetone dilutions and propylene oxide with gentle shaking on a Labline Junior Orbit Shaker at room temperature. Epon embedding was performed at $6O^0C$ for the last 36 h. Thin sections were cut with glass knives (45°) in a LKB Ultratome III and stained with uranyl acetate and lead citrate. Observations Were made in a Philips EM 300.

rESULTS AND DISCUSSION

Virus isolates. BYMV and PVY have been the most frequent Potyviruses recorded in annual crops (Table 1). Normal strains of the latter have been isolated from pepper *(Capsicum annuum* L.), tomato *(Lycopersicon esculentum* Mill.), tobacco *(Nicotiana tabacum* L. cv. Samsun) and potatoes *(Solanum tuberosum* L.), and a necrotic strain has been isolated only once from potatoes (BORGES, 1971).

TABLE 1

Potyviruses recorded in Portugal. Original hosts.

I [1]	
Bean Common Mosaic Virus (BCMV)	*Phaseolus vulgaris L.*[3]
Onion Yellow Dwarf Virus (OYDV)[2]	*Allium cepa* L.
II	
Bean Yellow Mosaic Virus (BYMV)	*Gladiolus* spp.
Several strains	*Lathyrus ochrus* (L.) DC.
	Lupinus albus L.
	Phaseolus vulgaris L.
	Trifolium pratense L.
	T. resupinatum L.
	Vicia faba L.[3]
	V. narbonensis L.
Beet Mosaic Virus (BMV)	*Beta vulgaris* L.[3]
Some strains	
III	
Carnation Vein Mottle Virus (CVMV)	*Dianthus caryophyllus* L.[3]
Potato Virus Y (PVY)	*Capsicum annuum* L.
Normal strains	*Lycopersicon esculentum* Mill.
	Nicotiana tabacum L. *
	Solanum tuberosum L.
Necrotic strains (rare)	*Nicotiana tabacum* L.[3]
	Solanum tuberosum, L.
Soybean Mosaic Virus (SBMV)[2]	*Glycine max* (L.) Merr.[3]
Sugar Cane Mosaic Virus (SCMV) [2] (only in a botanical garden)	*Saccharum officinarum* L.

[1] EDWARDSON sub-divisions of Potyvirus group.
[2] No ultrastructural observations have been made by the authors.
[3] Also propagation and assay species.

BYMV was found in numerous crops such as beans *(Phaseolus vulgaris* L.), broad beans *(Vicia faba* L.), clovers *(Trifolium resupinatum* L., Pl. I. Fig. 1), lupines *(Lupinus albus* L.), gladioli *(Gladiolus* spp.) and others. From these hosts different strains have been isolated, giving in broad

TABLE 2

Purification procedure

Disrupt and homogenize in a blender 100 g of infected leaves in 100 ml 0.1M tris-HCl pH 8.0 + 50 ml chloroform + 50 ml carbon tetrachloride + 1 ml 2-mercaptoaethanol. Centrifuge 2500 g, 10 min	
organic solvents phase	6000 8% (w/v). Stir 40 min. Centrifuge 3000g, 10 min
Discard supernatant	PELLET: resuspend in 100 ml 0.1M tris--HCl pH 8.0. Centrifuge 3000 g, 10 min
Discard pellet	SUPERNATANT: add Triton X-100 2.5% (v/v). Stir 40 min. Centrifuge 35 000 g, 120 min
Discard supernatant	PELLET: resuspend in 10 ml 0.01M borate pH 7.8. Centrifuge 3000 g, 10 min
	SION (partially purified)

beans very similar symptoms namely vein clearing followed by a bright mosaic. They present differences in host range and in serological relationships.

BMV is present in wild beet near the coast, BMV infected plants, showing mild mosaic symptoms (Pl. I, Fig. 2) have been found during the last years in sugar beet trials in Coruche, Bolão (Coimbra) and Braga.

BCMV was detected in bean fields (P. *vulgaris* L. cv. Princess of Holand) grown from imported seed. Symptoms were very severe and seed production very low. In glass-houses, inoculated beans showed a strong stunting, leaf mosaic and distortion (Pl. II, Fig. 1).

CVMV has been recognised recently in some imported carnations. Experimental infections developed in *Dianthus harbatus* L. vein chlorosis, mosaic and dwarfing (Pl. II, Fig. 2).

Soybean mosaic virus was isolated some years ago from soybean fields (VASCONCELOS, 1964) and onion yellow dwarf virus from onion fields (PEDROSO, 1969).

Sugarcane mosaic virus was recorded only in some sugarcanes in a botanical garden (Jardim do Ultramar, Lisboa) some years ago (SANTOS, 1962).

More detailed results will be published elsewhere for each of the viruses reported. However, it should be mentioned here that thermal inactivation point, dilution end point, longevity in vitro, as well as virions morphology and size, fall into the values attributed to the Potyvirus group (HARRISON *et al.*, 1971). The transmission by *Myzus persicae* Sulz, when investigated (BMV, BYMV, OYDV and SBMV), has been accomplished in a non-persistent manner.

Virus purification. There are several references to the difficulties of purifying Potyviruses. The main reason seems to be the tendency of the virions to aggregate resulting in subsequent losses during purification procedures. Spliting or fragmentation of the virions is another reason for unsuccess.

Besides the method described by BORGES (1966), several purification procedures have been tried (SEQUEIRA, unpublished data) with isolates of BYMV, BMV and PVY in order to devise a better method of purification. To reduce oxidation, the leaves were previously frozen at - 20° C for 24 to 48 hours and a strong reducing agent — 2-mercaptoethanol — was added during extraction. The removal of membranous material was enhanced with the use of Triton X-100. The purification flow-sheet (Table 2) shows the method finally adopted. With this method aggregation and fragmentation were reduced as observed under the electron microscope (Pl. m, Fig. 1).

Spectrum curves show a maximum absorption at 260 nm and a minimum one at 242 nm. The 260/280 nm absorption

ratio ranging from 1.46 to 1.53 seems too high to viruses supposed to have 5% nucleic acid. Since this fact is probably due to the presence of some impurities, further purification is therefore needed. However the method described besides preventing aggregation and fragmentation of the virions, is in addition adequate as far as preservation of virus infectivity, morphology and concentration are concerned.

Serology. Antisera for PVY, BYMV, BCMV and BMV have been tested with homologous and heterologous antigens.

Homologous titers, given as reciprocals, frequently range from 8000 to 256 000 for pVY, from 256 to 2000 for BYMV and from 128 to 1000 for BCMv and BMV antisera. Heterologous relationships were very weak and erratic. Positive reactions of BMV antiserum against BCMV and of BCMV antiserum against BMV were never observed.

Isolates tentatively referred to as BYMV strains were all serologically related but to differing degrees.

Host-virus ultrastructural relationships. According to EWARDSON (1974) «an additional criterion for placing viruses in the Potyvirus group is the ability to induce cylindrical inclusions in the cytoplasm of the host». The same author proposed three sub-divisions to the group, based on inclusions morphology.

In 1958, FERREIRA & BORGES presented this type of inclusions by the first time. Later, BORGES & DAVID-FERREIRA (1968) recognized the specificity of such inclusions as EDWARDSON (1966) had pointed out. In fact they observed pinwheels, bundles, scrolls, tubes and laminated aggregates (Pl. VI, Fig. 1) in cells of *Datura metel* L. infected with PVY and never in the ceils of the same host infected with potato virus X. Similar inclusions have been detected also in tobacco cells (ASCENSÃO *et al*, 1974) with the same virus. The inclusions observed are similar to those described by EDWARDSON to sub-division III. Although this author regards CVMV as also belonging to the same sub-division, *Dianthus barbatus* cells show inclusions (Pl. VI, Fig. 2) not as evident

as those observed in cells of *Datura metel* with PVY. The results of WEINTRATJB & RAGETLI (1970) do not elucidate this point either. Therefore the information so far obtained seems to permit only to assign CVMV to sub-division III with some reserves.

As far as BCMV, BMV and BYMV are concerned the results are more conclusive. Thin sections of beans (P. *vulgaris* L. cv. Princess of Holland) infected with BCMV show pinwheels and scrolls typical of sub-division I and no laminated aggregates (Pl. IV, Fig. 1). BMV and BYMV both ascribed to sub-division II induce very similar inclusions in beet and broad bean cells respectively (Pl. IV, Fig. 2; Pl. V, Fig. 1 and 2). These pinwheel inclusions are distinct from those present in group I and III, since pinwheel arms are less curved and longer. Laminated aggregates are often connected with pinwheel arms. Isolates of BYMV give similar inclusions in broad beans perhaps with differences in details. A study to elucidate this point is in progress.

From the ultrastructural aspects of PVY and BYMV the accumulation of filaments, interpreted as virions, near the mitochondria (Pl. III, Fig. 2; Pl. VT, Fig. 1), must be emphasized. Similar observations were described by BORGES & DAVID-FERREIRA (1968) and ANTUNES *et al.* (1974). This was also reported by KITAJIMA & LOVISOLO (1972) in *Datura stramonium* L. infected with henbane mosaic virus.

The different types of cylindrical inclusions observed support that all of them result from lamellar inclusions either isolated or assembled in different ways. The laminated agregates are obtained when the main surfaces are in contact and the pinwheels when the lamellar inclusions are attached by the edges to a common cylindrical axis. These lamellar inclusions are flat or curved sometimes scrolled or forming tubes, In transversal sections they are seen as pinwheels, scrolls and rings, while in longitudinal sections they appear as bundles or tubes.

Although, as WEINTRATJB *et al.* (1973) pointed out, the presence of these inclusions is not enough to ascribe a new-virus to the Potyvirus group, it is doubtless a useful

additional criterion for classification as proposed by EDWARDSON (1974).

As stressed by HARRISON *et al.* (1971) virus classification should be based on many characters to permit an arrangement of viruses in main groups. As far as Potyvirus group is concerned, where a large number of members could be included, a sub-division could be useful. EDWARDSON'S proposal should be considered as a working hypothesis and needs to be complemented with further data on serology and amino-acid and nucleotide composition. Extreme caution however, should be taken in the interpretation of serological results as pointed out by MOGHAL & FRANCKI (1976).

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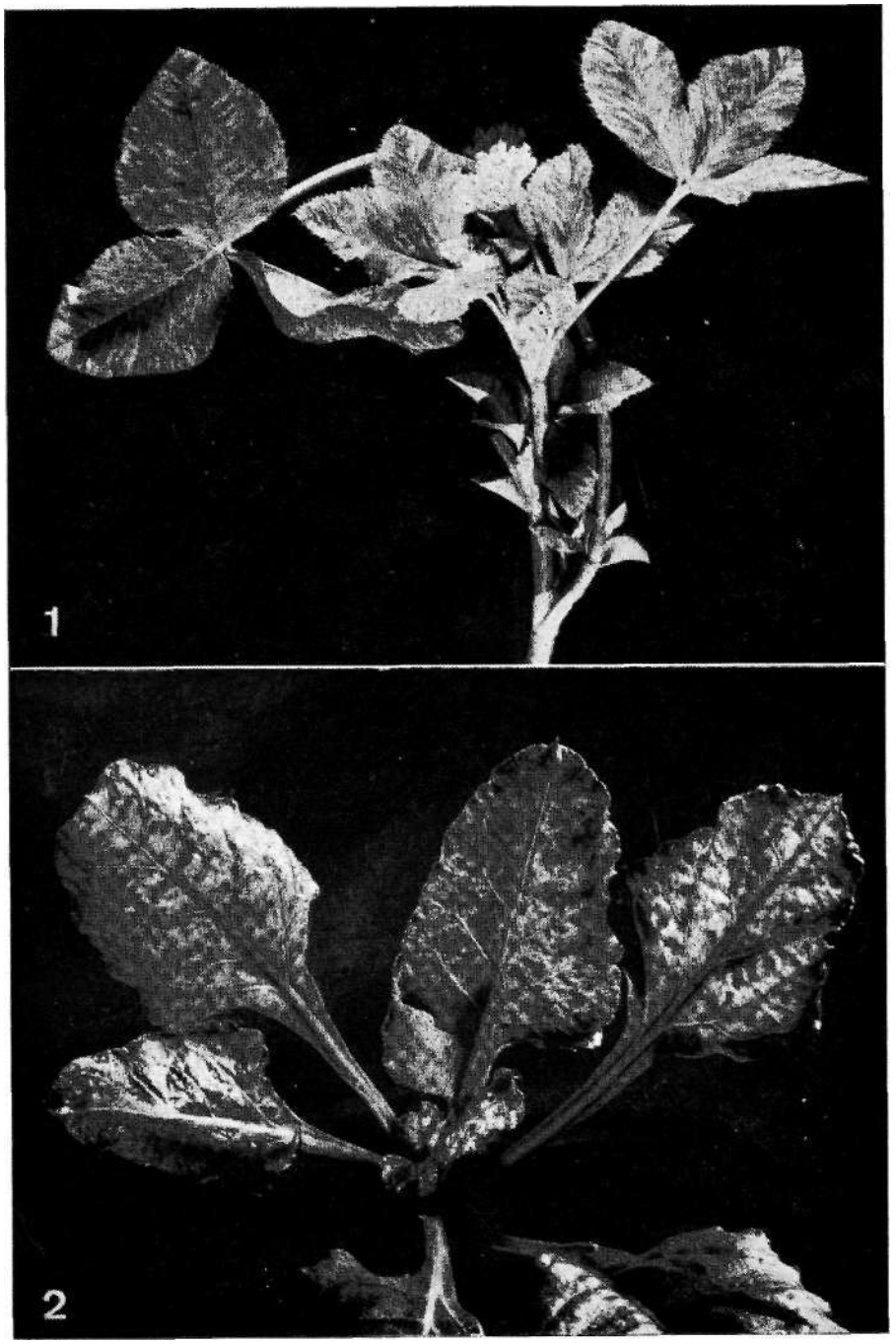

Fig. 1. — *Trifolium resupinatum* L. infected with bean yellow mosaic virus (BYMV).
Fig. 2. — *Beta vulgaris* L. infected with beet mosaic virus (BMV).

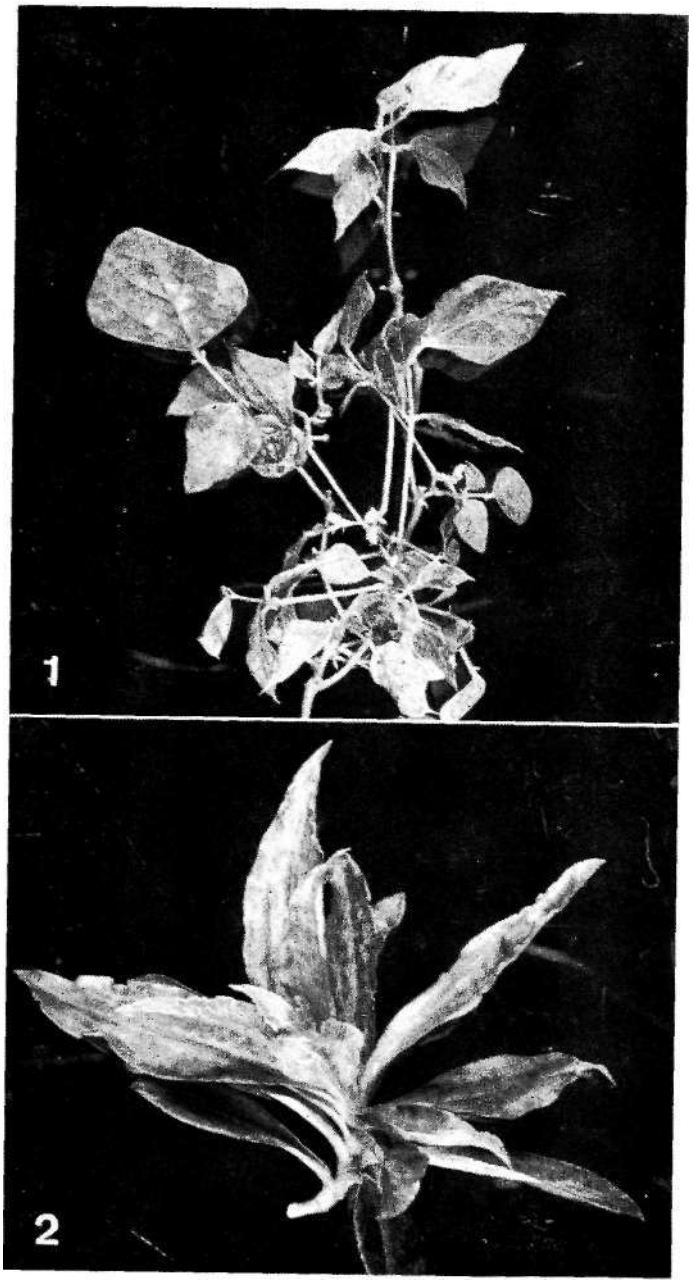

Fig. 1. — *Phaseolus vulgaris* L. infected with bean common mosaic virus (BCMV).

Fig. 2. — *Dianthus barbatus* L. infected with carnation vein mottle virus (CVMV).

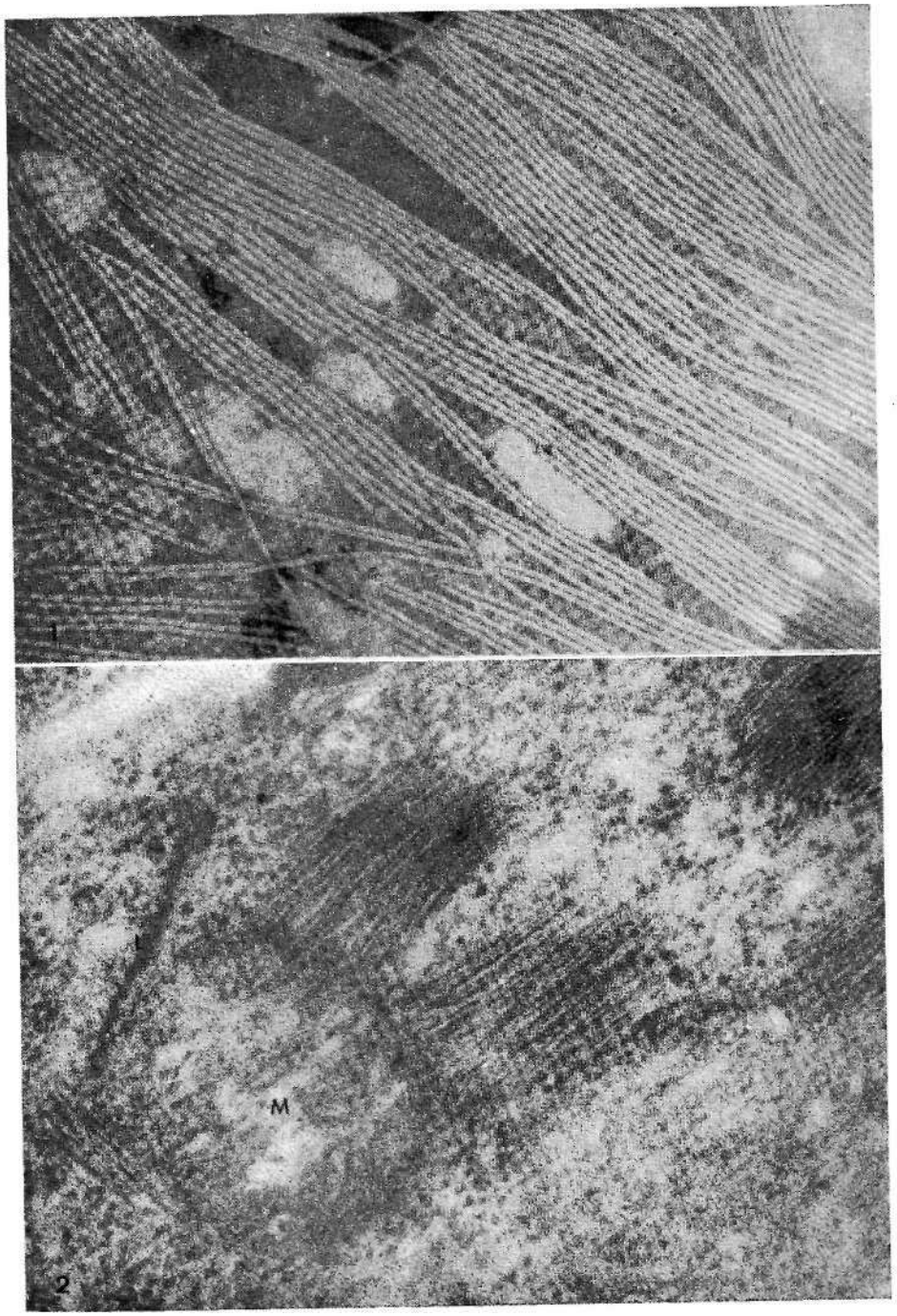

Fig. 1. — Negative stained preparation of purified BYMV X 80 000.
Fig. 2. — Mitochondria (M), a laminated aggregate (L) and filaments interpreted as BYMV, in a cell of *Vicia faba* L. X 80 000.

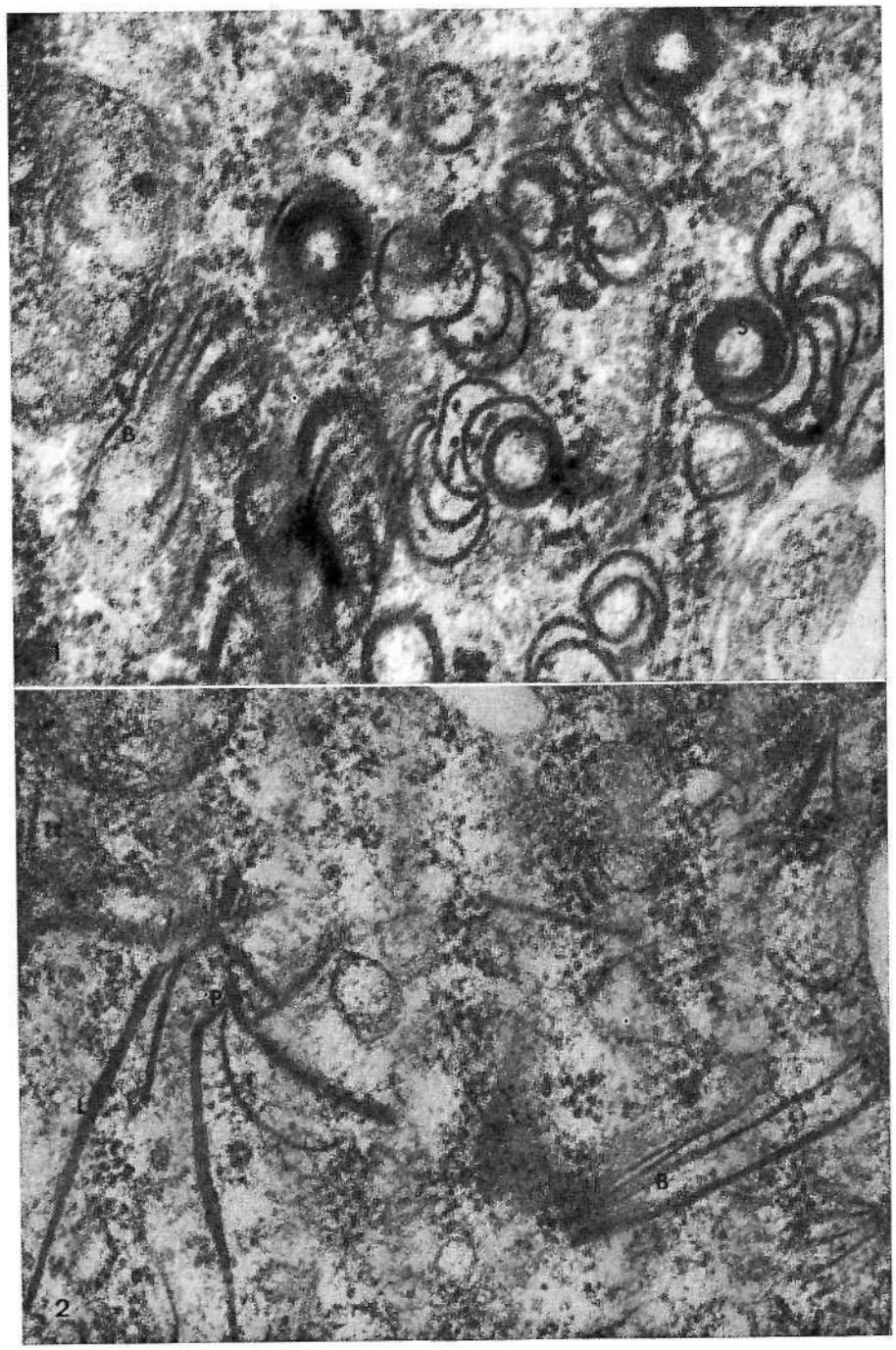

Fig. 1. — Pinwheels (P), scrolls (S) and a bundle (B) in a cell of *Phaseolus vulgaris* L. infected with BCMV. X 80 000. Sub-division I.

Fig. 2. — Pinwheels (P), laminated aggregates (L) and a bundle (B) in a cell of *Beta vulgaris* L. infected with BMV. X 60 000. Sub-division II.

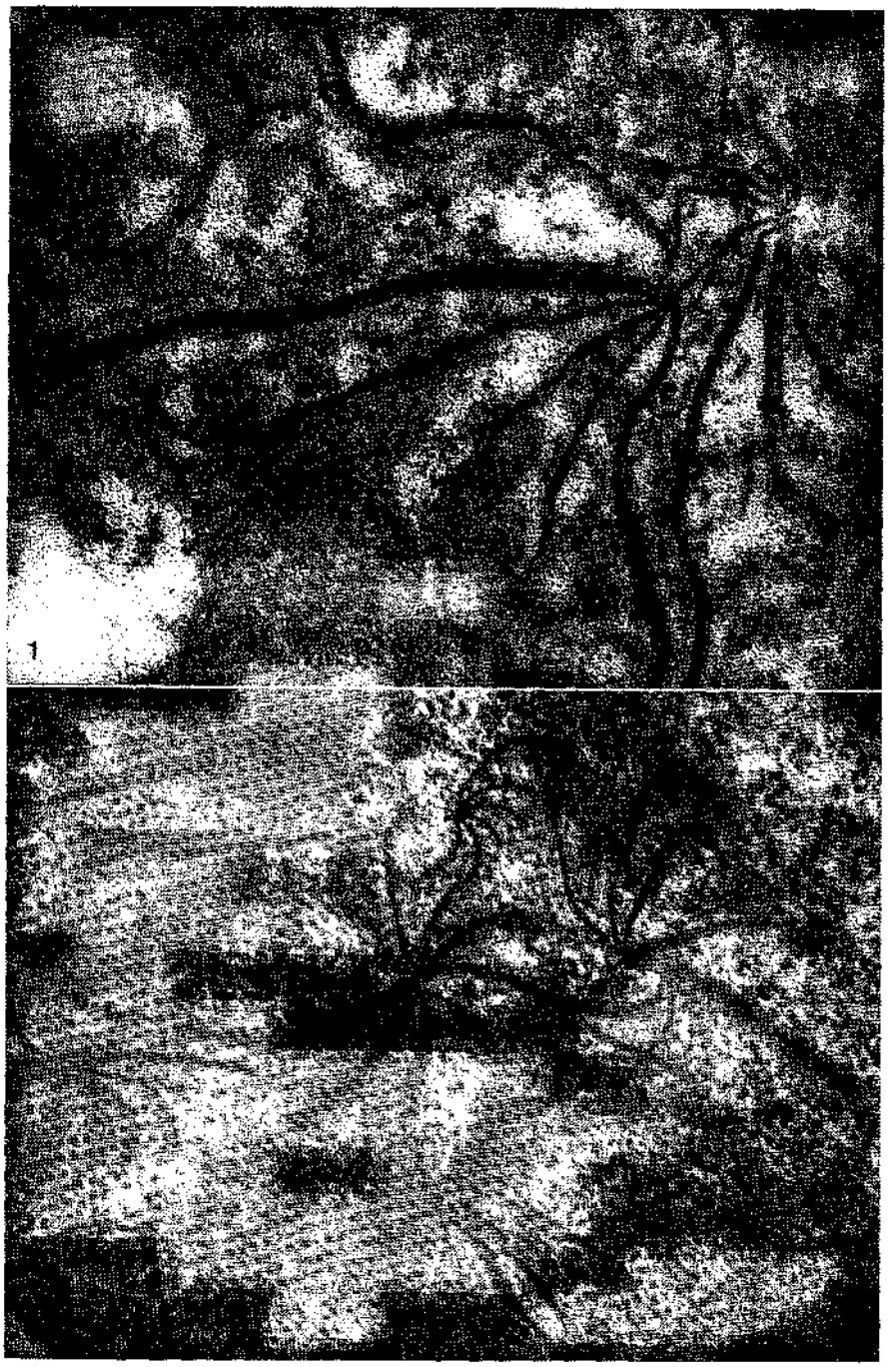

Fig. 1. — Pinwheels (P) and laminated aggregates (L) in a cell of *Vicia faba* L. infected with BYMV. *Trifolium resupinaturn* isolate. X 80 000. Sub-division II.

Fig. 2. — Pinwheels (P) and laminated aggregates (L) in a cell of *Vicia faba* L. infected with BYMV. *Vicia faba* isolate. X 60 000. Sub-division II.

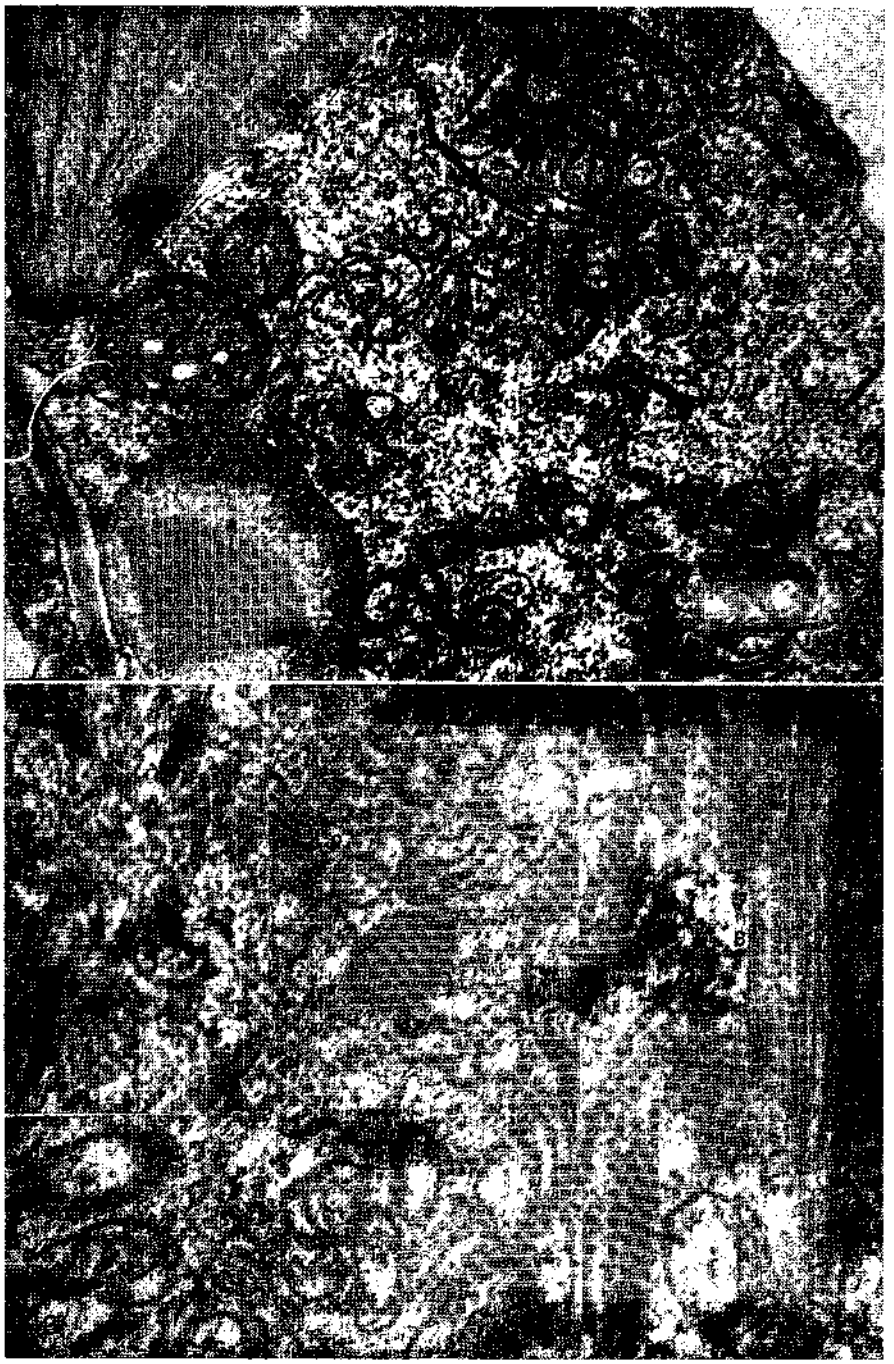

Fig. 1. — Pinwheels (P), scrolls (S) and laminated aggregates (L) in a cell of *Datura metei* L. infected with PVY. Filaments surrounding mitochondria. X 30 000. Sub-division III.

Fig. 2. — Pinweels (P), rings (R), bundle (B) and laminated aggregate (L) in a cell of *Dianthus barbatus* L. infected with CVMV. X 80 000. Sub-division III.

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THE ENDEMIC PLANTS OF METROPOLITAN PORTUGAL, A SURVEY

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THE Iberian peninsula as a whole is very rich in endemic plant species. Historically important contributing factors were the favourable climatic conditions during the last ice age, which allowed the area to act as a refugium for many species during that time, and the fact that in tertiary times it was the only large semi-arid land mass in Europe. This land mass was isolated to a large extent by the northern mountains, and this allowed the development of xerophytic and semi-xerophytic endemic taxa to take place.

In Portugal this development is very noticeable in the centre and south, where the light sandy soils are clothed in all the stages of mediterranean-atlantic vegetation, a good proportion of which contains either lusitanian or ibero-lusitanian endemics. This alone commends Portugal as an area of great interest for the study of endemics and their allies. However, no comprehensive work has been done in this field as yet, although the Council of Europe (1977) has published a list of names of rare, threatened, and endemic species. The foregoing highlights the fact that there is a lack of detailed information on the taxonomy, distribution, ecology, population biology, and cytology, of all the taxa concerned.

In this publication an attempt has been made to gather all the available information appertaining to the endemics of Portugal, and where possible lay the foundations for further studies. The work is largely based on Flora Europaea,

a publication which it is hoped will provide a basis for further cytotaxonomic and ecological research, and eventually the production of new detailed national floras. In Portugal, that commenced by FRANCO (1971) is unfinished, and the works of COUTINHO (1939) and SAMPAIO (1947) have been used as national sources.

This compendium contains only those endemics confined within the boundaries of mainland Portugal. The nomenclature used is that given in Flora Europaea. Where the source is other than this, the taxa are asterisked. Endemics are included to the sub-specific level, although wherever possible it is considered advisable to treat them as species, because taxa at lower ranks are neglected by some botanists, and existing differences remain concealed. Chromosome counts of a number of endemics are contained in both the recent and continuing series of cytological studies of material collected from natural Portuguese habitats (A. FERNANDES 1969-; and QUEIRÓS 1978-.). These excellent sources are used wherever possible. With regard to the endemic taxa, no particular cytological study has been made of the group, with the result that at the present time, only 55% of those listed here have been counted.

Taxonomic, and also distributional information on the Portuguese flora, including endemics, is often amplified in several recent series such as 'De Flora Lusitanica commentarii' (ed. P. SILVA, 1939-), 'Notas sobre a Flora de Portugal' (R. FERNANDES 1949-), and 'Notas de Florística' (MALATO-BELIZ 1951-) ; these in particular, have been a source of useful information. It is in the field of ecology that least appears to be known. This lack of information applies to most taxa, although the annuals as a group are particularly affected. The only detailled records which include Portuguese endemics are found in the series of papers by BRAUN-BLANQUET and his co-workers (1952, 1956, 1964, 1972), and those of P. SILVA (1952, 1958, 1968). Apart from these studies, and a few. others, little information is available regarding habitat and companion species. For this reason, few of the one hundred and twenty-seven endemic taxa considered here have any detailed ecological information.

Details of each taxon are set out in a regular format as follows. Name; life-cycle and form; chromosome number; distribution; habitat. Other relevant information. Where nothing is known about a particular aspect of a taxon, a dash is inserted in the appropriate place.

GYMNOSPERMAE

CUPRESSACEAE

Juníperas oxycedras L. Sp. Pl. 1038 (1753).

Perennial, woody phanerophyte; —; southern Europe; maritime sands, rocky hills, dry mountainous tracts.

Subdivided into three subspecies, two throughout the range, one endemic to Portugal.

Subspecies *transtagana* Franco, Feddes Repert. 68: 166 (1963).

Atlantic coast of south-west (south of the river Tejo); maritime sands.

ANGIOSPERMAE

CARYOPHYLLACEAE

Heniaria maritima Link in Schrader, Jour, für die Bot. 1: 57 (1799).

Perennial, hemicryptophyte; 2n = 108, 126 (BLACKBURN & MORTON 1957); south west and central west coasts, Minho, Beira, Estremadura, Alentejo; maritime sand-dunes. Recorded as present very rarely in the association *Stauracantho-Coremetum* Br. Bl., P. SILVA & ROZEIRA, on the dunes near the lighthouse at Sines (Br. Bl. *et aï.* 1964).

*Herniaria algarvica Chaudri, Med. Bot. Mus. Herb. Rykes, Utrecht No. 285: 346 (1968).

Annual, therophyte; —; south West; —.

*Hemiaria berlengiana (Chaudri) Franco, FI. Port. 1: 130 (1971).

Perennial, hemicryptophyte; —; Berlengas Isles; rocks and cliffs.

Very little is known about the above three species so far.

Loeflingia tavaresiana Samp, in Nobre, Anais Ci. Nat. (Porto) 10: 25 (1906).

Annual, therophyte; —; south, known from Vila Nova de Milfontes and Faro in the Algarve, also from the Alentejo coast; maritime sands and dune systems.

Silène cintrana Rothm. Bol. Soc. Brot. sér. 2, 13: 275 (1939).

Perennial, hemicryptophyte; —; Serra de Sintra only, apparently found mainly between 100 and 500 m. a. s. 1.; Crevices in granitic rocks. Apparently a calcifuge species, occurring on granitic soils only.

There is little known about this species apart from the fact that it appears to have affinities with *S. itálica* (L.) Pers. (CHATER & WALTERS 1964).

Silène rothmaleri P. Silva, Agron. Lusit. 9: 18 (1956).

Perennial, hemicryptophyte; —; south west (Sagres promontory) ; maritime rocks and calcareous maritime sands.

Both *S. cintrana* and *S. rothmaleri* appear to have affinities with S. *itálica* (L.) Pers., which is widespread in Europe. The common variant of *S. itálica* in Portugal is subsp. *coutirihoi* (Rothm. & P. Silva) Franco, and the two endemics could easily have evolved from this taxon. The chromosome number of *S. itálica* is 2n = 24 (FAVARGER 1946) ; this number does not appear to vary throughout the range. It is probable that both the Portuguese taxa are schizoendemics.

Silène elegans Link ex Brot. Fl. Lusit. 2: 185 (1804).

Perennial, hemicryptophyte; —; Serra da Estrela; rock crevices and rough grasslands above 1700 m. Not recorded by BRAUN-BLANQUET *et al.* (1952).

Allied to the very variable *S. ciliata* Pourret (CHATER & WALTERS L. *c*), *S. elegans* is on the whole smaller in all its parts and has only 1-2 flowers. The more widespread species, which occurs in the mountains of southern Europe, has a polyploid series of chromosome numbers ranging from 2n = 24 to 2n = c. 120 (KUPFER 1971, FAVARGER 1946, PEDROTTI & PEDROTTI 1971).

Silène bergiana Lindman, Acta Horti Berg. 1 (6) ; 3 (1891).

Annual, therophyte; —; south east and centre; corn-fields, vineyards, and fallow ground.

This species appears to be taxonomically related to *S. rubella* ssp. *rubella* L., which has the chromosome number 2n = 24 (FERNANDES & LEITÃO 1971). There is as yet no chromosome count for *S. bergiana,* but it may well be a schizoendemic.

*Dianthus marizii (Samp.) Samp. BoI. Soc. Brot. sér. 2, 1: 134 (1922).

Perennial, woody chamaephyte; 2n = 30 (FERNANDES & LEITÃO 1971); north east, Bragança region; crevices of serpentine rocks. Recorded by P. SILVA (1968) in the association *Genisto-Quercetum rotundifoliae* P. Silva, variant *serpentinicola,* between 600 and 800 m.a.s.l, and in the association *Cisto-Genistetum Hystricus* P. Silva, variant *serpentinicum.* The most common associated taxa on these serpentine rocks are *Alyssum serpyllifoliuni* Desf. ssp. *lusitanicum* Dudley & Silva, *Seseli peixotanum* Samp., *Santolina semidentata* Hoffmanns. & Lk., *Plantago radicata* Hoffmanns. & Lk. var. *radicata* P. Silva, *Jasione crispa* (Pourret) Samp, ssp. *serpentinica* P. Silva, *Dactylis glomerata* L. var. *microstachya* Webb.

There is a strong similarity between this species and *Dianthus laricifolius* Boiss. et Reuter, and it is not always considered specifically distinct. It is not listed in Flora Europaea, but given specific rank by FRANCO (1971). Experimental studies are needed to clarify the situation.

RANUNCUiACEAE

Ranunculus **henriquesii** Freyn., Bot. Centralb. 6 (3) : 21 (1881).

Perennial, hemicryptophyte ; —; northern areas, Minho, Trás-os-Montes, Douro Litoral, and Beira; roadside verges, grassy places.

A close relative of *R. gregarius* Brot., from Which it differs by narrow acute villous distant leaf-lobes, and densely pubescent receptacles (TUTIN 1964). Until a reappraisal of the nature of the variability within *R. gregarius* has been undertaken, it cannot be finally decided whether *R. henriquesü* is an endemic species.

PAPAVERACEAE

Corydalis claviculata (L.) D.C. in Lam. & D.C., Fl. Fr. ed. 3, 4: 638 (1804).

Annual, tendrilled climber, therophyte; 2n=32 (GADELLA & KLIPHUIS 1966); western Europe; hedges, fields, woods.

Two subspecies, one widespread, the other endemic to Portugal.

Ssp. *picta* (Samp.) P. Silva & Franco, Feddes Repert. 69: 56 (1964).

Northern areas, Beira, Minho, and Trás-os-Montes.

CRUCIFERAE

Murbeckiella sousae Rothm., Bot. Not. 1939: 474 (1939).

Perennial, hemicryptophyte; —; central and northern areas (Serra da Lousã, Serra da Freita, Serra do Marão) ; rocky areas.

Isatis platyloba Link ex Steudel, Nomencl. Bot. 1: 440 (1821).

Annual, therophyte; —; region of the upper Douro; crevices in granitic cliffs, and granitic uplands e. 650 m.a.s.l.

This species is apparently quite distinct, and confined to the NB of Portugal.

Erysimum linifolium (Pers.) Gay, Erysim. Nov. 3 (1842).

Perennial, chamaephyte; 2n = 14 (QUEIRÓS, 1973); Spain and Portugal; dry places and fissures in rocks.

An Iberian endemic divided into four subspecies, one of which is confined to Portugal.

*Subspecies *filifolium* cout., Fl. Port. 303 (1939).

Eastern districts, Beira.

BALL (1964) recognised the existence of this taxon but not give it formal status. The chromosome count is for ssp. li*nifolium.*

Malcolmia lacera D.C. Reg. Veg. Syst. Nat. *2:* 445 (1821).

Annual therophyte, biennial or perennial chamaephyte; —; Spain and Portugal; dry areas.

Two subspecies, one confined to Portugal (FRANCO 1971).

*Subspecies *gracilima* (Samp.) Franco Nov. Fl. Port. 1: 207 (1971).

Perennial, herbaceous chamaephyte; —; south-west, Estremadura, lower Alentejo, Algarve; heathlands and other dry areas. The species has been recorded as an occasional in the association *Cistetum Bourgeani* Rothm. between Faro and Armação da Arábia (BRAUN-BLANQUET et al. 1964). Ssp. *gracilima* is the only entirely perennial subspecies in the species.

Arabis lusitanica Boiss., Diagn. Pl. Or. Nov. 3 (1) : 20 (1853).

Biennial, hemicryptophyte; 2n = 16 (QUEIRÓS 1973); western-central areas, Beira Litoral (Coimbra), Estremadura, Alentejo; dry calcareous slopes, rocks, and walls. Recorded

by BRAUN-BLANQUET *et al.* (1956) on north facing slope at 220 m.a.s.l, growing in a brown forest soil (pH 6.5-7) overlying basalt, in degraded forest at Caneças near Lisbon. The species occurred in the association *Arisareto-Quercetum faginae* B.-Bl., P. Silva & Rozeira, sub-association *Vincetosum,* growing with the characteristic spp. of this association *Quercus faginea* Lam., *Vinca difformis* Pourr., *Aristolochia longa* L., *Genista tournefortii* Spach., and also other woodland species.

The endemic is taxonomically related to *A. sagittata* (Bertol.) D.C. (JONES 1964), with which it shares the same chromosome number, and most of its morphological characteristics. The relationships between these two ssp. need clarification.

Arabis sadina (Samp.) Cout, Fl. Port. 253 (1913).

Short lived perennial, hemicryptophyte; 2n = 32 (Trrz 1976); central western and southern areas (Serra da Arrábida, Serra de Montejunto, Serras de Aire, Mira, and Minde); dry calcareous slopes and calcareous dunes.

Related to the widespread and polymorphic *A. hirsuta* (L.) Scop, sens Str. (JONES L. *C),* a species complex which has a large number of genetically distinct variants with different chromosome numbers, usually 2n = 16 or 2n = 32 (CZAPIK & NOVOTNA 1969).

Arabis juressii Rothm., Agron. Lusit. 2: 79 (1940).

Perennial, hemicryptophyte; —; northern areas (Serra do Gerês); —.

The relationships of this species are not clear. JONES *(l. c.)* has suggested that it may be allied to *A. aïlionii* D.C., or perhaps to *A. muralis* Bertol. Both field work and cytotaxonomic studies are needed to clarify the situation.

Alyssum serpyllifolium Desf., Fl. Atl. 2: 70 (1798).

Perennial, herbaceous to slightly woody chamaephyte; 2n = 32 (PuECH 1968); south-western Europe; —.

A very variable species, presently divided into two subspecies (BALL, & DUDLEY 1964), one general, the other endemic to Portugal.

*Subspecies *lusitanicum* Dudley and P. da Silva, Agron. Lusit. 28: 72 (1967); 2n = 16 (QUEIRÓS 1973); north east, confined to the area round Bragança; uncultivated ground, and on thin soils overlying serpentine rocks.

Tolerant of high nickel levels, and often found growing with *Taeniatherium caput-medusae* (L.) Nevski ssp. *crinitum* (Schreb.) P. Silva in the serpentine association *Taeniatherion-Alyssetum lusitanica* P. Silva, between 500 m. and 850 m.a.s.l. This association is very variable, the most constant associates are the two species given above, however varying proportions of *Dianthus marizii* (Samp.) Samp., *Seseli peixotanum* Samp., *Plantago radicata* Hoffmanns. & Lk., var. *radicata, Santolina semidentata* Hoffmanns. & Lk., and *Jasione crispa* (Pourret) Samp., subsp. *serpentinica* P. Silva, are found. These often make up the major part of the other vegetation present (P. SILVA 1968). Subspecies *lusitanica* also ocurs in other serpentine associations (DUDLEY 1967).

Two chromosome counts are available for *Alyssum serpyllifolium* from outside Portugal, both of 2n = 32 (PUECH 1968). It is interesting that the Portuguese endemic is a diploid. Further cytological and genecological studies of Iberian populations would be of value.

Ionopsidium acaule (Desf.) Reichenb., Pl. Crit. 7: 26, t. 649 (1829).

Annual, therophyte; 2n = 24 (CHIARUGI 1928); south-west and central areas; on mainly calcareous dry and sandy soils.

Very little appears to be known about this annual.

Iberis procumbens Lange, Ind. Sem. Horto. Haun. 1861: 29 (1861).

Perennial, herbaceous chamaephyte; —; north-west Spain and Portugal; coastal areas and sea shores.

This Iberian endemic has two subspecies, one throughout the range, the other confined to Portugal.

Subspecies *microcarpa* Franco & P. Silva, Feddes Repert. 68: 195 (1983). 2n = 14 (QUEIRÓS 1973) ; western areas, from Cabo Mondego to Serra da Arrábida; calcareous slopes near the sea.

This subspecies appears to be ecologically distinct from the seashore ssp. *procumbens* (Lange) P. Silva & Franco, but further study is needed here.

Iberis sampaiana Franco & P. Silva, Feddes Repert. 68: 195 (1963)".

Annual, therophyte; —; south-west coast, lower Alentejo; sandy and red calcareous loamy maritime soils.

Very little is known about this annual.

Biscutella vincentina (Samp.) Rothm. ex Guinea, Feddes Repert. 69: 148 (1964).

Perennial, hemicryptophyte with woody rhizomes; —; south-west (Cape St. Vincent) ; Mainly sandy areas on higher parts of the coast. Recorded by BRAUN-BLANQUET, *et al.* (1964), at Sagres, near the road to the fort on thin, sandy, pebbly soil (pH 7.0) overlying the rock. The species occurred in the association *Junipero-Cistetum Palhinhae* (Rothm.) Br.-BL, P. Silva & Rozeira, sub-association *Ulicetosum erinacei.* The most common accompanying species are *Cistus pálinhae* Ingram, *Armería pungens* Hoffmanns. & Lk., *Teucrium vicentinum* Rouy, *Thymus camphoratus* Hoffmanns. & Lk., *Juniperus phoenicea* L., *Astragalus massiliensis* Lam., and *Ulex argenteus* ssp. *erinaceus* (WeIw. ex Webb) Webb.

Diplotaxis vicentina (Cout.) Rothm., Agron. Lusit. 2: 84 (1940).

Annual, therophyte, or sometimes biennial hemicryptophyte; 2n = 20 (FERNANDES & QUEIRÓS 19716; south-west (Cape St. Vincent) ; sand dunes overlying rocks and cliffs.

Sometimes allied to *D. catholica* (L.) D.C. (HEYWOOD 1964). However the chromosome number is different (2n=18,

QUEIRÓS 1973), and the life form and morphology are also somewhat different.

Ehyncosinapis pseuderucastrum (Brot.) Franco, Anais. Inst. Sup. Agron. (Lisboa) 22: 172 (1959).

Short-lived perennial (occasionally annual), chamaephyte; 2n = 48 (HARBERD 1972, QUEIRÓS 1973); north-west Spain, and central and northern Portugal; hilly and mountainous areas.

This Iberian species is divided into three subspecies, one endemic to Portugal.

Subspecies *cintrana* (Cout.) Franco & P. Silva, Feddes Repert 68: 197 (1963).

West-central areas (Serra de Sintra) ; uncultivated land, on shallow and stony soils. This subspecies is the most southerly distributed of the three, and little appears to be known about it.

Rhyncosinapis johnstonii (Samp.) Heywood, Feddes Repert. 68: 196 (1963).

Perennial, woody hemicryptophyte ; 2n = 48 (HARBERD 1972, QUEIRÓS 1973); north-west litoral; maritime sands, occurring on the stabilised dunes in one of the three dune associations found on the coasts of N. W. Portugal (BRAUN-BLANQUET *et al.* 1972). This association is the *Scrophulario-Vulpietum* Br.-Bl, Rozeira & P. Silva, characterised by *Vulpia alopecuros* (Schousb.) Dumort., *Corynephorus canescens* (L.) Beauv., *Jasione lusitanica* A.D.C., *Hemiaria ciliolata* Meld., c*arex arenaria,* L., *Scrophularia frutescens* L., *Helychrysum angustifolium* (Lam.) D.C., and *Seseli tortuosum* ssp. *ramosissimum,* (Lk.) Alte. Occasionally the species is found elsewhere in the dune system, but this appears to be an uncommon occurence.

The chromosome count by HARBERD *(I. C.)* was made from plants grown from a seed collection from wild Portuguese plants. This wild material is tetraploid, but HARBERD also reports a diploid count from a Botanic Garden collection.

The count by QUEIRÓS *(l. c.)* is also tetraploid. The fact that two chromosome numbers have been reported make it necessary for further cytotaxonomic studies to be undertaken. This species is well isolated geographically, and uniform and distinctive with regard to its specific characteristics. Apart from the preliminary ecological information and the chromosome numbers however, little is known of the species.

Rhyncosinapis hispida (Cav.) Heywood, Feddes Repert. 66: 154 (1962).

Annual, therophyte; 2n W24 (HARBERD Z. c.); Spain and Portugal; sandy rocky places. Two designated subspecies, one endemic to Portugal.

Subsp. *transtagana* (Coutinho) Heywood, Feddes Repert. 66: 196 (1963). South-east, Alentejo; rocky places.

This subspecies is confined to the southern margin of the distribution range of the species as a whole, and there is little published information on it. The chromosome number for the species was obtained from a seed collection from wild Spanish plants. No location or subspecific category was given.

CRASSULACEAE

Sedum pruinatum Link ex Brot., Fl. Lusit. 2: 209 (1804).

Perennial, herbaceous chamaephyte; —; north and central mountains, Minho, Trás-os-Montes, upper Beira, also Alentejo and Algarve; dry rocky or stony ground.

Flowers and inflorescences of this species are very similar to those of *S. tenuifolium* (Sibth. & Sm.) Strobl (WEBB 1964), but no comparative experimental studies have been undertaken.

Sedum wilkommianum R. Fernandes, Bol. Soc. Brot. sér. 2, 34: 121 (1960).

Annual, therophyte; —; north (Serras de Montesinho, Lapa, Estrela, and Gardunha); gravelly places.

This species is taxonomically closely allied to *S. pedicellatum* Boiss. & Reuter, which is endemic to the Iberian peninsula (WEBB L. C). Biosystematic studies are needed to show how distinct the two species are.

SAXIFRAGACEAE

Saxífraga cintrana Kuzinsky ex WiIIk., Österr. Bot. Zeitschr. 39: 318 (1889).

Perennial, herbaceous cryptophyte; —; north and west of Lisbon, from Cintra to Bombarral; calcicóle, crevices in limestone rocks, and walls.

No other information available on this geographically very restricted species.

ROSACEAE

Prunus spinosa L. Sp. Pl. 475 (1753).

Perennial, phanerophyte; 2n = 32 (DARLINGTON 1930); Europe; woodland and scrub.

One subspecies endemic to Portugal.

Ssp. *institoides* (Fic. & Cout.) Franco, Nova Fl. Port. 1: 294 (1971).

West-central areas; woodland and scrub on calcareous soils.

LEGUMINOSAE

Anthyïlis lusitanica Cullen & P. Silva, Agron. Lusit. 30: 205 (1968).

Annual; 2n=12 (FERNANDES & QUEIRÓS 1978); Trás-os--Montes and Alto Douro; found between 350 and 810 m.a.s.l. in various open habitats and on several soil types (CULLEN & P. SILVA L. *c.).*

Very little is known about this recently described species.

Ulex densus WeIw & Webb, Ann. sci. Nat. sér. 3 (Bot.) 17: 291 (1852).

Perennial, phanerophyte; 2n = 64 (FERNANDES, SANTOS & QUEIRÓS 1977); west and southern central areas, from Lisbon to Aveiro; heathlands on dry calcareous soils.

Little is known about this species.

Ulex argenteus Welw. ex Webb, Ann. sci. Nat. sér. 3 (Bot.) 17: 291 (1852).

Perennial, phanerophyte; 2n = 64 (CASTRO 1941); 2n=96 (FERNANDES & QUEIRÓS 1978); southern areas; woodland and open scrub areas.

The endemic species here defined is rather variable, and is usually split into the three subspecies given below. Many plants are however difficult to assign to any of these categories (GUINEA & WEBB 1968).

Subspecies *argenteus* Algarve and S. W. Alentejo; xerophytic scrub.

The subspecies is found mainly in its own association, the *Cisto-ulicetum argentei* Br.-Bl, Silva and Rozeira, which has as its characteristic species *Ulex argenteus* ssp. *argenteus, Thymelaea villosa* (L.) Endl., and *Genista polyanthos* R. de Roemer, within the alliance *Ulicino cistion* (BRAUN-BLANQUET *et al.* 1964). The association occupies land up to 400 m.a.s.l., forming a light cover. The schistose rock on which it is usually found, bears a stony, reddish soil, which is neutral or slightly acid.

Subspecies *subsericeus* (Cout.) Rothm., Bot. Jahrb. 72: 96 (1941).

On the south coast, from Faro to the Rio Guadiana, most common around Faro; sandy soils near the sea.

No further ecological information available.

Subspecies *erinaceus* (Welw. ex Webb) D. A. Webb, Feddes Repert 74: 6 (1967).

2n = 32 (PIQUERAS & REJÓN 1976); south-west, occurring in one locality only in S. E. Spain; rocky headlands.

This subspecies has been recorded by BRAUN-BLANQUET *et al.* (1964) in the association *Junipero-Cistetum Palhinhae*

(Rothm.) Br.-Bl, P. Silva & Rozeira (as *Ulex erinaceus* WeIw.). It is the major constituent, along with *Astragalus massiliensis* Lam. of the sub-association *Ulicetosum erinacei* at Sagres near the road to the fort, and between Sagres & São Vicente. The plants were growing on thin, neutral, sandy soils overlying the rock platform, in both situations.

These subspecies form an interesting group that would reward further biosystematic study. Cytological work has shown that plants of 4x, 8x, and 12x chromosome levels exist. It would be very interesting to find out whether these were regularly distributed with regard to geography and ecology. The information presently available on the range of natural morphological variation which occurs, is not adequate to distinguish between the alternatives of intersub-specific hybridisation, or the presence of large amounts of ecotypic variation. The ecology of the species as a whole has not been thoroughly studied as yet. BRAUN-BLANQUET *et al.* (1984) recorded it without sub-specific designation in both the *Cistetum Bourgeani* Rothm. and the *Arbuto-cistetum populifolii* Br.-Bl., p. Silva & Rozeira. A careful study on similar lines to that of PROCTOR (1965) on British *Ulex* would be most illuminating.

EUPHORBIACEAE

Euphorbia monchiquensis Franco and P. Silva, Feddes Repert. "79: 56 (1968).

Perennial, phanerophyte; —; south west, Alentejo and Algarve; along the edges of water-courses.

Similar to *E. welwitschii* Boiss. & Reuter (SMITH & TUTIN 1968) with which it is sympatric. There is little information on either of these species.

Euphorbia transtagana Boiss., Diagn. Pl. Or. Nov. 3 (4): 88 (1859).

Perennial, herbaceous chamaephyte; —; central and southern areas, in the range of *Quercus suber* L.; heathlands and scrub.

Commonly found in the association *Erico-Quercetum lusitanicae* (Rothm.) Br.-Bl., P. Silva and Rozeira (BRATJN-BLANQUET *et al.* 1964). The dominant species is *Quercus lusitanica* Lam., with *Carex oedipostyle* D. Jouv., *Serratula pinnatifida* Poir., *Thymus villosus* L., *Daucus setifolius* Desf., *Leuzia longifolia* Hoffmanns. & Lk., and *Drosophyllum lusitanicum* (L.) Link. The soil under this association is usually acid (pH 5-6) and formed from siliceous rocks.

THYMELACEAE

Thymelaea broterana P. Cout, Bol. Soc. Brot. 24: 145 (1909).

Perennial, nanophanerophyte; —; north and central areas; mountain heaths.

Recorded in the Serra do Gerês from two sites with stony and sandy soils (pH 5.5-5.7), at 1170 and 1200 m.a.s.l. The species occurred in the association *Pterosparto-Ericetum australis* Br.-Bl., P. Silva and Rozeira. Characteristic companion species are *Erica australis* L., *Tuberaria globularifolia* (Lain.) Pers., *Asphodelus albus* Mill., *Pdlygala microphylla* L., *Carex asturica* Bss., *Allium ericetorum* Thore., *Iris boissieri* Henriq., *Pterospartum tridentatum* (L.) Wk. & Lge., *Erica umbellata* L., *Lithodora diffusa* (Lag.) Johnston ssp. *diffusa, Agrostis setacea* Curt., *Halimium alyssoides* (Lam.) Koch, and *Erica cinerea* L. (BRAUN-BLANQUET *et al.* 1964).

CISTACEAE

Cistus palhinha« Ingram, Gard. Chron. sér. 3. 114: 34 (1943).

Perennial, woody phanerophyte; 2n = 18 (LEITÃO & ALVES 1976); south west, west coast of the Algarve only; rocky places on high ground or slopes by the sea.

Recorded in the association *Junipero-Cistetum Palhinhae* (Rothm.) Br.-Bl., P. Silva & Rozeira (BRAUN- BLANQUET *et al.* 1964), at Cabo de São Vicente and Sagres on sandy soils (pH 7-8.5) overlying the rock platform, or growing in the crevices between rocks.

Halimium verticillatum (Brot.) Sennen, Monde Pl. 192: 39 (1931).

Perennial nanophanerophyte; 2n = 18 (LEITÃO & ALVES 1976); southwest, Estremadura; characteristic of low altitudes and gravelly Pliocene soils south of the river Tejo. There it occurs in the association *Halimio-Ulicetum parviflora* ined. (P. SILVA 1964).

According to PROCTOR & HEYWOOD (1968), this endemic is taxonomically related to *H. umbellatum* (L.) Spach., which is confined to S. W. and C. France, and the north of the Iberian peninsula, where it grows up to 1200 m.a.s.l.

Halimium lasianthum (Lam.) Spach. Ann. sci. Nat. ser. 2 (Bot.), 6: 366 (1866).

Perennial, nanophanerophyte; 2n = 18 (LEITÃO & ALVES 1976); Iberian Peninsula; sandy areas, heathlands and pinewoods. This species is split into two subspecies, one of which is confined to Portugal.

Subsp. *formosum* (Curtis) Heywood, Feddes Repert. 79: 59 (1968).

Algarve and the south-west; stony, schistose red-brown soils. Recorded in the association *Arbuto-Cistetum populifolii* Br.-BL, P. Silva & Rozeira (BRAUN-BLANQUET *et al.* 1964) growing under cork oak at São Brás de Alportel [as *H. formosum* (Curt.) Wk.]. As this was the only site on which it occurred out of the fifteen studied which contained this association. Further work on the ecology of the subspecies is needed.

Tuberaria major (Willk.) P. Silva and Rozeira, Agron. Lusit. 24: 168 (1964).

Perennial, nanophanerophyte; —; southern areas, mainly S. E. Algarve; coastal scrub, on soils that are usually poorly drained and acid. It occurs in the plant association *Nepetum Boivini* (Rothm.) Br.-BL, P. Silva and Rozeira. Characteristic plants of the association are *Stauracanthus boivini* (Webb)

Samp., *Tuberaria major,* and *Thymus cephàlotus* L, (BRAUN-bLANQuET *et al* 1964).

This species is rather like *T. globularifolia* (Lam.) Willk., which is endemic to the north-west of the Iberian peninsula (PROCTOR 1968). *T. major* is therefore disjunct from the main distribution area of a similar relative, and distinct ecologically. It is *always* found in the above mentioned plant association (SILVA & ROZEIRA 1964).

UMBELLIPERAE

Seseli peixoteanum Samp., Ann. sci. Nat. (Porto) 10: 36 (1906).

Perennial, hemicryptophyte; —; north east; invariably on serpentine rocks, often in association with *Arabis serpyllifolium.* subsp. *lusitanicum,* though it is not as common as this subspecies (DUDLEY 1967).

Morphologically somewhat similar to the southern Spanish endemic, S. *granatensis* Willk. (BALL 1968). Little else is known about S. *peixoteanum.*

Angelica angelicastrum (Hoffmanns. & Link) Cout., Fl. Port. 455 (1913).

Perennial, hemicryptophyte; —; Serra da Estrela; damp places above 800 m.a.s.l.

This species is not included in the plant lists published in BRAUN-BLANQUET *et al.* (1952), and little is known about it.

Ferulago capillaris (Link ex Sprengel) Coutinho, Fl. Port. 455 (1913).

Perennial, hemicryptophyte; —; south, around Tavira, Algarve; —.

There is little published information on this species.

PLUMBAGINACEAE

Armería pseudarmeria (Murray) Mansfield, Feddes Repert. 47: 140 (1939).

Perennial, woody chamaephyte; 2n = 18 (PHILLIPS 1938) ; the area around Cabo da Roca; pastures and scrubland on soils of granitic origin.

It has been suggested by SILVA (1972), that this species has affinities with the widespread and polymorphic *A. alliacea* (Cav.) Hoffmanns. & Link.

Armeria humilis (Link) Schultes, in Roemer & Schultes, Syst. Veg. 6: 772 (1820).

Perennial, woody chamaephyte; —; mountains of the north-west; in pastures, and in crevices in granitic rocks between 800 m. and 1400 m.a.s.l.

A species endemic to Portugal which is divided into two subspecies which are geographically sympatric over part of their range. There is no published information as to their ecology, so far. And the only published chromosome count is for subsp. *humilis.*

Subsp. *humilis* 2n = 18 (SILVA 1972) ; Serra da Amarela, Serra do Gerês.

Subsp. *odorata* (Samp.) P. Silva, Bot. Jour. Linn. Soc. 64: 377 (1971). Serra da Amarela, Serra da Arga.

Armeria arcuata Welw. ex Boiss. & Reuter, Pugillus 101 (1852).

Chamaephyte; —; south-west, around Vila Nova de Milfontes; moist pastures. Apparently now extinct.

Armeria eriophylla Willk., Bol. Soc. Brot. 2: 145 (1884).

Perennial, chamaephyte; —; north-east; dry pastures and rock crevices on serpentine. Occurring between 700 and 1070 m.a.s.l. in the association *Armerio-Arenarietum Fontiqueri* P. Silva, on soils of pH 5.9-7.0. The most common associated species are *Plantago radicata* Hoffmanns. & Lk. var. *radicata, Dianthus marizii* (Samp.) Samp., *Arenaria tetraquetra* L. ssp. *Fontiqueri* Silva, *Alyssum serpyllifólium*, Desf. ssp. *lusitanicum* Dudley & Silva, *Seseli peixotanum*

Samp., and also *Genista hystrix* Lge. var. vi*llosa, Hemiaria scabrida* Boiss., *Filago minima* Reichb., *Agrostis castellana* Boiss. & Reut, and *Poa bulbosa* L. (SILVA 1968).

Armeria eriophylla is like *A. giraräii* (Bernis) Litard., and may occur in western Spain, but this is not known.

Armería berlengensis Daveau, Bol. Soc. Geogr. Lisboa 4 (9) : 426 (1884).

Perennial, woody chamaephyte ; 2n = 18 (SUGIURA 1944) ; Berlengas Isles; granitic maritime slopes, particularly those facing north.

It has been suggested by BERNIS (1953) that because of its intermediate morphology, *A. berlengensis* is a stabilised hybrid from a cross between the more southern *A. welwitschii* Boiss., and the more northern *A. pubigera* (Desf.) Boiss. This is quite possible, but has not been experimentally confirmed, neither do we yet known the chromosome number of *A. pubigera,* although it is probable that this species has eighteen somatic chromosomes.

Armería welwitschii Boiss. in D.C. Prodr. 12: 676 (1848).

Perennial, woody chamaephyte; 2n = 18 (QUEIRÓS 1978) ; west-central coast, from Cabo Mondego to Cascais; maritime, sand and calcareous rocks.

On the coastal dune systems, the species is found in the association *Armerio-crucianelletum* Br.-Bl., Rozeira and P. Silva. This association occurs on the soft partly stabilised dunes, and has as its other characteristic species *Lotus creticus* L., *Reichardia gaditana* (Willk.) P. Cout., and *Iberis procumbens* Lge. ssp. *procumbens.* Other common companion spp. are *Artemisia crithmifolia* L., *Linaria polygalifolia* Hoffmanns. *&* Lk., *Crucianella maritima* L., *Ammophila arenaria* (L.) Lk., and *Ononis ramosissima* Desf. (BRAUN-BLANQUET et al. 1972).

A. welwitschii is very polymorphic, mainly in the width of leaves, size of outer involucral bracts, and pubescence. In the northern part of the species range, the variety *pla-*

typhylla Daveau, strongly resembles a small form of *A. berlengensis* (SILVA 1972). There is a good case here for experimental work to clarify the relationships between these two species.

Armería pinifolia (Brot.) Hoffmanns. & Link, Fl. Port. 1: 437 (1813-1820).

Perennial, chamaephyte; 2n = 18 (DONADILLO 1967); centre and south-west, from Ribatejo to Algarve; scrublands on sandy or gravelly soil, calcifuge.

Armería rouyana Daveau, Bol. Soc. Brot. 6: 168 (1889).

Perennial, chamaephyte ; 2n=18 (SUDA 1989) ; southwest, from Barriero to Cereal do Alentejo; scrublands on sandy soil, calcifuge.

This species, and *A. pinifolia* are morphologically quite similar and also geographically sympatric (SILVA 1972). BERNIS (p. 83, 1954) looked at the variation pattern found in *Armería* populations at Sines, and pointed out that there was a cline of variation between *A. rouyana* on the inland dunes, and *A. pungens* on the coastal sands. He gave his opinion that this was caused by hybridisation between the two species. There is no experimental information to support this thesis, neither is there any mention in the literature of hybrids between these two species. Either this has not been observed, or there are genetic and/or ecological barriers between them.

There is a very interesting evolutionary pattern awaiting study here. BERNIS (1950, 1953, 1954, 1956) has laid at least the foundations for further studies of the genocological basis of the widespread schizoendemism found within the genus in Iberia. An analysis of the genetic bases of the variation, with a parallel study of the ecological factors acting as selective agents, would be most illuminating.

Limonium auriculae-ursifolium (Pourret) Druce, Pl: List, ed. 2, *11* (1928).

Perennial, chamaephyte; 2n = 25, 26 (BAKER 1952); France, Iberian peninsula, Balearic Isles; maritime cliffs and salt marshes.

The species is divided into three subspecies by PIGNATTI (1971), two of which are endemic to Portugal.

Subsp. *lusitanicum* (Pignatti) Pignatti, Bot. Jour. Linn. Soc. 64: 367 (1971). Central west coast, Trafaria to S. Martinho do Porto.

Subsp. *multiflorum* (Pignatti) Pignatti loc. cit. (1971). South central west coast, Torres Vedras to Colares.

Limonium ovalifolium (Poiret) O. Kuntze, Rev. Gen. Pl. 2: 396 (1891).

Perennial, chamaephyte; 2n = 16 (BAKER 1952); W. France, Portugal, Morocco; coastal.

There are three designated subspecies, one of which is endemic to Portugal (PIGNATTI 1972).

Subsp. *lusitanicum,* Pignatti, Collect. Bot. (Barcelona) *1:* 318 (1962).

Coasts of Estremadura and Alentejo; maritime rocks.

Since Flora Europaea 3. was published, ERBEN (1978) has completely revised the classification of all the Atlantic species of *Limonium.*

BORAGINACEAE

Echram creticum L. Sp. Pl. 139 (1753).

Herbaceous biennial; 2n = 16 (SUGIURA 1936); west mediterranean region, and southern Portugal; roadsides and grassy slopes.

The species is subdivided into three subspecies (GIBBS 1972), one of which is a Portuguese endemic.

Ssp. *algarbiensis* R. Fernandes, Bol. Soc. Brot. sér. 2, 43: 154 (1969).

2n = 16 (FERNANDES & QUEIRÓS 1971b); south (Albufeira).

Echium tuberculatum Hoffmanns. & Link, Fl. Port. 1: 183 (1810).

Annual (therophyte) or biennial; 2n = 16 (GARDÉ & MALHEIROS GARDÉ 1953); central and southern Portugal; open and grassy places, recorded in the *Melilotas* meadows around Lisbon in the association *Gladioleto-Phalaridetum Melilotetosum* Teles (TELES 1953).

GIBBS (1972) does not describe any separate subspecies, but COUTINHO (1939) designated three. Only one of these appears restricted enough to be regarded as a Portuguese endemic.

*Ssp. *densiflorum* P. Cout. Fl. Port, 595 (1939).

2n = 16 (FERNANDES & LEITÃO 1972) ; Cabo da Roca; maritime sands.

Recorded by BRAUN-BLANQUET *et al.* (1964), as a companion subspecies in the *Cisto-Ulicetum humilis* Br.-Bl., P. Silva & Rozeira *(l. c.)* grouping, on the littoral between Leça de Palmeira and Boa Nova near Porto, and on the Landes at Praia de Santa Cruz, Torres Vedras. The chromosome count of the subspecies was also determined from three separate Portuguese collections, none of which was from Cabo da Roca. These later records extend the original distribution area, and should be considered in the light of later comments.

Echium rosulatum Lange, Ind. Sem. Horto Haun. 1857: 22 (1857).

Perennial, hemicryptophyte ; 2n = 32 (FERNANDES & LEITÃO 1972); Portugal and N. W. Spain; maritime sands, fields and wastelands, roads and riversides.

COUTINHO (1939) designated four subspecies of *E. rosulatum,* only one of which appears likely to be a clearly distinct Portuguese endemic.

*Ssp. *davaei* (Rouy) P. Cout. Fl. Port. 596 (1939).

Berlengas Islands. No other information available.

It is with some hesitation that I include these three subspecies. As pointed out by GIBES *(l. c),* the taxa of

Echium are extremely variable in morphological characteristics, and those differentiating species are still critical «in a number of cases»; for example in the separation of E. *creticum* L., and *E. tuberculatum* Hoffmanns. & Link. There are considerable problems in these situations, because individual taxonomists draw specific boundaries at different points, may or may not delineate subspecies, and may undertake biosystematic studies or work entirely from herbarium specimens. With critical groups, the latter are inadequate, and can lead to erroneous conclusions, and unsatisfactory classifications. *Echium,* as can be seen from the above comments, is one taxon clearly in need of biosystematic studies.

LABIATEAE

Ajuga pyramidalis L. Sp. Pl. 561 (1753).

Perennial, rhizomatous hemicryptophyte; 2n = 32 (FAVArGER 1953); Europe, to N. Portugal, N. Italy, and Bulgaria; shady, damp places. One endemic subspecies.

*Subsp. *meonantha* (Hoffmanns. & Link.) R. Fernandes, Bol. Soc. Brot. sér. 2, 34: 131, 1960.

Northern areas; occurs in the *Holceto-Quercetum* Br.-BL, P. Silva and Rozeira, on poor acid soils at altitudes above 720 m.a.s.l. The other characteristic species of the association are *Quercus pyrenaica* Willd., *Erythronium dens-canis* L., *Poa nemoralis* L., and *Carduus gayanus* Dur. (BRAUN-BLANQUET *et al.* 1956), and also in the association *Myrtilleto-Quercetum roboris Myrtilletosum* R. R. D. Barretos, on the Serra da Peneda, between 850 and 1060 m.a.s.l. The arborescent layer is mainly *Quercus robur* L., and the other species characteristic of the association are *Vaccinium myrtillus* L., *Erythronium dens-canis* L., and *Brachypodium pinnatum* (L.) P. Beauv. (BARRETOS 1958).

In both eases the endemic was recorded as *Ajuga occidentalis* Br.-Bl. The chromosome number of the Portuguese material has not been recorded. BALL (1972) is of the opinion that this subspecies only merits varietal rank, but

in view of the fact that little is known of the range of cytological and morphological variation in Portugal, this is perhaps a premature decision.

Teucrium salviastrum Schreber, Pl. Vert. Unilab. 38 (1773).

Perennial, woody chamaephyte; —; central mountain ranges (Serra da Estrela, Serra de S. Macario); —.

Noted as frequent in the Serra da Estrela by COUTINHO (1939), but not recorded by BRAUN-BLANQUET *et al.* (1952).

*Teucrium algarbiensis P. Cout., Espoco Fl. Lenh. Portug. ed. 2: 262 (1936).

Perennial, woody chamaephyte; 2n = 52 (PUECH 1978); Algarve, central and western parts of the coast (Castro Marim, Tavira, Faro); rocky places and hills near the sea.

This species has been merged into *T. polium* subsp. *vincentinum* Rouy, by TUTIN & WOOD (1972). As *T. polium* shows a wide range of chromosome numbers, with the recent count of 2n = 80 (FERNANDES & QUEIRÓS 1971) different from the others, there appears to be a case for cytotaxonomic reappraisal here, and perhaps reinstatement of the endemic Portuguese species, as suggested by PUECH (1978).

Thymus cephalotos L. Sp. PL, 592 (1753).

Perennial, woody chamaephyte; —; Algarve; dry heathlands. Found in the association *Stauracanthum boivini* (Rothm.) Br.-Bl., P. Silva and Rozeira, in the alliance *Ulieino-Cistion* (Br.-Bl.). The other characteristic species of this association are *Tuberaria major* Wk. P. Silva & Roz., and *Stauracanthus boivini* (Webb) Samp., with *Lavandula stoechas* L. ssp. *luisieri* Roz., *Thymelaea villosa* (L.) Endl., *Calluna vulgaris* (L.) Hull, *Cistus crispus* L., and *Pulica odora* (L.) Rehb. The soils beneath the association are acid, and shown impeded drainage (BRAUN-BLANQUET *et al.* 1964).

Thymus villosus L. Sp. PL 592 (1753).

Perennial, woody chamaephyte; —; south-west Spain & Southern Portugal; —.

An Iberian endemic, which has one putative subspecies endemic to Portugal.

•Subspecies *lusitaniens* (Boiss.) Cout., Bol. Soc. Brot. 23: 87 (1907).

Central and southern areas; *Thymus vülosus* (incl. *T. lusitaniens* Bss.) has been recorded on sandy and stony soils (pH 5-6), in the association *Erico-Quercetum lusitanicae* (Rothm.) Br.-Bl., P. Silva and Rozeira. The other characteristic species of the association being *Quercus lusitanica* Lam., *Gar ex oedipostyla* D. Jouv., *Serratula pinnatifida* Poir, *Daucus setifolius* Desf., *Leuzia longifolia* Hoffmanns. & Lk., *Euphorbia transtagana* Bss., and *Drosophyllum lusitanicum* L. Lk. (BRAUN-BLANQUET *et al.* 1964).

According to JALAS (1972), the species as a whole shows considerable polymorphism in the shape of its bracts and length of the corolla tube. He suggests that the endemic subspecies is but one node of variation within a continuously varying population. Further cytotaxonomic and autecological work is necessary before any final conclusions can be drawn.

Thymus capitellatus Hoffmanns. & Link, Fl. Port. 1: 125 (1809).

Perennial, woody chamaephyte; —; south, Alentejo, usually close to the sea; heathlands, and other sandy areas. A species of the association *Stauracantho-Corematum* (Rothm.) Br.-BL, P. Silva and Rozeira, in the alliance *Stauracantho-Coremenion.* The main associated species being *Corerna album* (L.) D. Don., and *Stauracanthus genistoides* (Brot.) Samp. Less common in the association, but also characteristic of it, are *Leontodón taraxacoides* (Vill.) Merat, *Santolina rosmarinifolia* L., and *Helichrysurn angustifolium* (Lam.) DC.

Usually this grouping follows behind the Ammophilion on the dunes. The sand is well aerated, occasionally slightly

saline and of approximately neutral pH (BRAUN-BLANQUET *et al* 1964).

Thymus camphoratus Hoffmanns. & Link, Fl. Port. 1: 131 (1809).

Perennial, woody chamaephyte; —; south, lower Alentejo near the coast, and Algarve; dry heathlands and other sandy places. A common species in the association *Junipero-Cistetum pálhinhae* (Rothm.) Br.-Bl., P. Silva and Rozeira. This association contains *Cistus palhinhas* Ingram, *Armería pungens* (Link) Hoffmanns. & Link., and *Teucrium polium* ssp. *vicentinum* Rouy. It is usually divided into two; sub association *typique,* which contains in addition *Juniperus phoenicea* L., and *Helychrysum angustifolium* (Lam.) D. C, and sub association *Ulicetosum erinacei,* having two different associate species, *Ulex argenteus* subsp. *erinaceus* (Webb) D. A. Webb, and *Astragalus massiliensis* (Miller) Lam. The soils are usually alkaline (pH 7-8.5), and overly rock platforms, or are contained in wide crevices between rocks, especially in the area of Cabo S. Vicente and Sagres.

T. camphoratus and *T. capitellatus* are morphologically rather similar (JALAS Z. c.) ; however the available published information shows that their ecology is quite different. How close they are genetically is not known.

Thymus carnosus Boiss. Voy. Bot. Midi Esp. 2: 490 (1841).

Perennial, woody chamaephyte; —; south, Alentejo and Algarve; maritime sands.

No other information available.

These endemic *Thymus* species are not well known scientifically. Biosystematic work on plants such as these is not easy, due to the size of the flowers, and the slow growth of the plants. However, experimental garden and field studies would reveal how morphological patterns vary, and how they are related to habitat, if at all. Cytologieal studies are also difficult, due to the smallness and often high number of the chromosomes in *Thymus.* Chromosome counts of these

species would help to clarify their relationships. It is interesting that the only putative natural hybrids reported by cOUTINHO (1939) are *T. camphoratus* X *T. mastichina* L., and *T. carnosus* X *T. mastichina L.* The chromosome number of this latter species is 2n = 56 (BONNET 1967), which suggests that the other species may also have high numbers.

Lavandula stoechas L. Sp. Pl. 573 (1753).

Perennial, woody chamaephyte or nanophanerophyte; 2n = 30 (GARCIA 1942); southern Europe; —.

Subdivided into six subspecies by GUINEA (1972), two of which are endemic to Portugal.

Subsp. *lusitanica* (Chaytor) Rozeira, Agron. Lusit. 24: 173 (1964).

Central and southern areas; dry sandy soils. An important component of the littoral alliance *Stauracantho-Coremion* (Rothm.) Br.-Bl. P. Silva & Rozeira, the subspecies occurs in the association *Stauracantho-Corernetum* sub-association *Helichrysetosum* in central Portugal (see p- 968 for details), and the *Cisteum bourgeani* Rothm. amend. The distinctive species of this association, which is characteristic of the south coast, are *Thymus tomentosus* Willd., *Euphorbia baetica* Boiss., *Centaurea áspera* L. subsp. *stenophylla* (Duf.) Wk., *Cistus bourgeanus* Coss., and *Armería macrophylla* Boiss. et Reut. (BRAUN-BLANQUET *et al.* 1964).

Subsp. *luisieri* (Rozeira) Rozeira, Agron. Lusit. 24: 173 (1964).

Central and southern areas; dry schistose, loamy soils. Found in the alliance *Ulicino-Cistion* Br.-BL, P. Silva & Rozeira *(l. c)*, from sea level to 200 m.a.s.l., and in the algarve sometimes up to 900 m.a.s.l. (Serra de Monchique, Fóia). This is a characteristic subspecies of the alliance and is much more widely distributed than subsp. *lusitanica.* It is recorded in eight of a total of nine associations, all of which are found on the plains and lower mountain slopes of Beira Baixa (to the south of Serra de Estrela).

SCROPHULARIACEAE

Verbascum litigiosum Samp., Lista Esp. Herb. Port. **108** (1913).

Biennial, hemicryophyte; —; southern and central areas; sandy places by the sea.

According to FERGUSON (1972), this species is like the widespread and polymorphic *V. thapsus* L. There is no other information on cytology or ecology.

Scrophularia grandiflora D.C., Cat. Pl. Horti. Monsp. **143** (1813).

Perennial, chamaephyte; west-central Spain and north-central Portugal; woods, walls, roadsides, and damp rocky places.

An Iberian endemic with two subspecies, one of which is confined to Portugal, the other to Spain.

Subsp. *grandiflora* 2n = 58 (FERNANDES, QUEIRÓS & SANTOS 1977); central areas; woods, walls and roadsides.

Anarrhinum longipedicellatum R. Fernandes, Bol. Soc. Brot, sér. 2, 33: 14 (1959).

Biennial, chamaephyte; 2n = 18 (FERNANDES, QUEIRÓS & SANTOS 1977); central areas, Beira Alta and Douro Litoral (Vale de Vouga); shady places and the banks of dry watercourses.

Very little is known about this recently described species, but it appears to have some affinities with the widespread *A. bellidifolium* L., with which it shares the same chromosome number and a similar karyotype.

Antirrhinum lopesianum Rothm., Feddes Repert. (Beih.) **136:** 65 (1956).

Perennial, chamaephyte; —; north, Trás-os-Montes (Vimioso, Bragança); calcareous rock 1000-1400 m.a.s.l.

This species has been separated from *A. molle* L. which has the chromosome number 2n = 16 (BAUR 1932). There is no record for the endemic.

Antirrhinum majus L., Sp. Pl. 617 (1753).

Perennial, chamaephyte; 2n = 16 (HEITZ 1927); south-west Europe, and east to Sicily; dry, open areas.

Subdivided into four subspecies (WEBB 1972), of which one is endemic to Portugal.

Subspecies *linkianum* (Boiss. & Reuter) Rothm., Feddes Repert. 54: 19 (1944). 2n= 16 (FERNANDES, QUEIRÓS & SANTOS 1977), recorded as *A. linkianum*, Boiss. & Reuter; west central areas; rocky places, walls, hedgerows, scrubland and cornfields. Recorded in the association *Meliceto-Cocciferetum* Br.-BL, P. Silva & Rozeira, which often occurs in degraded *Quercus faginea* Lam. areas now dominated by *Quercus coccifera* L. scrub. The association occupies calcareous soils (pH 6.5-8.5) at low altitudes (30-150 m.a.s.L). Other characteristic species of the asociation are *Mélica minuta* L., *Lonicera implexa* Ait., *Origanum virens* Hoffmanns. & Lk., *Bupleurum paniculatum* Brot., *Salvia sclareoides* Brot., & *Silene patula* Desf. (BRAUN-BLANQUET *et al.* 1956).

Chaenorrhinum serpyllifolium (Lange) Lange in Willk. & Lange, Prodr. Fl. Hisp. 2: 578 (1870).

Annual, therophyte; —; central Spain i& south-west Portugal; ruderal.

An Iberian endemic, divided into two subspecies (R. FERNANDES 1972), one of which is endemic to Portugal.

Subsp. *lusitanicum* R. Fernandes, Bot. Jour. Linn. Soc. 64: 223 (1971).

South-west; calcareous sandstone by the sea.

No other information available.

Linaria ficalhoana Rouy, Naturaliste (Paris) 5: 285 (1883).

Annual or biennial, therophyte or hemicryptophyte; —; south-west; maritime sands.

Linaria aigarviana Chav., Monogr. Antirrh. 142 (1833).

Annual, therophyte; —; south-west (West Algarve); dry sandy places, heaths and vinyards.

Linaria lamarckii Rouy, Naturaliste (Paris) 5: 351 (1883).

Perennial, chamaephyte; 2n = 12 (FERNANDES & QUEIRÓS 1971b; south, coasts of Estremadura, Alentejo, and Algarve; maritime sands only.

Linaria coutinhoi Valdês, Rev. Esp. Eur. *Linaria* 183 (1970).

Annual, therophyte; —; valley of the Rio Douro; —.

This species is similar to *L. diffusa* Hoffmanns. & Link, and occupying at least in part, the same geographical area (CHATER, VALDÊS & WEBB 1972).

Linaria ricardoi Coutinho, Bol. Soc. Brot. 2: 131 (1906).

Annual, therophyte; —; south, upper and lower Alentejo; cornfields, on calcareous soils.

The recent monograph on *Linaria* (VALDÊS 1970) has gone a considerable way toward clarifying the taxonomy and distribution of many species but a great deal has yet to be learnt about their cytology, and general ecology.

Digitalis purpurea L. Sp. PL 621 (1753).

Biennial or perennial, hemicryptophyte; 2n = 56 (CARPIÓ 1957); west, south-west, and west-central Europe; woodlands, heaths, rocky places.

A very variable species subdivided into four subspecies, one of which is endemic to Portugal (HEYWOOD 1972).

Subspecies *heywoodi* P. & M. Silva, Agron. Lusit. 20: 239 (1959). 2n = 56 (M. NORONHA-WAGNER 1959) ; south east, apparently restricted to around Reguengos de Monsaraz, alto Alentejo; granitic rocks, in crevices and fissures.

Veronica micrantha Hoffmanns. & Link, Fl. Port. 1: 286 (1813-1820).

Annual, therophyte ; 2n = 16 (FERNANDES, QUEIRÓS & SANTOS 1977) ; north, Trás-os-Montes, Douro, Minho, Beira and Ribatejo; damp and shady places in general. Little is known about this species.

DIPSACACEAE

Succisella carvalhoana (Mariz) Baksay, Ann. Hist. Nat. Mus. Hung. ser. 6: 174 (1955).

Perennial, chamaephyte; —; north west and central Portugal (Porto to Coimbra) ; wet places.

This species is not very well known, and needs further investigation.

CAMPANULACEAE

Campanula primulifolia Brot., Phyt. Lusit. 9 (1800).

Perennial, chamaephyte ; 2n = 36 (GADELLA 1964) ; southern areas, Minho, southern maritime Beira, Alentejo & Algarve, damp or shady places.

Campanula lusitanica L. ex Loefl., Iter. Hisp. 111 (1758).

Annual, therophyte ; 2n = 18 (FERNANDES 1962) ; Spain and Portugal; hedges, cornfields, pastures, shady places, sandy areas. Recorded by P. SILVA (1971) on sandy soils of granitic origin (pH 5-5.8), in vinyards of the region of Dão, upper Beira.

Subspecies *transtagana* (R. Fernandes) Fedorov, Bot. Jour. Linn. Soc. 67: 281 (1973). 2n = 20 (FERNANDES 1962); south and south central areas, south of the river Tagus; on dry slopes, under hedges, and on the margins of streams and rivulets on clay soils. Originated from the type by tetrasomy (FERNANDES 1962).

Jasione lusitanica A.D.C., Monogr. Camp. 105 (1830).

Perennial, chamaephyte; —; north-west and central Portugal (Porto to Sintra); maritime sands.

Recorded on the stabilized dune areas in the association *Scrophulario-Vulpietum* Br.-BL, Rozeira, and P. Silva. This association, just inland of the *Ammophiletum* where the sand is not leached and highly acid, has as its characteristic species *Vulpia alopecuros* (Schousb.) Dumort., *Helichrysum angustifolium* (Lam.) D.C., *Corynepfiorus canescens* var. *maritima* Godr., *Seseli tortuosum* ssp. *ramosissimum* (Lk.) Alte, *Herniaria ciliolata* Melderis, *Cerastium diffusum* Pers., *Scrophularia frutescens* L., *Carex arenaria* L., *Euphorbia portlandica* L., and *Anthyllis dillenii* Schultes ex Loudon. Other common species are *Leontodón taraxacoides* ssp. *crassifolius* (Mariz) P. Silva, *Artemisia crithmifolia* L., *Grucianella maritima* L., *Malcolmia littorea* (L.) R. Br., and some *Ammophilla arenaria* (L.) (BRAUN-BLANQUET *et aï.* 1972).

Jasione crispa (Pourret) Samp., Ann. sci. Acad. Polyt. Porto 14: 161 (1921).

Perennial with stout woody stock, chamaephyte; 2n = 36 (FAVARGER & KUPFER 1968, KUPFER (1969a & *b),* 2n = 48 (KUPFER 1971) both recorded as *J. humilis* (Pers.) Lois.; south-west Europe; mountain rocks and screes.

Divided into ten subspecies, one endemic to Portugal.

Subspecies *serpentinica* P. Silva, Agron. Lusit. 30: 225 (1970). —; north-east; ultra-basic and serpentine rocks.

Recorded by P. SILVA (1968) in the Bragança region, most often in the association *Armerio-Arenarietum Fontquerii* n. nom., on stony calcareous soils (pH 6.5-7.3) between

690 and 870 m.a.s.L, with *Plantago radicata* Hoffmanns. & Lk. var. *radicata, Dianthus marizii* (Samp.) Samp., *Arenaria tetraquetra* L. ssp. *Fontiqueri* P. Silva, *Atyssum serpyllifolium* Desf. subsp. *lusitanicum* Dudley & P. Silva, *Seseli peixotanum Samp.,* and *Armería eriophylla* Willk. The subspecies has also been recorded in the association *Gisto-Genistetum Hysteris* n. nom. var. *serpentinicum* on slightly acidic serpentine rocks (pH 5.7-6.3) with *Cistus ladanifer* L., *Genista hystrix* Lge. var. *villosa* Lge., *Lavandula stoechas* L. ssp. *Sampaiana* Roz., and the *Plantago* and *Atyssum* mentioned above.

No chromosome count has yet been published for the endemic subspecies. Considering those quoted above, this would be interesting.

COMPOSITAE

Dittrichia viscosa (L.) W. Greuter, Exsicc. Genev. 4: 71 (1973).

Perennial, chamaephyte; 2n=18 (FERNANDES & QUEIRÓS 1971a, 2n = 34 KLIPHUIS & MENNEGA 1966), as *Inula viscosa* (L.) Ait.; southern Europe; dry and arid places, heaths and pine woods.

Two subspecies, one endemic to Portugal.

Subspecies *revoluta* (Hoffmanns. & Lk.) P. Silva & Tutin, Bot. Jour. Linn. Soc. 67: 282 (1973). South-western areas; dry hills, margins of fields and roads.

Tanacetum mucronulatum (Hoffmanns. & Lk.) Heywood, Agron. Lusit. 20: 214 (1958).

Perennial, chamaephyte; —; north-east, and east-central Portugal (Bragança, Covilhã, Fundão, Portalegre) ; open scrub and rocky places.

This species bears some resemblance to the widespread *T. corymbosum* (L.) Schulz Bip., but does not grow as tall (HEYWOOD 1976).

Leucanthemum lacustre (Brot.) Samp., Lista Esp. Herb. Port. 132 (1913).

Perennial, chamaephyte; —; west-central areas, Estremadura region; edges of ditches, rivers, marshes.

Part of the species complex of *L. vulgare* Lam. (HEYWOOD 1976), this complex is extremely variable both in its morphology and its cytology. Although the above species is included here, no firm conclusions can be drawn as to its geographical status until further work is carried out on the Iberian part of the complex.

»Senecio cespitosas Brot., Fl. Lusit. 1: 390 (1804).

Perennial, hemicryptophyte; —; Serra da Estrela (Malhão, Covão das Vacas); grassy and rocky places. Not recorded by BRAUN-BLANQUET *et al* (1952).

Merged into the variable *S. pyrenaicus* L. by CHATER & WALTERS (1976). According to these authors, there is not a clear enough morphological difference between *S. caespitosus* and the putative ancestral stock to maintain either specific or sub-specific status. A careful cytotaxonomic study may refute the suggestion.

S. pyrenaicus L. was previously known as *8. tournefortii* Lap. Chromosome counts are given under this name as 2n = 20 (FAvARGER & KUPFER 1968), and under *S. pyrenaicus* L. from Serra da Estrela & lower Beira as 2n = 40 (QUEIRÓS 1978).

Senecio doronicum (L.) L. Syst. Nat. ed. 10, 2: 1215 (1759).

Perennial herb, hemicryptophyte; 2n = 40, 80 (FERNANDES & QUEIRÓS 1971a, AFZELIUS 1949); mountain areas, dry places, calcicole.

»Subspecies *lusitanicum* Cout. Fl. Port. 641 (1913).

Central areas (Serra de Montejunto).

The above subspecies has been placed in *S. lagascanus* D.C., by CHATER & WALTERS (1976). Further information is urgently needed on all aspects of the biology of the Portuguese endemic in order to clarify its status.

Cynara algarbiensis Cosson ex Mariz, Bol. Soc. Brot. 10: 236 (1893).

Perennial, hemicryptophyte; —; southern areas, lower Alentejo and Algarve; cultivated land, or open waste ground. Recorded as a companion species in the association *Arbuto-Cistetum populifolii* Br.-BL, P. Silva & Rozeira assoc. nova, in three relevés between 460 and 700 m.a.s.l. in the Serra Monchique (BRAUN-BLANQUET *et al.* 1984). These sites were considered to have been originally under cultivation.

Leuzia longifolia Hoffmanns. & Lk., Fl. Port. 2: 217 (1825).

Perennial chamaephyte; 2n = 26 (FERNANDES & QUEIRÓS 1971a) as *Centaurea longifolia* (Hoffmanns. & Lk.) P. Cout.; southern regions, Beira Litoral, Estremadura, Alentejo and Algarve; damp scrub. See *Thymus villosus* for ecology.

Centaurea fraylensis Schultz Bip. ex Nyman, Consp. 420 (1879).

Perennial, chamaephyte; —; south west areas, lower Alentejo and Cabo St. Vincente; —.

Centaurea rothmalerana (J. Arènes) Dostal, Bot. Jour. Linn. Soc. 71: 199 (1976).

Biennial, hemicryptophyte; —; north-central areas (Serra da Estrela); mountain pastures.

Bears some resemblance to c. *urgellensis* Sennen, which is endemic to the Pyrenees (DOSTAL 1976).

Centaurea aristata Hoffmanns. & Lk., Fl. Port. *2:* 226 (1820--1828).

Biennial, hemicryptophyte; —; Portugal and north-west Spain. Split into four subspecies, two of which are endemic to Portugal (DOSTAL 1976).

Subspecies *exilis* (J. Arènes) Dostal, Bot. Jour. Linn. Soc. 71: 199 (1976). East-central regions (Monfortinho).

Subspecies *geresensis* (J. Arènes) Dostal, *I. c*- (1976).

Northern areas (Serra do Gerês) ; —; Recorded as an occasional in one relevé, in the association *Ulicio-Ericetum Umbellatae* (as *C. paniculata* L. ssp. *geresensis)* (BRAUN-BLANQUET *et al.* 1964).

Centaurea micrantha Hoffmanns. & Lk. Fl. Port. 2: 220 (1820--1828).

Biennial, hemicryptophyte ; —; north-west Spain and north-central Portugal; —.

Divided into three subspecies (DOSTAL 1976), one of which is endemic to Portugal.

Subspecies *herminii* (Rouy) Dostal Z. c. (1976).

North-central areas (Serra da Estrela) ; —.

The above four endemic taxa are all split from *C. paniculata* L. (DOSTAL 1976), the chromosome number of which is 2n = 18 (FERNANDES & QUEIRÓS 1971a).

Centaurea sphaerocephala L. Sp. Ph 916 (1753).

Perennial, chamaephyte; 2n = 44 (GUINOCHET & FOISSAC 1962); west mediterranean region and Portugal; sandy ground, mainly by the sea.

Three designated subspecies ,one endemic to Portugal (DOSTAL L. C).

Subspecies *lusitanica* (Boiss. et Reuter) Nyman, Consp. 432 (1879).

2n = 22 (FERNANDES & QUEIRÓS 1971a) ; central and southern areas; edges of fields and roads, yards and rubbish tips.

Centaurea nigra L., Sp. Pl. 911 (1753).

Perennial, chamaephyte. 2n = 44 (GADELLA & KLIPHUIS 1970) ; Europe eastwards to Sweden and central Italy; dry grasslands.

Three designated subspecies, one endemic to Portugal.

Subspecies *rivularis* (Brot.) Cout. Fl. Port. 655 (1913).

2n = 22 (FERNANDES & QUEIRÓS 1971a); north and central areas, Trás-os-Montes, Minho, Beiras, upper Alentejo; dry, grassy places. A species which shows a great deal of natural quantitative variation, and is difficult to classify unless a great many plants are studied both in the field and experimental garden.

All these endemic *Centaurea* taxa are in need of a scientific appraisal of all aspects of their biology.

Picris algarbiensis Franco, Bot. Jour. Linn. Soc. 71: 268 (1976).

Biennial or short-lived perennial, hemicryptophyte or chamaephyte; —; southern areas; woodland (on schistose soils under cork oak).

Little information appears to be available on this species, although it may have affinities with *P. comosa* (Boiss.) B. D. Jackson (SKtL 1976).

Picris spinifera Franco, 1. c. (1976).

Biennial, hemicryptophyte; —; central and east-central areas; dry waste ground.

Taraxacum duriense Van Soest, Agron. Lusit. 13: 67 (1951).

Perennial, hemicryptophyte; —; Douro Litoral, Beira Litoral, Estremadura; maritime sands, meadows, roadsides, waste ground. Little is known of this species except that it resembles *T. càlocephàlum* Handel-Mazzetti emend. Dahlstadt.

LILIACEAE

Asphodelus bento-rainhae P. Silva, Agron. Lusit. 18: 20 (1956).

Perennial, with sessile swollen roots, geophyte; 2n = 28 (CASTRO, ex SILVA 1956); central areas (Serra da Gardunha); pastures and cultivated fields, on granitic soils.

ScMa beirana Samp., Bol. Soc. Brot. sér. 2, 7: 125 (1931).

Perennial, bulbous geopraphyte; 2n = 20 (BARROS-NEVES 1973) as *Sc. ramburei* Boiss.; north east, Trás-os-Montes, upper Beira; —.

Hyacinthoides vicentina (Hoffm. & Lk.) Rothm., Feddes Repert. 53: 15 (1944).

Perennial, bulbous geophyte; —; south-western areas, Alentejo; woods and rocky places.

HEYWOOD (1980) considers that this species may probably be only varietally distinct from *H. itálica* L. Rothm.; further ecological work is certainly needed as well as a careful taxonomic appraisal.

Bellevalia hackelii Freyn, Österr. Bot. Zeitschr. 27: 289 (1877).

Perennial, bulbous geophyte; —; southern areas; dry open habitats.

Allium schmitzii Cout., Bol. Soc. Brot. 13: 103 (1896).

Perennial, bulbous geophyte; 2n = 16 (BARROS-NEVES 1973); eastern Portugal, Trás-os-Montes and upper Douro, lower Beira, and Alentejo; crevices in rocks, and river banks.

Allium pruinatum Link ex Sprengel, Syst. Veg. 2: 35 (1825).

Perennial, bulbous geophyte; 2n = 16 (BARROS-NEVES 1973); central and southern areas, maritime Beira, Ribatejo, Estremadura, Alentejo, Algarve; pine-woods, scrub-lands and sandy heaths.

Narcissus fernandesi G. Pedro, Bol. Soc. Brot. sér. 2, 21: 60 (1947).

Perennial, bulbous geophyte; 2n = 14, 28 (FERNANDES 1970); south-central areas (Monte da Aderna, Pé de Galinha);

grassy margins of ditches, on compacted sandy soils liable to flooding.

Known from 2 populations in south-central Portugal. In most characters intermediate between *N. wilkommii* (Samp.) A. Fernandes, and *N. gaditanus* Boiss. & Reuter (WEBB 1980).

Narcissus calcicola Mendonça, Bol. Soc. Brot. sér. 2, 6: 318 (1930).

Perennial, bulbous geophyte; 2n = 14 (FERNANDES 1939); west-central areas (Porto de Mós); crevices of limestone rocks at higher altitudes.

Narcissus scaberulus Henrique, Bol. Soc. Brot. 6: 45 (1888).

Perennial, bulbous geophyte; 2n = 14 (FERNANDES 1939); north-central areas, Mondego valley; uncultivated and rocky places.

IRIDACEAE

Iris lusitanica Ker-Gawler, Bot. Mag. 18: 5. 679 (1803).

Perennial, bulbous geophyte; 2n = 34 (SIMONET 1932); central areas, Trás-os-Montes, upper Douro, Beira, Ribatejo, Estremadura, and upper Alentejo; dry and stony hillsides.

According to WEBB & CHATER (1980), this species is a yellow flowered form similar to *I. xiphiurn* L.; however there appear to be enough differences to keep the two taxa distinct.

Crocus serotinus Salisb., Parad. Lond. 5: 30 (1806).

Perennial, bulbous geophyte; —; Spain and Portugal; pinewoods, scrub, and rocky grassland.

Three subspecies ,one endemic to Portugal.

Subspecies *serontinus* 2n = 22, 23 (BRIGHTON, MATHEW & MARCHANT 1973); central and southern areas.

Romulea ramiflora Ten., subspecies gaditana (G. Kunze) Marais, was recorded as occurring in Portugal, Spain, and Morocco by MARAIS (1975), but as occurring «in the west and southern parts of the Iberian peninsula», in Flora Europaea 5 (1980). The geographical status of this taxon is thus not clear.

JUNCACEAE

Juncus acutiflorus Ehrh. ex Hoffm., Deutschl. Fl. 125 (1791).

Perennial, herbaceous chamaephyte; 2n = 40 (TIMM & CLAPHAM 1940); West, central, and southern Europe; wet meadows and marshes.

One subspecies endemic to Portugal, although SNOGERUP (1980) does not consider this worthy of the rank given to it.

•Subspecies *rugosus* (Stendel) Cout., Fl. Port. 118 (1913).

South-central and southern areas; wet meadows, marshes, and woods.

GRAMINEAE

Festuca brigantina (Markgraf Dannenb.) M.-D., Bot. Jour. Linn. Soc. 76: 328 (1978).

Perennial, herbaceous chamaephyte; —; north-east (Serra de Nogueira); serpentine rocks and soils.

Recorded by SILVA (1968) between 840 and 1070 m.a.s. l., in fissures in serpentine rocks (as *F. ovina* L. ssp. *brigantina* M. D.).

Festuca henriquesii Hackel, Monogr. 126 (1882).

Perennial, herbaceous chamaephyte; —; north-central areas (Serra da Estrela); grasslands.

Dactylis glomerata L. Sp. Pl. 71 (1753).

Perennial, herbaceous chamaephyte; 2n = 14, 28, 42 (JONES, CARROLL & BORRILL 1961); Europe, Asia, N. Africa,

Macaronesia; woodlands, grasslands, dunes, cliffs, and alpine screes.

At least eleven subspecies, one endemic to Portugal.

Subspecies *lusitanica* Stebbins & Zohary, Univ. Calif. Pub. Bot. 31 (1): 9 (1969).

2n = 14 (FERNANDES & QUEIRÓS 1969); northern and central areas; pine and oak forests, up to 900 m.a.s.l.

This subspecies does not appear to have constant ecological requirements, and its full distribution is not yet known; it appears to be rare and local in its occurrence (STEBBINS 1961).

Avenula occidentalis (Gervais) J. Holub, Folio Geobot. Phytotax. (Praha) 11: 295 (1976).

Annual, therophyte; —; central and south-west Portugal; dry scrub, and pine-woods.

This endemic is divided into two subspecies.

Subspecies *occidentalis.*

2n = 42 (HOLUB 1980); central areas.

Subspecies *stenophylla* Franco, Bot. Jour. Linn. Soc. 76: 359 (1978).

Southern areas, Algarve; heathlands.

Avenula deliculata Franco, Bot. Jour. Linn. Soc. 76: 359 (1978).

Annual, therophyte; —; north-east (Miranda do Douro, Montalegre, Bragança); arid mountain areas.

Avenula hackellii (Henriq.) J. Holub, Folio Geobot. Phytotax. (Praha) 11: 295 (1976).

Perennial, herbaceous chamaephyte; —; Alentejo and Algarve; dry places.

There is little information available about any of the above three recently described species. The whole taxon is in need of further investigation. In Portugal this may give endemic status to *Avenula marginata* (Lowe) Holub. from

the north-east, and certainly clarify the relationships between the endemics already described.

Pseudarrhanatherum palîens (Link) J. Holub, Taxon 15: 167 (1966).

Perennial, herbaceous chamaephyte; 2n = 14 (QUEIRÓS 1973a) as *Arrhenatherum longifolium* (Thore) Dulac; central areas; calcicole, dry grasslands.

Koeleria caudata (Link) Steudel, Syn. Pl. Glum. 1: 293 (1854).

Perennial, herbaceous chamaephyte; 2n =14 (FERNANDES & QUEIRÓS 1969) ; east-central areas, Trás-os-Montes, Minho, Beira; open woods and dry mountain grasslands.

Holcus setigiumis Boiss. & Reuter, Diagn. Pl. Nov. Hisp. 27 (1842).

Annual, therophyte ; 2n = 14 (QUEIRÓS 1973a) ; southern Europe; fields and other grassy places.

Two subspecies, one throughout the range, the other endemic to Portugal (TUTIN 1980a).

Subspecies *duriensis* P. Silva, Agron. Lusit. 18: 11 (1956).

2n - 14 (CASTRO, ex SILVA 1956) ; north-eastern areas, Trás-os-Montes, upper Douro and Beira.

Agrostis litigans Steudel, Syn. PL Glum. 1: 162 (1854).

Annual, therophyte; —•; southern areas (Serra da Arrábida) ; dry soils.

A very rare species, which certainly needs investigating. It has been suggested that it is a variant of *A. tenerrima* Trin. (TUTIN 1980b).

ORCHIDACEAE

Ophrys speculum Link in Schrader, Jour, für die Bot. 1799 (2): 324 (1800).

Perenial, tuberous geophyte; 2n = 36 (SHIMOYA 1956); Mediterranean region and central and southern Portugal, Beira to the Algarve; grassy places.

Two subspecies (Soó 1980), one endemic to Portugal, the other throughout the range of the species.

Subspecies *lusitanica* O. & A. Danesch, Orchidée 20: 21 (1969). West central areas (Coimbra).

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CHROMOSOME NUMBERS OF FUEGIAN ANGIOSPERMS

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ABSTKACT

Chromosome numbers are given for 117 of the 384 species of flowering plants native in Tierra del Fuego. 71 species have not previously been counted, including the first reported chromosome counts for the genera *Arjona, Bolax, Chiliophyllum, Donatio,, Dysopsis, Eriachaenium, Huanaco., Lepidophyllum, Misoäenärum, Nardophyllum, Phaiophleps, Phïlesia* and *Plagiobothrys,* as well as the family Misodendraceae. The list includes species at the highest levels of polyploidy yet recorded in 3 species, with perhaps, aneuploidy in another species. About 64 % of Fuegian angiosperms known cytologically are polyploid, thus being closely comparable to similar data from other southern cool temperate areas.

TIERRA del Fuego, the complex archipelago to the south of the Estrecho de Magallanes, is the most southerly area reached by a significant segment of a continental flora. Most of the 416 species of vascular plants (MOORE, 1974, unpub.) native in Tierra del Fuego have their affinities with temperate Argentina and Chile north to *c.* 40° S. lat. In addition, a significant and interesting proportion of the species belong to elements showing links across the tropics with cool temperate areas of the Northern Hemisphere and, via the circum-Antarctic islands, with the New Zealand region. Any understanding of the underlying causes of these affinities depends, among other things, upon a sound modern

* Dedicated to Professor Dr. ABÍLIO FERNANDES, distinguished student of plant chromosomes.

taxonomic basis. Whilst some of the groups present in Tierra del Fuego have been the subject of biosystematic and chemotaxonomie studies, much of our knowledge of the flora is at the level of «alpha-taxonomy», based largely on exomorphology. Cytologieal data are essential for understanding the evolution and relationships of closely related groups of plants, and information on chromosome numbers constitutes a first step in this direction. For this reason such information, related to taxonomic studies of plant groups or floristic studies of regions, is important. In the Southern Cool Temperate Zone the New Zealand region is to some extent covered by the surveys of RATTENBURY (1957), HAIR (1963), 1967a, *b, 1968,* 1970), HAIR and BEUZENBERG (1958, 1959, 1980, 1966, 1968), BEUZENBERG and HAIR (1959, 1963), GROVES and HAIR (1971), HAIR, BEUZENBERG and PEARSON (1967), BEUZENBERG (1970), and MOORE (1960); the South American sector, however, has received much less attention (e. g. RAHN, 1960; MOORE, 1967). For this reason it seems useful to put on record data on chromosome numbers which have been collected so far as part of a continuing study of the flora of Tierra del Fuego, botanically one of the most interesting cool temperate areas of the western hemisphere.

METHODS AND RESULTS

The material used consisted of buds collected in the field, or either buds or root-tips taken from plants cultivated at Reading from field collections of seeds or, rarely, living plants. Material was fixed in one of the modifications of Carnoy's solution and subsequently stored in 70 % ethanol at - 5° C. Meiotic configurations were examined in anther-squashes and mitosis was observed in squashes of root-tips pretreated with paradichorobenzene; all preparations were stained with aceto-orcein. Voucher herbarium specimens for all chromosome-counts are deposited in the herbarium of the University of Reading, as are permanent slides and photographs or drawings of the cytological preparations.

Chromosome numbers have been determined on Fuegian matetrial for 117 of the 384 species of flowering plants native in Tierra del Fuego; these are documented in Table I. The nomenclature used throughout is that adopted by MOORE (1974, unpub.); the sequence of families and genera being that of the forthcoming «Flora of Tierra del Fuego» (MOORE, unpub.), while the species within genera are in alphabetical order.

COMMENTS

Chromosome numbers are available for *c.* 42 % of the Fuegian angiosperm species, of which *c. SO %* have been determined from material originating in Tierra del Fuego (Table I), the remainder being derived from material from the Falkland Islands or the area immediately north of the Estrecho de Magallanes (MOORE, 1967). On these data about 64% of the Fuegian angiosperm species appear to be polyploid. There is thus a remarkably close agreemen with 63 % polyploidy in the Falkland Islands flora (mooRE, 1967), 63 % in the New Zealand flora (HAIR, 1966) and *c.* 62 % in the flora of the sub-Antarctic Macquarie Island (MOORE, 1960).

Genera **not previously counted**

As far as I am aware, chromosome numbers for 13 genera are reported here for the first time: *Arjona, Bolax, CMUophyllum, Donatio,, Dysopsis, Eriachaenium, Huanaca, Lepidophyllum, Misodendrum, Nardophiyílwm, Phaiophleps, PMlesia* and *Plagiobothrys.* The first count for the mistletoe-like family Misodendraceae suggests it has a basic chromosome number of $x = 6$, in contrast to the related hemiparasitic Loranthaceae, in which $n = 8$ is the lowest number (e. g. WIENS, 1964). The Fuegian endemic species of *Chiliophyllum* has the same chromosome number (n = 27) as *Chiliotrichum,* probably its closest relative, and *Nardophyllum,* all according with the commonest basic number ($x = 9$; SOLBRIG *et al,* 1964) known in the Compositae-Astereae, to which they belong. *Lepidophyllum* ($n = 20$), which is also included in

the same tribe may be based on $x = 5$ or 10 (SOLBRIG *et al.*, 1964; GRAU, 1977) which occur in some members of the Astereae. In the Umbelliferae, *Bolax* is a tetraploid based on $x = 8$, like some species of the related genus *Azorelta,* while *Huanaco,* is the second genus, after *Laretia,* in the tribe Mulineae to have a basic number of $x = 9$ (MOORE, 1971). Of the other genera *Donatia* (n = 24) contrasts with the few other members of the Stylidiaceae for which data are available, all having $n = 15$ (e. g. HAIR and BEUZENBERG, 1959), while *Philesia* (n = 6) differs ehromosomally from another genus *Luxuriaga* (n = 10), with which it is sometimes included in the segregate family Philesiaceae (MOORE, 1967). *Plagiobothrys* ($n = 34$) is apparently based on x — 17, not hitherto reported for the Boragmaceae and *Arjona* ($n = 14$) is presumably based on $x = 7$, reported once in the Santalaceae, for a species of *Thesium* (BAKSAY, 1961). *Dysopsis* ($n = 14$) has a number which is rather widespread in the Euphorbiaceae and, similarly, that of *Phaiophleps* (n = 9) is common in related genera of the Iridaceae.

Species not previously counted

In addition to those considered above a further 58 of the species whose chromosome numbers are documented in Table I have not been counted previously. The majority of these merit no further discussion here since they have chromosome numbers which are either well-known in the genera, e. g. *Astragalus* (GOMEZ-SOSA, 1979), *Ribes* (ZiELiNSKI, 1953), *Lagenophora* (ARANO, 1965), *Jaborosa* (RATERA, 1944) and *Alstroerneria* (FEDEROV, 1969), or accord with the basic numbers that can be deduced for the genera from previous data. Thus, for example, *Samolus spathulatus* ($n = 39$) is the first hexaploid reported for the genus, in which the basic number appears to be a;=13 (FEDEROV, 1969).

A few species are, however, worthy of comment. *Perezia magellanica,* with $n = 19$, provides a new basic number in the genus, for which $n = 4$, 8, 12 and 27 have previously been reported (VUILLEUMIER, 1969). *Cardamine geraniifolia* ($2n = 144$) shows the highest level of polyploidy (18x)

accurately determined for the genus, although BORGMANN (1964) reported a diploid number of *c.* 150 for an undetermined species from New Guinea. MOORE (1967) gave an approximate count of *2n* = *c.* 200-220 for Falkland Islands material of *Cotula scariosa;* this is now shown to have been very imprecise since good preparations from Fuegian collections show the species to be 20-ploid based on *x* = 13, which is characteristic for subgenus *Leptinella* (HAIR, 1962). As pointed out by MOORE (1973), the previous report of *2n* = *c.* 20 for *Drapetes muscosus* (MOORE, 1967) from the Falkland Islands must be corrected; the species clearly has 2*n* = 18 in Fuegia, thus according with the basic number (*x* = 9) prevalent in the Thymelaeaceae.

Species previously counted

The chromosome numbers of 46 species documented in this paper have been counted previously and all the earlier records are confirmed here. Eleven of these species are temperate bipolar disjuncts and this study confirms the chromosomal similarity between Northern and Southern Hemisphere populations of *Anemone multifida, Armería maritima, Carex magellanica, C. microglocMn, Galium aparíñe, Hippuris vulgaris, Koenigia islándica, Microsteris gracilis, Phleum alpinum, Plantago maritima, Polemonium micranthum* and *Triglochin palustris.* Interestingly, while counts of 2n = 22, 44, 66 and 88 are available for Northern Hemisphere material of *Galium aparine* (FEDEROV, 1969), only the hexaploid has so far been encountered in Tierra del Fuego.

Intraspecific chromosomal variation

Four species show intraspecific variation in chromosome number. Whilst most species of *Leucheria* for which there are data have *n* = 20 (CRISCI, 1976), *L. hahnii* shows an euploid variation with both 19 and 20 occurring as haploid numbers. This sort of variation may explain the report of *n* = 19 or 20 for *L. achillaeifolia* by CRISCI (1974). Two species of *Senecio* show euploid variation. *S. acanthifolius,* a distinctive and not particularly variable species, is normally

tettraploid (*n* = 20) but in one locality the octoploid was also present. *S. tricuspidatus* is a conspicuously variable species within which tetraploid, octoploid and 16-ploid populations have been found in Tierra del Fuego; so far no correlation has been detected between the variation in chromosome number and that in morphology or habitat or geographical factors. *Acaena magellanica* is the only member of the genus to show intraspecific variation in chromosome number. As noted by MOORE (1972), *A. magellanica* is «diploid» (*2n* = 42) on the sub-Antarctic islands and in the climatically similar regions of easternmost Tierra del Fuego, while «tetraploids» (*2n* = 84) are found in the forested regions of Fuegia, S. Chile and the Falkland Islands, which floristically belong to the deciduous forest areas along the Andes, and may perhaps chart the migration from open to more closed vegetation-types.

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TABLE I

Documented chromosome numbers of Fuegian angiosperms

Species	n	*2n*	Locality t	Collector + and number
Berberidaceae				
Berberís buxifolia Lam.	14		A: Isla Grande; Ea Harberton, Peninsula	G 853
ilicifolia L. f.	14		A: Isla Grande; Ea Harberton	G 3886
Ranunculaceae				
Caltha sagittata Cav.	24		A: Isla Grande; Ea Harberton, near lake	G 1803
Ranunculus fuegianus Speg.	24		A: Isla Grande; Ea José Menendez, Arroyo Guanaco	M 2527
minutiflorus Bert, ex Phil.		32	A: Isla Grande; Ea Harberton, Campo Afuera	G 1029
peäuncularis Sm.	16		A: Isla Grande; Ea Cullen, Río Alfa	M 2601
	16		C: Prov. Magallanes; Ea Cerro Guido	M 1009
unifloras Phil, ex Reiche		32	C: Isla Grande; Ea Cameron, Torcido Chico	M 1065
Anemone multifida Poiret		32	A: Isla Grande; Ea Cullen, Arroyo Beta	G 3242
Fagaceae				
Nothofagus pumilio (Poeppig & Endl.) Krasser		26	A: Isla Grande; Ea Punta Segunda	M s. n.
Caryophyllaceae				
Silène magellanica (Desr.) Bocquet	12		A: Isla Grande; Ea Cullen, Río Alfa	M 2592
Chenopodiaceae				
Chenopodium antarcticum (Hooker f.) Bentham & Hooker	8		C: Isla Grande; Porvenir, 10 km S.	M & G 55
		16	A: Isla Grande; Las Violetas; Cabo Domingo	M 2508
Salicornia ambigua Michx	9		A: Isla Grande; Ea Cullen, Campo Playa	G 1894
Suaeda argentinensis Soriano	18		C: Isla Grande; Ea Río Hondo, Río Marazzi	M 2409
patagonica Speg.	18		A: Isla Grande; Ea Cullen, Campo Playa	G 1895
Portulacaceae				
Calandrinia fuegiana Gandoger		24	A: Isla Grande; Ea, San José; Río Rasmussen	M 2537
Polygonaceae				
« olruvt /7ir*rt. T.		28	C: Isla Grande; Río Azopardo, 2 km from mouth	M 2295

I *Cálandrinia fuegiana* Gandoger	I A: Isla Grande; Ea San José; Río Rasmussen	M	2537
I Polygonaceae			
Koenigia islándica L.	C: Isla Grande; Rio Azopardo, 2 km from mouth	M	2295
Rumex magellanicus Campd.	A: Isla Grande; Bahia Thetis	G	2370
Plumbaginaceae			
Armería maritima L. ssp. *andina* (Poeppig ex Boiss.) D. M. Moore & Yates	A: Isla Grande; Ea Harberton, head of bay	G	1039
Cruciferae			
Sisymbrium magellanicum (Pers.) Hooker f.	A: Isla Grande; Ea Harberton, Campo Cutalataca	G	991
C*ardamine geraniifolia* (Poiret) DC.	C: Isla Grande; Puerto Yartou, sawmill	M	2451
glacialis	A: Isla Grande; Lago Fagnano, Fabrica Kami	M	2630
	A Isla Grande; Ea Harberton, near lake	G	1806
	C: Isla Grande; Caleta Josefina to Río Chico	M	2419
	C: Isla Capitán Aracena; Bahía Morris	M	2771
Lepidium pseudodidymus Thellung	C: Isla Grande; Punta Catalina, Cuarto Chorillo	M	2343
Primulaceae			
Primula magellanica Lehm.	C: Isla Grande; Ea Cameron, Torcido Chico	M	1077
Samolus spathulatus (Cav.) Duby	C: Isla Grande; S. of Porvenir, Lago Barrosa	G & L	66
Rosaceae			
Geum magellanicum Comm. ex Pers.	A: Isla Grande; Bahía Thetis	G	2356
	C: Isla Grande; Puerto Yartou, sawmill	M	2449
parviflorum Comm. ex Sm.	A: Isla Grande; Montes Martiales	G	2794
Acaena antárctica Hooker f.	C: Isla Grande; Río Azopardo	M	2301
magellanica (Lam.) Vahl	A: Isla Grande; Bahía Thetis	G	2283
	A: Isla Grande; Ea Harberton,	G	1844
pinnatifida Ruiz & Pavón	C: Isla Grande; Punta Catalina, Cuarto Chorillo	M	2345
Saxifraguceae			
Ribes magellanicum Poiret	A: Isla Grande; Ea Harberton, Peninsula	G	845
Leguminosae			
Astragalus palenae (Phil.) Reiche	A: Isla Grande; Ea Los Flamencos, Laguna Miranda	G	2512
	A: Isla Grande; Ea Las Violetas, Cabo Domingo	M	1497

TABLE I

(Continuation)

Species	*n*	*2n*	Locality	Collector -(- and number
Vicia bijuga Gillies ex Hooker & Arn.		14	C: Isla Grande; Punta Catalina	M 2387
		14	A: Isla Grande; Ea San Martín, Bahía San Sebastian	M & G 389
		14	A: Isla Grande; Ea San Martín, Bahía San Sebastian	G 4290
		14	A: Isla Grande; Río Grande, Punta Popper	G 3295
		14(21)	A: Isla Grande; Ea San Julio, Cerro Hongo	M 2557
Vida magellanica Hooker f.		28	C: Isla Grande; Ea Vicuña, near settlement	M s. n.
		28	A: Isla Grande; Ea CuUen, Arroyobeta	G 4318
		28	A: Isla Grande; Ea San Martín, Bahía San Sebastian	M & G 382
	14	28	A: Isla Grande; Ea Harberton, 1st West Creek	G 2659
		28	A: Isla Grande; Ea Harberton, Isla Uru Waru	G 3580
Lathyrus magellanicus Lam.		14	C: Isla Grande; Punta Espora	M 2362
		14	A: Isla Grande; Ea José Menendez, Punta Maria	G 1771
	7		A: Isla Grande; Ea San Martín, Bahía San Sebastian	G 2580
Hippuridaceae				
Hippuris vulgaris L.		32	A: Isla Grande; Ea Harberton, Harberton swamp	G 83
Thymelaeaceae				
Drapetes muscosus Banks ex Lam.	9		A: Isla Grande; Ea Moat, Laguna Moat	G 886
		18	C: Isla Grande; Caleta Josefina to Río Chico	M 2422
Onagraceae				
Fuchsia magellanica Lam.	22		A: Isla Grande; Ea Harberton, Peninsula	G 2719
Epilobium australe Poepp. & Hausskn. ex Hausskn.	18		A: Isla Grande; Ea Harberton, mte Spion Kop	M 2803
Proteaceae				
Embothrium coccineum Forster & Forster f.	11		A: Isla Grande; Ea Harberton, Peninsula	G 850
Santalaceae				
Arjona patagónica Hombron & Jacq.	14		A: Isla Grande; Ea Los Flamencos, Laguna Miranda	G 2525
	14		C: Isla Grande; Punta Espora	M 2366
[Misodendraceae				
Misodendrum vunctülatum Banks & Sol. ex|				

[1] Misodezidraceae			UMj
Mísodendrum punctulatum Banks & Sol. ex Forster f.			
Euphorbiaceae			
Dysopsis glechomoiäes (A. Richard) Muller Arg.			
Rhamnaceae			
Discaria serratifolia (Vent.) Bentham & Hooker			
Umbelliferae			
Azorella caespitosa Cav.	*>.. ^xa, «""iuc, ^a uua i laxiiKiico», alaguna Miranda	G	2525
	C: Isla Grande; Punta Espora	M	*2366*
filamentosa Lam.	A: Isla Grande; Ea Harberton, Peninsula	*G*	835
lycopodioides Gaudich.	C: Isla Grande; Caleta Josefina to Río Chico	M	2421
selago Hooker f.	A: Isla Grande; Ea Harberton, Peninsula	G	899
	A: Isla Grande; Ea Harberton, Peninsula	G	2505
trifurcata (Gaertner) Hooker f.	C: Isla Grande; Porvenir to Caleta Josefina	M & G	47
	A: Isla Grande; Ea Harberton	CONSTANCE	*et* t0ï., 1971
	A: Isla Grande; Ea José Menendez	CONSTANCE	*et al.*, 1971
	A: Isla Grande; Ea Harberton, Campo Cutalataca	G	875
	A: Isla Grande; Ea Harberton	CONSTANCE	*et al.*, 1971
	A: Isla Grande; Montes Martiales, below Glacier	G	2785
Bolax gummifera (Lam.) Sprengel	A: Isla Grande; Ea Harberton	CONSTANCE	*et al.*, **1971**
Huanaca acaulis Cav.	A: Isla Grande: Ea Viamonte	OOSNTANCE	*et al.* **1971**
Osmorrhiza depauperata Phil.	A: Isla Grande; Montes Martiales, below Glacier	G	2616
	A: Isla Grande; Ea Los Flamencos, Laguna Miranda	G	2528
D(MtCMs *montanus* Humb. & Bonpl. ex Sprengel	C: Isla Grande; Punta Espora	M	2363
Oreomyrrhis hookeri Mathias & Const.	C: Isla Grande; Caleta Josefina, Aserr. Las Golondrinas	M	2426
Apiwm australe Thouars	A: Isla Grande; Lago Fagnano, Aserr. Las Lengas	M	2629
	C: Isla Grande; Ea Cameron, Río Mayo	M	2234
	A: Isla Grande; Ea Harberton, Peninsula	G	309
	C: Isla Capitán Aracena; Bahía Morris	M	**2773**

TABLE I

(Continuation)

Species	*n*	*2n*	Locality t	Collector -j- and number
Gentianaceae				
Gentianella magéllanica (Gaudich.) Fabris ex D. M. Moore	18		A: Isla Grande; Ea Harberton, Campo Tropilla	M 1331
Solanaceae				
Jaborosa magéllanica Griseb.	12		A: Isla Grande; Ea Cullen, Campo Playa	G 2538
	12		C: Isla Grande; Punta Catalina, W. side	M 2390
		24	A: Isla Grande; Ea Las Violetas, Cabo Domingo	M 1494
Polemoniaceae				
Polemonium micranthum Bentham		18	A: Isla Grande; Ea San Martín, Bahía San Sebastian	G 2582
		18	C: Isla Grande; Punta Espora	M 2358
Microsteris gracilis (Douglas ex Hooker) Greene	7		A: Isla Grande; Ea José Menendez, Punta María	G 195
Hydrophyllaceae				
Phacelia magéllanica (Lam.) Coville	11		A: Isla Grande; Ea Los Flamencos, Laguna Miranda	G 2521
	11		C: Isla Grande; Punta Catalina, Cuarto Chorillo	M 2351
Boraginaceae				
Plagiobothrys calanärinioides (Phil.)	34		A: Isla Grande; Rio Grande, Misión	G 516
Plantaginaceae				
Plantago barbata Forster f.	24		A: Isla Grande; Sierra Sorondo, Paso Garibaldi	M 1596
maritima L.		12	C: Isla Grande; Ea Cameron, Bahía Inutil	M 1083
		12	A: Isla Grande; Ea Cullen, Campo Playa	M 1470
		12	A: Isla Grande; Ea Santa Ana, Cabo San Pablo	M 1514
		12	A: Isla Grande; Ea Las Violetas, Cabo Domingo	M 1490
	6		A: Isla Grande; Ea Policarpo	G 2269
		12	A: Isla Grande; Seno Almirantazgo, Isla Tres Mogotes	M s. n.
		12	C: Isla Navarino; Caleta Wulaia	B 50-30
Serophuíarlaceae			Talo nranrl»' *V,a* Viftmonte	M 1274

	A. !»ia urauus; seno Almirantazgo, Isla Tres Mogotes	M	s. n.
	C: Isla Navarino; Caleta Wulaia	B	50-30
Scrophulariaceae			
Limosella australis R. Br.	A: Isla Grande; Ea Viamonte	M	1274
Calceolaria uniflora Lam.	C: Isla Grande; Punta Espora	M	2364
Hebe elliptica (Forster f.) Pennell	C: Islas Wollaston; Isla Bayley (Cult. Ea Harberton)	G	1863
Stylidiaceae			
Donatio fascicularis Forster & Forster f.	A: Isla Grande; Bahía Thetis	G	2243
Rubiaceae			
Galium aparine L.	C: Isla Grande; Bahía Inutil, Puesto Maria	M	2432
fuegianum Hooker f.	C: Isla Grande; Porvenir to Caleta Josefina	M & G	26
Valerianaceae			
Valeriana carnosa Sm.	A: Isla Grande; Ea Viamonte	G	939
	C: Isla Grande; Punta Espora	M	2365
Calyceraceae			
Boopis australis Decne	C: Isla Grande; Punta Baja	M	2372
	C: Isla Grande; Ea Río Hondo, Río Marazzi	M	2410
Compositae			
Lagenophora hariotii Franchet	C: Isla Grande, Puerto Yartou	M	2467
Lepiäophyllum cupressiforme (Lam.) Cass.	C: Isla Grande; Punta Catalina, Cuarto Chorillo	M	2355
Nardophyllum bryoiäes (Lam.) Cabrera	C: Isla Grande Penin. Juan Mazia, Bahía Lee	M	2339
Chiliotrichum diffusum (Forster f.) O. Kuntze	A: Isla Grande Bahía Thetis	G	2307
	C: Isla Grande; Altos de Boquerón	M & G	132
	A: Isla de los Estados; Puerto San Juan	DGC	912
	C: Isla Grande; Ea Cameron	M	1082
Ohiliophyllum fuegianum O. Hoffmann	A: Isla Grande Ea Cullen, Río Alfa	M & G	356
Erigeron patagonicus Phil.	C: Isla Grande; Ea Río Hondo, Río Marazzi	M	2411
Aster vahlii (Gaudich.) Hooker & Arn.	A: Isla Grande Bahía Thetis	G	2371
Baccharis magellanica (Lam.) Pers.	C: Isla Grande; 10-km S. Of Porvenir	M & G	65
Gamochaeta spiciformis (Seh. Bip.) Cabrera	A: Isla Grande Ea Harberton, Cerro No Top	M	1388
	A: Isla Grande Ea Harberton, Mte. Hueheupen	M	2840
	C: Isla Grande; Caleta Josefina to Rio Chico	M	1638

TABLE I

(Continuation)

Species	*n*	*2n*	Locality *f*	Collector 4- and number
Eriachaenium magellanicum Sch. Bip		c. 36	A: Isla Grande Ea José Menendez, Punta Maria	G 975
Cotula scariosa (Cass.) Franchet	131		A: Isla Grande Bahía Thetis	G 2296
	130		C: Isla Grande Ea Rio Hondo, Rio Marazzi	M 2408
Artemisia magellanica Sch. Bip.		22	A: Isla Grande Ea Las Violetas, coast	M & G 318
Senecio acanthifoMus Hombron & Jacq.	20		C: Isla Grande Caleta Josefina to Río Chico	M 2427
	20		C: Isla Grande Puerto Yartou	M 2455
	20		A: Isla Grande Sierra Sorondo, Paso Garibaldi	M 1582
	20		A: Isla Grande Bahía Thetis	G 2378
	40		A: Isla Grande Bahía Thetis	G 2308
candidans DC.	20		A: Isla Grande Bahía Thetis	G 2254
	20		C: Isla Grande Puerto Arturo to Puerto Yartou	M 2459
eightsii (Hooker f.) Cabrera	40		A: Isla de los Estados; Puerto Cook	DGC 827
humifums (Hooker f.) Cabrera	60		A: Isla Grande; Montes Martiales, below Glacier	G 2790
laseguei Hombron & Jacq.	20		A: Isla Grande; Ea Cullen, Campo Playa	M 1457
magellanicus Hooker & Arn.	40		A: Isla Grande; Ea La Marina	M 69085 *
patagonicus Hooker & Arn.	40		A: Isla Grande; Ea Harberton, Twin Islands	M 1811
	40		C: Isla Grande; Altos de Boquerón	M & G 90
	40		A: Isla Grande; Ea Cullen, Arroyo Beta	M & G 326
	40		A: Isla Grande; Ea San Sebastian, coast	M 2577
smithii DC.	20		C: Isla Capitán Aracena; Bahía Harris	M 2734
tricuspidatus Hooker & Arn.	20		A: Isla Grande; Ea Harberton, Campo Cutalataca	M 1401
	20		C: Isla Grande; Ea Cameron, Lago Blanco	M 2159
	20		C: Isla Grande; Ea Río Hondo, Río Marazzi	M 2406
	40		A: Isla Grande; Ea Harberton, Río Varela	M 1359
	40		A: Isla Grande; Ea Harberton, Cerro No Top	M 1746
	60		A: Isla Grande; Ea Harberton, Valle Lashifashaj	M 2078
trifurcatus (Forster f.) Less.	20	40	A: Isla de los Estados; Puerto Parry	DGC 1718
websteri Hooker f.		40	I -A-: Grande; Bahía, Tlietis	^^^^^^^ I
Nassauvia darwinii (Hooker & Arn.) O. Hoffm. & Dusén			A: Isla Grande; Ea San Sebastian, Barrancas Carmen Sylva	M & G 262 l

	trifurcatus (Forster f.) Less.	i A: isia urande; Ea Harberton, Valle Lashifashaj	M	2078
	websteri Hooker f.	A: Isla de los Estados; Puerto Parry	DGC	1718
	Nassauvia darwinii (Hooker & Arn.) *O. Hofim.* & Düsen	I A: Isla Grande; Bahía Thetis		
	Nassauvia magellanica J. F. Gmelin	A: Isla Grande	Ea San Sebastian, Barrancas Carmen Sylva	M & G 262
	Leucheria hahnii Franchet	A: Isla Grande	Ea Harberton, Cerro No Top	M 1731
		C: Isla Grande	Caleta Josefina to Río Chico	M 2415
		C: Isla Grande	Altos de Boquerón	M & G 182
		A: Isla Grande	Ea Harberton, Cerro No Top	M 2785
		A: Isla Grande	Ea José Menendez, Arroyo Guanaco	M 2515
		A: Isla Grande	Ea Cullen, S. of settlement	M 2586
		A: Isla Grand?	Ea San Julio, Cerro Hongo	M 2545
	purpurea (Vahl) Hooker & Arn.	C: Isla Grande	Ea Río Hondo, Río Marazzi	M 2403
	Peresia magellanica (L. f.) Lag.	A: Isla Grande	Ea Harberton, Mte Spion Kop	M 2797
		C: Isla Grande	Lago Blanco, Cerro Cuchillo	M 69051 *
	pilifera (D. Don) Hooker & Arn.	A: Isla Grande	Ea José Menendez, Arroyo Guanaco	M 2514
		C: Isla Grande	Ea Rio Hondo, Rio Marazzi	M 2404
	recurvata (Vahl) Less.	A: Isla Grande	Ea Punta Segunda	M 69047 *
	Hypochoeris arenaria Gaudich.	A: Isla Grande	Ea San Julio, Cerro Hongo	M 2549
		A: Isla Grande	Ea San Sebastian, Barrancas Carmen Sylva	M & G 268
		C: Isla Grande	Ea Río Hondo, Río Marazzi	M 2407
		C: Isla Grande	Punta Baja	M 2373
	Hypochoeris incana (Hooker & Arn.) Macloskie	A: Isla Grande; Ea Harberton, Campo Afuera		M 1382
	Taraxacum gilliesii Hooker & Arn.	A: Isla Grande; Ea José Menendez, Cabo Peñas		G 1675
		C: Isla Grande; Seno Almirantazgo, Isla Tres Mogotes		M 69011 *
		A: Isla Grande; Ea Harberton, Peninsula		M 69009 *
		A: Isla Grande; Bahía Aguirre, Puerto Español		M 69008 *
		C: Isla, Grande; Punta Catalina, W. side		M 2381
	Agoseris coronopifolium (D'Urv.) Chambers ex D. M. Moore	C: Isla Grande; Ea Vicuña, settlement		M 2193
		A: Isla Grande; Ea Las Violetas, Cabo Domingo		M 2505
	Hieracium antarcticum D'Urv.	A: Isla Grande; Ea Harberton, Campo Afuera, Cerro Flat Top		M 1377
	Juncaginaceae			
	Triglochin concinna Davy	A: Isla Grande; Ea Harberton, head of bay		G 1041
	palustris L.	A: Isla Grande; Ea Harberton, Campo Lagunas Abajo		M 1345

TABLE I

(Continuation)

Locality	*n*	*2n*	Locality f	Collector +• and number
Gramineae				
Agropyron antarcticum Parodi		*28*	A: Isla Grande; Lapatia	HUNZIKEE, 1966
magellanicum (Desv.) Hackel		28	A: Isla Grande; Ea San Martin, Bahía San Sebastian	HUNZIKER, 1966
Elymus antarcticus Hooker f.		28	A: Isla Grande; Ushuaia	HUNZIKER, 1966
Phleum alpinum		28	A: Isla Grande; Ea Harberton, Campo Afuera, Cerro Flat Top	M 1394
Cyperaceae				
Carex magellanica Lam.		58	A: Isla Grande; Ea Harberton, Campo Lagunas Abajo	M s. n.
microglochin Wahlenb.		58	A: Isla Grande; Ea Harberton, Campo Lagunas Abajo	M 1343
Iridaceae				
Sisyrinchium patagonicum Phil, ex Baker		64	C: Isla Grande; Porvenir	M 2314
Phaiophleps biflora (Thunb.) R. C Foster		18	A: Isla Grande; Ea Remolino	M 1997
Liliaceae				
Philesia magellanica J. P. Gmelin		12	C: Isla Dawson, Coll. O. Magens	M 2316
Amaryllidaceae				
Alstroemeria patagónica Phil.		! 16	A: Isla Grande; Ea Las Violetas, coast	G 2589

t Locality. C = Chile; A = Argentina.

+ Collectors' abbreviations: DGC — DUDLEY, GOODALL and CROW; G — GooDALL; B —BARRETT; G & L — GOODALL and LUTKEN; M — MOORE; M & G — MOORE and GOOD ALL.

* Culture numbers of living plants held in garden cultivation.

Bol. Soc. Brot., Sér. 2, 53(2): 1013-1036 (1981).

APORTACIONES AL ESTUDIO DE LA FLORA DE ANDALUCÍA ORIENTAL: PROVINCIA DE JAEN (ESPAÑA)

por

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SUMMARY

In this paper we comment on the écologie, chorologic and syntaxonomic behavior of several taxa in the Iberian Peninsula and specially in the province of Jaén (Spain). The majority of these taxa are reported for the first time in this province; *Digitalis purpurea* L. subsp. *heywoodii* P. & M. Silva is reported for the first time in Spain. The chromosome numbers and karyotypes for 3 taxa are studied. The chromosome numbers are as follows: *Digitalis purpurea* L. subsp. *heywoodü* P. & M. Silva has 2n = 56; *Catananche lutea* L. subsp. *carpholepis* (Schultz Bip.) Nyman has 2n = 18; *Sternbergia lutea* (L.) Ker-Gawler ex Roemer & Schultes has 3x = 30; the possible basic number of the genus *Sternbergia* is discussed.

rESUMEN

En el presente trabajo se estudia la ecología, corología y sintaxonomía de varios táxones de la Peninsula Ibérica y especialmente en la, provincia de Jaén (España). La mayoría se citan por vez primera en dicha provincia; *Digitalis purpurea* L. subsp. *heywoodii* P. & M. Silva se cita por primera vez para España. Asimismo se estudian los números cromosómicos y cariotipos de tres táxones; los números cromosómicos son los siguientes: *Digitalis purpurea* L. subsp. *heywoodü* P. & M. Silva tiene 2n = 56; *Catananche lutea* L. subsp. *carpholepis* (Schultz Bip.) Nyman, 2n = 18; *Sternbergia lutea* (L.) Ker-Gawler ex Roemer & Schultes, 3x = 30; se discute el posible número básico del género *Sternbergia.*

INTRODUCCIÓN

EN este trabajo señalamos la presencia de una serie de táxones en la provincia de Jaén que consideramos de interés por ser nuevas citas o encontrarse de manera escasa; una de ellas es a la vez primera cita para España.

En aquellos casos que se ha creido conveniente se ha confeccionado un mapa corológico en el que se señalan las citas incluidas en el Atlas Florae Europeae y además las existentes en los herbarios MA, MAF, SEV, GDA y GDAC; la cita aportada por nosotros va señalada con una flecha en los mapas correspondientes.

Para el estudio de los números cromosómicos y cariotipos se han utilizado meristemos radicales obtenidos por cultivo de los especímenes en macetas. Pretratamiento con 8-hidroxiquinoleína 0,002 M durante 2-6 horas. Las preparaciones se obtuvieron por aplastamiento y se tiñeron con orceína acética. La terminología empleada para la descripción de los cariotipos es la de LEVAN, FREDGA & SANDBERG (1954). Todos los testigos están depositados en el herbario de la Facultad de Ciencias de Granada (GDAC).

Ophioglossum lusitanicum L., Sp. Pl.: **1063** (1753).

JAÉN: 30SVH3125. Carretera del Rumblar, Baños de la Encina, XII-1979, *F. Valle* (GDAC 6301 y 6302).

Son pocas las citas de este taxon en Andalucía Oriental; aunque creemos no ha sido citado con anterioridad en la provincia de Jaén no dudamos su existencia en otras localidades del Norte de la misma.

También queremos señalar la existencia de un pliego de esta especie en MAF herborizado en Sierra Nevada (Granada) por RIVAS MATEOS, localidad ésta no recogida en el Atlas Florae Europeae (J. JALAS & J. SNOMINEN, 1972).

Distribución peninsular: Provincias corológicas Atlántica, Luso-Extremadurense y Gaditano-Onubo-Algarviense, así como localidades disyuntas en la Bética y costa mediterránea (Figura 1).

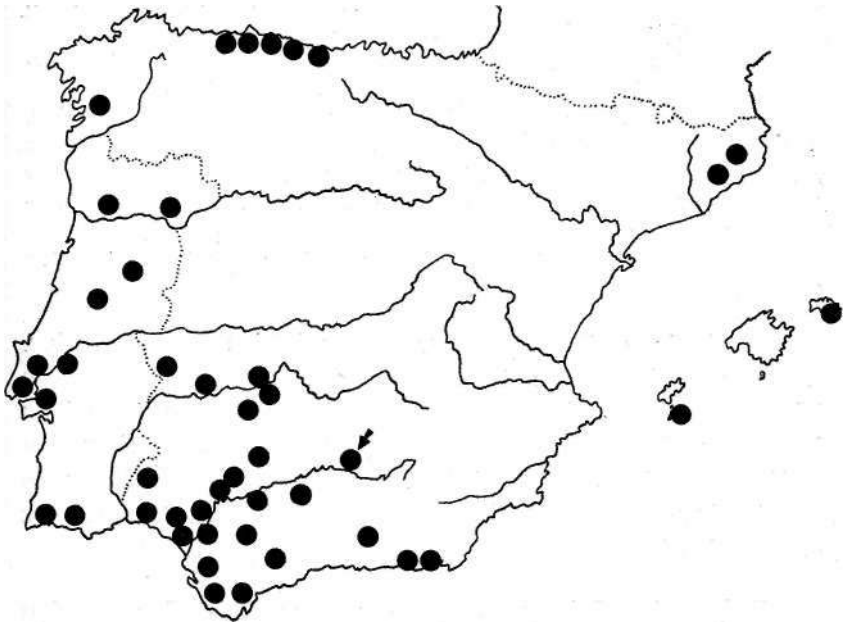

Fig. 1. — Distribución peninsular de *Ophioglossum lusitanicum* L.

Exsiccata

ASTURIAS: Gijón, viii-1928, *Miranda* (MA 1); Posada de Llanera, sin fecha, *Mayor & al.* (MA 185526).

BADAJOZ: Entre San Pedro de Mérida y Trujillo, 15-XI-1976, *Costa & al.* (MA 207578); Albuquerque, 26-XI-1972, *Rivas Goday & Ladero* (MAP 83620) ; entre Trujillanes y San Pedro de Alcántara, 24-XI-1972, *Rivas Goday & Ladero* (MAF 83621); Dehesa de Trujillanos, 2-XI-1976, *Ladero, Pérez Chiscano & Rivas Goday* (MAP 95515); Presa de Orellana, 14-XI-1976, *Peres Chiscano* (MAF 99382).

BALEARES: Formentera, 27-X-1920, *Gros* (MA 7).

CADIZ: Chipiona, 3-XI-1978, *Galiano & al.* (SEV 41799); Bornos, 17-111-1974, *Silvestre* (SEV 16814); Algeciras, 9-II-1977, *Mólesworth* (SEV 616); Chiclana, 28-III-1968, *Galiano & al.* (SEV 1413).

GERONA: La Sellera, sin fecha, *Casares Gil* (MA 2).

GRANADA: Sierra Nevada, sin fecha, *Rivas Mateos* (MAF 72862).

HUELVA: Cala, Mezquita, 1-1870, *Rodriguez* (MA 3); Hinojos, 19-III-1976, *Galiano & al.* (SEV 24085); Paimogo, 20-III-1976, *Galiano & al.* (SEV 24084); Aljaraque, 29-XII-1967, *Sánchez Jurado* (MAF 101196); Doñana, 6-II-1966, *Galiano* (SEV 23551); El Granado, 2-iii-

-1977, *Cabezudo & Silvestre* (SEV 27454); entre Almonte y el Rocío, 9-III-1975, *B. Valdês* (SEV 23546).

MALAGA: Sierra de Ronda, sin fecha, *Rivas Mateos* (MAF 72863).

PONTEVEDRA: Sin localidad ni fecha, *Merino* (MA 4).

SEVILLA: Entre Venta del Alto y el Ronquillo, 27-H-1972, *Galiano, Silvestre & B. Valdês* (MAF 98706); entre Puebla de Infantes y Constantina, *Cabezudo & B. Valdês* (SEV 32497) ; entre Bollullos y Aznalcazar, l-IV-1969, *P. Gibbs & al.* (SEV 7315); Gantillana, 27-XI--1969, *Domínguez* (SEV 3600).

PORTUGAL: Alrededores de Porto, Leça, XI-1909, *Johnston* (MAF 9).

Ecología y Fitosociología: Suelos ácidos muy húmedos, a veces encharcados; también se ha indicado en arenas húmedas soportando incluso una gran salinidad. Nosotros la hemos observado en comunidades de la al. *Tuberarion guttatae* Br.-Bl. 1931 en tránsito a otras más nitrófilas de la al. *Taenianthero-Áégilopion geniculatae* Rivas Martínez & Izco 1977.

Polypodium interjectum Shivas, Jour. Linn. Soc. London (Bot.) 58: 29 (1961).

Polypodium vulgare L., Sp. Pl.: 1085 (1753) var. *.prionodes* Ascherson in Ascherson & Graebner, Syn. *Mitteleur. Fl.* 1: 94 (1896).

Polypodium vulgare L. (1. c.) subsp. *prionodes* (Ascherson) Rothm., Mitt. Thür. Bot. Ver. 38: 106 (1929).

Polypodium serratum sensu Hess, Landolt & Hirzel, Fl. Schweiz 1: 106 (1967); nom Willd. (1810).

JAÊN: 30SVH3442. Tres Hermanas, El Centenillo, 6-IV-1977, *G. Blanca, J. A. Gil & F. Valle* (GDAC 3232 y 3233).

Aunque frecuente en el Sur de la Península (E. SALVO com. pers.) son muy escasas las citas que tenemos de ella en nuestra región; la nuestra sería la primera dada para este taxon en la provincia de Jaén.

Distribución peninsular: Muy mal conocida. Aparece en toda la Península, aunque menos frecuente en áreas más influenciadas por el clima mediterráneo (Figura 2).

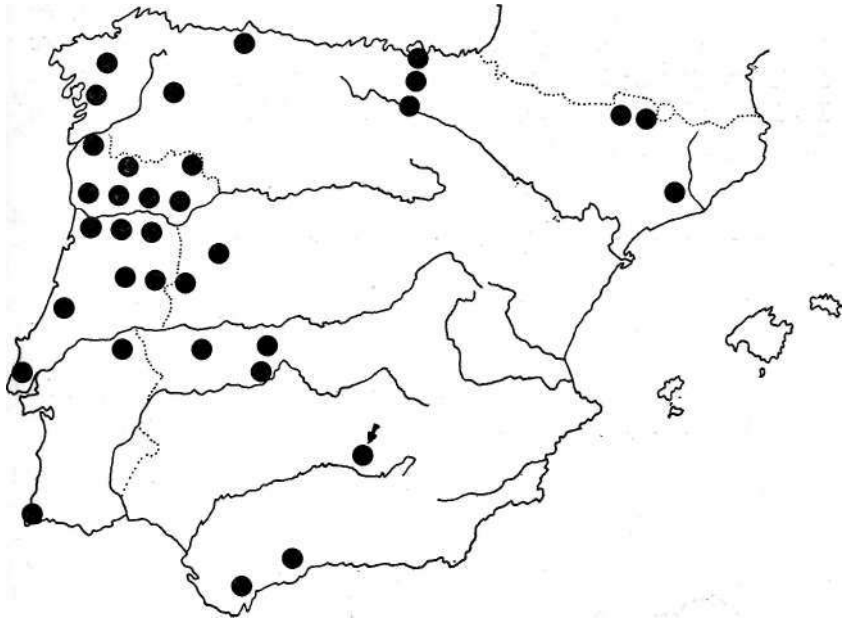

Fig. 2, — Distribución peninsular de *Polypodium interjectum* Shivas

Exsiccata

ÁLAVA: Altos de Encía, 13-VI-1926, *Ruiz de Azúa* (MA 1918).

CÁCERES: Navatrasierra, 7-II-1976, *Ladero* (MAF 80577); S.» de Altamira, Carrascalejo, 13-VI-1966, *Ladero* (MAF 84045); Torrejón el Rubio, 15-1-1977, *Pérez Chiscano* (MAF 103909).

CORUJA: Santiago de Compostela, 25-IV-1975, *A. Mijares & Losa Quintana* (MAF 101693).

MALAGA: S.ª de Aguas, 11-IV-1972, *G. López* (MAF 89402).

OVIEDO: Valle de Belmonte, 23-IV-1970, *Rivas Goday* (MAF 83429).

PONTEVEDRA: Moaña, 23-III-1970, *Castroviejo* (MAF 76957).

VIZCAYA: Begoña, 5-1-1926, *Ruiz de Azúa* (MAF 1919).

PORTUGAL: Eirol, Beira Litoral, 22-IV-1965, *A. Fernandes & al.* (MAF 72788).

Ecología y Fitosociología: Al no tener grandes requerimientos ecológicos tiene mayor poder colonizador que otras

especies del género *(P. cambricum* L. y *P. vulgare* L.), se presenta en roquedos, muros y taludes, generalmente sobre sustrato silíceo; tiene preferencia por las estaciones umbrías. Lo hemos herborizado en comunidades de la al. *Cheilanthion hispanícete* Rivas Goday 1955.

Oleome violácea L., Sp. **PL: 672 (1753).**

JAEN: 30SVH2528. Navamorquín, Baños de la Encina, IV-1978, G. *Blanca, O. Socorro & F. Valle* (GDAC 6540 y 6541).

Según la bibliografía consultada, nuestra cita sería la primera dada para este taxon en la provincia de Jaén.

Distribución peninsular: Sur y Oeste de la Península Ibérica (Fig. 3).

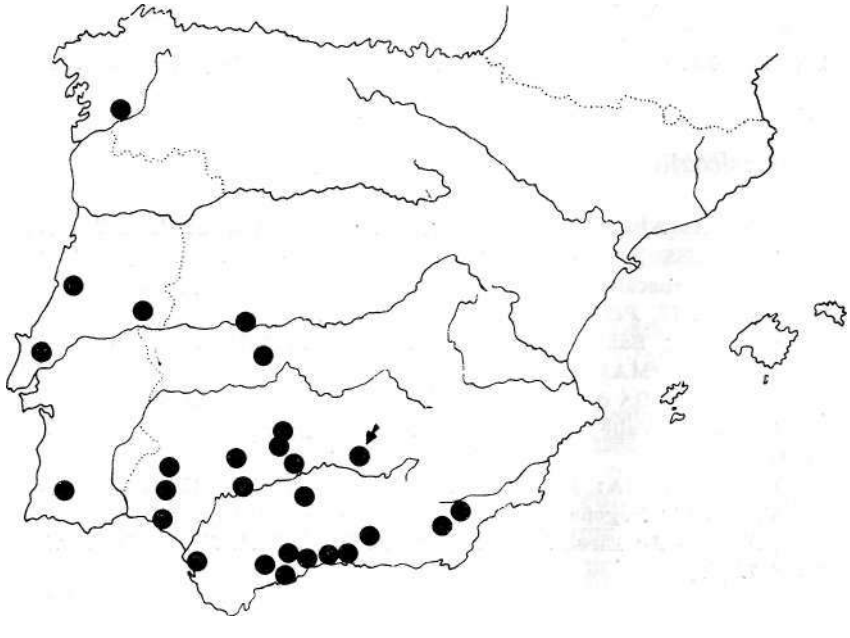

Fig. 3. — Distribución peninsular de *Cleome violácea* L.

Exsiccata

ALMERÍA: Uleila, 4-IV-1929, *Gros* (MA 43633); S de Filabres, V-1959, Losa *España* (MAF 88722); Huércal-Overa, 11-VI-1974, *E. Domínguez & Talavera* (SEV 19768).

BADAJOZ: Zorita, 20-VI-1952, s. colect. (GDA 1260).

CÁCERES: Cedavín, VI-1925, Aterido (MA 147396); entre Peralejo y Carrascalejo, 20-VIII-1968, *Ladero* (MAF 80439); ídem, 6-VIII--1969, *Ladero* (MAF 78236); ídem, 21-IX-1969, *Ladero* (MAF 74119).

CADIZ: Pto. da Santa María, sin fecha, *J. Rodrigues* (MA 43630).

CORDOBA: Pie de la Sª de Córdoba, 28-VI-1935, *R. Sagredo* (MA 43632 y GDA 1257) El Toril, Villaviciosa, 13-V-1920, *Pau* (MA 436310); los Pedroches, 9-VIII-1976, *Devesa* (SEV 34230); Montoro, l-VIII-1963, *Galiano* (SEV 1537).

GRANADA: Granada, V-1837, *Boissier* (MA 43634).

HUELVA: Niebla, 8-VI-1974, *Cabezudo & Talavera* (SEV 25607); Linares de la Sierra, 22-VI-1942, *Vicioso* (MA 43629); Alosno, 26-V--1942, *Vicioso* (MA 43628); Moguer, 9-V-1942, *Vicioso* (MA 43623).

MALAGA: S' Bermeja, 19-V-1919, *Gros* (MA 43643); Sª de Cártama, 19-VI-1888, *Reverchon* (MA 43644); Málaga, sin fecha, *Colmeiro* (MA 43647); ídem, *Clemente* (MA 43645); ídem, VI-1831, s. colect. (GDA 1256); S* de los Angeles, VI-1831, *Prolongo* (MA 43646); entre Vélez y Canillas de Albaida, 5-VI-1919, *Gros* (MA 43642); S» Tejeda, 7-VII-1935, *Estremera* (MA 43641); Competa, 31-V-1931, *Ceballos* (MA 43640); ídem, 9-VI-1965, *Rivas Goday* (MAF 96303); Carratraca, 20-VI-1930, *Vicioso* (MA 43639); Sª de Aguas, 7-V-1973, *G. López & E. Valdês* (MAF 89154); Sª Almijara, sin fecha, *Laza Palacios* (MAF 8506); ídem, 23-VI-1935, *Laza Palacios* (MAF 3522); Frigiliana, 15-V-1953, *Muñoz Medina* (GDA 1261); entre Alora y Carratraca, 23-V-1971, *Galiano* cê *al.* (SEV 15512).

ORENSE: Entre Santiago y El Barco, 20-VIII-1971, *Lain»* (SEV 9672).

SEVILLA: Lora del Río, 19-X-1970, *Galiano & al.* (SEV 18667); Guadalcanal, 20-VI-1970, *Silvestre* (SEV 9552).

PORTUGAL: Baixo Alentejo, 12-V-1959, *M. Beliz & J. Guerra* (MA 200576); ídem, 8-VI-1962, *M. Silva* (MA 199933); Beira Litoral, entre Coimbra y Geria, 9-VIII-1958, *M. Silva* (MA 171789); Beira Baixa, Monfortinho, 15-VI-1948, *B. Rainha* (MA 162390); Val de Rosei, VI-1891, *J. Débeau* (MA 43622); ídem, VII-1920, *L. Fernandes* (MA 43624 y MAF 58919); Estremadura, Almada, 4-VI-1944, *B. Rainha* (GDA 1255).

Ecología y Fitosociología: Especie silicícola que vive sobre suelos poco profundos o pedregosos, en comunidades pertenecientes al orden *Phagnalo-Rumicetalia indurati* Rivas Goday 1972.

Ononis speciosa Lag., Gen. Sp. Nov.: 22 (1816).

JAÉN: 30SVG2278, Cerca de la Bañizuela, Torredelcampo, IV-1978, *G. Blanca, O. Socorro* & *F. Valle* (GDAC 6544).

Ya fué citada en la provincia de Jaén por CUATRECASAS (1929) ; la nueva localidad que señalamos sería la más interior de la especie en la Península Ibérica; la presencia de este taxon termófilo en dicha zona iría ligada a la influencia climática del Valle del Guadalquivir.

Distribución peninsular: Provincia corológica Bética y algunas localidades en la Murciano-Almeriense (Figura 4).

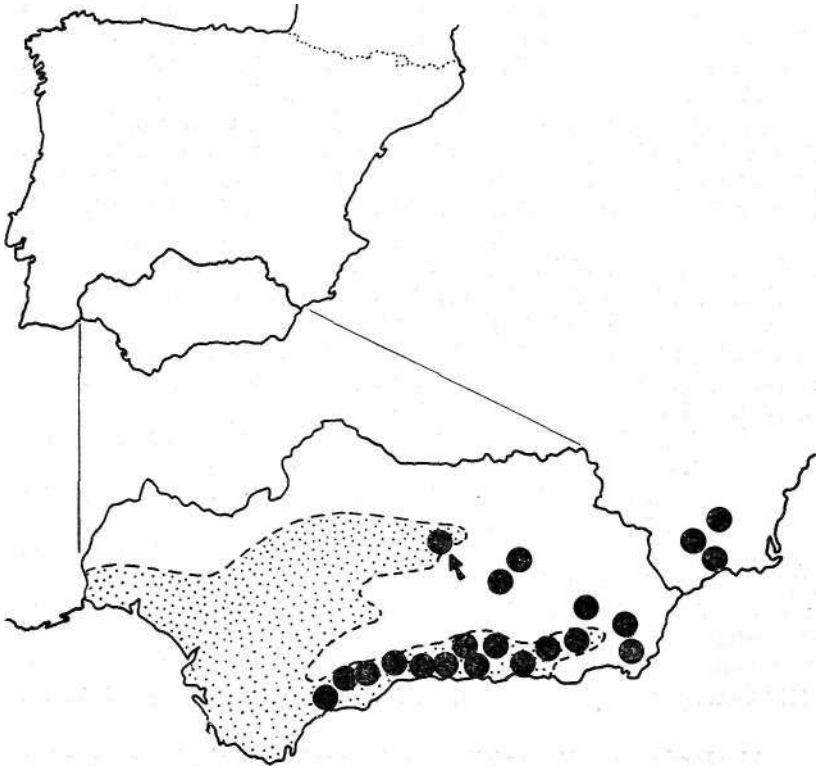

Fig. 4. — Distribución peninsular de *Ononis speciosa* Lag. La zona punteada corresponde al área virtual del orden *Phlomidetalia purpurae.* Rivas Goday & Rivas Martínez 1967.

Exsiccata

ALMERÍA: Sª de Gádor, NE de Berja, sin fecha, *Ball & al.* (SEV 2086); Sª Alhamilla, 19-VI-1958, *Losa España & Rivas Goday* (MAF 90479); Berja, 22-V-1959, *Rivas Goday* (MAF 66465).

GRANADA: Entre Zafarraya y Alcaucín, Sª Tejeda, 6-VIII-1973, *Cabezudo & B. Valdês* (SEV 22S08); Sª de Baza 4-VI-1975, *Galiano & al.* (SEV 30189); ídem, 4-VIII-1975, *Galiano & al.* (SEV 25574); La Herradura, 30-IV-1919, *Gros* (MA 61901); Granada, sin fecha, l*opez Seoane* (MA 6190O); Valle del Darro, 16-VI-1876, *Sáinz* (MA 61915); Pampaneira, 31-V-1902, *Pau* (MA 61903); entre Granada y Motril, VI-1963, *Borja* (MA 178782); Granada, VII-1876, *Campo* (MA 61905); Gavcin, 28-IV-1931, *Ceballos* (MA 61917); Cádiar, 21-IV-1957, *Ruis de la Torre* (MA 167828); Torvizcón, 30-VI-1959, *Ruiz de la Torre* (MA 167829); Puerto de Zegrí, IV-1961, *Borja* (MAF 70551 y 101947); Sª de Baza, 4-VII-1973, *Galiano* (MAF 97082) ; entre Granada y Motril, VI-1963, *Borja* (MAF 67618); Alhambra, VII-1953, *Sáinz* (MAF 40681); túnel de Izbor, V-1977 (GDAC 3360, 3361 y 3362); Tello, Lanjarón, 9-VII-1944, *Muñoz Medina* (GDAC 6177).

JAÉN: Puente de Cambil, 4-VI-1928, *Cuatrecasas* (MAF 40682); río Jandulilla, *c.* Venta del Capataz, 24-VI-1926, *Cuatrecasas* (MAF 40683).

MALAGA: Gaucín, 28-IV-1931, *Ceballos* (MA 61927); Canillas de Albaida, 30-V-1931, *Ceballos* (MA 61918); Genalguacil, 17-V-1932, *C Vicioso* (MA 61916); S" de Cártama, VI-1888, *Reverchon* (MA 61919); Frigiliana, Sª Tejeda, s. d., *Gros* (MA 61920); cerros de Cimbre, *Vélez,* s. d., *Isern* (MA 61921); Sª de Aguas, 16-V-1972, *G. López* (MAF 89190); Sª Almijara, 23-VI-1935, *Laza Palacios* (MAF 40684); Carratraca, 23-VI-1926, *Laza Palacios* (GDA sin reg.).

MURCIA: Rambla de la Cueva del Buitre, 29-V-1978, *Hernández* (MA 209663); Lorca, 5-VI-?, *Cánovas* (MA 61906); ídem, s. d., *Guirao* (MA 61907); Sª Espuña, 17-V-1927, *Hno. Jerónimo* (MA 61908); S* de Carrascoy, 22-V-1891, *Porta y Rigo* (MA 61909); río Ramonete entre Águilas y Mazarrón, 19-V-1969, *Borja* (MAF 74139 y 80012).

Ecología y fitosociología: Lugares muy térmicos y soleados sobre sustrato calizo. Característica del orden *Phlomidetália purpúrae* Rivas Goday & Rivas Martínez 1967 en el que se engloban las comunidades termófilas del Sur de la Península Ibérica, ricas en especies endémicas muchas de ellas residuales de la flora terciaria por haber sobrevivió o a la influencia de las glaciaciones; como se puede observar en la Figura 4, el área de distribución de la especie se encuadra en su mayor parte dentro del área virtual de este

orden, teniendo su óptimo en comunidades de la as. *Bupleuro-Ononidetum speciosae* Rivas Goday & Rivas Martínez (1. a), desarrolladas sobre suelos profundos ricos en bases de los sectores corológicos Alpujarro gadorense y Malacitano almibárense.

En la provincia Murciano-Almeriense vive en comunidades del orden *Anthyllidetália terniflorae* Rivas Goday & al. 1961. Al internarse en la Península aparece fuera de sus comunidades naturales, pero siempre en los lugares más protegidos y soleados (condiciones más térmicas).

Hay que resaltar el comportamiento edáfico observado en la S[a] de Filabres, Tética de Bacares (FERNANDEZ CASAS, 1975) donde fue herborizada sobre suelo pedregoso silíceo.

La nueva localidad que indicamos está situada en el sector Hispalense y es climáticamente idónea para el desarrollo de comunidades del orden *Phlomidetalia purpúrae,* pero como indican RIVAS GODAY & RIVAS MARTÍNEZ (1. c.) no están bien representadas probablemente por la gran extensión que alcanzan los cultivos. La hemos recolectado en un encinar muy aclarado rico en elementos termófilos *(Olea europea* var. *silvestris, Asparagus albus, Smilax áspera,* etc.).

Digitalis purpurea L., Sp. Pl.: 621 (1953) subsp. heywoodii P. & M. Silva, Agron. Lusit. 20: 239 (1959).

JAÉN: 30SVH2528, Navamorquín, Baños de la Encina, 5-IV-1977, *J. A. Oil, G. manca & F. Valle* (GDAC 3244 y 3245); ídem, IV-1978, *G. Blanca, O. Socorro & F. Valle* (GDAC 6542 y 6543).

Hasta ahora sólo era conocida de la localidad en que fue descrita (Reguengos de Monsaraz, Alto Alentejo, Portugal) ; nuestra cita es la primera para España y aumenta considerablemente el área potencial del taxon, ya que es una indicación clara de que se extiende hacia oriente a través de S[a] Morena.

Las diferencias morfológicas entre algunas de las subspecies de la *Digitalis purpurea* L. son a veces muy exiguas y existen numerosos pasos intermedios; la subsp. *Heywoodii* tiene un área de distribución que coincide en parte con la

subsp. *mariana*, por lo que hemos creído oportuno reseñar aquí las principales diferencias de tipo morfológico entre ambos táxones:

subsp. *mañana* (Boiss.) Rivas Goday	subsp. *heywoodii* P. & M. Silva
Planta verde-tomentosa.	Planta densamente blanco-tomentosa.
Brácteas florales menos de $^{1/3}$ de la longitud de los pedúnculos.	Brácteas florales más o menos igualando la longitud de los pedúnculos.
Pedúnculo 1,5 a 3 veces de largos que el cáliz, recubiertos de pelos que alcanzan como mucho $^{1/5}$ del grosor de dichos pedúnculos.	Pedúnculos 1(1,5) veces de largos que el cáliz, recubiertos de pelos que superan el grosor de los mismos.
Divisiones del cáliz redondeado-obtusas ó acuminadas.	Divisiones del cáliz agudas más o menos lanceoladas.
Corola purpúrea.	Corola blanca ó amarillo pálido.
Corola glabra en la superficie extema (sólo presenta pelos en los bordes).	Corola pelosa en la superficie externa, sobre todo en su tercio superior.

Palinologia

Ademas de la localidad ya reseñada hemos estudiado las siguientes:

Digitalis purpurea L. subsp. *heywoodii* P. & M. Silva. Reguengos de Monsaraz, Alto Alentejo (Portugal), 23-V-1975, *G. Blanca & J. L. Rosua* (GDAC 5866).

Digitalis purpurea L. subsp. *heywoodii* P. & M. Silva. Reguengos de Monsaraz, Alto Alentejo (Portugal), 23-V-1975, *A. R. Pinto da Silva, M. H. Ramos Lopes* & *M. A. Pina* (GDAC 5690).

Para todas las medidas se han utilizado granos acetolizados tratados de idéntica manera y montados en glicerogelatina. El número de medidas por carácter es de 30 de

las cuales se han hallado la media aritmética y la desviación standard.

Los caracteres utilizados para la descripción mediante examen con el microscopio óptico son (Cuadro 1) : P (longitud polar), E (anchura ecuatorial), P/E, t (lado del triangulo polar formado por el ápice de los tres colpos), t/E, M (distancia intercolpal ó mesocolpia), la anchura del colopo medida en la zona ecuatorial, el espesor total de la exina y la relación entre el grosor de la sexina y nexina.

Como se aprecia en dicho cuadro, los pólenes de ambos táxones son muy similares entre sí; son de tamaño menor, isopolares, elípticos en vista meridional, prolado-esferoidales ; en vista polar subcirculares con tendencia a ser subtriangulares (Fig. 5).

Poseen tres aberturas colpadas (tricolpados) ; anguloaperturados; colpos muy largos dejando zona apocólpica pequeña, lisos y no llevan partículas sexínicas, de margen no diferenciado. La notación NPC (N = número, P = posición y C = caracter de las aperturas; según ERDTMAN, 1969) es 343.

Exina muy fina, ligeramente más gruesa que la intina; nexina de menor grosor que la sexina. Superficie exínica finamente reticulada.

Citogenética

Hemos determinado el número cromosómico de la *Digitalis purpurea* L. subsp. h*eywoodii* P. ¡& M. Silva en la población de Baños de la Encina (Jaén) : 2n = 56 (Fig. 6) por lo que nuestro resultado concuerda con la determinación de M. NORONHA WAGNER (in P. & M. SILVA, 1959: 240) sobre material de Portugal.

Los cromosomas son muy pequeños y según hemos podido observar poseen en su mayor parte constricciones medianas.

Como indican nuestro homenajeado y colaboradores (A_4 FERNANDES, M. QUEIRÓS & M. FÁTIMA SANTOS, 1977), este género se caracteriza por un alto grado de poliploidía, de modo que si la mayor parte de las especies poseen 2n = 56,

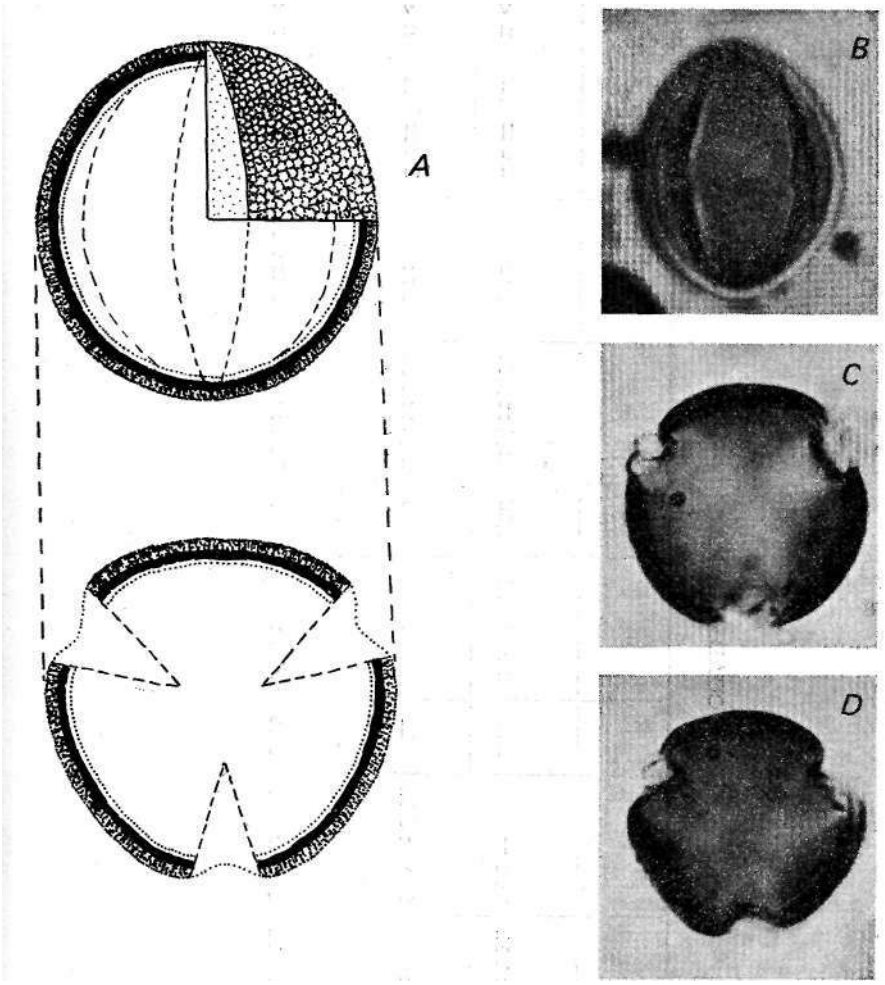

Fig. 5 — Polen de *Digitalis purpurea L.* subsp. *heywoodii* P. & M. Silva: A, palinograma; B, vista meridiana; C y D, vista polar.

se puede decir que 28 es un número de base secundario derivado de 7, por lo que *D. purpurea* L. subsp. *heywoodii* P. & M. Silva sería un octoploide. Los números somáticos 14 y 28 correspondientes, respectivamente, a las formas diploides y tetraploides, no han sido encontrados hasta ahora en este género, por lo que es probable que sólo las formas octoploides hayan persistido.

CUADRO 1

	P	E	P/E	t	t/E	M	Anchura del colpo	Grosor exina	
Digitalis purpurea subsp. *mañana*	23,3 ± 1,11	21,4 ± 0,79	1,09	3,9 ± 0,56	0,18	13,6 ± 0,59	4,9 ± 0,50	1,4 ± 0,39	n < s
Digitalis purpurea subsp. *heywoodü* (ESPANHA)	24,3 ± 0,78	21,3 ± 0,84	1,13	4,0 ± 0,67	0,19	13,8 ± 0,84	5,3 ± 0,25	1,2 ± 0,33	n < ß
Digitalis purpurea subsp. *heywoodü* (PORTUGAL)	24,0 ± 1,14	21,0 ± 0,97	1,14	4,í±0,81	0,19	13,5 ± 0,48	5,2 ± 0,33	1,3 ± 0,35	n < s

Ecología y Fitosociología: Es un taxon silicícola que vive en grietas de rocas graníticas o en suelos arenosos muy permeables y poco profundos. Hemos analizado una muestra de este suelo obteniendo un pH de 6,45 y ausencia total de carbonatos.

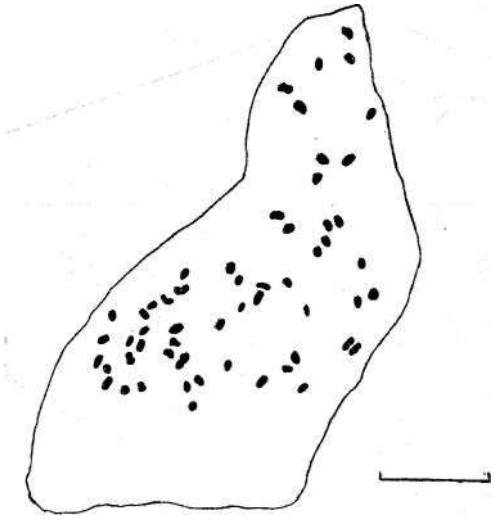

Fig. 6. — Metafase somática de *Digitalis purpurea* L. subsp. *hywoodii* P. & M. Silva 2n = 56 (escala 10 u).

Tanto en Reguengos de Monsaraz como en la nueva localidad que señalamos existen afloramientos de rocas graníticas que hemos esquematizado en la Fig. 7. A grandes rasgos podemos distinguir dos unidades distintas: los batólitos existentes a la izquierda de la falla AB que se caracterizan por ser pequeños, muy numerosos y bastante heterogéneos en su composición, y los de la derecha de la falla que son mayores y más homogéneos.

Nuestra cita estaría en el extremo Sur del llamado «batólito de los Pedroches» y se encuadraría en la hoja 1:50 000 perteneciente a La Carolina (884//19-35); se compone de granitos en masas irregulares de color rosáceo o gris cuando no están alterados, y aspecto granudo.

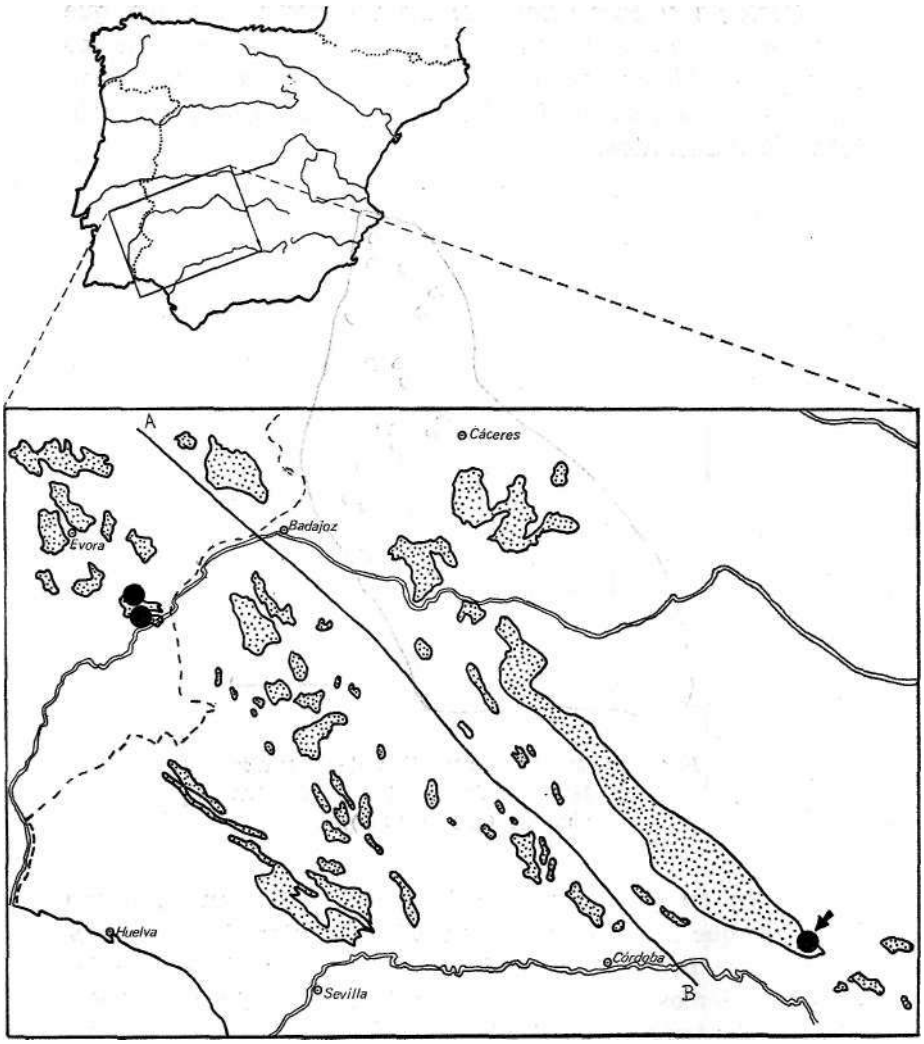

Fig. 7. — Distribución de *Digitalis purpurea* L. eubsp. *heywoodii* P. & M. Silva (las zonas punteadas corresponden a los afloramientos graníticos).

Tienen textura alotriomorfa de grano medio y están formados por cuarzos, feldespato potásico ligeramente caolizado, plagioclasa en cristales subidiomorfos débilmente sensitizada; el mineral máfico (biotita) es sustituido por com-

pleto por moscovita de origen neumatolítico, desprendiéndose óxidos de hierro que se concentran entre las láminas de mica.

Hacemos hincapié en estos datos geológicos ya que P. & M. SILVA (1959) indicaban que el área de este taxon parecía corresponder con notable precisión a la mancha granítica del Silúrico inferior que aflora en Reguengos de Monsaraz y se preguntaban si servía este hecho para explicar su aislamiento geográfico. Tal vez sea posible localizar esta subespécie en alguna de los numerosos asomos graníticos que existen entre esta zona y la que indicamos como nueva, que bien podían ser precisamente los limites de área del taxon.

La hemos recolectado en comunidades poco específicas del orden *Phagnalo-Rurnicetalia indurati* Rivas Goday 1972; también suele aparecer como fisurícola situándose siempre en aquellas condiciones más umbrías; se presenta en el dominio de la as. *Sanguisorbo-Quercetum suberis* Rivas Goday 1959 em. Rivas Martínez 1975, en sus etapas más degradadas o aclaradas *(Myrteto-Quercetum rotundifoliae* silicíneo bético Rivas Goday 1959).

Digitalis purpurea L. es una especie subatlática europea que se encuentra diversificada a nivel intraespecífico en tres subespécies (V. H. HEYWOOD in Flora Europaea 3: 240 (1972), lo cual puede ser debido a la cantidad de habitats y climas distintos con que se encuentra en su amplia área de distribución, ya que la podemos encontrar tanto en ambientes realmente euatlánticos como en otros mediterráneos. Para explicar esta diversidad tal vez sea preciso recurrir a fenómenos de tipo radiación adaptativa e incluso de esquizoendemismo (FAVARGER & CONTANDRIOPOULOS, 1971).

Catananche lutea L., Sp. Pl. 812 (1753) subsp. carpholepis (Schultz Bip.) Nyman, Consp.: 472 (1879).

Piptocephalum carpholepis Schultz Bip. in Bonplandia 8: 369 (1860).

JAÉN: 30SVG2081. Torredelcampo, VI-1975, *G. Blanca* (GDAC 2013); ídem, V-1976, *G. Blanca* (GDAC 2260 y 2261).

Fue indicada por nosotros en esta localidad (MORALES & al., 1978), por tratarse de la primera cita de este taxon en la provincia de Jaén y ser muy escasa en la Península Ibérica.

Gitogenética

En la población señalada hemos encontrado el número cromosómico 2n = 18, por lo que nuestro resultado concuerda con el obtenido por STEBBINS, JENKINS & WALTERS (1953) para material cultivado en el Botanical Garden de Stockholm y de Copenhagen. Por lo tanto es la primera vez que se estudia el número cromosómico de este taxon en material silvestre. Como ya apuntaron dichos autores el cariotipo es esencialmente simétrico; el estudio detallado del mismo da como resultado (Fig. 8) la siguiente formula cromosómica: 8m + l sm, es decir, ocho pares de cromosomes con centrómero mediano y un par con centrómero submediano (par 8) ; los pares 4 y 9 son satelíferos.

Distribución peninsular: Tiene su óptimo en la provincia Bética, llegando de forma disyunta a los depósitos calizos del sector Mariánico monchiquense en la Luso-Extremadurense (LADERO, 1974).

Ecología y Fitosociología: Planta termófila que vive sobre suelos básicos; la hemos herborizado en comunidades de la al. *Thero-Brachypodion* Br.-Bl. 1925 en faciès algo nitrificádas.

Sternbergia lutea (L.) Ker-Gawler ex Roemer & Schultes, Syst. Veg. 7 (2): 795 (1830).

Amaryllis lutea L, Sp. PL: 292 (1753).

JAÉN: 30SVG2278. Torredelcampo, 1-1-1974, G. *Blanca* (GDAC 21); ídem, 2-XI-1975, *G. Blanca* (GDAC 353, 354 y 355).

Se trata de una especie subespontánea escasamente representada en la Península Ibérica. Nuestra cita sería la primera para la provincia de Jaén.

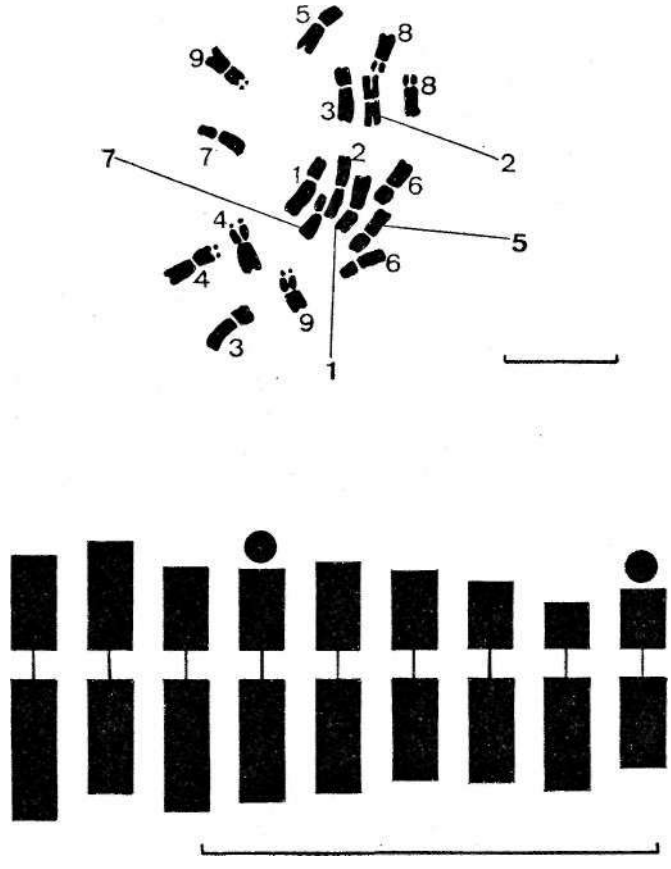

Fig. 8. — Metafase somática (2n = 18) y cartograma de *Catananche lutea* L. subsp. *carpholepis* (Schultz Bip.) Nyman (escala 1u.).

Citogenética: Los números cromosómicos encontrados para esta especie hasta ahora son los siguientes:

2n = 12 YAMAMOTO in KIHARA, YAMAMOTO & HOSONO (1931).
2n = 16 NAKAJIMA (1936).
2n = 18 DELAY (1947).
2n = 20 MOOKERJEA (1955) ; SHARMA (1956).

2n = 22 INARIYAMA (1937); SATO (1938); LA COUR in DARLINGTON & JANAKI-AMMAL (1945); BATTAGLIA (1949); MOOKERJEA (1955); SHARMA (1956); PIZZOLONGO (1964); LOVKA, SUSNIK, LÖEVE & LÖEVE (1971).

2n = 23 BATTAGLIA (1949).

2n = 24 AMICO (1947).

n = 9, 10, 12 KAPiNOS (1960).

Se ha estudiado el cariotipo en la localidad indicada más arriba y se ha podido comprobar que se trata de un triploide, con un número cromosómico 3x = 30 (Fig. 9); según la bibliografía consultada es la primera vez que se estudia material español de este taxon y además es el nivel más alto de ploidía encontrado para el mismo. El número básico parece ser por tanto x = 10, hecho apoyado además por los recuentos en los que se ha encontrado 2n = 20; por otro lado FERNANDEZ CASAS, PAJARON & RODRÍGUEZ PASCUAL (1978) encontraron 2n = 20 para una especie próxima, la *Sternbergia colchiciflora* Waldst. & Kit. de lo que deducimos que x = 10 sea posiblemente el número básico del género. Luego en la evolución de *St. lutea* deben haber intervenido fenómenos de poliploidía y aneuploidía.

La fórmula cromosómica es 1 m + 3 st + 6 t: un par de cromosomas con centrómero mediano (par 1), tres pares con centrómero subterminal (pares 2, 6 y 10) y seis pares con centrómero terminal (pares 3, 4, 5, 7, 8 y 9).

Distribución peninsular: Aparece como subespontánea en localidades aisladas entre sí del Centro y Sur le la Península (Fig. 10).

Exsiccata

CÁCERES: Almaraz de Taja, 5-X-1976, *E. Rico* (MA 203634). CADIZ: Benamahoma, 2-X-1976, *Casaseca & Fernández Diez* (MA 208290); S[a] de Grazalema, 2-X-1976, *Pérez Chiscano* (MAF 95073). GRANADA: Cerros del Avellano, 6-X-1955, s. colee. (GDA 276). MADRID: Alcalá de Henares, IX-1895, *Mas y Guindal* (MAP 63671).

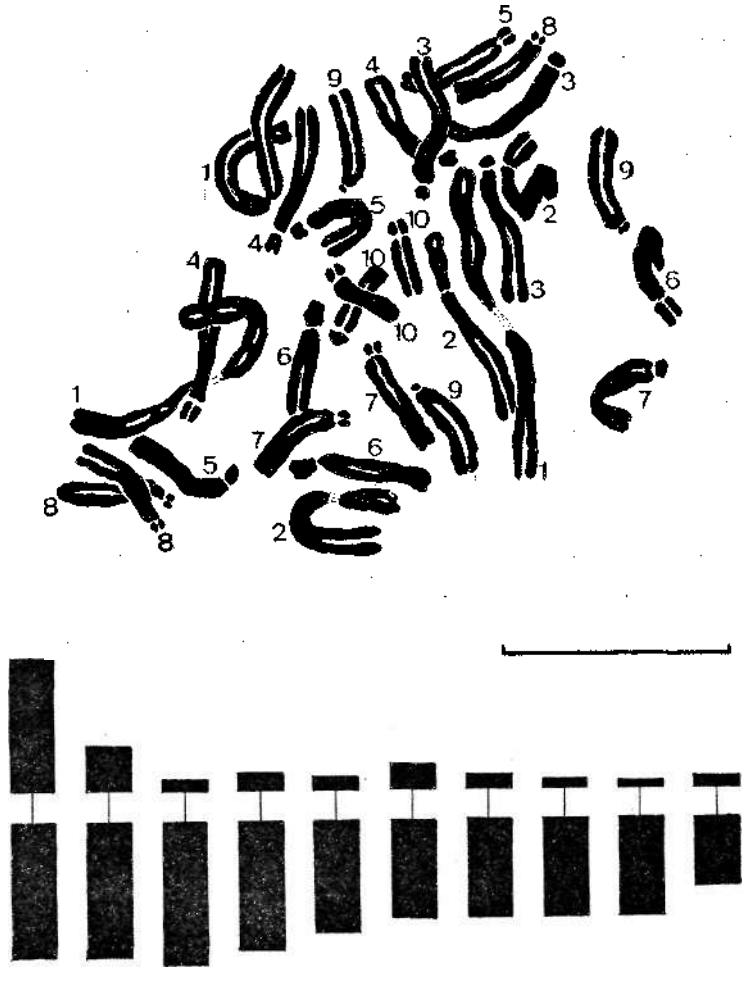

Fig. 9. — Metafase somática (3x = 30) y cariograma de *Sternbergia lutea* (L.) Ker-Gawl (escala 10 u).

MALLORCA: Avenal, 17-IX-1945, *Palau Ferrer* (MA 22509); Sóller, Bianor, 20-IX-1910 (MA 22511); Eunyola 29-IX-1950, *Palau Ferrer* (MAF 838 y GDA 277).

SEVILLA: Cazalla de la Sierra, 4-X-1931, *C. Vicioso* (MA 22510); San Pedro, IX-1900, *Rivas Mateos* (MAF 839).

TOLEDO: Carretera de Toledo a Navahermosa, 11-X-1972, *Ruis Ramos* (MAF 83590).

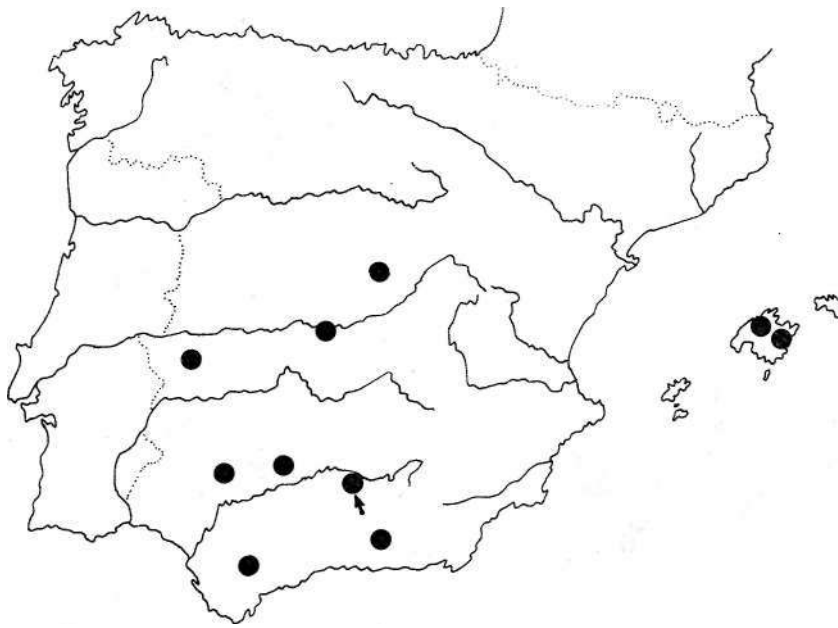

Fig. 10.—Distribución peninsular de *Stembergia lutea* (L.) Ker-Gawl ex Roemer & Schuttes.

AGRADECIMIENTOS

Queremos expresar nuestro más sincero agradecimiento a los profesores A. R. PINTO DA SILVA y MANUEL DA SILVA por el envío de un isótipo de la *Digitalis purpurea* L. subsp. *heywoodii* P. & M. Silva, y también al profesor A. E. SALVO TIERRA por su ayuda en la determinación y corología de *Ophioglossum lusitanicum* L. y *Polypodium interjectum* Shivas.

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DONNÉES CHiMïOTAXINOMïQUES SUR QUELQUES ARMOISES ENDÉMIQUES DU MAROC

par

8. NÈGRE [1], Melle A. OUYAHYA [2] & M. DE **BOTTON** [3]

RÉSUMÉ

Cinq taxons d'*Artemisia* endémiques du Maroc sont spécifiquement distincts par la composition de leurs huiles essentielles. Aux constituants majoritaires déjà connus (thuyone et isothuyone, camphre, cinéol), s'ajoutent ici les «- et ß- piñenes, le bornéol et surtout des sesquiterpenes. La nature de ces derniers semble en rapport avec la microécologie; de même que la nature des constituants majoritaire paraît en rapport avec les conditions altitudinales et peut-être plus directement les basses températures.

*D e*nombreuses études ont été consacrées déjà à la chimie des armoises et plus spécialement à celles des huiles essentielles de ces dernières, mais sans poursuivre des buts strictement taxinomiques. Parmi les plus récentes, celles de GUERIB (1967), GEISMAN (1970-1974), BANTHORPE (1971), FENARDJI (1974), HALLIGAN (1975) ont apporté de très intéressantes vues sur ce groupe complexe et fourni de plantes méditerranéo-sarmatiques. En particulier 37 constituants ont pu être mis en évidence dans les essences des 25 espèces d'armoises étudiées comparativement par BANTHORPE.

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Parmi ceux-ci, les principaux sont: la thuyone, l'iso-thuyone, le 1-8 cinéol, l´*artemisia-cêtone,* le camphre et le bornéol. Les huiles essentielles de toutes les espèces présentent des compositions différentes mais on peut les grouper en fonction du constituant majoritaire CM comme l'indique le tableau n° 1.

TABLEAU N° 1

Classification des 25 espèces du genre *Artemisia* en fonction du constituant majoritaire présent dans l'huile essentielle, . d'après BANTHORPE *et al.* (1971)

ESPECES	Constituant majoritaire (C. M.)	% de C. M. dans l'huile essentielle
A. abrotanum L.	thuyone	72
A. kurramensis Qazilb.	»	51
A. nobilis L. var. *aurea*	»	59
A. maritima L.		53
A. serótina Burg.		63
A. rutaefolia Steph.	»	40
A. verlotorum Lamotte	»	83
A. lactiflora Wall.	»	63
A. arborescens L.	»	43
A. austriaca Jacq.	»	41
A. campestris L.	»	38
A. filifolia Torr.		33
A. absinthium L.	isothuyone	83
A. nutans Willd.		69
A. pontica L.	Artemisia- cétone	43
A. annua L.		61
A. chamaemelifolia ViIl.	>	51
A. procera WiUd.	camphre	37
A. cana Pursh.		61
A. frigida Willd.	1,8 cinéol	58
A. californica Less.		33
A. vulgaris L.	linalol	65
A. porrecta Krasch.	acétate de linalyle	24
A. dracunculus L.	nérol	67
A. dracunculoides Pursh.	sabinène	30

Le présent article regroupe quelques uns des résultats obtenus par l'analyse des cinq taxons suivants endémiques des moyenne et haute montagnes marocaines:

Artemisia flahaultii Emb. et Maire
A. ifranensis Did.
A. mesatïuntica Maire
A. negrii Ouyahya
A. atlántica Coss. et Dur. var. *maroccana* (Coss.) Maire

Les analyses ont porté sur dés échantillons composites obtenus à partir des récoltes de terrain concernant au minimum 100 individus appartenant à des populations homogènes morphologiquement et écologiquement. Les stations respectives sont, dans l'ordre, situées sur le versant N du Jbel Bou Naceur 2450 m, sur son versant S à 2500 m, route d'Ifrane au Michlifène à 1600 m, à 34 km d'Ifrane sur la route de cette localité à Boulmane, au bord de la piste d'Agoudal à Mesemrir à **2750** m d'altitude et 26 km d'Agoudal, Gorges du Dadès 1730 m d'altitude au N de Boumalne.

1. TECHNIQUES

Les principes volatils ont été analysés par la technique proposée par STAHL (1975) consistant à utiliser le micro-four TAS (Thermomikro-Abtreen Transfer und Auftrage Verfahren— Procédé thermique de micro-extraction, de transfert et de dépôt) couplé avec la Chromatographie sur couches minces (CCM). Les huiles essentielles ont été analysées, après leur isolement, par Chromatographie sur couche mince (CCM) et par Chromatographie en phase gazeuse (CPG).

Avant d'être soumis aux techniques d'extraction les capitules et les tiges ont été finement pulvérisés à l'aide d'un broyeur électrique (FORPLEX Foo).

1.2.— Extraction des huiles essentielles

Nous avons adopté pour recueillir les huiles essentielles la technique préconisée par la Pharmacopée Française (VIII^{0} Edition p. 1554) et utilisé le matériel décrit dans cet

ouvrage. Deux modifications y ont été cependant apportées, la première consiste à utiliser un ballon de 2000 ml de capacité permettant de mettre en oeuvre 100 g de poudre végétale et 1000 ml d'eau distillée, la seconde correspond au remplacement sur le tube de recueillement du bouchon rodé par un réfrigérant à reflux ce qui conduit à une très bonne régularisation de la condensation de la vapeur d'eau et évite la variation de pression dans le dispositif qui, sans cela, fonctionne en circuit fermé.

1.3. — Chromatographie sur couches minces

Toutes nos chromatographies sur couches minces ont été réalisées sur gel de silice Merk 60 F 254 (fluorescente) de 0,2 mm d'épaisseur déposé sur feuille d'aluminium (20 X 20 cm).

Nous avons utilisé comme phase mobile un mélange de benzène et d'acétate d'ethyle (95:5 v/v). Le dépôt des échantillons a été fait sur une ligne de départ située à 15 mm de la partie inférieure de la plaque et, afin d'éviter les effets de bord, les deux dépôts extrêmes ont placés à 25 mm de la partie gauche et 25 mm de la partie droite du support.

Les dépôts ont été réalisés à 15 mm les uns des autres et les quantités de solutions analysées ont été pour chaque dépôt de 3 à *4 ul.* Le solvant de migration a parcouru la plaque sur 10 cm au-dessus de la ligne de départ.

Nous avons utilisés plusieurs techniques de révélation des composés séparés.

a — Après evaporation du solvant sous un léger courant d'air les chromatoplaques ont été observées sous lumière UV (lampe Universek CAMAG type TL-900) à la longueur d'onde de 254 nm.

b — Les révélations par les techniques colorimétriques ont mis en oeuvre les réactifs et les méthodes suivants :

b-1 — *Réactif à l'anisdldêhyde sulfurique:* A 0,5 ml d'anisaldéhyde on ajoute successivement 10 ml

d'acide acétique glacial, 85 ml de methanol et 5 ml d'acide suifurique concentré ; on asperge la plaque de CCM d'une manière régulière puis on porte la plaque à l'étuve réglée à 100°-110° pendant 10 minutes.

b-2 — *Réactif à l'acide phosphomolybique :* 20 g d'acide phosphomolybdique sont dissouts dans 100 ml d'éthanol. On pulvérise la plaque avec ce réactif, puis on porte à l'étuve réglée à 105°-110° pendant 5 minutes.

1.4. — Chromatographie en phase gazeuse

Nous avons disposé d'un appareil TRACOR 550 à détecteur à ionisation de flamme. Les séparations ont été réalisées sur une colonne en acier inoxydable de 6' de long (1824 mm) et de $^{1/4}$" (6,25 mm) de diamètre remplie de phase stationnaire constituée de 0,5 p. 100 d'OV-1 sur chromosoorb W HP (60-80 mesh).

Le gaz vecteur est l'azote, la température de l'injecteur a été fixée à 200° C et celle du détecteur à 250° C. Les séparations ont été effectuées avec programmation linéaire de température entre 71° C et 220° C à une vitesse de 5° C par minute.

2. RÉSULTATS

2.1. — Chromatographie des principes volatils suivant la méthode T. A. S.

Les figures 1 et 2 illustrent les chromatogrammes. Le tableau n° 2 rassemble les colorations et les valeurs des Rf obtenus, les produits obtenus à partir d'*Artemisia absinthium* du commerce servant de base de référence.

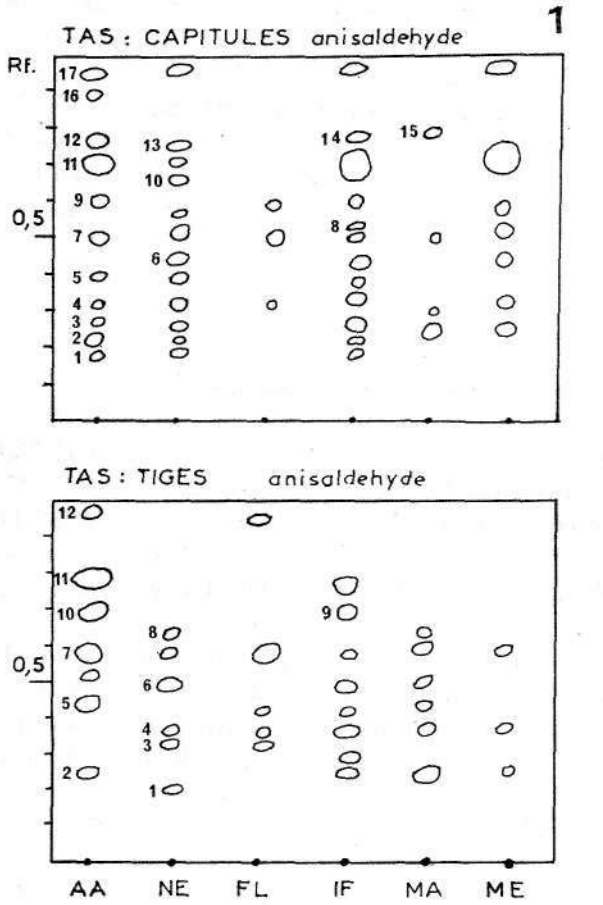

Fig- 1. — Chromatographie (T. A. S. selon STAHL) des principes volatils de cinq armoises marocaines — *(A. absinthium* pris comme référence: AA) (NE: *A. negrii,* FL: *A. flahaultii,* IF: *A. ifranensis,* MA: *A. altantica* var. *maroccana,* ME: *A. mesatlantica)* (révélation: anisaldehyde)—n° des spots: **voir tableau n° 2.**

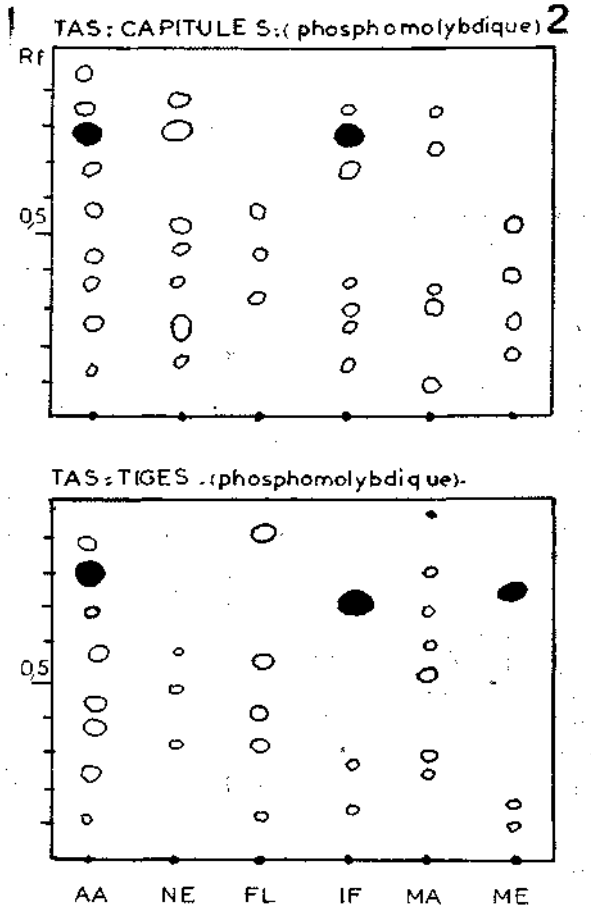

Fig. 2. — Chromatographie (T. A. S. selon STAHL) des principes volatils de cinq armoises marocaines — *(A. absinthium* pris comme référence: AA) (NE: *A. negrii,* FL: *A. flahaultii,* IF: *A. ifranensis,* MA: *A. atlántica* var. *maroccana,* ME: *A. mesatlantica)* (révélation acide phosphomolybdique)—taches noires: thuyone et/ou isothuyone.

TABLEAU N° 2

Données chromatographiques relatives aux principes volatils des capitules et das tiges des armoises (Techniques T. A. S.) — révélation au R. à l'anisaldéhyde (AA: *A. absinthium* — NE: *A. negrii*— Fl: *A. flahaultii*— IF: *A. ifranensis* — MA: *A. maroccana* — ME : *A. mesatlantica)*

— *A: capitules*

Spot n°	Rf	coloration	AA	NE	FL	IF	MA	ME
1	0,20	gris	+	+		+		
2	0,23	gris-rouge	+	+		+		+
3	0,28	gris-jaune	+	+		+	+	
4	0,34	violet-rose	+	+	+	+	+	+
5	0,40	violet-rose	+	+		+		
6	0,45	gris-jaune		+		+		+
7	0,54	rouge	+	+	+	+		
8	0,56	jaune-vert		+		+		+
9	0,60	orange	+		+	+		+
10	0,66	gris		+				
11	0,70	rose	+	+			+	+
12	0,77	rose	+			+		
13.	0,77	gris						
14	2,77	violet		+		+		
15	0,77	rose						
16	0,89	bleu-violet	+					
17	0,96	bleu-violet	+			+		+
				+			+	
		— *B: tiges*						
1	0,20	gris		+		+		
2	0,25	gris-rouge	+			+	+	+
3	0,32	gris-jaune		+	+			
4	0,37	violet-rose		+	+	+	+	+
5	0,44	violet-rose	+		+	+	+	
6	0,48	gris-jaune	+	+		+	+	
7	0,54	rouge	+	+		+		+
8	0,64	orange		+			+	
9	0,68	gris			+	+	+	
10	0,68	violet	+					
11	0,79	rose	+			+		
12	0,98	bleu-violet	+					
					+			

2.2. — Chromatographie par couche mince

Les quantités d'huiles essentielles extraites par la vapeur d'eau sont faibles en ee qui concerne les tiges (de l'ordre de 0,3 ml d'huile essentielle pour 100 g de matériel sec), plus importantes pour les capitules:

A. atlántica var. *maroccana*	1 ml/100' g de matériel sec (vert)
A. mesatlantica	1 ml/100 g de matériel sec (vert)
A. infranensis	2 ml/100'g de matériel sec (jaune)
A. negrii	2 ml/100 g de matériel sec (jaune)
A. flahaultii	0,5 ml/100 g de matériel sec (jaune-vert)

Les résultats qui concernent les huiles essentielles de tiges sont présentés sous forme de graphiques (figure 3) ; pour les huiles essentielles des capitules, le chromatogramme est analogue.

Le tableau n° 3 rassemble des données concernant les Rf et la couleur des spots mis en évidence au moyen du réactif à l'anisaldéhyde.

TABLEAU N° 3

Principes volatils reconnus par CCM

n° des spots	Rf	coloration	AA	NE	FL	IF	MA	ME
1	0,27	gris						+
2	0,30	gris-jaune	+	+	—	+	—	—
3	0,45	gris-violet	+	+	+	+	+	+
4	0,51	bleu-violet ,						+
5	0,57	gris-jaune	—	—	—	—	+	+
6	0,64	rouge	+	+	+	+	+	+
7	0,73	jaune-gris	—	+	+	—	+	—
8	0,90	rose-gris	—	—	+	—	—	—
9	0,98	bleu-violet	+	+	+	+	+	+

De l'examen de ces résultats il ressort que:

I°) Les huiles essentielles d'*A. negrii* et d'*A. infranensis* présentent des caractéristiques voisines de celles *d'A. absinthium;* cependant la thujone et/ou l'iso-

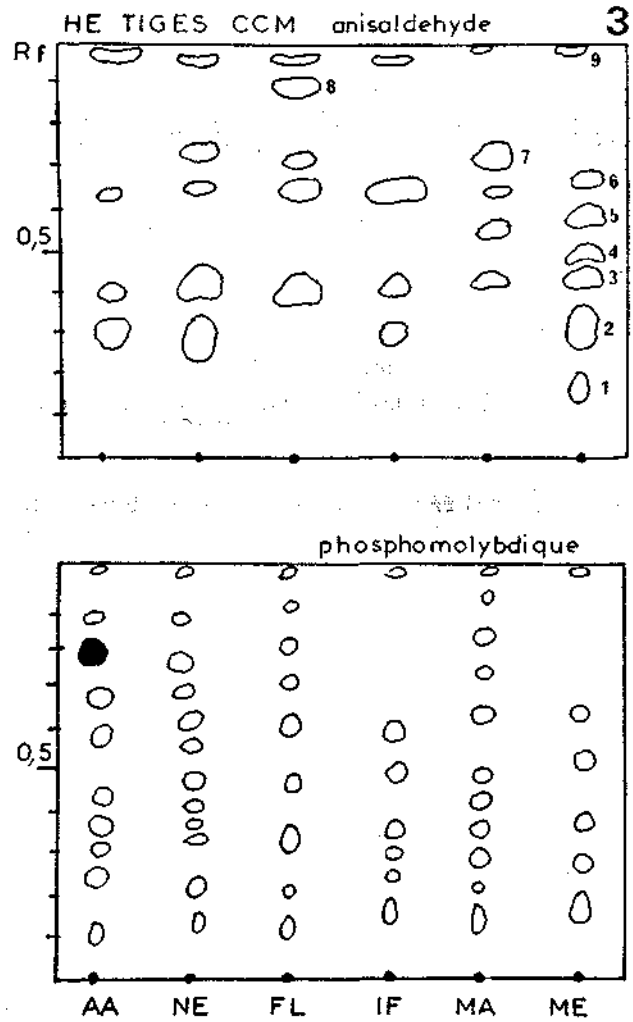

Fig. 3. — Chromatographie sur couches minces des huiles essentielles d'Armoises (AA: *A. absinthium*, NE: *A. negrii*, FL: *A. flahaultii*, IF: *A. ifranensis*, ME: *A. mesatlantica*, MA: *A. atlántica* var. *maroccana* (révélation au réactif à l'anisaldéhyde et révélation au réactif à l'acide phosphomolybdique).

thujone n'est mise en évidence par Ia révélation au réactif phosphomolybdique que dans l'huile essentielle *d'A. ifranensis.*

De ce point de vue, cette dernière est également voisine *d'A. mesatïantica.*

2°) L'huile essentielle *â'A. flahaultii* contient un principe donnant avec le réactif à l'anisaldéhyde un spot très fortement coloré en rouge violacé qui semble être le constituant principal.

3°) Toutes les huiles essentielles sont différentes entre elles et différentes de celle d'*A. absinthium.* Elles semblent présenter une complexité beaucoup moins grande que celle observée dans le cas des principes volatils étudiés par la méthode T. A. S. de STAHL.

2.3. — Chromatographie en phase gazeuse

Les figures 4 à 7 reproduisent les graphes obtenus.

La figure 4 représente les chromatogrammes des huiles essentielles de capitules et de tiges d'*A. ifranensis* et d'*A. mesatïantica.* On notera une certaine concordance entre les documents résultants de la séparation chromatographique des huiles essentielles de tiges et de capitules; en règle générale, et cela est surtout évident en ce qui concerne *A. mesatïantica,* les huiles essentielles de tiges contiennent des composés plus lourds et en plus grandes quantités que ceux des huiles essentielles des capitules. Les composés élues de la colonne à une température supérieure à 180° C, dans les présentes conditions opératoires, peuvent être considérés comme des composés de nature sesquiterpénique.

L'huile essentielle *d'A. ifranensis* contient quatre constituants dont les deux principaux sont la thuyone et l'isothuyone, cette dernière étant en une concentration environ 5 fois supérieure à la concentration de son isomère. Ont été également identifiés le camphre et le bornéol.

L'huile essentielle *d'A. mesatïantica* est à peu près exclusivement constituée, en ce qui concerne les capitules, d'isothuyone. Dans l'huile essentielle de tige celle-ci est

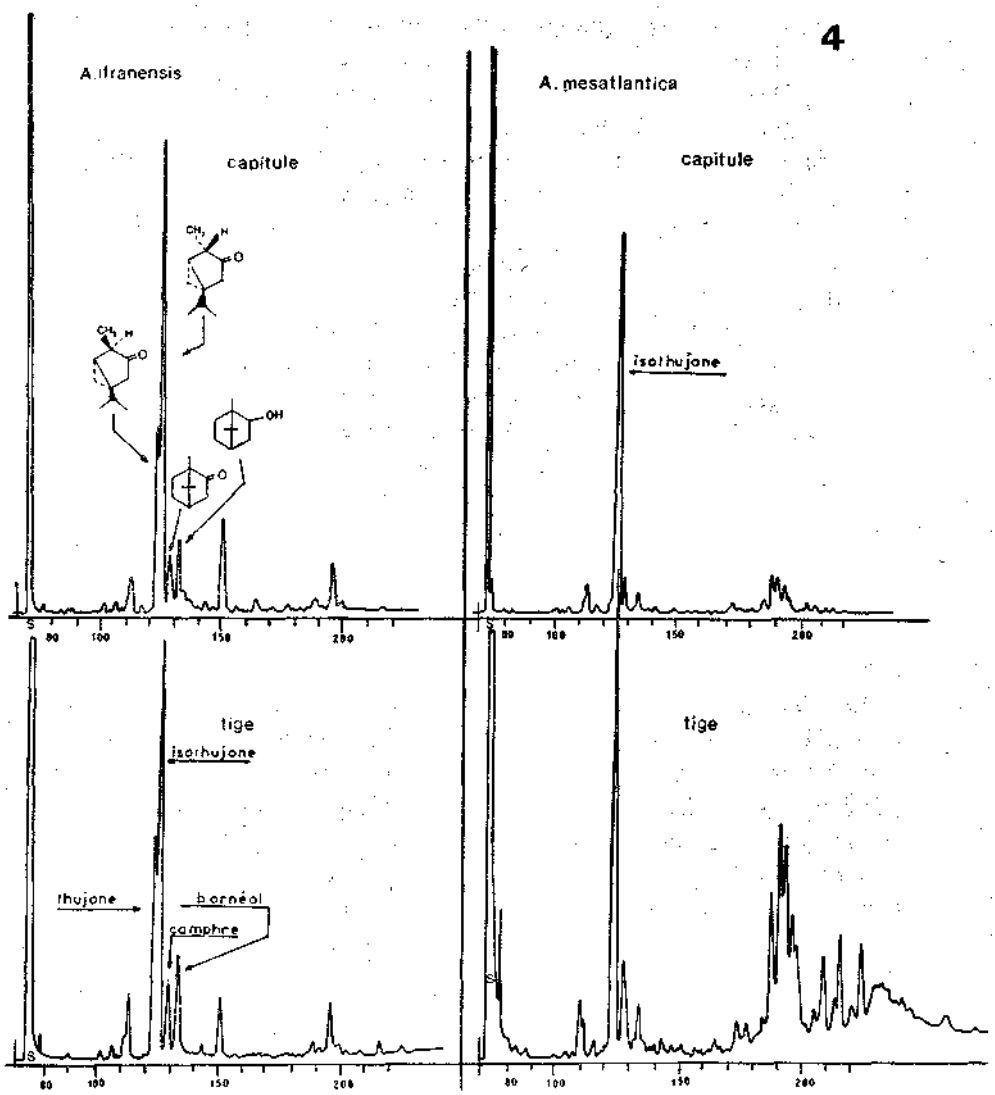

Fig. 4. — Chromatographies en phase gazense (OV_1) des huiles essentielles de tige et de capitules *d'A. ifranensis* et *d'A. mësatlantica.*

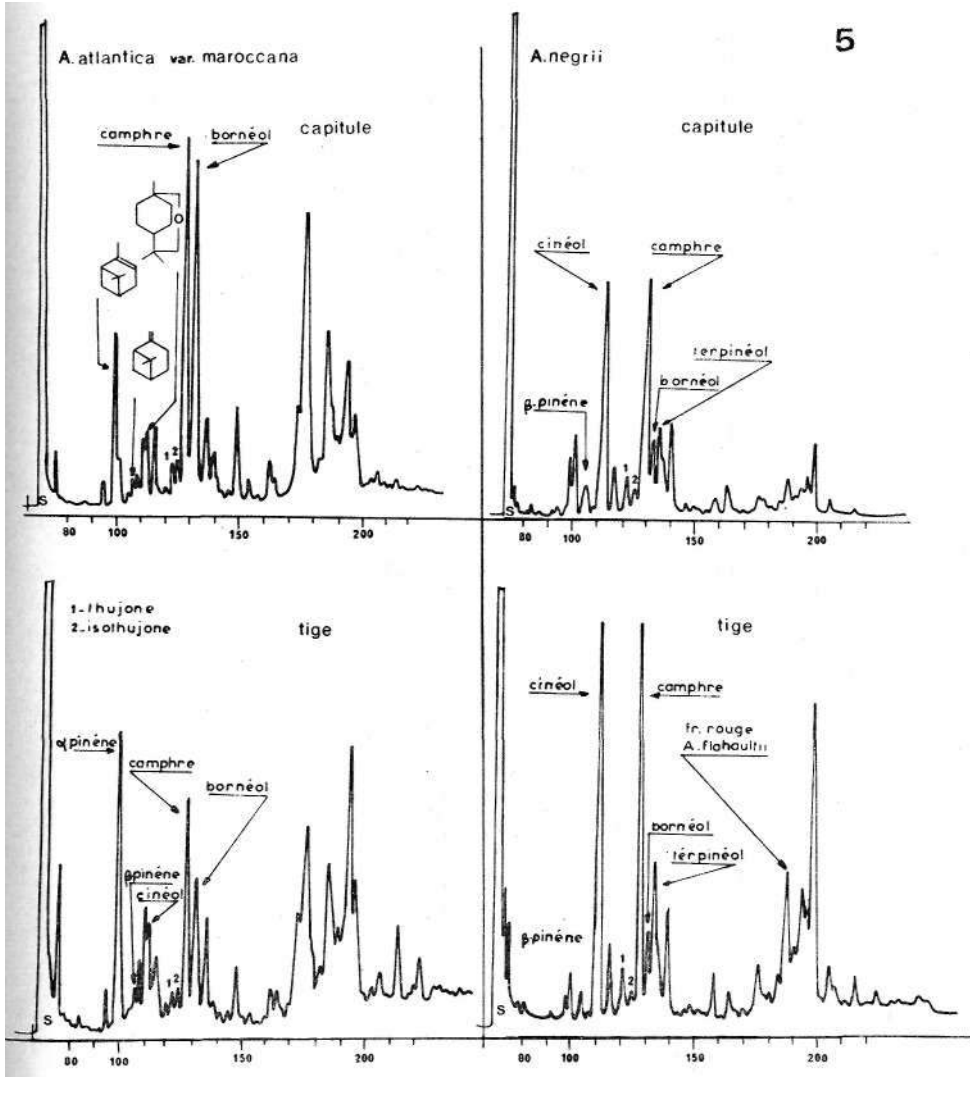

Fig. 5. — Chromatographies en phase gazeuse (OV_1) des huiles essentielles de tiges et de capitules *d'A. atlántica* var. *maroccana* et *d'A. negrii.*

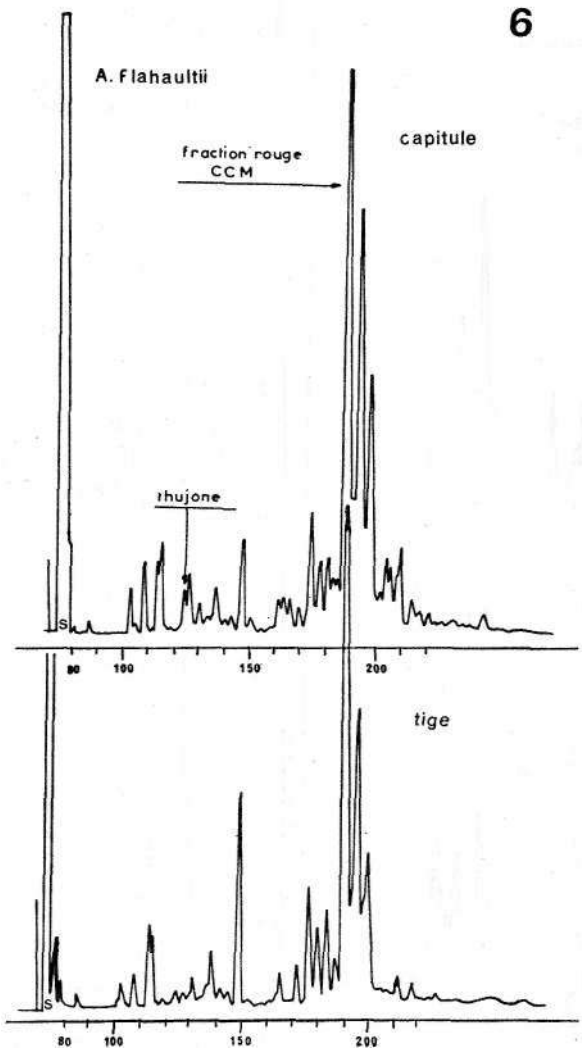

Fig. 6. — Chromatographies en phase gazeuse (OV_1) des huîles essentielles de tiges et de capitules d'A. *flahaultii* (provenant du versant N du Jebel Bou Naceur).

accompagnée d'une quantité notable de composés de nature sesquiterpénique.

Les huiles essentielles *á'A. atlántica* var. *maroccana* et *a'A. negrii* (figure 5) montrent, également, une bonne concordance entre les chromatogrammes relatifs aux tiges et

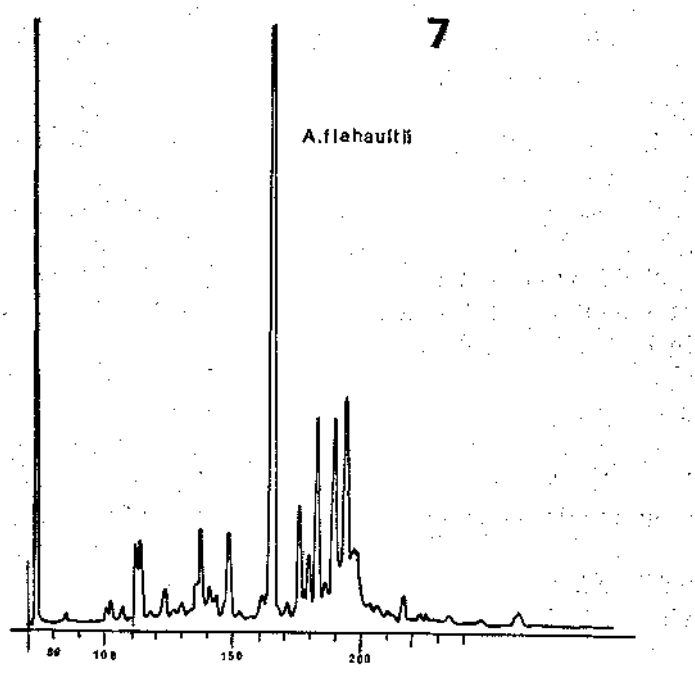

Fig. 7. — Chromatographies en phase gazeuse (OV_1) des huiles essentielles de tiges et de capitules d'*A. flahaultii* (provenant du versant S du Jebel Bou Naceur).

aux capitules. Dans les huiles essentielles de tiges on trouve cependant une quantité supérieure de composés à volumes de rétention élevés correspondant aux sesquiterpenes. Ceci est en particulier très net dans le cas *d'A. negrii.*

En ce qui concerne *A. atlántica* var. *maroccana* on note la quasi-absence de thuyone et d'isothuyone. Les constituants principaux sont l'a-pinène, le camphre et le bornéol. Le b-pinène et le cinéol sont également présents mais en faibles proportions.

En ce qui concerne *A. negrii,* les deux constituants principaux sont le cinéol et le camphre. On a pu également identifier le bornéol et le terpinéol ainsi que le /?-pinène, ce dernier se trouvant en très faibles proportions. En outre l'existence du composé de coloration rouge mis en évidence par Chromatographie sur couches minces par le réactif à l'anisaldéhyde a pu être caractérisée dans *A. negrii* et aussi dans *A. atlántica* var. *muroccana.* Il ne représente qu'une faible portion des composés à volumes de rétention élevés (T^0 de sortie dans les conditions opératoires: 190^0C). Il est surtout abondant dans l'huile essentielle de tiges.

Sur la figure 6 les chromatogrammes obtenus pour les tiges et les capitules *d'A. fláhaultii* récoltés sur le versant N du Jebel Bou Naceur montrent que les deux huiles essentielles contiennent avant tout des fractions lourdes; le constituant principal se trouve être le composé repéré en Chromatographie sur couche mince (révélation: anisaldéhyde). Un peu de thuyone est aussi présente en faible proportion.

Dans les échantillons récoltés au versant sud du même Jebel (fig. 7), les sesquiterpenes précédents sont encore présents mais en proportions plus faibles ; les fractions légères se retrouvent à peu près identiques. Par contre, un autre constituant terpénique fortement majoritaire s'observe aux environs de 165°; ce constituant qui est à peine visible dans les échantillons du versant N n'a pas encore non plus pu être identifié.

Toutes les autres conditions (de végétaux, de substrats, de pente et d'exploitation) étant identiques, cette différence de constitution des huiles essentielles doit être mise en lien avec les différences microclimatiques des deux stations. Une telle constatation souligne à la fois la sensibilité des taxons à la microécologie et les précautions à prendre lors de la récolte d'échantillons en vue de leur analyse chimiotaxinomique; peut-être permettent-elles également d'envisager l'existence de races ou de types chimiques, ce qui devra être vérifié ultérieurement.

En résumé ce bref aperçu sur les constituants des huiles essentielles présentes chez les cinq endémique étudiées met en évidence leur spécificité chimique.

Au plan général et par rapport aux données de la bibliographie actuelle, cette recherche apporte la conviction que les *a*- et *ß*- piñenes sont présents dans les armoises du Maroc, que l'isothuyone et la thuyone peuvent coexister en grande abondance dans le même végétal et surtout que d'abondants sesquiterpenes, dont la nature reste encore à préciser, constituent le constituant majoritaire *d'A. flahaultii*. Il semble que ces constituants aient été mis en évidence ici pour la première fois.

Au plan spécifique, chaque taxon est bien particulier.

A. flahaultii ne renfermant pas de produits volatils mais seulement des sesquiterpenes se classe nettement à part des autres.

A. mesatlantica et *A. ifranensis* se séparent par leur abondance en thuyone et isothuyone et parce qu'elles contiennent un peu de camphre à côté d'une petite quantité de bornéol; en outre *A. ifranensis* contient l'isothuyone alors que *A. mesatlantica* n'en renferme pas, et celle-ci est assez riche en sesquiterpene (ce qui l'allierait à *A. flahaultii)* au contraire de la première.

A. atlántica var. *maroccana* et *A. negrii* se rapprochent au contraire par leur pauvreté en thuyone et isothuyone, leur richesse en cinéol et bornéol d'une part, *a*- et /3-pinène d'autre part, tous caractères qui les distinguent des deux autres groupes; cependant la présence de la fraction sesquiterpénique reconnue chez *A. flahaultii* peut constituer un lien de parenté avec celle-ci. En outre, ces deux taxons diffèrent nettement l'un de l'autre par leurs teneurs respectives en cinéol et bornéol qui paraissent antinomiques l'un de l'autre.

Dans les cinq armoises étudiées, trois groupes apparaissent donc: l'un à sesquiterpenes dominants l'autre à thuyone et isothuyone dominants et le troisième à bornéol, cinéol, piñenes dominants. Ces trois groupes s'ordonnent suivant la figure 8 en fonction de l'altitude (donc du froid) et des étages de végétation. Cet étagement semble assez correspondre avec ce qu'on connaît de la répartition des principales espèces étudiées par BANTHORPE *(l. c),* les thuyone et isothuyone semblent dominantes chez les plantes

planitiaires et juxtamaritimes, le camphre et le cinéol surtout chez les espèces de pays plus froids.

Bien sûr, il est infiniment trop tôt pour tenter de tirer la moindre conclusion de cette constatation tant sur le plan biogéographique, que sur le plan phylogénétique mais on peut déjà, d'une part, avancer l'hypothèse que ces trois groupes pourraient correspondre à trois souches particulières (certes affines les unes des autres par l'intermédiaire des

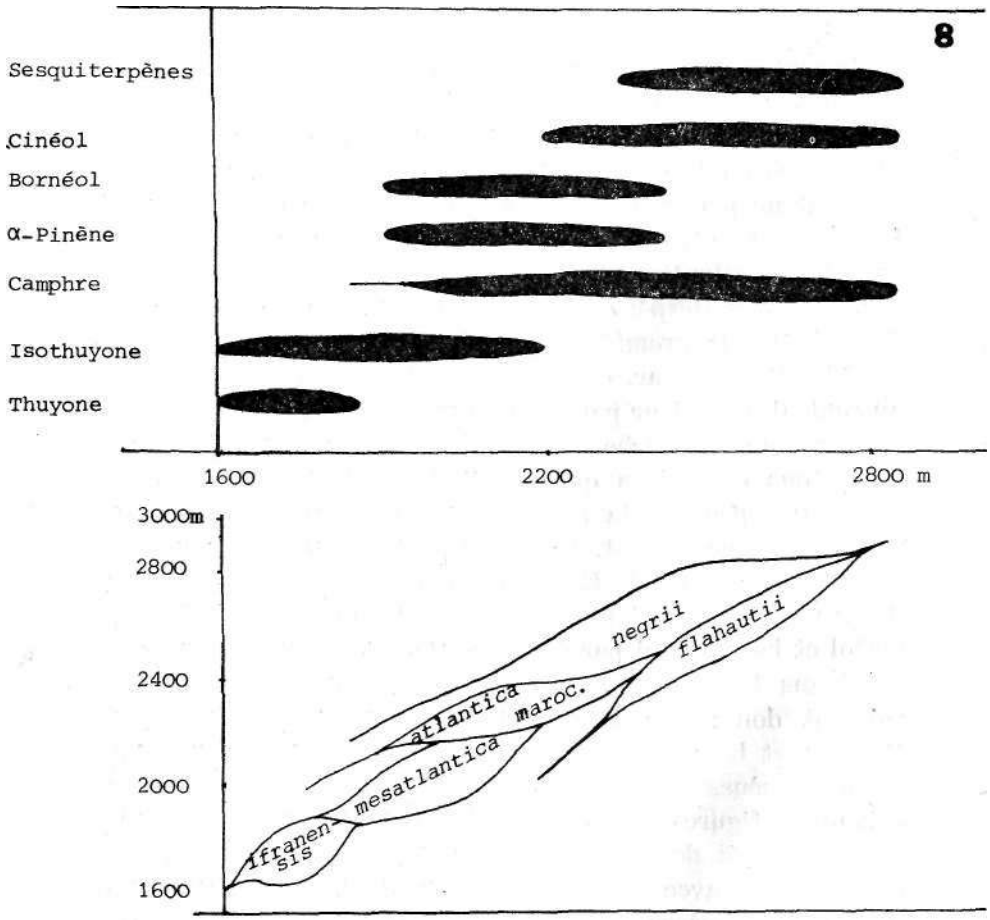

Fig. 8. — Répartition des taxons et des constituants des huiles essentielles en fonction de l'altitude.

sesquiterpenes mais néanmoins bien distinctes), d'autre part, confirmer la validité au plan spécifique, des deux endémiques *A. mesatlantica* et *A. negrii* que d'aucuns pourraient contester au vu de leurs caractères phénologiques d'ensemble. Enfin au plan de l'autoécologie, il n'est pas sans intérêt de constater que des conditions de milieu très différentes entraînent une modification sensible du spectre.

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KARYOMORPHOLOGICAL STUDIES ON MERISTEMATIC CELLS IN *SPIRANTHES SINENSIS*

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SUMMAKY

Observations of the morphology of chromatin in each meristematic cell were made from the root tip of *Spiranthes sinensis,* 2n = 30. The nucleus of each cell cycle showed characteristic features of the chromatin.

1. It was found that the interphase nuclei can be grouped into types according to size and shape of chromatin, i. e., diffused to granular, dark condensed chromatin blocks to faint ones, and round to rod-shaped blocks.
2. Some of the dark condensed chromatin blocks appeared to encircle the nucleolus.
3. At prophase the chromatin tends to take shape as mitotic chromosomes, showing euchromatic segments in the distal region and heterochromatic segments in the proximal region. The dark condensed blocks later become the heterochromatic segments. Thus, these blocks can be said to be the prochromosomes.

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INTRODUCTION

THE somatic chromosomes in *Spiranthes sinensis* are observed to form rod-shaped condensed blocks in the resting nuclei of velamen cells in root tips. From such shape it has been observed that it is of the prochromosomal type nucleus (TANAKA 1971). Some of the prochromosomes were found to convert into diffused chromatin accompanied by the speciating change in this species (TANAKA 1989a). In a study on the synthesis of DNA it was found that the chromosomal blocks were of the early replicating type (TANAKA 1965). Therefore, to study the morphological changes of the chromosomes which take place during the somatic cell cycle is of interest.

One of the authors, HIRAHARA (1979) has differentiated the resting nuclei in each tissue throughout the organs, and a karyomorphological study has been reported. As a result, the resting nuclei can be divided into various types, each tissue possessing its own typical nucleus. However, studies on the mitotic cells of the meristematic cells have not yet been made. Thus, karyomorphological observations on the mitotic cell cycle of the somatic cells will be reported.

MATEKIAJL AND METHOD

The clones of *Spiranthes sinensis* were collected from Hatsukaichi, Saeki-gun, Hiroshima Prefecture. The karyotype of these clones is 2n = 30 = 20¾ + *She* + *2e* (TANAKA 1969a, 19696).

Somatic chromosomes were observed in the meristematic cells of root tips. The root tips were first fixed in 45% acetic acid at 15 C for 10 min and macerated in a mixture of 45% acetic acid and 1 N HCl (1:2) at 60 C for 20 sec, and then squashed in 1 % aceto-orcein.

OBSERVATIONS

Observations were made on various stages in the meristematic region and the results are described as follows:

1. interphase stage

The chromatin in the interphase nuclei appeared as strongly condensed blocks in many nuclei and in some nuclei as rod-shaped to crescent blocks, which are lightly stained only at the edges in the large blocks but the entire chromatin blocks were lightly stained in the small condensed ones. Besides these condensed chromatin blocks, faint, diffused and granular chromatin to slender fiber-like chromatin could be observed (Fig. 1).

The shape of the chromatin blocks differed depending on the nucleus. In some nuclei, all or parts of the chromatin were diffused. In these nuclei several granular chromatin blocks appeared forming fiber-like structures with edges being irregular or rough, whereas, in others not entirely but partially condensed. In such nuclei a layer of granular chromatin could be seen attached to the nucleoli which enables one to easily locate the nucleoli. The size of these nuclei was comparatively small (Fig. 1 A-C, L).

In another interphase nuclei, a few strongly condensed chromatin blocks ranging from 6 to 12 in number could be seen. Other condensed blocks could also be seen but appeared faint. In these nuclei faint condensed blocks ranging from 8 to 10 in number appeared surrounding the nucleoli (Fig. 1 D-H).

Strongly condensed chromatin blocks with edges being quite irregular could be seen in some nuclei (Fig. 1 I). There were more than 20 strongly condensed blocks per nucleus. In this type of interphase nucleus the nucleoli were very indistinct. However, from the circular arrangement of some of the irregular chromatin blocks the nucleoli could be easily located. Such nuclei appeared to be the largest in size.

In another interphase nuclei the condensed chromatin blocks were round to rod-shaped (Fig, 1 J). The chromatin blocks had enlarged and edges were smooth. Some of these condensed chromatin blocks were arranged in a circular form which appeared as if the nucleolus was located within, but, distinction could not be made. There were approximately

30 chromatin blocks, which is equivalent to the number of somatic chromosomes.

Another interphase type had strongly condensed chromatin blocks of round to rod-shaped (Fig. 1 K). These chromatin blocks morphologically appeared as prochromosomes. There were about 30 such blocks measuring 1 u*m* to 3 um in diameter. Faintly stained granular chromatin were diffused throughout these nuclei. In some nuclei the round to rod-shaped chromatin blocks appeared to form a ring. Within the round rings it was more translucent and clear compared to the other parts of the nucleus.

From these observations it was revealed that the nuclei can be grouped according to the shape and size of the chromatin. As shown in Fig. 1 A-C and L, granular chromatin blocks could be seen throughout the nuclei with a layer of granular chromatin around the nucleoli. The size of such nuclei is somewhat small, i. e., about 20 um in diameter. These nuclei gradually showed strongly condensed chromatin blocks of irregular shape. In such nuclei a few round chromatin blocks appeared as if attached to the nucleoli as in Fig. 1 D-H. Eventually, the partially condensed chromatin blocks appeared larger and other parts within the nucleus somewhat more granular as shown in Fig. 1 I-K. The size of the nuclei as well as the nucleoli appeared larger than those observed in other cells mentioned above.

2 Resting stage in differentiated cells

In a few parts of the meristematic region several enlarged or elongated cells were observed. The cells were regarded as the differentiated cells at resting stage. In these cells a distinct layer of chromatin with the edges quite irregular could be seen around the nucleoli (Fig. 2 A, B). Chromatin within the nuclei were also irregular in form, although in some nuclei the chromatin formed round or rod-shaped blocks with rough surface.

In comparatively larger cells, the nucleoli were observed to be also quite enlarged (Fig. 2 C, D). Around these nucleoli a layer of chromatin surrounded the nucleoli, which made

the nucleoli distinct. In these nuclei the chromatin were round and condensed with various size. Granular chromatin were diffused throughout the nucleus. In some nuclei the location of the nucleoli was faint and as the condensed chromatin blocks became very distinct the nucleoli could no longer be seen (Fig. 2 E, F). This type of nucleus with both small and large condensed round or rod-shaped chromatin blocks distributed throughout the nucleus correspond to Type A, which was observed from the resting nuclei by one of the authors (HIRAHARA, 1979).

3. **Mitotic stage**

As already reported by TANAKA (1969a, 1969b), the standard karyotype of *Spiranthes sinensis* is 2n=30=20h + + *She* + 2e and the deheterochromatinization is found in the prophase chromosomes. Twenty chromosomes were heterochromatic while 8 had a small heterochromatic segment in the proximal region and a large euchromatic segment in the distal region of the long arm, while the short arm was euchromatic and relatively long. The remaining 2 chromosomes were euchromatic. This observation is shown in Fig. 3.

In the chromosomes from middle prophase to metaphase the chromosomes were counted to be 2n = 30, which confirmed the previous reports (cf. TANAKA and KAMEMOTO, 1974). The primary constrictions in the mitotic chromosomes were quite distinguishable and the location of heterochromatic regions could be observed (Fig. 3 A-C). Some segments at the distal region were still slightly extended and faintly stained showing euchromatic regions at late prophase. At anaphase to telophase the euchromatic regions were extended, while the heterochromatic regions remained condensed (Fig. 3 D-F).

Morphological changes of chromosomes in the mitotic cycle of *Spiranthes sinensis* can be summarized as follows: Two daughter nuclei appeared as pairs within two daughter cells at early interphase (Fig. 4 A). The size of each nucleus was about 15 um. One or two small round nucleoli could be seen within the nucleus. Around the periphery of the

round nucleoli a few irregular projections of dark stained chromatin blocks were attached to the nucleoli, which enabled one to easily located the nucleolus. Within the nucleus partially condensed chromatin threads, which were made up from small granules, spread in a network pattern. Besides these threads, light fiber-like strands and small granules were diffused throughout the nucleus (Fig. 4 B).

In some nuclei large condensed chromatin blocks of irregular shape with edges quite indistinct could be seen. In these nuclei either faint condensed blocks or strongly condensed chromatin blocks appeared surrounding the nucleolus. Faint granular chromatin was diffused throughout the nucleus (Fig. 4 C, D). Next, the condensed chromatin blocks became round or rod-shaped. The chromatin blocks were enlarged and edges were smooth. Some of these condensed chromatin blocks were arranged in a circular form which appeared as if the nucleolus was located within. There were approximately 30 chromatin blocks, which is equivalent to the number of somatic chromosomes (Fig. 4 E).

At prophase the condensed blocks extended gradually and changed into slender threads becoming progressively more clearly visible as chromosomes, but among the dark stained chromosomes some were faintly stained (Fig. 4 F). It has already been reported that at this stage some chromosomes appear euchromatic or partially euchromatic (TANAKA 1969a, 1969b). In the chromosomes from middle to late prophase the primary constrictions were quite distinguishable and the location of heterochromatic regions could be observed. Some segments at the distal region were still slightly extended and faintly stained showing euchromatic regions (Fig. 4 G).

The chromosomes at mitotic metaphase were located at the equatorial plane of the cell (Fig. 4 H). As already reported by TANAKA (1969b) the chromosome number was confirmed to be $2n = 30$. Only one proximal heterochromatic segment occurred within a chromosome. The chromosomes were of relatively small size, the longest being 3.1 um.

Anaphase follows metaphase in the mitotic cycle. The centromeres divided so that each chromatid had its own

centromere (Fig. 4 I). They then moved apart from each other to initiate a slow movement that would take sister chromatids to the opposite pole. The daughter chromosomes reached almost simultaneously to the pole by their own centromere at first, then leaving the long arms at the middle part of the spindle to the pole where the daughter chromosomes formed a chromosome complement at the two poles. At early telophase the daughter chromosomes in the condensed state located themselves gradually forming round daughter nucleus (Fig. 4 J). At middle telophase the chromosome diffused to become slender threads again. The diffusion occurred at both ends of the chromosomes. The chromocenters and nucleoli made their appearance at middle telophase. At the equator a new cell wall was formed (Fig. 4 K). Two daughter nuclei took on an interphase character, i. e., densely packed chromatin blocks with diffused chromatin throughout the nucleus (Fig. 4 L).

TANAKA (1965) has shown by autoradiography that in the mitotic cells of *Spiranthes sinensis* the above two nuclei, diffused nuclei and partly condensed nuclei, like in Fig. 4 B and C, respectively, are in the DNA synthetic period. From the observations it can be presumed that the nuclei like in Fig. 4 A, K and L are in the G_1 period and those in Fig. 4 D and E in the G_2 period.

DISCUSSION

A great many observations on the morphological transformation of chromosomes during the somatic cell cycle have been reported since the review of BELAR (1928). These studies are mainly dealt on the mitotic stage and only a few on the morphological transformation of the interphase nuclei. During mitosis, the interphase chromosome transforms morphologically when undergoing DNA synthesis (KUROIWA and TANAKA 1971) and C-bodies, as a form of chromosomes, also transform (KOMATSU and TANAKA 1978). However, generally, in plants and animals the interphase chromosomes undergo a complicated morphological transformation and thus, it is often times difficult to grasp all

the detailed structures (TSCHEEMAK-WOESS 1963, LAFONTAINE 1974).

Since the material used in the present study, *Spiranthes sinensis,* takes a prochromosomal type form of interphase (TANAKA 1969¾ 1971), it is comparatively easy to trace the interphase chromosomes. As a result, at interphase almost the entire prochromosomes transform into a diffused type and this finding coincides with the observations on the DNA synthesized nuclei reported by TANAKA (1965). From the observations that a portion of the prochromosome surrounds the nucleolus, it can be said that several chromosomes take part in the formation and function of the nucleolus by encircling it.

On the other hand, during interphase, prochromosomes undergo an extreme morphological change, in other words, become diffused, granular, condensed, round, rod-shaped or fiber-like in structure. Furthermore, in each of the forms the surface appeared either smooth or irregular. The morphological change in the prochromosomes appear to be influenced by the physiological conditions of the cell. Further study is needed to elucidate this observation.

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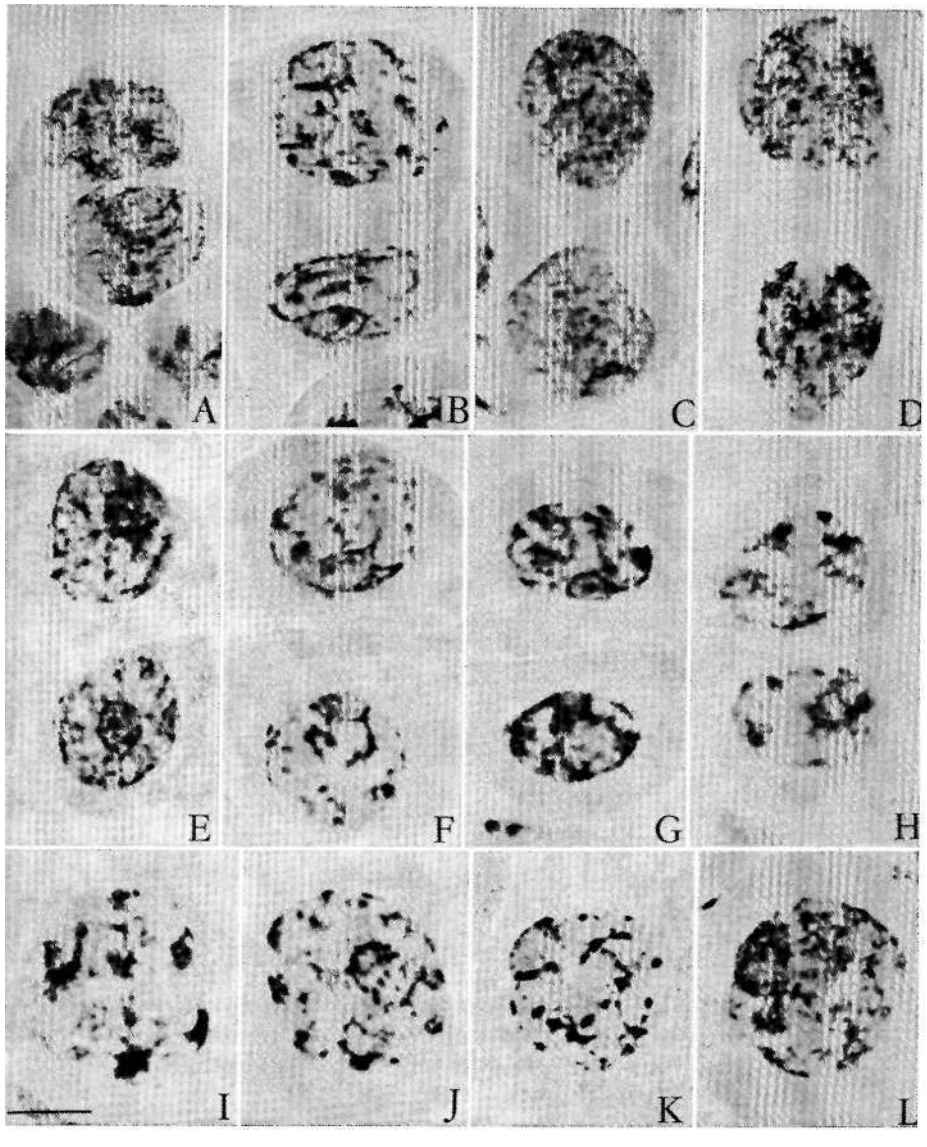

Fig. 1. — Photomicrographs showing variation in the morphology of the chromosomes at the interphase stage observed in the meristematic region. Bar indicates 10 $_{u}$m.

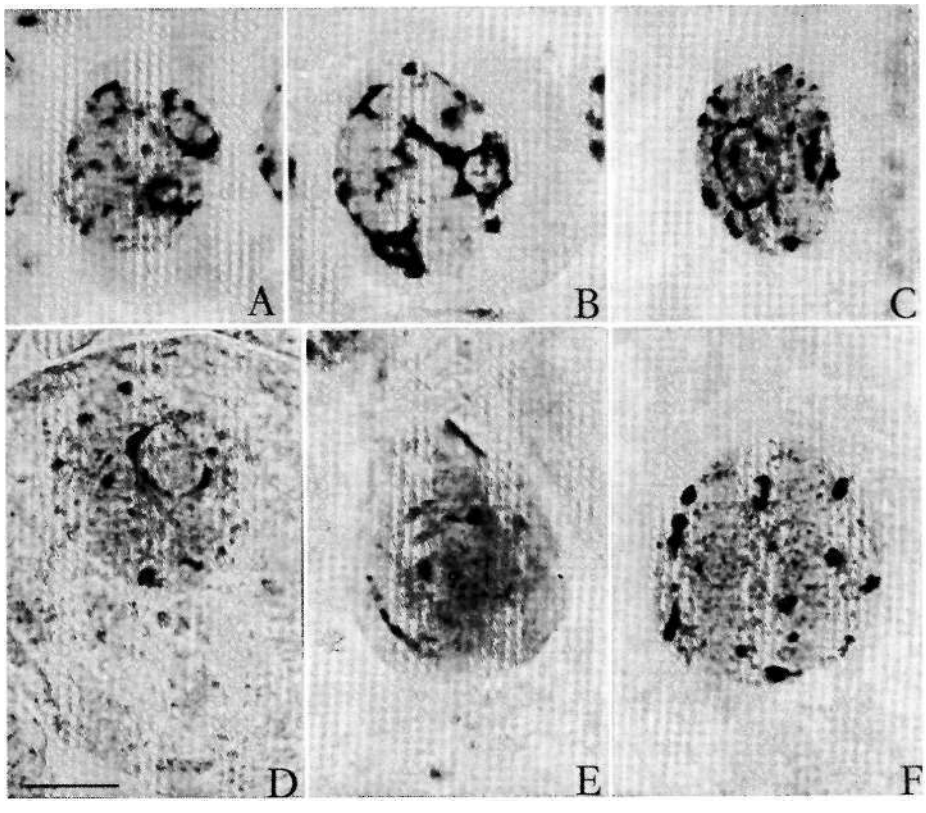

Fig. 2. — Photomicrographs showing variation in the morphology of the chromosomes at the resting stage in differentiated cells of the meristematic region. Bar indicates 10 u.m.

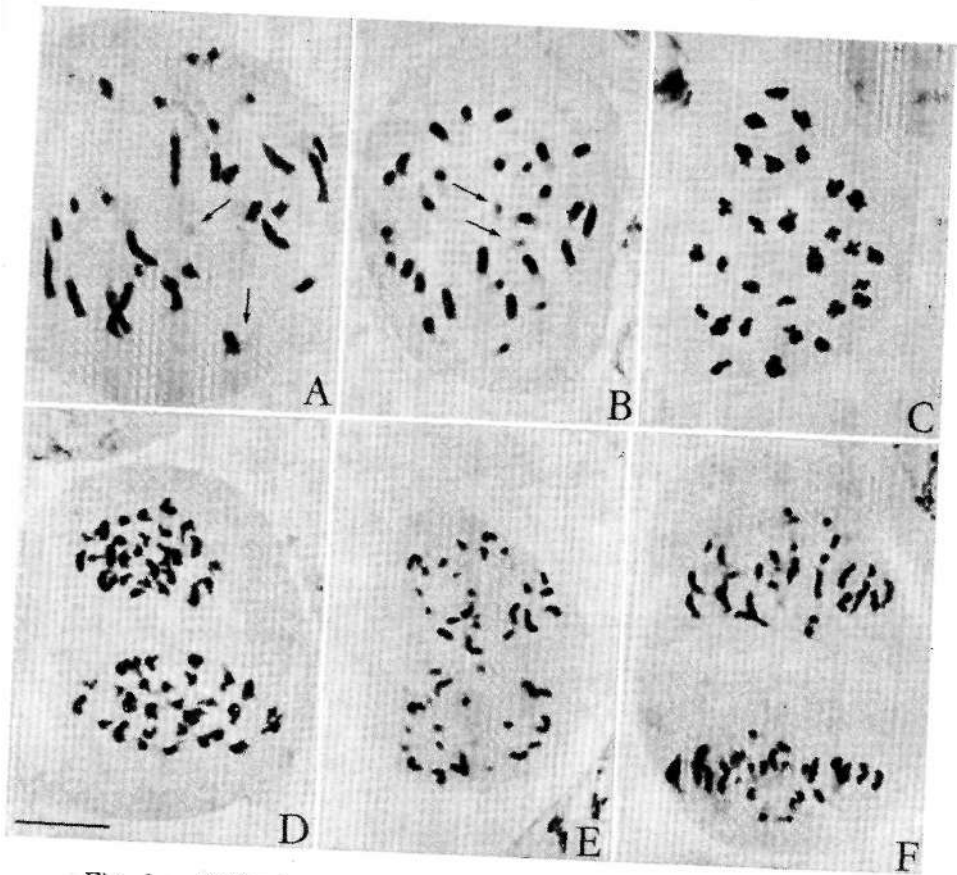

Fig. 3. — Photomicrographs showing variation in the morphology of the chromosomes at the mitotic stage observed in the meristematic region. Arrows indicate *e* (euchromatic) chromosomes. Bar indicates 10 μm.

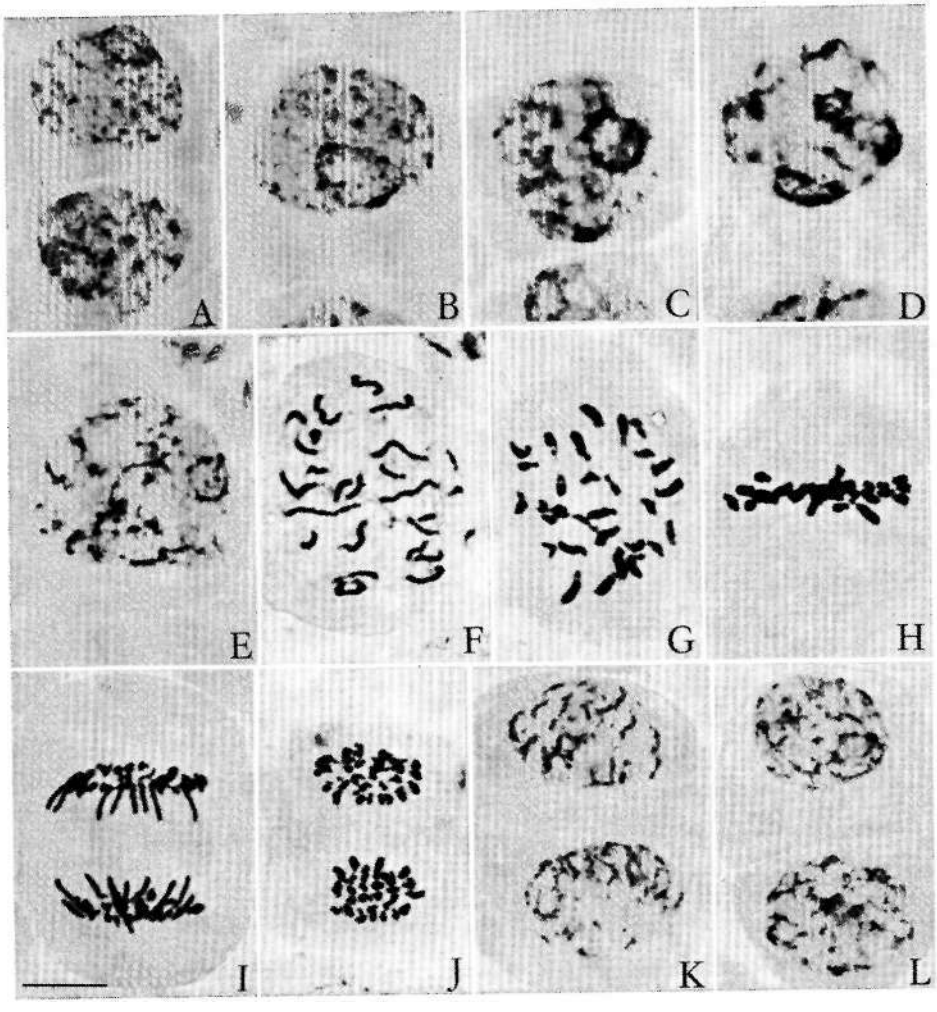

Fig. 4. — Photomicrographs showing variation in the morphology of the chromosomes in the mitotic cycle observed in the meristematic region. A, K, L are presumed to be in G_1 period, B, C in the DNA synthetic period, and D, E in the G_2 period. Bar indicates 10 $_u$m.

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BINUCLEATE CELL FORMATION IN A PUTATIVE HYBRID BETWEEN 4X *LOTUS TENUIS* AND *L. EMEROIDES*

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ABSTRACT

Interspecific hybridization was attempted between two widely diverse species of *Lotus* having different basic chromosome numbers, namely a synthetic autotetraploid form of *L. tenuis* Waldst. et Kit. (2n = 4x = 24) of the Eulotus group and the more primitive species *L. emeroides* R. P. Murphy (2w == 4x = 28) of the section Endolotus. *Lotus tenuis* served as the female parent. Three pods were obtained out of 128 cross-pollinated florets. Ten viable embryos were recovered, transferred to embryo culture, but did not develop further. Binucleate cells, the first to be reported in the genus, were repeatedly observed in the embryonic tissues. While the two species are quite dissimilar, it is considered that a viable hybrid might be obtained with modified techniques.

INTRODUCTION

INTERSPECIFIC crosses between two species of the same genus have been attempted and successfully implemented in other legumes; the purpose of which has been mainly to study species interrelationships and to attempt a transfer of germplasm from one species to another for characters which may be of economic value (QUESENBERRY and TAYLOR, 1978; MOK *et al,* 1978).

In the genus *Lotus* a number of studies have been undertaken between different species for cytotaxonomic evaluations of the species and for studying the genetics of

the interspecific hybrids (DE NETTANOOURT and GRANT, 1963; SOMAROO and GRANT, 1971). GRANT *et al.* (1962) successfully hybridized three diploid species *(2n=2x=12)* closely related to the tetraploid species *Lotus corniculatus* L. (2»= 2x=24) which is a commercial forage legume. SOMAROO and GRANT (1971) hybridized seven species of *Lotus* which were diploid (2n — *2x* = 12), as well as 67 combinations of amphidiploid and autoploid derivatives of those seven diploids (SOMAROO and GRANT, 1972) all of which were on the tetraploid level (2n = 4x = 24). The latter tetraploid taxa were hybridized in combination with *L. corniculatus* in an attempt to establish interspecific relationships between the species which are closely related to *L. corniculatus.* A similar study was carried out on a lesser scale by CHEN (1968) where a diploid *(2n* = 12) and a tetraploid (2n = 24) form of *Lotus pedunculatus* L. were hybridized and a triploid (2n = 3x = 18) hybrid was successfully produced.

In these studies species closely related to *L. corniculatus,* all of which have a basic chromosome number of *x* = 6, were used. This study attempted to hybridize two species of the genus *Lotus* with different basic chromosome numbers and, hence, of more diverse phylogenetic origin. *Lotus tenuis* Waldst. et Kit. has a basic chromosome number of *x* = 6 and belongs to the Eulotus group, and *Lotus emeroides* R. B. Murray has a basic chromosome number of *x* — 7 and belongs to the Endolotus group. Autotetraploids of *L. tenuis (2n — 4x* = 24) have been produced through colchicine treatment, whereas *L. emeroides {2n* = 4a? = 28) is a natural tetraploid.

MATERIAL, AND METHODS

The species used in this study were 1) a synthetic autotetraploid form of *Lotus tenuis (2n* = 4a? = 24) obtained from the Plant Introduction Station, Geneva, New York, and 2) *Lotus emeroides (L. borzii Pitard),* originating from Gran Canaria, Teneriffe, Spain, which is a natural tetraploid form with a chromosome complement of 2n = 4a? = 28 (LARSEN, 1958).

In our study we arbitrarily used *L. emeroides* as the male parent, and *L. tenuis* as the female. Single plants of *L. emeroides* and *L. tenuis* were grown simultaneously in the greenhouse with a photoperiod of 18 h and a temperature of 12° to 25° C. Crosses were carried out when both species were in full bloom.

Florets of *L. tenuis* were emasculated using the technique of GRANT *et al.* (1962) with the following modifications: a slit was made vertically along the entire length of the keel to expose all anthers, and anthers were removed with an air-suction device; pollination with pollen from *L. emeroides* was conducted immediately after emasculation of the *L. tenuis* plants. A total of 128 florets were emasculated on three separate occasions (48, 46, and 34 florets, respectively). As controls, a total of approximately 300 florets were tagged and left unpollinated; pollinated florets were tagged with colored tape as a means of identification. In addition, reciprocal crosses between *L. tenuis* plants and also between *L. emeroides* plants were made on ten florets per species.

Nineteen days after fertilization from the first set of pollinations, one abortive pod was fixed in modified Carnoy's fixative (3:2:1 95% ethanol: chloroform: glacial acetic acid) and stained using the Feulgen technique. The pod was then placed in 5 % pectinase for 2 h, after which five embryos were dissected, macerated, squashed in 45 % aqueous acetic acid and examined under a microscope. A second non-abortive pod was removed 22 days after pollination, viable embryos were dissected and cultured using the embryo-culture technique of MuRiSHiGE and SKOOG (MSl) (1962). After three weeks, two embryos were found to be viable and were in turn transferred to a modified version of MSl medium, which entailed the addition of 5 ppm kinetin and 0.2 ppm napthalene acetic acid (NAA) to induce root formation. A third pod was obtained from the third cycle of pollination. One viable embryo was dissected and cultured in modified MSl medium.

Ten embryos were obtained from five pods in the *L. tenuis* X *L. tenuis* crosses and 12 embryos were obtained from

four pods in the crosses of *L. emeroides* X *L. emeroides.* Embryos from both crosses were fixed and stained in the same manner as previously described.

RESULTS AND DISCUSSION

Table I indicates the number of pods obtained from the crosses between *L. tenuis* and *L. emeroides;* no pods were produced on unpollinated florets indicating the self-incompatible nature of 4x *L. tenuiis,* as been noted previously (BUBAR, 1958; DE NETTANCOOURT, 1963).

SOMAroo and GRANT (1972a) found pod set to vary widely in crosses of a number of synthetic amphidiploids related to *L. tenuis* and *L. corniculatus.* They found pod set to range from 6.25 % for *(L. filicaulis* X *L. schoelleri)*^{2} X *L. corniculatus* to 85.15 % for *(L. japonicus* X *L. alpinus)*^{2} X *L. corniculatus.* Our results of 2.3 % for *L. tenuis* X *L. emeroides* falls outside the lowest value of this range, owing largely to the cytological and genetic diversity of these two species.

From an examination of embryonic tissue, no actively dividing cells were found; therefore a chromosome count of the embryo was not possible. We found that our cultured embryos showed either no growth or extensive necrosis. This is in sharp contrast to the fairly high success rate obtained by SOMAROO and GRANT (1971) in culturing hybrid embryos from a number of hybrid crosses having *L. tenuis* as one parent.

Figure 1 shows a binucleate condition found in some cells of all the embryos examined. On crossing *L. tenuis* with *L. tenuis* the embryos obtained did not show such a binucleate condition; the same was found for the cross between *L. emeroides* X *L. emeroides;* this suggests that the binucleate cell condition arose as a result of the unstable chromosome complements arising in the putative hybrids.

The binucleate condition has never been reported in *Lotus.* This is indicative that the species *L. emeroides* and *L. tenuis* are quite dissimilar and that a viable hybrid between these two species may be difficult to obtain. Such

crosses should be further attempted in order to be able to successfully transfer desirable characters from widely diverse species in the genus.

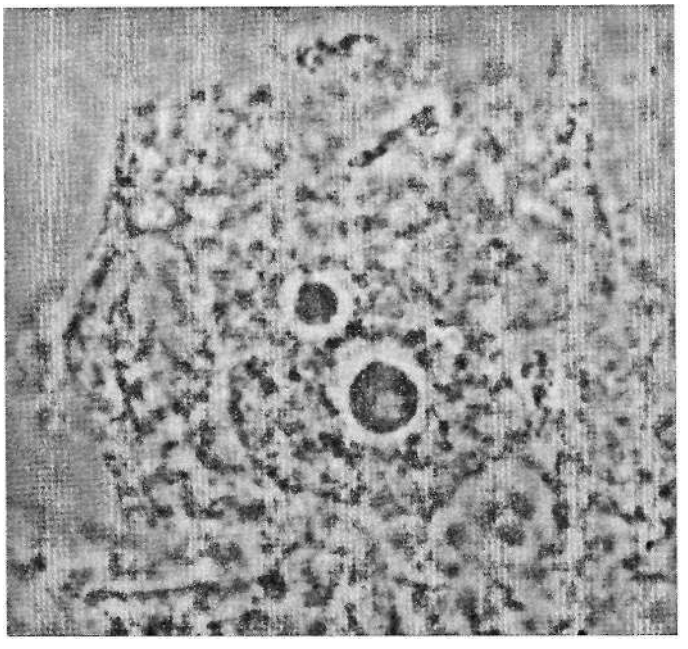

Fig. 1. — Binucleate cell in hybrid between 4*x Lotus tenuis* and *L. emeroides.* Nuclei are not in the same plane of focus.

TABLE I

Production of pods from cross-pollination with *L. tenuis* X L. *emeroides*

No. of triais	No. of florets	No. of pods produced
(unpollinated)		
1	300	0
(pollinated)		
1	48	2
2	46	0
3	34	1

ACKNOWLEDGEMENT

The financial assistance of the Natural Sciences and Engineering Research Council of Canada is gratefully acknowledged.

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MEMECYLON FERNANDESIORUM JAC-FEL. ESPÈCE NOUVELLE DE MADAGASCAR

par

H. JACQUES-FÉLIX

Museum national d'Histoire naturelle de Paris

UN *Memecylon* de Madagascar, récolté par H. PERKIER DE LA BÂTHIE, et opportunément nommé *M. pterocarpum* par lui-même, présente cette particularité d'avoir un fruit orné d'ailes méridiennes. C'est probablement en raison d'un caractère aussi singulier pour le genre, qu'un spécimen de cette espèce, recueilli bien antérieurement par A. DUPETTT-THOUARS, dû d'être relégué parmi les *incertae sedis* et de n'être pas étudié par C. NAUDIN, ainsi que le furent les autres espèces rapportées, tant de Madagascar que des Mascareignes, par ce célèbre botaniste explorateur.

Le matériel relatif aux *Memecylon* s'étant considérablement enrichi depuis H. PERRIER, notamment grâce aux vigoureuses prospections du Service forestier de Madagascar, animé par R. CAPURON, il convenait de mieux étudier cette espèce, dont la description est incomplète, afin de vérifier si d'autres caractères sont associés à celui, quelque peu aberrant, du fruit. Malheureusement, il se trouve que les récoltes complémentaires, de même provenance régionale, ne portent également que des infrutescences, de sorte que nous n'en connaissons toujours pas les fleurs. Par contre, un *Memecylon* nouveau, récolté plus au nord, avec des spécimens complets, doit nous permettre de mieux comprendre ce qu'est le *M. pierocarpum*, en raison des affinités étroites entre ces deux espèces.

Memecylon fernandesiorum Jac.-Fél., sp. nov.[1]

A M. pterocarpi, fructo non alato differt.

Arbuscula; ramis junioribus acute quadrangularibus, vel sursum internodorum alatis. Folia subsessilia, utrinque nitidula; petiolo 1-2 mm longo; anguste elliptico-lanceata, usque 3,5 X 12-15 cm; basi rotundata, deinde petiolum subcordata; apice obscure obtuseque acuminata, acumine 1-2 cm longo; nervo mediano supra impresso, infra prominenti; nervis pennatis 14-18 paribus, subperpendicularibus, supra obsoletis, infra modice prominentibus; nervis convergentibus obsoletis, submarginalibus. Cymae solitariae vel ternatae ad nodos foliatos nuper defoliatosque; sessiles vel stipite robusto, 3mm longo; simplices, 3(1) floribus sessilibus; bracteis scariosis; bracteolis late ovato-cordatis, 3 X 3 mm, imbricatis, ad hypanthium applicatis. Flos campanulatus, demum urceolatus ob calycem auctum; lobis calycis membranaceis, limbum aequantibus, truncatis, 2,5X1 mm, imbricatis; petalis violaceis, 4 X 4,5 mm, rhombo-unguiculatis, vel subhastatis, ungui crasso. Stamina basifixa, anthera 2-2,2mm longa; connectivo basin versus praecipue crasso, subparallelo, basi obtuso, eglanduloso; filamento 6-7 mm long. Ovarium 6-8 ovulatum; cavité epigyne crateriformi, parietalibus valde sulcatis. Stylus 10 mm longus. Fructus globosus, 1,5 cm diameter; corona calycis erecta, 3 mm alta; semine late adherenti. Embryo cotyledonibus foliaceis, convolutis; hypocotylo elongato; radicula valde invaginata.

Tab. I.

Madagascar: regione boreo-orientali, in sylvis littoralibus arenosis, circum Sambavam, *Humbert* 24386 (P), 28.XI-3.XII. 1950; *Capuron* SF. 24922 (p; TAN), 20.X.1966; *Capuron* SF. 27698 (holotypus, P; isotypus, TAN), 1-10.IV.1967.

[1] Dédié à ABÍLIO et ROSETTE FERNANDES, dont les beaux travaux ont considérablement amélioré notre connaissance des Melastomataceae d'Afrique.

Quelques caractères communs et distinctifs nous permettent de séparer le *Memecylon fernandesiorum* du *M. pterocarpum,* et de les considérer, l'un et l'autre, comme suffisamment apparentés, et opposés aux autres espèces, pour constituer une petite unité infragénérique, dont nous traiterons ultérieurement dans le cadre d'une étude générale des *Memecyleae* de Madagascar.

Tout d'abord ces deux espèces ont sensiblement même aspect général, par leurs rameaux quadrangulaires-ailés; par leurs feuilles présentant le même type de nervation, et le même type de sclérites filiformes, dont on peut reconnaître l'existence à la déchirure fibreuse des limbes. Cependant, les rameaux sont plus robustes, et les feuilles généralement plus grandes, chez le *M. pterocarpum.*

Avec des fleurs sessiles, immédiatement sous-tendues par une paire de bractéoles, les inflorescences sont également du même type. Toutefois, chez le *M. fernandesiorum,* les cymes sont peu fleuries, principalement situées aux aisselles foliaires et noeuds récemment défeuillés, alors que chez le *M. pterocarpum,* elles sont plus copieuses, et se renouvellent sur les noeuds du vieux bois, en formant d'épais coussinets.

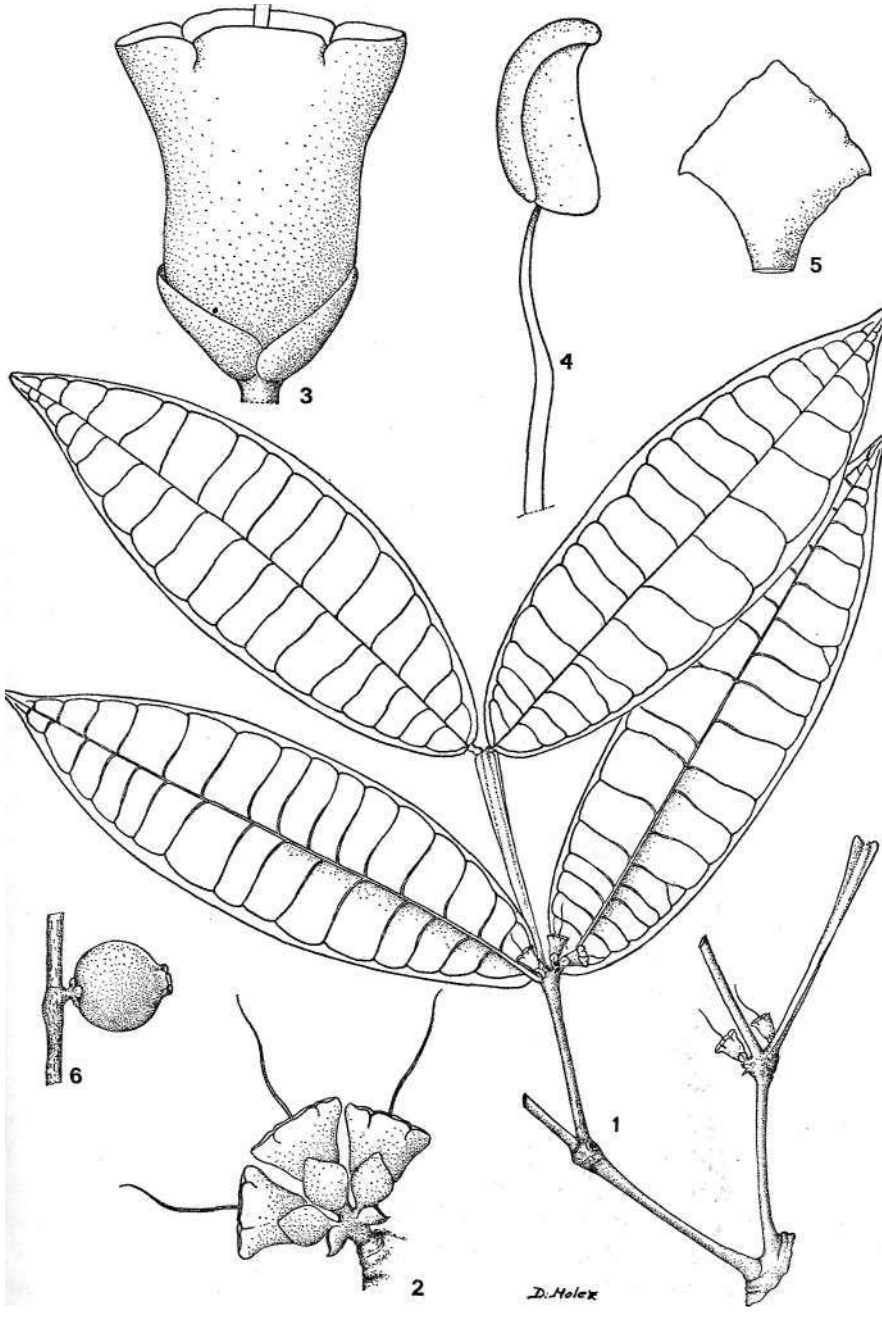
Notons encore la grande taille des fruits, et surtout la large adhérence de la graine au placenta, déjà signalée par H. PERRIER dans sa description du *M. pterocarpum.* Pour très prononcé qu'il soit, ce dernier caractère n'est pas exclusif. Une large cicatrice hilaire, dont le tissu parenchymateux contraste avec les parois crustacées de la graine, et favorise probablement la germination, s'observe chez d'autres *Memecylon.*

Les précédents caractères sont donc surtout utiles à la spéciation, mais ne suffisent pas pour affirmer l'originalité de nos deux espèces parmi leurs congénères. Avec leur connectif peu divergent, et dépourvu de glande, les étamines du *M. fernandesiorum* sont déjà plus significatives. Cependant, l'absence de glande, considérée indépendamment de la forme de l'anthère, n'est pas exceptionnelle non plus chez les *Memecylon* de Madagascar, et H. PERRIER la cite pour plusieurs de ses sections.

Les *Memecylon*, tels qu'ils sont compris après le rétablissement du g. *Warneckea*, sont, à une exception près[1], caractérisés par un calice entier, ou sinué, ou lobé-valvaire. Or, le calice de nos deux espèces, tel que l'on peut l'observer sur la fleur du *M. fernandesiorum*, et tel qu'il persiste sur le fruit du *M. pterocarpum*, est identique, avec des lobes bien différenciés et imbriqués. C'est bien ce caractère qui nous permet de confirmer, même en absence de fleurs, que le *M. pterocarpum*, malgré son fruit ailé, est étroitement allié au *M. fernandesiorum*, que nous connaissons mieux.

En conclusion, bien que spectaculaire et exceptionnelle dans le genre, l'alature du fruit chez le *M. pterocarpum*, nous apparaît comme un caractère secondaire, seulement de valeur spécifique.

[1] Le *Memecylon humbertii* Perr. (Sect. Humbertocylon Perr.) est décrit comme ayant également des lobes calicinaux imbriqués. Il s'agit d'une espèce tout à fait différente de celles que nous avons examinées ici.



Memecylon fernandesiorum Jac.-Fél. : 1, rameau fleuri X ⅔ ; 2, cyme de trois fleurs X 2; 3, fleur et ses bractéoles (pétales et étamines enlevés) X 6; 4, étamine X 12; 5, pétale X 6; 6, fruit X 2/3
(1 à 5, *Capurón* SF. 27698; 6, C*apuron* SF. 24922).

Bol. Soc. Brot., Sér. 2, 53 (2): 1077-1095 (1981).

CYTOTAXONOMIC STUDIES ON *GALIUM HARCYNICUM* WEIG.

by

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SUMMARY

Galium harcynicum Weig. is an European-Atlantic species in which two cytotypes occur, a diploid (2n = 22) and a tetraploid (2n = 44). The tetraploid is widely distributed throughout the whole area of the species. The diploid has a restricted, eccentric, southern distribution.

The difference in chromosome number is correlated with quantitative morphological differences, including the size of the pollen grains.

Vegetative characters are influenced by the environment. Floral characters are stable or only slightly modifiable.

SEM-micrographs of the fruits did not show a difference in the fine-structure of the fruit-epidermis of both cytotypes.

Preliminary results of a phytochemical investigation of flavonoids show a different pattern in the chromatograms of the diploid and the tetraploid.

INTRODUCTION

Galium harcynicum Weig. is an European-Atlantic species commonly distributed from the South of Scandinavia to the North of Spain and Portugal, and to the British Isles, and extends eastwards across Europe into Bohemia and locally to the Karelian Isthmus and the Central Carpathians.

The species is strictly calcifuge. It prefers poor, moist to dry soils and generally grows in open woodlands, along forest-edges and in open habitats such as heaths, moors

and grasslands. It is hermaphrodite, protandrous and self-incompatible and is pollinated by flies and other small insects.

Galium harcynicum belongs to the section *Leptogalium* Lange and within this section it is characterized by well defined morphological characters. Two cytotypes are known. A diploid with 2n = 22 chromosomes (KLIPHUIS, 1962, 1967, **1972;** KUPFER, 1969) and a tetraploid with 2n = 44 chromosomes (FAGERLIND, 1934,1937; EHRENDORFER, 1956; KLIPHUIS, **1962, 1967, 1972;** GADELLA and KLIPHUIS, 1963; PIOTROWICZ, **1964).** Whereas the tetraploid is distributed throughout the whole area of the species, the diploid seems to be restricted to the North-Western parts of the Iberian Peninsula (KLIPHUiS, **1972).**

The chromosome number is correlated with quantitative morphological differences, the diploid being more fragile with slender shoots, shorter internodes, fewer flowers per inflorescence, smaller leaves, flowers and fruits.

Hybrids of the two cytotypes are not known. Crossing experiments were unsuccessful. This indicates a reproductive barrier between the two levels of ploidy (KLIPHUIS, 1972). This phenomenon is also known from other Galium species. Inside the genus there is a strong reproductive barrier between various levels of ploidy, intra- as well as interspecifically. These barriers are extremely effective in lower polyploid levels, but become less effective in the higher ploidy levels (FAGERLIND, 1934,1937; EHRENDORFER, 1955; KLIPHUIS, **1970,** 1972, **1973, 1974).**

According to their morphological, geographical and karyological characteristics the two cytotypes have been defined as subspecies, the diploid as subspecies *vivianum* Kliph., the tetraploid as subspecies *harcynicum* (KLIPHUIS, **1972).** HOLUB (1974) is of the opinion that there are sufficient arguments for classifying the diploid as an independent species, e. g. *Galium vivianum* (Kliph.) Holub.

Galium harcynicum is generally described as uniform in its morphology. In most Floras it is not subdivided. An exception is made by ROUY (1903) in his «Flore de France». He distinguishes four varieties. COUTINHO (1939)

mentioned two of these for Portugal e. g. var. *genuinum* Rouy and var. *riparium* Rouy. Their descriptions do not agree with any of the cytotypes (KLIPHUIS, 1972). Probably they are nothing but extremes of morphology connected by all possible intermediates, as suggested by FONTES (1948).

The present study is a continuation of an investigation the result of which was described in an earlier paper (KLIPHUIS, 1972). More material was obtained and, therefore, more was available for investigation. The results of palynological and phytochemical studies will be given here in addition to cytological and morphological data.

MATERIAL AND METHODS

Plant material

Plants obtained directly from the wild as well as plants grown from seed which was collected in the field were cultivated under uniform conditions in an experimental plot of the Botanical Garden of the State University of Utrecht.

From each seed-sample 3-5 plants were used for cytological purposes.

Cytological studies

Chromosome counts were made from root-tip mitosis of potted plants. The tips were fixed in Karpechenko's fixative, embedded in paraffin wax, sectioned at 15 micron and stained according to Heidenhain's haematoxylin method.

Scanning electron microscopical studies

A Cambridge stereoscan 600 M was used to perform SEM studies on fruits sputtered with gold.

The fruits were cleaned in water for 10 seconds by means of ultrasonic waves in a sonicator (Sonicor, SC 50-22).

Palynological studies

Pollen grains of both cytotypes were obtained from herbarium specimens only. The herbarium specimens origi-

nated from the collection of living plants cultivated in the experimental plot.

The pollen grains were acetolysed and mounted in glycerine-jelly according to the methods described by REITSMA (1969).

Phytochemical studies

Flavonoids were extracted with 1% HCl in 70% methanol from petals and vegetative parts of the plants from the experimental plot as well as from plants in the wild. The extracts were stored at 4° C. untill needed.

Two dimensional paper-chromatography was carried out on Whatmann no. I or Schleicher and Schull in BAW = n-butanol : acetic acid : water (4:1:5, V/V/V, upper phase) and water successively.

Larger quantities necessary for ultra-violet spectra recording and sugar identification were isolated by means of two dimensional paper-chromatography on Whatmann no. III, first in BAW and then in water. After the flavons had been eluted with methanol the eluate was concentrated at reduced pressure in the presence of diagnostic reagents to MABRY *et al.* (1970).

The flavonoids were visible under longwave ultra violet (366 m), some of them only after they had been sprayed with 5% sodium carbonate.

RESULTS

I. Cytology

The results of the counts are represented in an appendix at the end of this paper. The material is arranged according to the alphabetical order of the countries of provenance, and the place of origin and plant number are given. Plants obtained from seed samples are indicated by an asterisk.

Diploids (2n = 22) and tetraploids (2n = 44) were observed. The tetraploids were found throughout the whole area of the species. Diploids were encountered only in the North of Portugal and in one locality in the South of

Galicia in the North-West of Spain. Fig. 1 shows the distribution of the localities of the collections. The diploid is represented by a cross, the tetraploid by a black dot. Records published in an earlier paper (KLIPHUIS, 1972) are also included in this figure.

In all, 203 localities have been registered, 98 of which are in the central part of the area: the Netherlands.

II. Morphology

Diploid and tetraploid plants show a remarkable uniformity in characters such as growth habit, the shape of the leaves on both flowering and non-flowering stems, petal shape and number of leaves per node.

Galium harcynicum is a mat-forming perennial with filiform rooting stolons, prostrate non-flowering branches and ascending flowering stems, the stems being quadrangular, glabrous, smooth and much branched; leaves in whorls of 6-7, on non-flowering shoots obovate, on flowering shoots the lowermost leaves obovate, obtuse, the middle ones obovate-lanceolate and the upper ones lanceolate to oblong-lanceolate, mucronate, the margin almost antrorsely ciliolate, weakly revolute and midrib slender; flowers in terminal, rather ovoid panicles, white, with flat, acute, patent lobes; pedicels straight, divaricating in fruit; fruit densely verruculose scabrous.

The diploid differs from the tetraploid in having rather a slender appearance, shorter internodes, smaller flowers and fruits, fewer flowers in the inflorescences and generally smaller leaves.

The difference in habit of the two cytotypes is shown in figure 2. This figure is a photograph of a diploid and a tetraploid potted plant. The diploid is a plant from Portugal, Trás-o-Montes, near Montalegre (collection number K 106), the tetraploid is a plant from Denmark, Jylland, near Holstebro (collection number K 1433).

The diploid plants can be distinguished from the tetraploid plants by the above mentioned quantitative morphological characters provided they are cultivated under similar

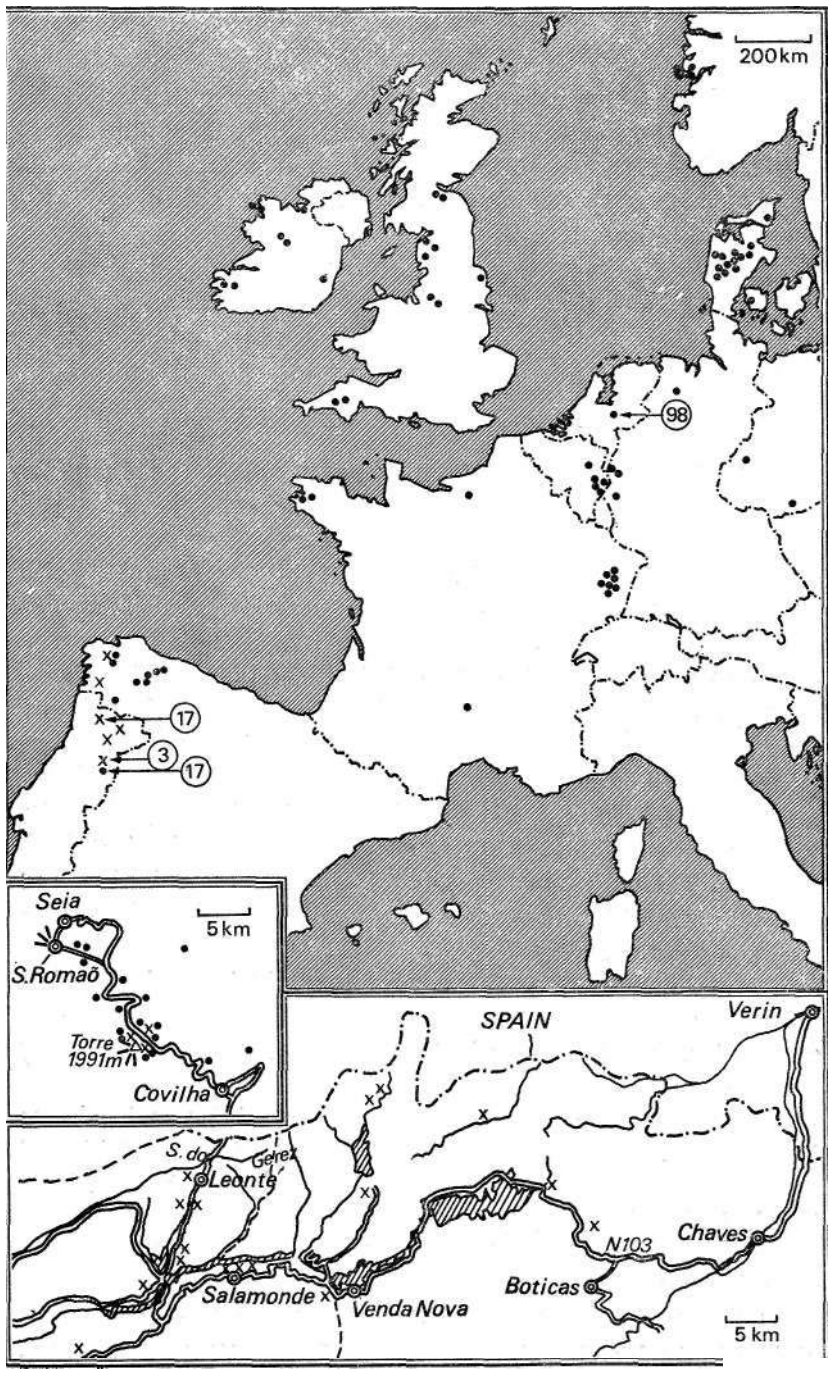

Fig. 1. — Localities of the cytologically investigated plants of *Galium harcynicum* Weig. The diploid is represented by a cross, the tetraploid **by a black dot. Records** published in an earlier paper (KLIPHUIS, **1972) are also included.**

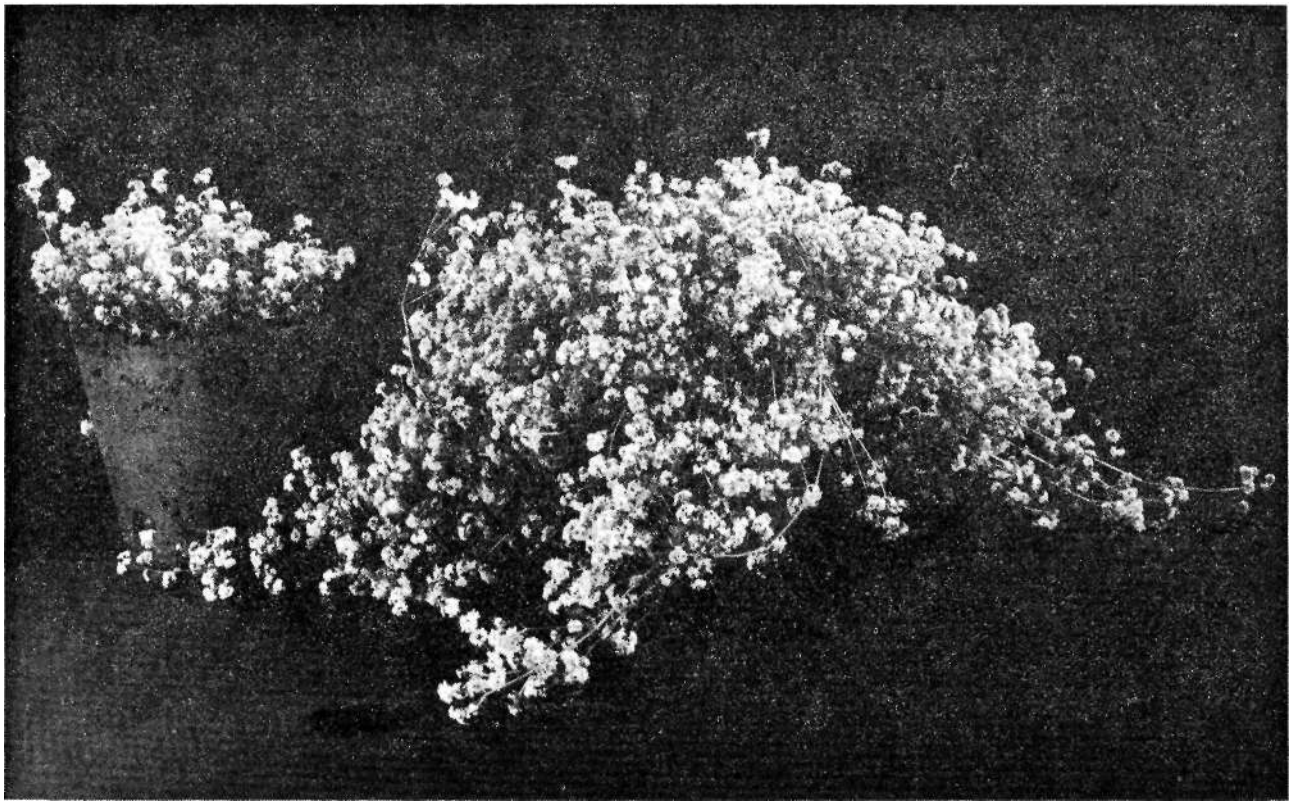

Fig. 2. — A photograph of a diploid and a tetraploid potted plant. The diploid is a plant from Portugal, Trás--os-Montes, near Montalegre (coll. no.: K 106), the tetraploid is a plant from Denmark, Jylland, near Holstebro (coll. no.: K 1433).

conditions. Diploids cultivated under favourable circumstances often but not always resemble tetraploids grown under adverse conditions in characters such as height of the plant, length of the internodes, size of the leaves, but are more robust. The plasticity of these vegetative characters is not always clear-cut. It differs from individual to individual and may be even absent.

The environment has no or hardly any influence on characters such as the size of the flowers and fruits and the number of flowers in the inflorescences.

III. **The structure of the fruit epidermis**

Fruit character is often of importance for the classification of taxa within the genus Galium. Usually this is the indûment, as in the species of the section KOLGYDA Dum. or as in the varieties of *Galium boréale* L. (KLIPHUIS, 1973). The structure of the exocarp may also be of importance, as was demonstrated by STERNER (1944) in his classification of the North-West European representatives of the *Galium pumilum* group.

Scanning electron microscopical micrographs were taken of the fruits of the two cytotypes to ascertain whether there is a difference in the structure of the epidermis. Figure 3 shows a photograph of the fruit of a diploid from Portugal (collection number K 1281) and a tetraploid from Spain (collection number K 1914).

As can be seen from the micrographs the fruit epidermis has a very similar structure in both cytotypes. The fruits are ovoid, two grooves dividing the surface into three parts. The surface is covered with closely spaced, rather elongated, broad, irregular elevations in longitudinal rows. The elevations, as can be seen at higher magnification, are finely ribbed, the ribs being separated by irregular furrows.

The difference between the fruits of the two cytotypes is of a quantitative character. The fruits of the diploids are smaller.

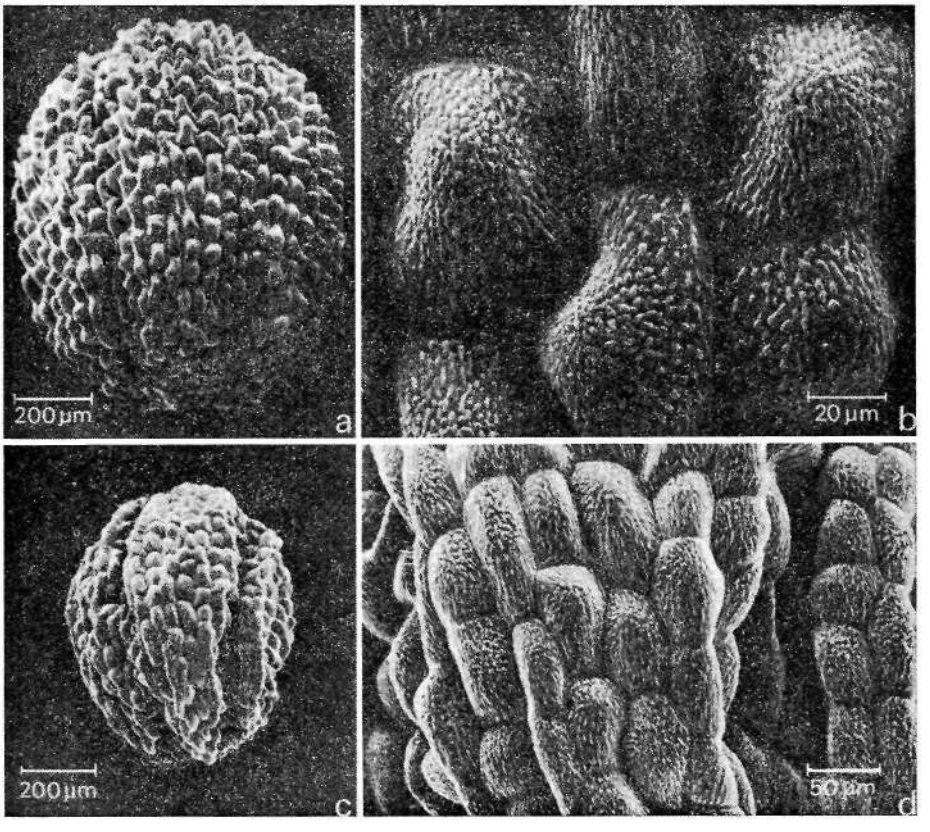

Fig. 3, — MES micrographs of the fruit of a diploid (c and d) and of a tetraploid plant (a and b). The diploid is a plant from Portugal (coll. no.: K 1281), the tetraploid is a plant from Spain (coll. no.: K 1914). For explanation see text.

The structure of the fruit-epidermis is very similar in both cytotypes. Even the size of the elevations is about the same.

The fine structure of the exocarp, therefore, cannot be used as a distinguishing character.

IV. **Pollen** grains

For both cytotypes the size of ten pollen grains of three plants from three subsequent years of cultivation was measured.

The average length of the pollen of the diploid turned out to be 19.50 micron and of the tetraploid 22.59 micron. The minimum and maximum values were 17.16-22.88 micron and 20.20-24.31 micron, respectively.

A nested analysis of variance showed a significant difference in the size of the pollen grains of the two cytotypes, but no significant variation within the cytotypes between plants and within plants from various years. The results are given in Table I. In this table is SS te sum of squares, df the degrees of freedom, MS the mean square, F the values from F-test and NS means not significant.

The pollen grains are prolate to spheroidal and have 7-9 colpi in both cytotypes.

TABLE I

Analysis of variance. Pollen size of diploids and tetraploids. SS = the sum of squares; df = degrees of freedom; MS = mean square; F = values from F-test, NS = Not significant. 1 = 2.86 micron

Source of variation.	SS	df	MS	F
Cytotype	13.58	1	13.58	32.14 **
Plants within the cytotypes	1.69	4	0.42	0.74 NS
Years within plants	6.81	12	0.57	1.73 NS
Error	53.12	162	0.33	
Total	75.20	179		

** $P < 0.005$

V. Phytoehemical studies

To arrive at a better insight into the interrelation of cytotypes within a polyploid complex it may be important to obtain information about biosynthetic processes of the cytotypes concerned.

Flavonoids are, by their frequent occurrence and their variation, very suitable for obtaining insight into such relations.

On account of its isolated position within the section *Leptogalium* and the fact that it has diploids and tetraploids only, the Galium harcynicum complex seems to be an appropriate subject for such a phytochemical investigation of flavonoids.

In the chromatograms of the diploids seven clear spots are always visible, in the chromatograms of the tetraploid only two can be seen. The chromatograms are shown in fig. 4, In this figure the spots visible under U. V. before treatment with 5% sodium carbonate are indicated by Roman numerals, the spots after spraying by Arabic numerals. In both cytotypes a spot that is missing in some of the chromatograms is denoted by A.

In some of the tetraploids two other spots, B and C, are visible. These spots resemble those of the compounds III and IIa of the diploid.

A varying number of other compounds may also be present: in the diploid 2-5, and in the tetraploid 1-3.

The results were obtained on material from the experimental plot. To see if these also apply to material in the field, 60 plants, all tetraploids, from 32 populations occurring in the Netherlands, were investigated. Almost all plants showed the pattern characteristic for the tetraploid (spot I, II and A). A few of them, however, had in addition to these spots the compounds B and C, as described above.

Within the tetraploid glycosides of two aglycones seem to be present. The diploid probably has more than two different glycosylated aglycones.

Preliminary data indicate that one of the aglycones is the flavonol quercetine. Only identification of the compounds concerned can answer the question as to whether the difference between the two cytotypes is based on a difference in aglycones or if it is caused by differences in glycosylation.

The compounds of the diploid and tetraploid were isolated. At the moment an attempt is being made to identify

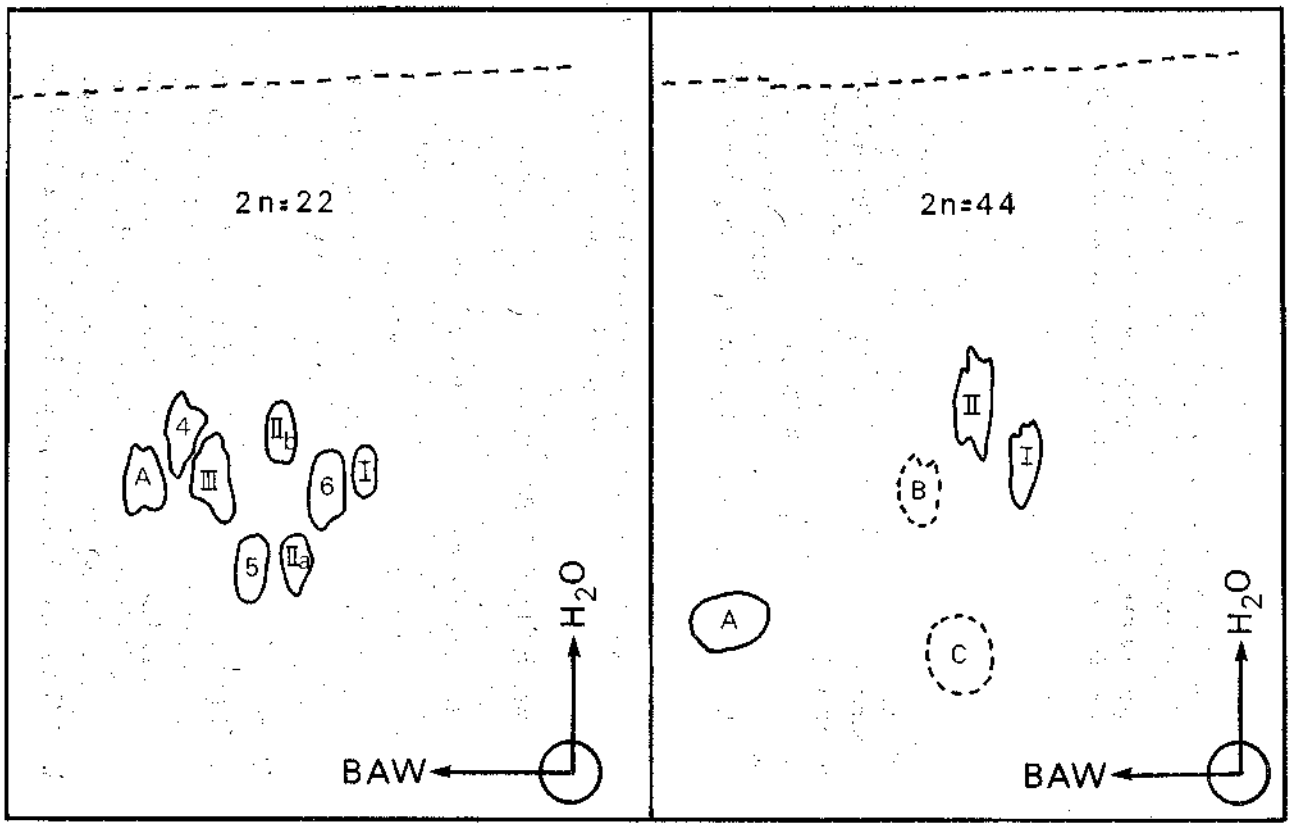

Fig. 4. — A chromatogram of a diploid and tetraploid plant of *Galium harcynicum* Weig. The diploid is a plant from Portugal (coll. no.: K 1281), the tetraploid is a plant from England (K 1226).
For explanation see text.

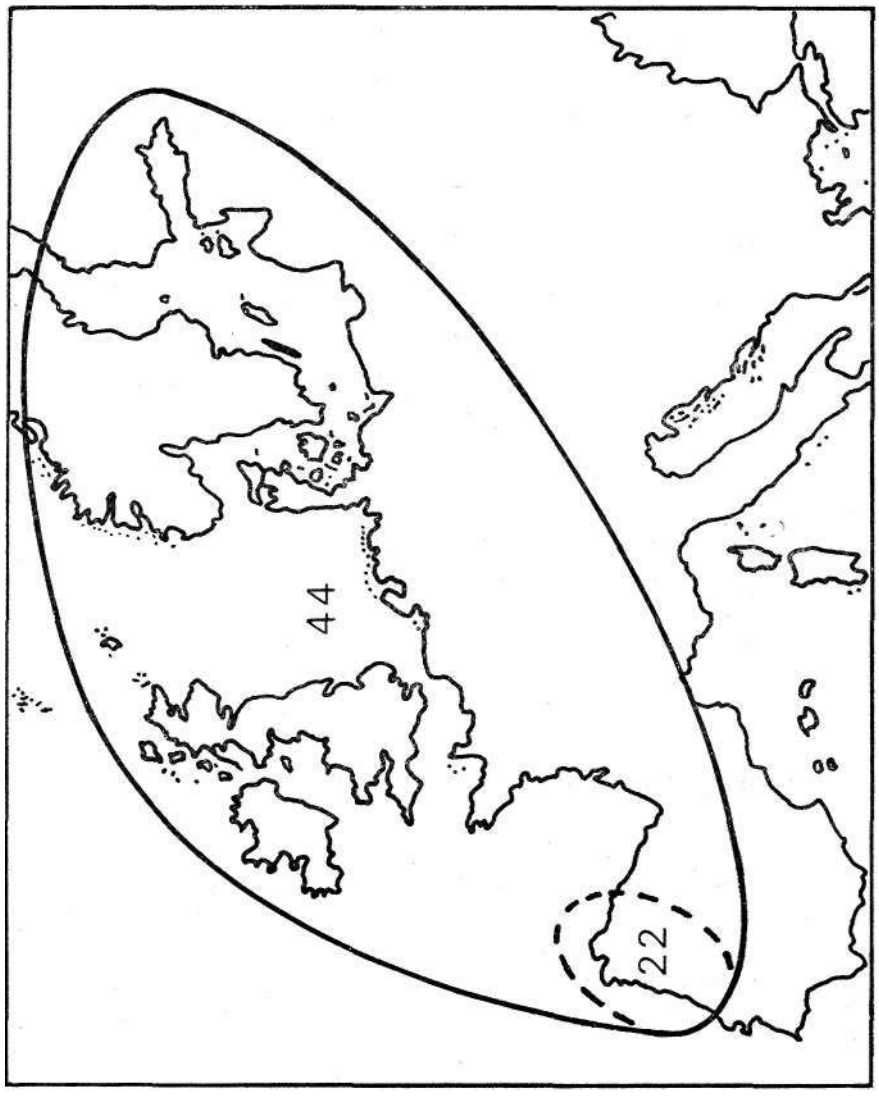

Fig. 5. — Distribution pattern of diploids and tetraploids of *Galium harcynicum* Weig.

these compounds, the results will be published in a forthcoming paper.

DISCUSSION

The *Galium harcynicum* complex is represented by two cytotypes, a diploid (2n = 22) and a tetraploid (2n = 44), which both show a difference in their distribution pattern. In this respect the present study simply confirms the results obtained in an earlier investigation (KLIPHUIS, 1972).

The tetraploid is widely distributed throughout the whole area of the species. The diploid is a plant of the North-West of the Iberian Peninsula. Geologically this part of the Peninsula belongs to the Iberian Massif, where ancient blocks of granite and gneiss and some Palaeozoic sediments form dissected plateaux. Here the old-fold mountains are found: the Serras of North Portugal and the Sierras of Central Spain. These are Hercynian foldings, which were reactivated during the Tertiary. Botanically they are of importance because they served as refuges for plants and, afterwards, as centres of diversification and distribution. In this area the distribution of the diploid could be determined more precisely.

In the Serra da Estrela three localities of diploids were found among a majority of tetraploids at an altitude of 1600-1750 m. The climate, especially on the summits, is rough with extremes of temperature in summer and winter and with a rainfall of over 2500 mm a year.

The Serra da Estrela is the westernmost chain of the Central Sierras of the Peninsula. KUPFER (1969) reported finding a diploid on of these Sierras, the Sierra do Gredos.

In the Serra do Gerês, in particular on the lower slopes, and in the surrounding of this chain only diploids were observed. Here the habitats are open with grasslands and light forests. The climate is influenced by the Atlantic, with moderate to high rainfall and humidity and mild summers and winters.

Not very much can be said about the situation in the northern parts of the Iberian Massif because there is a

lack of cytological data. In East Galicia only tetraploids were encountered in six localities, five in the province of Lugo and one in the province of Orense. In the western parts of Galicia, Galium harcynicum is not a very common plant. Two tetraploids and one diploid were recorded from the same area between La Coruña and Santiago de la Compostela (KLIPHUIS, 1972). In the present study one locality with diploids was found in the province of Pontevedra, North of the river Minho, near Pineiro. It has not yet been possible to investigate material collected in Galicia in the autumn of 1979, so nu further information is available at the moment.

Compared to the tetraploid the diploid has a limited, eccentric ,southern distribution (see figure 5). It occupies an area which served as a refuge for plants during the last Quaternary glacial period. This picture is in agreement with one of the possible distribution patterns of a diploid and the corresponding polyploids as given by FAVARGER (1987). *Galium harcynicum,* in that case, is an example in which the diploid has a southern, but not a Mediterranean distribution.

Plants cultivated under different circumstances show a certain degree of phenotypic plasticity in their vegetative characters. When they are not in flower it is not always easy to distinguish the cytotype with certainty. This was also the case in the field, in particular with the tetraploid.

Floral characters are much more stable. Flower size and fruit size and the size of the pollen grains and the number of inflorescences are good discriminating characters.

A difference in the size of the pollen grains in diploids and polyploids within a series is reported by many authors, although others mentioned some exeptions. An overall clear-cut separation between cytofypes is not always possible.

The pollen grains of the diploid and tetraploid do not show any difference in the number of colpi. This is not in agreement with the situation in another polyploid complex of the genus e. g. in the *Galium palustre* complex. In the latter the diploid differs from the tetraploid and octoploid in mainly having 6 colpi, whereas the tetraploid and octoploid mainly have 7 or 8.

In the phytochemical investigation intraspecif ic variation is demonstrated. The tetraploid shows in its chromatogram less variation than the diploid. The chromatograms of both cytotypes show a regular, rather constant pattern. However, in the tetraploids exceptions are found. If spots B and C are present the pattern is similar to the diploids. To what extent this indicates a relation between the phytochemical processes of the diploid and tetraploid and what kind of relation this may be, can be concluded only when more is known about the identity of the of the compounds concerned.

In view of the similarities between the morphological characters on the one hand and the quantitative differences on the other, and the geographical distribution of the diploid and the tetraploid the classification into subspecies is in our opinion much more reliable than the classification into species which HOLUB (1974) prefered.

The tetraploid has originated from the diploid, probably through autopolyploidy as well as through allopolyploidy and has expanded successfully with time to occupy its present area.

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APPENDIX

Cytologically investigated material of *Galium harcynicum* Weig.

Diploids. 2n = 22 (ssp. *vivianum).*

PORTUGAL: K 1252 — S. da Estrela, vic. of Penhas da Saúde; K 1275* K 1282* — S. do Gerês, between Gerês and Leonte K 1276* — vic. of Rindufinho; K 1280* — vic. of Cerdeirinhas; K 1281* — vic. of Vilar da Veiga; K 1286* — S. da Estrela, 3.5 km after Torre direction Covilhã; K 1288*—near Torre, alt. ca. 1750 m; K 1290* — between Salamonde and Ruivas; K 1292* — vic. of Paradela; K 1294* — Trás--os-Montes, vic. of Venda Nova; K 1296* — vic. of Salamonde; K 1902* — S. do Gerês, near the Pousada; K 1903* — vic. of Ruivais; K 1904* — vic. of Gralhos, St. Vincente; K 1905* — 6 km North of Sapiaos.

SPAIN: K 3278—Galicia, prov. of Pontevedra, vic. of Pineiro.

Tetraploids. 2n = 44 (ssp. *harcynicum).*

BELGIUM: K 1642* — vic. of la Roche en Ardenne, Ardennes.

DENMARK: K 1212* — between Vrads and Hjollund, Jylland; K 1433 — vic. of Bur, near Holstebro, Jylland; K 1435 — between Stjenberg and Sonderhä, Jylland; K 1458 — S0nder Sorâ, between Asa and Saeby, Jylland.

PRANCE: K 1570* — Chaumes du Haut Chitelet, Vosges; K 1576* — vic. of Remiremont, Vosges; K 1578* —vic. of Cornimont, Vosges.; K 1901* — Entraygues sur Truyère, Barrage de Couesque, Aveyron.

GERMANY-WEST: K 1554*. K 1997* —vic. of Mansholt, Oldenburg; K 1979* — between Belscheid and Krautscheid, Eifel.

GREAT-BRITAIN: K 1182 —vic. of Ullswater, Lake district, England; K 1221 — Culloden Moor, Iverness shire, Scotland; K 1223 — vic. of Clara Lodge Hotel, Culloden Moor, Iverness shire, Scotland; K 1226 — Haisty Banks, Yorkshire, England.

THE NETHERLANDS: K 3047 —K 3049 —«de Schovenhorst», Putten, prov. of Gelderland; K 1821 — vic. of Rijssen, prov. of Overijssel; K 1822 •— Sprengenberg, prov. of Overijssel; K 1823, K 1824, K 3030—K 3032 — Haarlerberg, prov. of Overijssel; K 1825, K 3035 — Noetselerveld, prov. of Overijssel. K 1827 — vic. of Diepveen, between Lhee and Spier, prov. of Drenthe; K 1828 — vic. of Spier; K 1829—vic. of Hooghalen, prov. of Drenthe; K 1830 de Moere, near Grollo, prov. of Drenthe; K 1831 —vic. of Grollo; K 1836 — vic. of Ommen, prov. of Overijssel; K 3036 — den Treek, prov. of Utrecht; K 3037 — K 3043 — Speulderveld, prov. of Gelderland; K 3044 —Laage Vuursche, vic. of «Drakensteijn», prof .of Utrecht; K 3045 — Groot Kievitsdal, prov. of Utrecht; K 3046 — «Wildforster» between Putten and Garderen, prov. of Gelderland; K 3050, w 3051 — Uddelermeer, prov. of Gelderland; K 3052, K 3053 — vic. of Kootwijkerveen, prov. of Gelderland; K 3054 — K 3074 — Laage Vuursche, prov. of Utrecht.

PORTUGAL: K 1259, K 1251, K 1256* — S. da Estrela, near Seia; K 1253*, K 1254* — S. da Estrela, 1km before Torre; K 1277*, K 1283* — S. da Estrela, between Seia and Torre; K 1278*, K 1291* — S da Estrela, near Torre, alt. ca. 1750 m; K 1295*, K 1297* — S. da Estrela, between Torre and Covilhã, near Torre alt. ca. 1350 m; K 1469* — S. da Estrela, between Torre and Covilhã, 7 km before Covilhã; K 3273 — S da Estrela, Ponte de Cabaços.

SPAIN: K 1907* —vic. of Xiam, prov. of Lugo, Galicia; K 1910* — 20 km North of Lugo, Galicia; K 1911* — 5 km South of Lugo, Galicia; K 1913* — Marco de Alvare, alt. 575 m, prov. of Lugo, Galicia; K 1914* — vic. of Marco de Alvare, Galicia; K 3272 — near Vieiro, vic. of Bande, alt. 850 m, prov. of Orense, Galicië.

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STUDIES ON THE INITIATION OF CALLUS FROM VARIOUS EXPLANTS SOURCES IN RYE *(SÉCALE CEREALE* L.)

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ABSTRACT

Callus initiation was obtained in rye from mature excised embryos and segments both of seedling coleoptiles and roots, using the medium B5 containing 1 mg/1 2,4-D. The anatomy of callus initiation, after 7 days of incubation was studied in longitudinal and transversal serial sections. It was concluded that: a) the callus in excised embryos arose only from the coleoptile tissue and in a much less degree from the coleorhiza; b) the anatomy of the callus derived from isolated coleoptiles segments is identical with the ones from the embryos; c) in cultured root segments the callus originates by proliferation of the cortex cells although there is at the pericycle region the formation of abnormal lateral root primordia.

INTrODUCTION

TISSUE culture of cereals has shown an increased interest in recent years (for revision see YAMADA, 1977; GREEN, 1978). However from the published papers we can concluded that only a few give information on the anatomy of callus initiation. Such a study is however important as it can resolve the controversial question if the supposed redifferentiation of shoots and roots and also the regeneration of whole plants in subcultured callus is really a *de novo* phenomenon or is simply due to meristems already present along with the callus of the primary expiant.

The purpose of the present work was to study the anatomy of callus initiation in various expiants (mature embryo, coleoptile and root) in a cereal, rye.

MATERIALS AND METHODS

Seeds of *Sécale céréale* L. c. v. Montalegre were obtained from the Instituto Universitário de Trás-os-Montes e Alto Douro. For the isolation of embryos the seeds were washed with 70 % ethyl alcohol for 2 minutes, sterilized with 10 % sodium hypochlorite for 20 minutes, then rinsed three times in sterile distilled water. After that the seeds were soaked in sterile distilled water for 3-5 hrs. Thereafter the embryos were aseptically excised by cutting the embryo with the scutellum attached.

For the isolation of segments (±1 c m long) of roots and coleoptiles, seeds sterilized as described above were put aseptically on wet filter-paper in a glass Petri dish and germinated for 2-3 days at ± 8° C.

All the expiants (embryos, coleoptiles and roots) were individually traisferred to cultures tubes, each one containing 20 ml of solid culture medium. Cultures were incubated at 27° C. in the dark. '

The basic culture medium was that of GAMBORG, MILLER and OJIMA (1968) (B5 medium) containing 1 mg/1 2,4-D and 3 % sucrose. The pH of the medium was adjusted to 5.6 before adding Difco-Bacto agar at 0.8%.

The medium was then autoclaved at 120° C. during 20 minutes.

For studying the anatomy of callus initiation we have used mainly cultures aged 7 days. The tissues were fixed in formalin-acetic acid-alcohol, dehydrated, embeded in parafin wax and serially cut at 15 u. The staining was by the safranin fast green method.

KESUETS

Callus initiation from excised embryos

Calluses formed readily from over 90% of all excised embryos and were cleary visible after 3 days incubation.

Calluses aged 7 days show a membranous folded surface (Plate II, fig. 1 and Plate III, fig. 1). They are yellowish and friable. The sections of these cultured embryos showed that the only parts of the embryo structure that give rise to a callus are the coleoptile and, to a much less degree the coleorhiza (Plate I, fig. 2 and 3; Plate II, fig. 2). In fact the growth and differentiation of the embryo axis is supressed. The callus arising from the coleoptile is a multi--folded structure (Plate III, fig. 2). It is clear from the photographs that neither the cotyledonary node nor the scutellum take part in the formation of the callus.

Although the present paper is confined to the study of the initiation of the calluses it must be referred that these calluses have been successful subcultured, in the same medium as the one used for the expiants, for more than 2 years.

Callus initiation from coleoptiles and root segments

Callus developed at any place along the coleoptile segment (Plate IV, fig. 1 and 2). Its morphological aspect is quite similar to the callus originated in the cultured embryos. This is not strange as its origin is the same: the tissues of a coleoptile. Section of one of these calluses after 7 days incubation shows (Plate IV, fig. 3) that it has the same peculiar folding as the ones arising in the embryos. This figure also shows (arrow) that the enclosed immature leaf maintains its structure without any traces of tissue proliferation.

Callus developing on root segments can occur as scattered nodules or almost covering the segment (Plate V, fig. 1 and 2). Sections of these calluses show (Plate V, fig. 3) that the parenquima cells of the cortex proliferate and as a result there is a rupture of the epidermis. At the pericycle region there is also production of abnormal lateral roots, densely packed and of limited growth (Plate V, fig. 4, arrow).

DISCUSSION

From Table I we can see that only CARAMIELLO and MoNTACCHiNi (1970) and O'HARA and SHEET (1978) have

TABLE I

Callus induction In cereal plants

Cereals	Plant part	Anatomical study of callus iniciation	References
Wheat	EMBRYO (cotyledonary node)	NO	TriONE *et al.* (1968)
Wheat	ROOT	NO	ShiMADA *et al.* (1969)
Wheat	EMBRYO	YES	CAEAMIELLO and MONTA-OCHINI (1970)
Wheat	ROOT	NO	KAO *et al.* (1970)
Wheat	ROOT	NO	SHIMADA (1971)
Wheat	ROOT	NO	DUDITS *et al.* (1975)
Wheat	ROOT	NO	BHOJWANI and HAYWARD (1977)
Wheat	EMBRYO	NO	CHIN and SCOTT (1977)
Wheat	EMBRYO (mesoeotyl)	NO	BENNICI and D'AMATO (1978)
Wheat	EMBRYO ROOT Coleoptile	YES	O'HARA and STREET (1978)
Rye	EMBRYO	NO	CAEEW and SCHWArTING (1958)
Rye	ROOT	NO	MULLIN (1970)
Oat	EMBRYO	NO	WEBSTEE (1966)
Oat	ROOT	NO	CAETEE *et al.* (1967)
Barley	ROOT	NO	CmN and SCOTT (1977)
Barley	EMBRYO ROOT	NO	KAETEL and MANESHiNA (1977)
Rice	ROOT	NO	YATAZAWA *et al.* (1967)
Rice	EMBRYO	NO	MAEDA (1969)
Maize	SEEDLiNGS Mesocotyl Coleoptile	NO	VUILLAUME and DESHAYES (1977)

have studied the anatomy of the iniciation of callus in mature excised embryos of a cereal, wheat. So, our observations will be compared with the ones reported by those authors although our material is different, rye instead of wheat. CARAMIELLO) and MONTACCHINI studied sections of embryos with 8 days incubation and concluded that the callus arises from the cotyledonary node and to a much less extent from the coleorhiza. O'HARA and SREET studied sections of 7 days cultured embryos and concluded that «The coleoptile and enclosed leaves retained their normal form and that callus arose at the cotyledonary node and in the region of the radicle as previously reported by CARAMIELLO and MONTACCHINI (1970». It is evident that ours results are at variance with these conclusions because as we have seen, the callus arises, in rye from the coleptile and not from the cotyledonary node. Proliferation of the coleorhiza is small compared with that of coleoptile. As rye and wheat are closely related genera and the growth substance used by those authors and ourselves for inducing the callus was the same, viz., 2,4-D it is difficult to find an explanation for this discrepancy. The work of CAREW and ScHWARTiNG (1958) on rye embryos is not directly comparable to ours because they have used excised immature embryos 15-20 days old. However the authors are of the opinion, based on a rather incomplete study, that the callus tissue originated from the scutellum. This conclusion, again, differs from ours as we shown that the scutellar tissue never proliferates.

It is interesting to note the fact that the effect of 2,4-D is to supress the growth and differentiation of the embryo axis, and to promote enhancement of proliferation of the coleoptile and coleorhiza tissue. So, the meristematics tissues are arrested in its growth and the tissues with a natural limited potentiality for growth, as are the coleoptile and coleorhiza, are activated. In tissue culture research this apparent paradoxical effect of 2,4-D has received little atention, but in our opinion it should be clarified, especially as we are now starting to understand the mode of molecular action of this growth substance (GUILFOYLE: *et al.,* 1975).

With reference to our results on the formation of callus in the segments of coleoptile excised from 2-3 days old seedlings they are also not coincident with the ones obtained by O'HARA and STREET as these authors report than «callus seemed to arise from the bases of the enclosed immature leaves». As we have seen the enclosed leaves never show traces of celular proliferation and the origin of the callus exclusively from the coleoptile tissue is evident.

Finally our results on the initiation of callus on root segments agree with the conclusions of O'HARA and STREET in that there is an intense proliferation of the cortex cells. It seems however that the abnormal densely packed lateral root primordia are of limited growth contrary to what happens in the potato roots where they proliferate to form the callus (MONTEZUMA-DE-CARVALHO and M. L. GUIMARÃES, 1976).

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PLATES

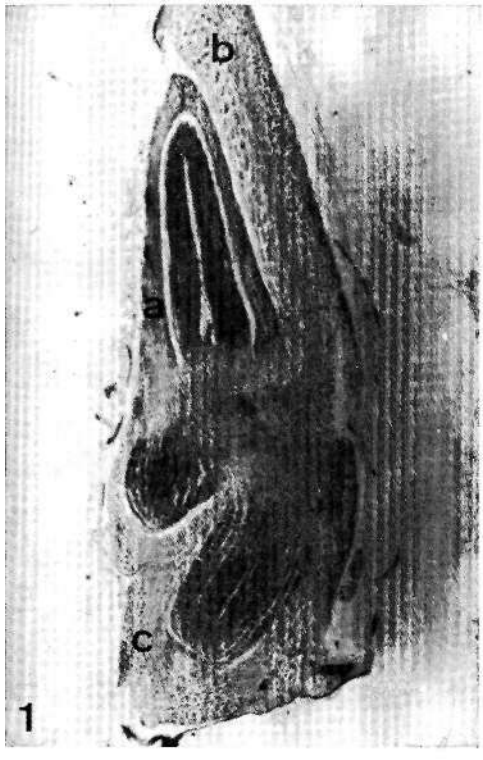
PLATE; I

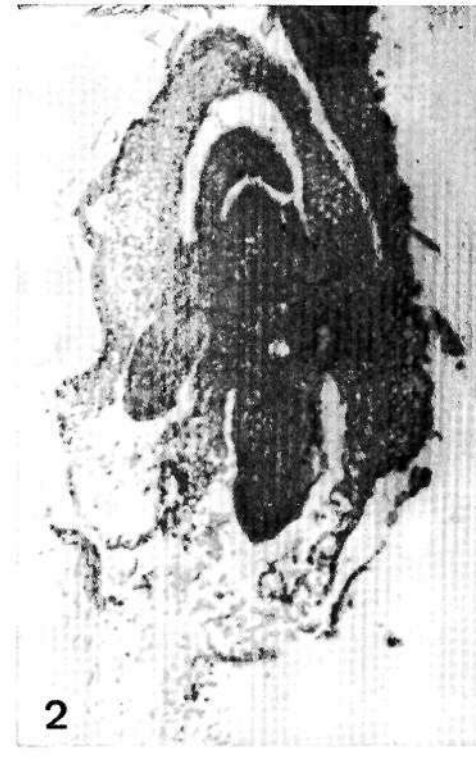
Anatomical study of callus initiation in mature excised embryos of Rye *(Sécale céréale* L.) cultured in medium B5 + 1 mg/1 2,4-D

Fig. 1. — Longitudinal section of an excised mature embryo, after soaked in water for 5 hrs, before planting it on the culture medium. Note: *a* (coleoptile), 6 (scutellum), *c* (coleorhiza). X 30.

Pig. 2. — Longitudinal section of an embryo after 3 days of culture. Note proliferation of the cells of the coleorhiza and cell enlargement in the coleoptile. X 30.

Fig. 3. — Longitudinal section of an embryo after 7 days of culture. Note the intense proliferation of the coleoptile tissue compared with the slight proliferation of the coleorhiza. Growth and differentiation of the embryo axis is supressed. X 30.


b
a
c
1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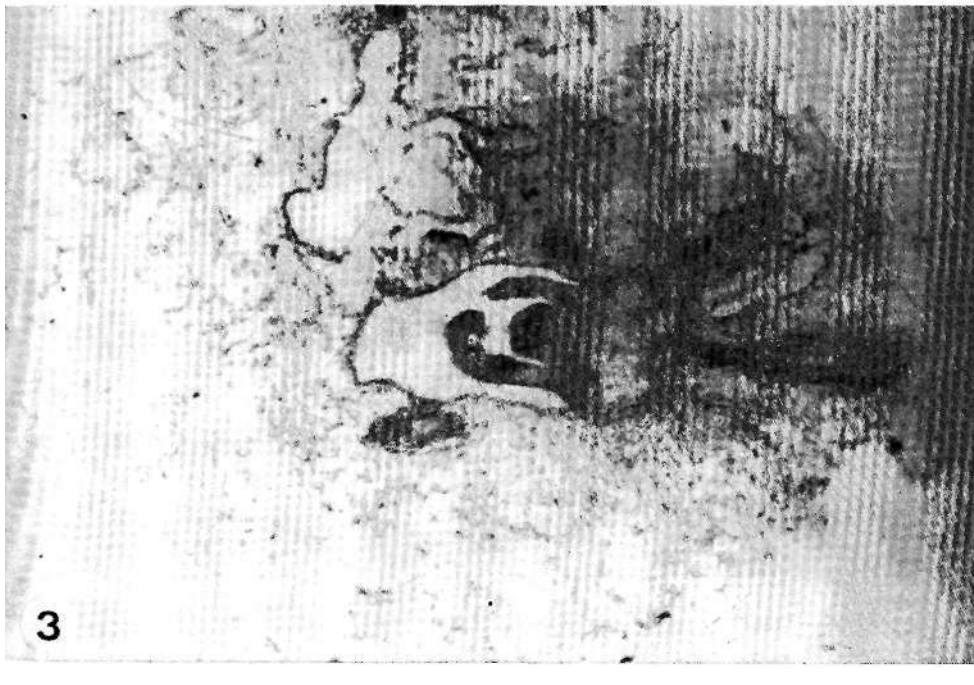
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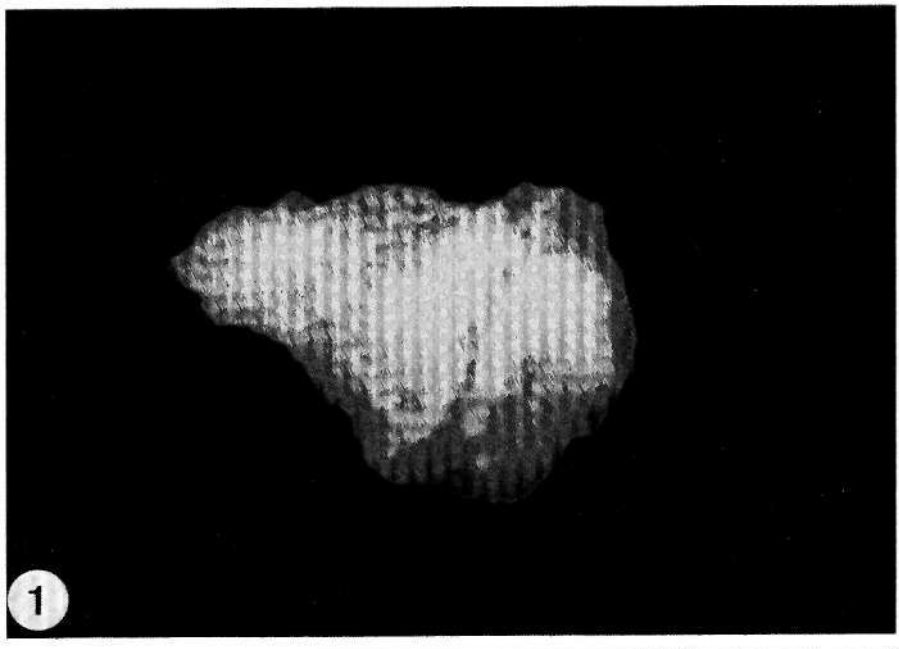
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PLAtE II

Anatomical study of callus initiation in mature excised embryos of Rye {*Sécale céréale* D.) cultured in medium B5 + 1 mg/l 2,á-D

Fig. 1. — Morphological aspect of a cultured embryo. T days incubation. X 9.5.

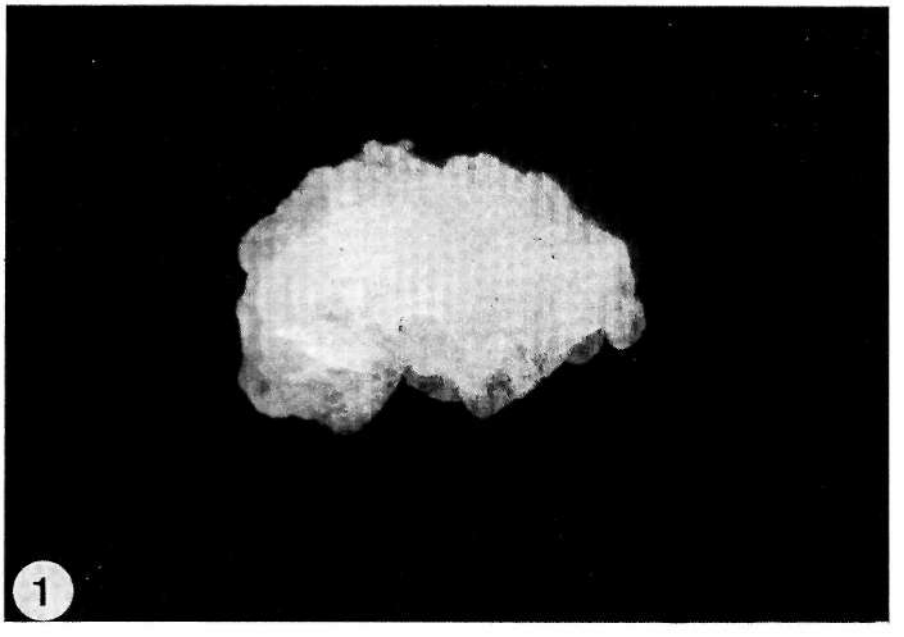
Fig. 2. — Longitudinal section through the embryo represented in Fig. 1. Note that the proliferation of the coleoptile tissue is accompanied by its intense folding; this folding is also cleary visible on the surface of the intact embryo (Fig. 1). Note the supressed elongation of the embryo axis and also the non proliferation of the scutelar tissue (arrow). X 30.

PLATE III

Anatomical study of callus initiation in mature excised embryos of Rye *(Sécale céréale* L.) culture in medium B5 + 1 mg/1 2,4-D

Fig. 1. — Morphological aspect of a cultured embryo. 7 days incubation. X 9-5.

Fig. 2. — Transversal section, at the region of the eoleoptile, of the embryo represented in Fig. 1. Note the intense folding of the proliferating coleoptile tissue. The scutellum (arrow) does not show any proliferation. X 30.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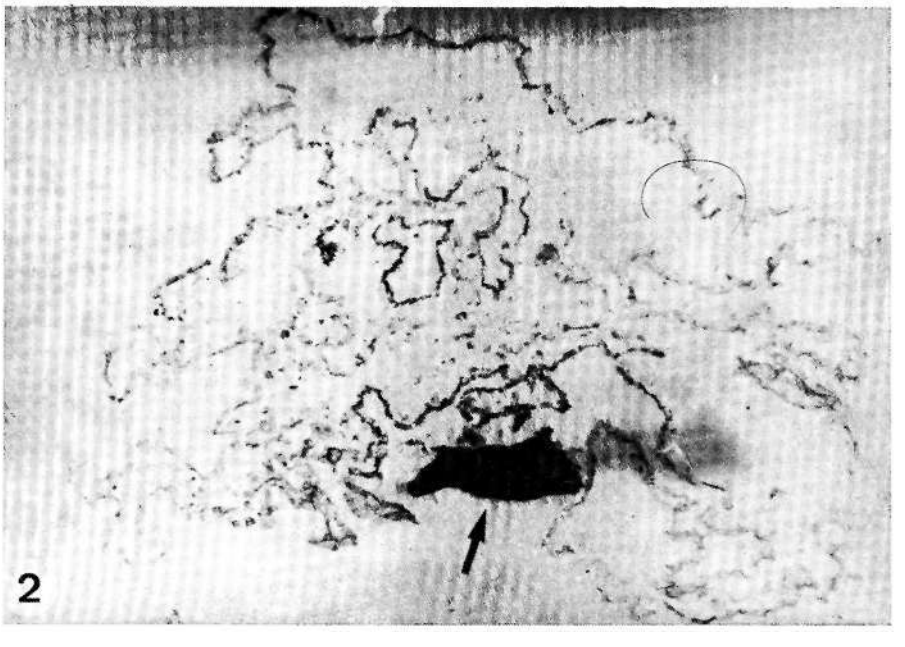
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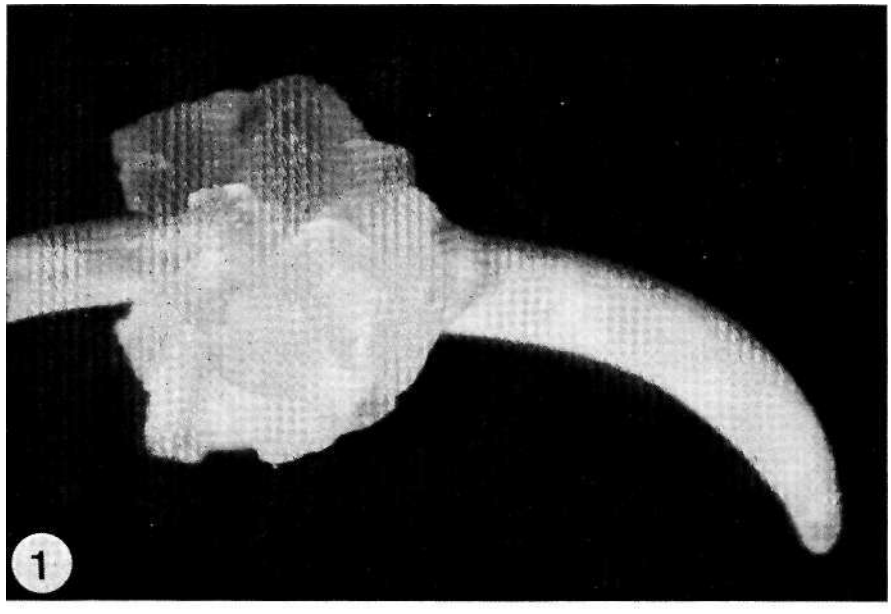
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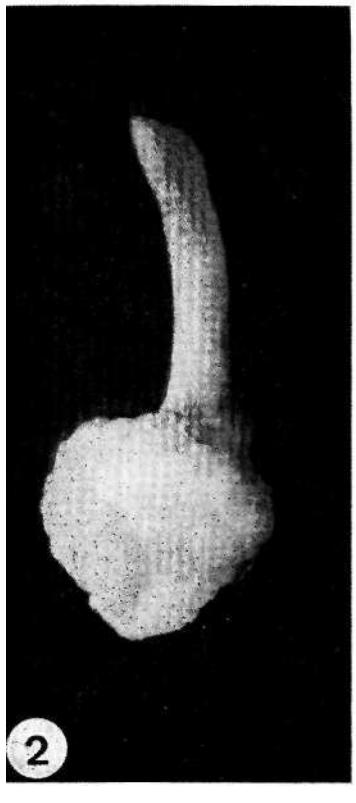
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PLATE IV

Anatomical study of callus initiation in excised coleoptiles of Rye (*Sécale céréale* L.) cultured in medium B5 + 1 mg/1 2,4-D

Fig. 1. — Morphological aspect of a callus arising in the middle region of the excised coleoptile. 7 days of incubation. X 9.5.

Fig. 2. — Another example of a callus developed at the basal cut end of the excised coleoptile. 7 days of incubation. X 9.5.

Fig. 3. — Transversal section of the callus represented in Fig. 1. The folding of the proliferating coleoptile tissue is identical with the one observed in cultured excised embryos (see Plate I, fig. 3; Plate II, fig. 2 and Plate III, fig. 2). The enclosed immature leaf (arrow) does not show any trace of proliferation. X 30.

PLATE V

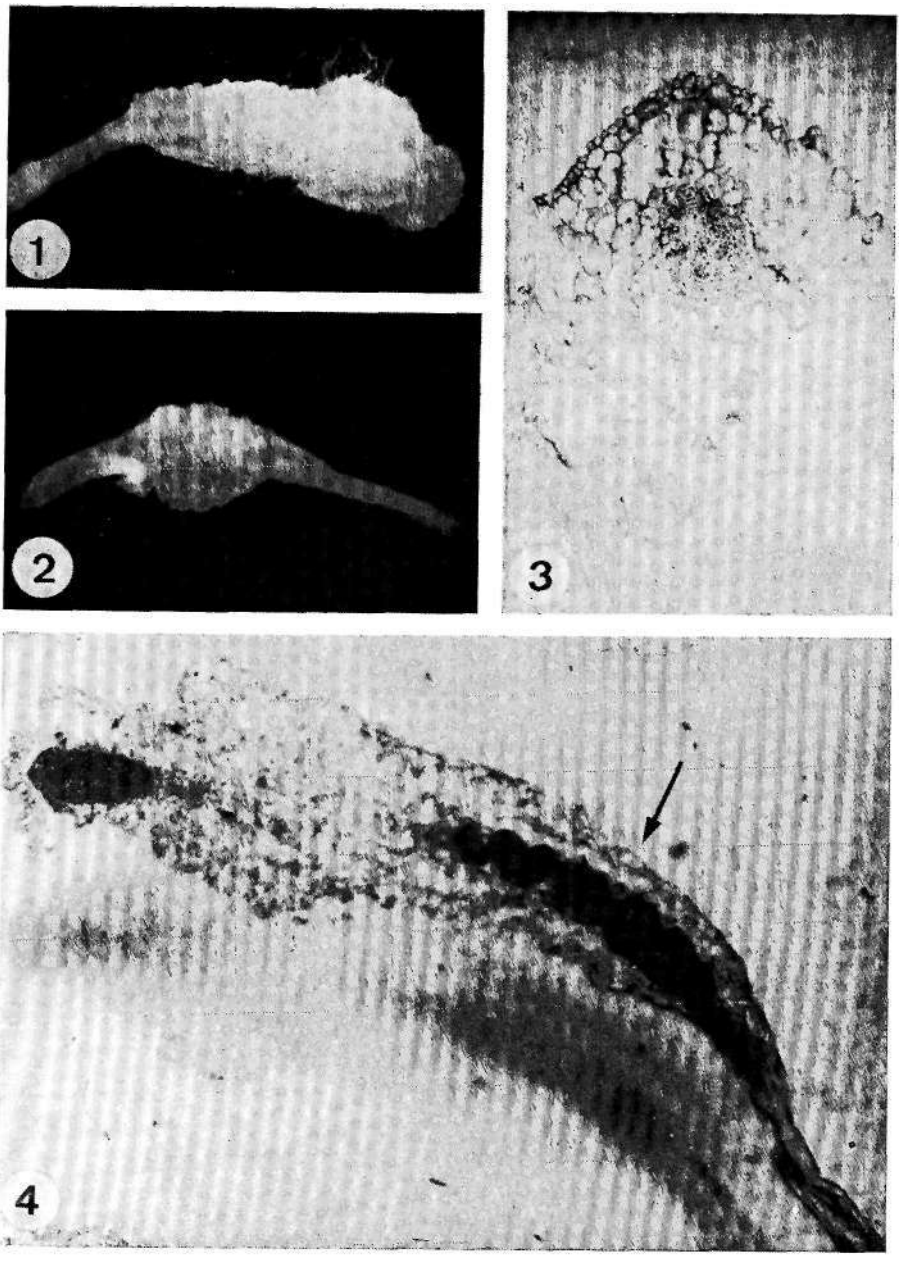
Anatomical study of callus initiation in excised root segments of Rye *(Sécale céréale* L.) cultured in medium B5 + 1 mg/1 2,4-D

Fig. 1 and 2. — Morphological aspect of calluses arising in the excised roots after 7 days of incubation. X *9.5.*

Fig. 3. — Transversal section of the callus represented in Fig. 2. Note proliferation of the cortex parenquima and formation of several abnormal root primordia. X 30.

Fig. 4. — Longitudinal section of the callus represented in Fig. 1. Note the proliferation of the cortex parenquima and the formation of several abnormaly broad and packed lateral root primordia (arrow). X 30.

PLATE V



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LYCIUM BARBARUM L. EN MENORCA (BALEARES)

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SUMMARY

The author state for the first time the presence of *Ltycium barbarum* L. in the Balearic Islands: Torret (Sant Lluís, Menorca). The species, long ago cultivated, now is naturalized. It is very possible that, from RAMiS (1814), a mistake was made by different authors with this species and *L. europaeum* L. at Menorca.

Lycium barbarum L., Sp. PL: 192 (1753) (= *L. halimifolium* Miller, *L. vulgare* Dunal) es una especie cultivada desde hace muchos años en Menorca. Popularmemnte se la denomina *ullastre d'ase.*

Desde 1814, año en que fué citada por primera vez de la isla por RAMIS (1814), parece que *L. barbarum* se ha venido confundiendo con *L. europaeum* L. Oleo (1859) considera a dicha planta como espontánea en Menorca aunque sin indicación de localidad; en cambio RODRÍGUEZ FEMENÍAS (1904) y KNOCHE (1921-1923) afirman que sólo la han visto cultivada. Por otra parte, BARCELÓ (1879-1881) dice haberla encontrado en estado silvestre en Andratx y Montcaire (Mallorca).

J. CAMBESSEDES (1829), M. WILLKOMM (1876) y P. PORTA (1887) no incluyen dicho taxon en sus respectivas obras relativas a la Flora Baleárica y MARES & VIGINEIX **(1880)** repiten la cita de BARCELÓ.

DUVIGNEAUD (1979) incluye en su catálogo *L. europaeum* L. en Mallorca y en Menorca.

Nosotros hemos encontrado *L. barbarum* L. en estado silvestre en Torret (Sant Lluís, Menorca) junto a los muros de piedra seca, característicos del paisaje menorquín, que separan las fincas y los campos de cultivo e, incluso, en el interior de dichos muros los cuales, cuando este hecho ocurre, se derrumban. Creemos, pues, que *L. barbarum* L. en Menorca puede considerarse como subespontánea.

Nuestros pliegos de herbario se encuentran depositados en el Herbario del Instituto Botánico de Barcelona (BC).

L. barbarum, originario de China, se encuentra en casi toda Europa en forma cultivada o naturalizada (STEARN 1972).

En la Península Ibérica es relativamente frecuente en Castilla la Nueva, norte de Aragón y de Navarra, País Vasco, Asturias, Galicia y Norte de Portugal según WILLKOMM & LANGE (1870). Sin embargo, PEREIRA COUTINHO (1939) en su Flora de Portugal, únicamente cita *L. barbarum* de Cintra y entre Setúbal y Palmela.

Asso (1781), por su parte, cita dicho taxon de Senegués, cerca del río Gallego. Manifesta su extrañeza respecto a que dicha planta, muy espinosa en estado natural, pierda las espinas al ser cultivada.

La cita anterior parece dudosa dado que en la localidad citada abunda *Hippophae rhamnoides* L., arbusto mucho más espinoso que *L. barbarum.* Por otra parte, *L. barbarum* se encuentra cultivado con cierta frecuencia en la región. Agradecemos dicha sugerencia al Dr. P. MONTSERRAT, del Centro de Biología Experimental de Jaca.

El área de distribución de *lycium europaeum* L. comprende la región mediterránea y parte de Portugal; en la Península Ibérica se desarrolla preferentemente en la zona oriental y meridional desde el este de Aragón y Cataluña hasta Andalucía, el Algarbe, el Alentejo, Extremadura portuguesa y Beira litoral (WillKOMM 1893; WILLKOMM & LANGE 1870; PEREIRA COUTINHO 1939 y SAMPAIO 1947).

Por otra parte, conviene señalar que *L. barbarum* se encuentra cultivado con frecuencia en Córcega donde se

encuentra también subespontáneo (cerca de Ajaccio). En cambio, *L. europaeum* es muy raro en dicha isla; sólo se ha citado, por lo que sabemos (BRIQUET & OE LITARDIÊRE 1910-1955) de Macinaggio, en las arenas de la playa situada al norte de dicha ciudad.

No es de extrañar, dada el área de distribución de ambas especies, que *L. barbarum* fuera confundido, como parece, con *L. europaeum* en Menorca.

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CHLOROPLAST MICROTUBULES IN SOME CAM-PLANTS[1]

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INTRODUCTION

REFERENCES to microtubules in the stroma of chloroplasts are few and far between. They were first reported in algal chloroplasts, namely *Oedogonium* (HOFFMAN, 1967), *Chara*, *Vólvox*, and *Nitella* (PICKETT-HEAPS, 1968), *Leathesia difformis* (COLE *et al.*, 1968) and *Bulbochaete* (RETALLACK & BUTLER, 1972), and later described in chloroplasts of a higher plant, *Sedum telephium* L. (BRANDÃO & SALEMA, 1974) where they assume the form of huge paracrystalline inclusions. Later on it was found that microtubules could be forced to form by subjecting CAM-facultative plants to salt stress (SALEMA & BRANDÃO, 1978), conditions which prompted the functioning of the referred type of photosynthesis, thus relating such structure to CAM activity.

These results pointed to the interest to search for the presence of microtubules in the stroma of chloroplasts of various plants known to belong to the above mentioned group. Results of such study are reported in the present paper, together with some considerations concerning the location and diurnal behaviour of the structure.

[1] Part of the results presented were extracted from the Ph. D. thesis of Dr. ISABEL SANTOS.

* Presented in honour of Professor Dr. ABÍLIO FERNANDES of the Botany Institute of Coimbra.

MATERIAL AND METHODS

Young as well as mature leaves of *Umbilicus rupestris* (Salisb.) Dandy, *Kàlanchoë fedtschenkoi* Hamet & Perr., *K. Quartiniana* Rich. X *K. Blossfeldiana* Porlen, *Sedum spectàbïlis* Bor., *Lithops* sp., *Haya carnosa* R. Br., *Aloe arborescens* Mill, and *Euphorbia trígona* Haw., grown under open green-house conditions, were fixed for electron microscopy with 2.5% glutaraldehyde, followed by *2%* osmium tetroxide in NaOH-PIPES buffer (SALEMA & BRANDÃO, 1973). Dehydration took place through an acetone series and the specimens were embedded in Epon through propylene oxide (LUFT, 1961). Sectioning was done with an 1KB Ultrotome III using diamond knife. Sections were contrasted with uranyl acetate (satured solution on alcohol at 50 '% with 1 % of acetic acid) (VALENTINE, personnal communication, 1965) and/or lead citrate (REYNOLDS, 1963). Observations were carried out with either an AEI EM 6G or a Siemens Elmiskop 1A, at 80 kv, using a 200 um condenser and a 50 um objective aperture. Agfa Gevaert 23D 50 cut film was used for photographic recording.

RESULTS

Chloroplasts of *Umbilicus rupestris* are large, with abundant thylakoids, which form numerous grana. Laying on the stroma, among the membranous system, microtubular aggregates were observed, in general more or less centrally located in the plastid (Fig. 1). Very often 2-4 aggregates could be seen adpressed against each other (Fig. 1), interestingly enough the elements which make them up all displaying the same orientation. Cross-sectional views (inset, Fig. 1) clearly show the characteristic hexagonal arrangement.

In the stroma of *K. fedtschenkoi* chloroplasts various aggregates are common, each of them formed by some 20-50 microtubules, laying close together, some touching, others nearby (Fig. 2). The spatial orientation of these aggregates is not the same and, for this reason, in one

section they can be intercepted in various ways (Fig. 2). Again, hexagonal packing is clearly observed (Fig. 2, inset).

Microtubular aggregates were also observed in chloroplasts from *K. Quartiniana* X *K. Blossfeldiana* (Fig. 3) ; generally one aggregate is seen in each chloroplast section, and they are not very large, although they are regular and with parallel hexagonal packing. Only one aggregate in each section was also the rule with the chloroplast of *Sedum spectabilis,* and the microtubular inclusion if can not be said small can not also be classified as large (Fig. 4). Small groups of microtubules were found in the stroma of chloroplasts of *Lithops* sp. (Fig. 5). However, no such structures were observed in the chloroplasts of *Hoya carnosa, Aloe arborescens* and *Euphorbia trígona.*

DISCUSSION

Microtubules in chloroplasts of a higher plant were first reported for *Sedum telephium* (BRANDÃO & SALEMA, 1974) where they appear as huge aggregates, some of them with more than 10,000 elements. As a consequence of the parallel hexagonal packing assumed by them, each one is surrounded by six others. This arrangement and their number, clearly set them apart from the few reports of microtubules in algal chloroplasts (references under Introduction).

In the referred lower plants, microtubules always appear few in number and scattered in the stroma; in some cases they even have larger diameters.

Microtubules in *Sedum telephium* were first found in mesophyll chloroplasts and later also in plastids of other tissues, as stem cortex and stem growing region, nucellus, root cortex and root meristem. The number of microtubules for plastid ranks from various thousands in chloroplasts, to just few, isolated ones in proplastids. This pointed to a possible relationship with chloroplast activity and, since this plant uses CAM pathway, very likely with a special type of photosynthesis.

Added support to this expectancy came from experiments with CAM-facultative plants (SALEMA & BRANDÃO, 1978)

in which chloroplast microtubules could be induced, when such plants were forced to switch to the referred photosynthetic scheme, and allowed to vanish by releasing them from salt-stress.

In the literature there are references to periodic structures in the stroma of chloroplasts of *Kalanchoë pinnata* (LEE & THOMPSON, 1973) and *Opuntia basilaris* (FREEMAN, 1973), both of them CAM-plants, although the elements from which the inclusions are build up were not interpreted as microtubules. Interestingly enough, THOMPSON *et al.* in a later study (1977) considered the inclusion as made up from an enzyme of the CAM scheme.

Various CAM-plants have been under study in our laboratory, and data already available indicate that the protein from which chloroplast microtubules are build up very likely is an enzyme linked to the primary CO_2 fixation, possibly malic dehydrogenase (EC 1.1.1.37).

Searching various sections from leaf samples of *Hoya carnosa, Aloe arborescens* and *Euphorbia trígona* revealed no microtubules in their chloroplasts. It is well known that the absence of a given structure is much more difficult to prove than its occurrence, calling for a systematic survey of the different cell layers of the tissue under study, and even the observation of serial sections and, in the present ease, only random sections ¡from various blocks were made. Neverthless, since randomly cut sections have a statistical value (BERGER, 1969), the probability to found microtubules in the referred species seems to be rather low. However, it must be stressed that in the case of leaves of *K. Quartiniana* X *K. Blossfeldiana* microtubules were observed only on the third cell layer, counted from the abaxial face. If microtubules appear in minute aggregates, or isolated and located in the chloroplasts of specific cell layers, a possibility exists that they can be missed even with random sectioning. This situation is even worsened by the fact that the amount of microtubules in chloroplasts shows a dramatic daily variation (in preparation), a condition which was took into consideration when the material was collected for preparation. Even so, the aforementioned conditions point

to the necessity to deepen the analysis of this type of material, before a definite conclusion can be drawn. It is, however, interesting to note that we have so far found microtubules only in chloroplasts of species belonging to the CAM NADP-malic enzyme (EC 1.1.1.40) rich subgroup and that *Hoya carnosa*, *Aloe arborescens* and *Euphorbia trígona* all belong to a different subgroup (DITTRICH *et al.*, 1973), in which secondary CO_2 is obtained through PEP-carboxykinase (EC 4.1.1.32).

A survey of more species, from both groups, is needed, and is currently under way in our laboratory.

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PLATE

LEGENDS TO THE FIGURES

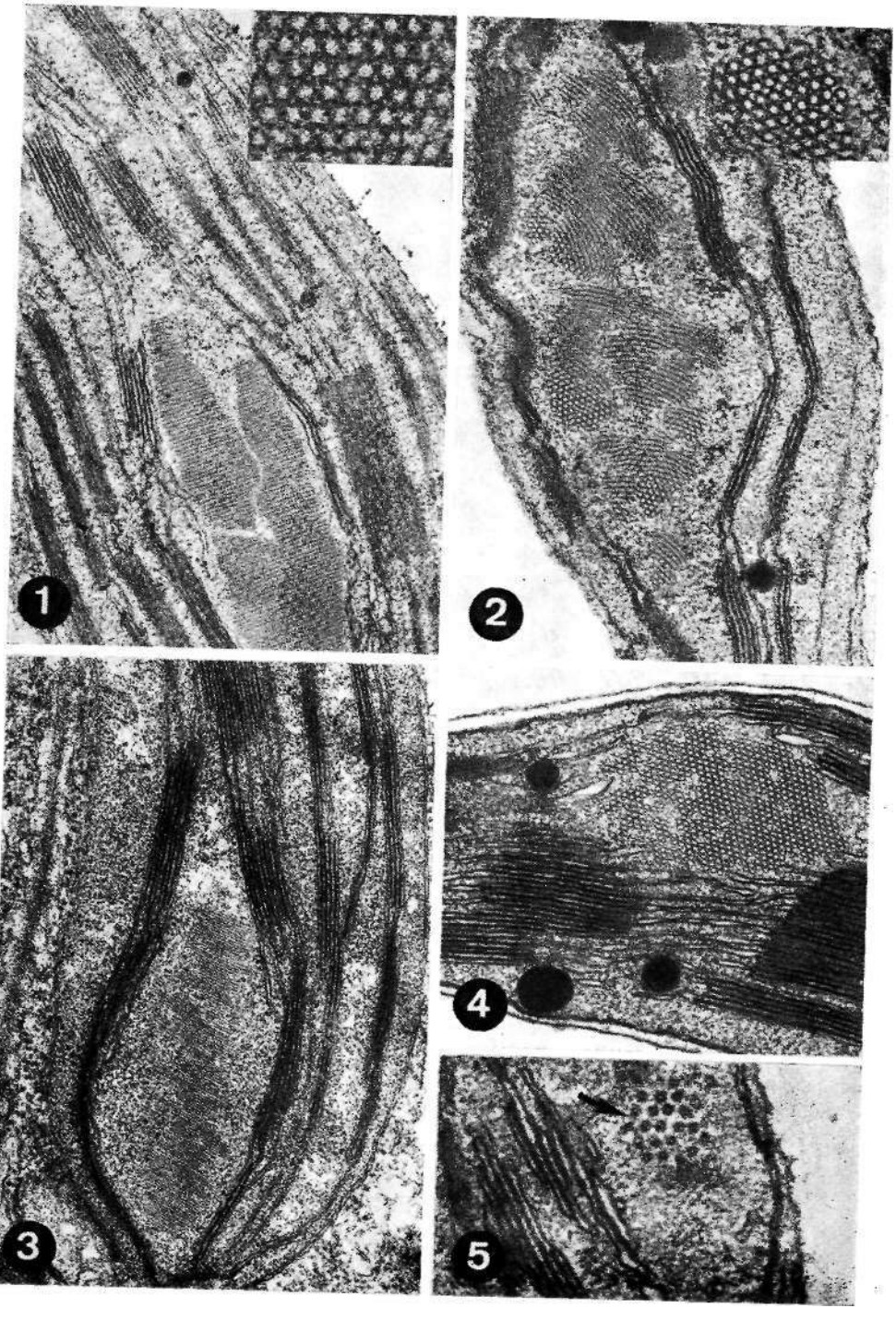
Fig. 1. — Chloroplast of *Umbilicus rupestris,* with microtubular inclusions, in longitudinal view. 28,500 X. Inset — Higher magnification of microtubules in transection, showing parallel hexagonal packing. 180,000 X.

Fig. 2. — Tipical situation encountered in chloroplasts of *Kalanchoë fedtschenkoi,* with microtubules associated in various groups, differently oriented. 45,000 X. Inset — Microtubules with hexagonal packing, seen in transection. 140,000 X.

Fig. 3. — Chloroplast from the hybrid *K. Quartiniana* X *K. Blossfeldiana,* showing a microtubular inclusion. It is noteworth that the periodicity observed (distance between microtubules) is smaller than in the other pictures, which is a consequence of the tilting of the specimen in relation to the electron beam. 36,500 X.

Fig. 4. — Transection view of a microtubular inclusion in the stroma of a chloroplast from *Sedum spectabilis* leaf. 48,000 X.

Fig. 5. — Small group of microtubules (arrow) in a chloroplast of *Lithops* sp., which although not tightly packed show hexagonal arrangement. 70.000 X.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UNE NOUVELLE MOUSSE DE MADÈRE *THAMNOBRYUM FERNANDESII* N. SP.

par

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rÉSÜMÉ

On décrit une nouvelle espèce de *Thamnobryum* (Musci) de la flore de Madère. On l'a nommée *T. fernandesii* en hommage au Prof. A. FERNANDES. Elle a été herborisée en trois localités à une altitude de 1200-1500 mètres par divers collecteurs. Se basant sur la distribution géographique des espèces du même groupe, l'Auteur essaye de donner des explications sur la discontinuité de l'aire du genre. Des types significatifs et semblabes d'aire disjointes sont cartographies. Une nouvelle combinaison — *Thamnobryum assimile* (Broth.) C. Sérg.— est établiée.

NOUS avons été amenés à reviser les échantillons de *Tharnnium angustifolium* indiqués par A. LUISIER (1953) à Madère, après une objection qui nous avait été posée par le Dr. GILBERT de Scheffield, concernant l'existence de cette espèce dans cette île.

Nous avons eu la possibilité d'examiner 'e spécimen de l'Herbier de A. LUISIER (IN) identifié comme *Thamnium angustifolium* et aussi quelques échantillons en plus de l'Herbier du Seminário do Funchal (MADS), de l'Herbier du Museu Municipal do Funchal (MADM) et de l'Estação Agronómica Nacional de Lisboa (LISE), quelques uns desquels n'étaient pas identifiés.

Nous avons eu la surprise de trouver parmi ces matériaux un *Thamnobryum* avec des feuilles à nervures très large à la base, aussi excurrente et du groupe indiqué par

BROTHERUS (1925) seulement dans les Iles Juan Fernandez près du Chili. Il était sans doute bien distinct de *T. angustifolium* par la forme de la feuille, nervure et denticulation de l´apice.

Nous avons hésité avant d'inclure cette mousse dans le groupe des *Thamnobryum* de l'Amérique du Sud, mais tout de suite, après l'examen du type de *Thamnium rigidum* de l'Herbier de Paris (PC) et des types de *T. assimile* et *T. proboscideum* de Stockholm (S), nous avons confirmé la ressenblance des échantillons de Madère avec ces espèces des îles Juan Fernandez.

Ã ce moment nous sommes sûrs de décrire une nouvelle espèce de *Thamnobryum* pour la flore de Madère, *T. fernandesii,* et de l'inclure dans le groupe des *Thamnobryum* à nervure excurrente, très large à la base, et bien proche de *T. proboscideum* et *T. assimile.*

Le degré de varibilité de quelques caractères morphologiques parmi les *Thamnobryum* est grand. Cependant, la forme et l'excurrence de la nervure et des apices des feuilles sont assez stables chez chaque espèce et considérées comme des caractères taxonomiques importants.

Le anciens taxa ont été en général décrits par la méthode classique, les descriptions ne comportant aucune ou peu d'indications sur l´anatomie de la section de la feuille et de la nervure. Ä ce jour, il est bien indispensable de recourir à l'examen des types pour aboutir à une conclusion.

Ainsi, après avoir examiné les types, nous avons eu de grands doutes sur l'inclusion de *Thamnium assimile* Broth, dans la synonime de *Thamnobryum rigidum* (Mitt.) H. Robinson, puisque la nervure de la deuxième espèce est assez étroite et elle n'est pas élargie à la base, ce qui arrive chez le groupe *T. assimile — T. proboscideum.* Ä notre avis, il s'agit certainement de trois espèces distinctes. KINDBERG (1902) a considéré *T. rigidum* comme synonime de *T. pumïlum* (Hooker et Wilson) Kindb. de l'Amérique australe et de l'Australie et Tasmanie. Cependant, cette synonimie n'est pas aussi exactement établie, puisque les deux espèces sont distinctes. V. F. BROTHERUS (1909 et 1924-1925) a indiqué trois espèces distinctes dans ce groupe: *T. assimile* Broth.,

T. crassinerviwm Broth, et *T. proboscideum* Broth. Donc, selon notre opinion, ce groupe sera constitué par *Thamnobryum proboscideum* (Broth.) H. Robinson, *T. rigidum* (Mitt.) H. Robinson (T. *crassinervium* Broth, inclus.), *T. assimile* (Broth.) C. Sérg.[1] et maintenant en plus *T. fernandesii C.* Sérg.[2] dont la description se suit :

Thamnobryum fernandesii, sp. nov.

Plantae steriles usque ad 15 cm altae, dendroideae, robustae, rigidae, brunneae vel viridi-brunnescentes. Caulis primordialis repens, rhizomatosus, brevis, crassiusculus, stoloniferus. Caulis secundarais erectus, inferne simplex, superne ramosus, rami irregulariter pinnati, omnes in eandem directionem curvati. Folia caulina inferiora squamiformia, scariosa, minima, adpressa, remota; caulina superiora et ramealia majora, 1,5-3 mm longa et 0,3-0,5 mm lata, linearilanceolata, subulato-acuminata, margine plana et integra sed prope apicem remote ac irregulariter, breviterque denticulata, erecto-patula, ± contorta et in apicem caulis ramorumque incurvata, laxe imbricata; lamina in mediana parte utroque latere 15-25 seriebus cellularum constituta (in 1-2 stratis dispositis) ; costa valida, sursum angustata et ad apicem subexcurrens vel excurrens, basin versus ampliata, in basi ipsa 100-200 u, lata, $^{1/2\text{-}2/3}$ latitudinis folii occupans, fere homogénea, in sectione transversale 6-9 seriebus stereidarum instructa, haec autem a lamina paullo distincta et in eadem basi fere usque ad utramque marginem in 2-3 strata

[1] Le nom *Thamnium* a été employé tout d'abord par KloTzSCH (in Linnaea 12: 223, 1838) pour un genre d'*Ericaceae*, le nom *Thamnium* B. S. G. (Bryol. Eur. 5: 211, 1852) étant donc un hom. Illeg.

Thamnobryum a été crée pour substituer *Thamnium* B. S. G. et les combinaisons nouvelles respectives ont été faites à l'exception de celle concernant *Thamnium assimile* Broth, que nous faisons ci-dessous.

Thamnobryum assimile (Broth.) C. Sérg., comb. nov.

Basion.: *Thamnium assimile* Broth, in Skottsb. Nat. Hist. Juan Fernandez Islands 2 (3): 433 (1924).

[2] Nous sommes heureuses de dédier cette espèce à M. le Prof. Dr. A. FERNANDES de Coimbra, à l'occasion de l'hommage qui lui a été prêté.

gradatim transiens. Cellulae superiores et mediane laminae 15-30 X 5-12 u, interdum in stratis 2 dispositae, irregulares, triangulares, usque rhombeo-hexagonales, eae versus basin folii paullo elongatiores. Plantae fertiles ignotae.

Habitat in Insula Madeira, in saxis innundatis rivuli «João Fernandes», pr. pagum vulgo dictum «Boaventura», ubi, die 27-IX-1951 a *Nobrega* sub n.^{0} 263, collectum. Holotypus in MADS; isotypus in LiSU et in IN.

Specimina altera.

Insula Madeirae: «Vereda do Pico Ruivo para Caldeirão Verde», IX-1950, *Nobrega* (MADS; LISU) ; «Levada do Caldeirão Verde para Sant'Ana», IX-1941, *J. M. Carvalho* (LISE; Lisu); s. 1., s. d. *Costa* 116 (MADS; LISU).

T. proboscidei et *T. assimile* proximum sed ab eis foliis angustioribus et plus acuminato-subulatis ; seriebus cellularum laminae minus numerosis (utroque latere 15-25 necque 50-55 seriebus) ; costa a lamina non distincte delimitata, in utroque latere in 2-3 strata cellularum a basin marginem attingentia, praecipue differt.

Ab *T. proboscidei* cellulis foliorum 2-3 nec 1-straficata differt at ab *T. assimile* basi foliorum fere costa reducta nec costa in lamina distincte producta.

Plantes stériles jusqu'à à 15 cm de haut, dendroïdes, robustes, rigides, brunes ou d'un vert-brunâtre. Tige primaire rampante, rhizomateuse, courte, un peu épaisse, stolonifère. Tige secondaire dressée, inférieurement simple, ramifiée vers le haut, à ramification ± pennée et à rameaux irréguliers, souvent tous les rameaux courbés et dirigés vers le même côté. Feuilles caulinaires inférieures très réduites, formées presque uniquement par la nervure, scamiformes, appliquées; feuilles caulinaires supérieures et celles des rameaux 1,5-3 X 0,3-0,5 mm, linéaires-lancéolées, subulées--acuminées, entières mais à quelques dents irrégulières et courtes vers le sommet, étalées-dressées, ± tordues, courbées à l'apex, lâchement imbriquées; limbe à 15-25 rangées de cellules (d'un et d'autre côté de la nervure) au milieu de

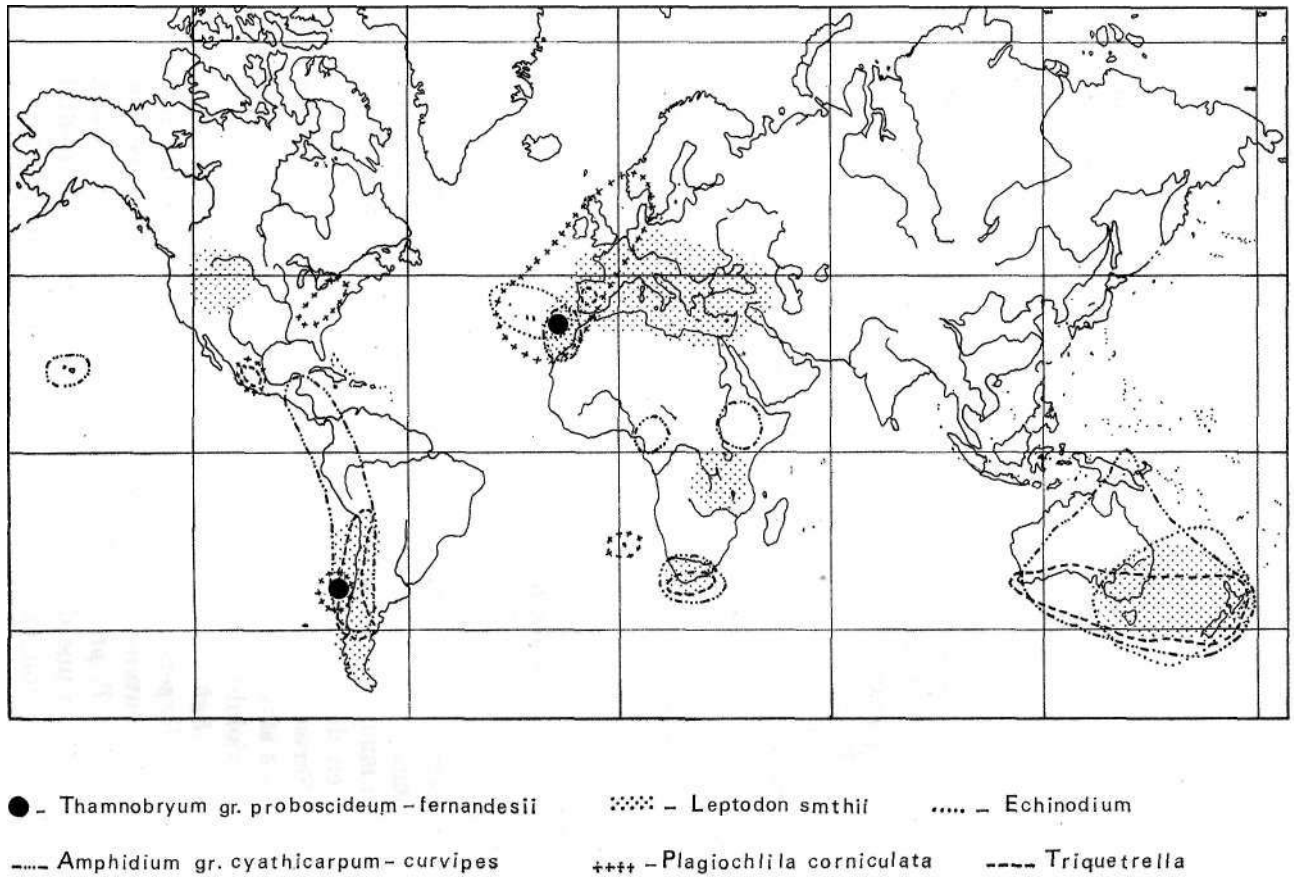
la feuille; nervure épaisse, s'amincissant ver l'extrémité, excurrente ou evanescente un peu au dessous du sommet, large de 100-200 u. à la base, occupant ici $^{1/2\text{-}2/3}$ du limbe et présentant en coupe transversale 6-9 séries de stéreides, presque homogène, peu distincte du limbe dans lequel elle se prolonge latéralement par 2-3 assises de cellules presque jusqu'à la marge. Cellules supérieures et médianes du limbe se disposant ça et là en 2 couches, 11-30 ,u X 15-12 u, irrégulieres, triangulaires à rhombiques-hexagonales, celles de la base un peu plus longues. Plantes fertiles inconnues.

Cette espèce se distingue de *T. proboscideum* et de *T. assimile* par les feuilles relativement moins larges; par le limbe possédant un nombre moins élevé de rangées de cellules de chaque côté de la région médiane de la nervure (15-25 et non 50-55 rangées) ; par la délimitation peu nette de la nervure le long de presque toute la feuille, la nervure se prolongeant latéralement en une zone à 2-3 assises de cellules d'un et d'autre côté.

Les cellules du limbe sont ça et là 2-stratifiées comme il arrive chez le *Th. assimile,* tandis que chez le *Th. proboscideum* elles sont toujours 1-stratifiées.

Ã présent, *T. fernandesii* doit être considérée comme une autre espèce endémique de Madère parmi d'autres exemples assez abondants.

On connaît quelques espèces de Madère avant des affinités avec d'autres éloignées géographiquement. Nous pouvons indiquer: *Amphidium curvipes* de Madère et des Canaries (A. LUISIER, 1930), bien proche de *A. cyathocarpum,* qui existe en diverses aires disjointes, mais aussi dans les îles Juan Fernandez (Carte fig. 1) ; *Bryoxiphium modérense* ayant des affinités avec *B. norvegicum,* qui a une distribution aussi disjointe mais dans l'hémisphère Nord (C. SÉRGIO, 1976) ; et *Echinodiiim spinosum,* endémique de la Macaronésie et très semblable à *E. hispidulum* de l'Australie, Tasmanie et Nouvelle-Zélande (A. LUISIER, 1938). Dans ce cas, comme chez *T. proboscideum, T. assimile* et *T. fernandesii,* ii y aurait eu une différenciation morphologique en résultat d'une adaptation et d'une évolution naturelle au cours des siècles. Ainsi, les caractères distinctifs de ces espèces sont



Carte 1. — Répartition de *Thamnobryum* gr. *proboscideum-fernandesii* en corrélation avec divers types d'aires disjointes de Bryophytes.

bien précis et nous admettons que les modifications proviennent d'une évolution en deux stations géographiquement éloignées et que les facteurs écologiques n'ont pu contribuer que dans une courte échelle à la différenciation des deux taxa.

On a beaucoup écrit au sujet des disjonctions, en ce qui concerne les Bryophytes (TH. HERZOG, 1926; P. ALLORGE, 1931; T. pocs, 1960; I. ABRAMOV, 1969; W. B. SCHOFIELD, 1969, 1972; R. M. SCHUSTER, 1979, etc.), les Lichens (W. CULBERSON, 1972; R. G. WERNER, 1973, 1975) et les Phanérogames (HULTEN, 1958, C. E. WOOD JR., 1972, VAN STEENIS, 1971, J. P. BRENAN, 1978, etc.)

Parmi les différentes interprétations pour chaque cas, telles que le transport par les vents, les courants, les oiseaux migrateurs ou l'action directe ou indirecte de l'homme, l'interprétation la plus probable pour certains des cas est basée sur des données géologiques en correspondance avec les conditions climatiques au cours de l'évolution des masses continentales.

Généralement, il est admis que les espèces ou les groupes d'espèces ou genres disjoints sont des types anciens, dont l'aire autrefois plus ou moins continue a été réduite par des phénomènes géologiques divers et en spécial par la dérive des continents.

Souvent les taxa disjointes appartiennent phytogéographiquement aux reliques du Terciaire.

Pour l'île de Madère il n'y a pas de doute que, au Miocène, ele était déjà formée, aussi bien que toutes les autres îles Atlantiques (C. N. TAVARES, 1965). Et même quelques genres de plantes vasculaires comme *Clethra* étaient déjà incluses comme reliques du Terciaire par CH. DARWIN (cf. C. N. TAVARES, 1965).

Les îles Atlantiques en spécial, aussi bien que la Péninsule Ibérique et le Nord de l'Afrique, sont des régions où les disjonctions sont à la foi très nombreuses et très frappantes.

Ce groupe de *Thamnobryum,* en particulier, sera un exemple de disjonction que nous appelerons de Macaronéso-Australe, un peu semblable à la disjonction Ibéro-Australe de P. ALLORGE (1931) de *Triquetrella (Pottiaceae)* et du

Fougère *Pleurosorus,* avec des espèces endémiques ibériques (T. *arapilensis* et *Pleurosorus hispanicus).*

Parmi les Bryophytes, il existe plusieurs exemples en plus avec des disjonctions analogues, comme *Leptodon smithii,* les genres *Echinodium* et *Amphidium* et *Plagiochïla cornicuïata* (syn. *P. tridenticulata),* mais ayant des microaires plus étendues.

Le parallélisme est très marqué et nous pouvons le vérifier chez les cinq exemples (carte 1) que possèdent deux ou même quatre aires en commun entre elles et que le groupe *T. proboscideum-T. assimile,* et T. *fernandesii* existe dans une de ces aires.

Nous remarquerions encore que ces trois espèces de *Thamnobryum* sont dépourvues d'organes de réproduction et que, chez les espèces à seule propagation végétative, les disjonctions des aires sont fréquentes, étant admis que ces espèces auraient perdu leurs moyens de dessimination au cours des âges géologiques (P. AlloRGE, 1931).

Il est intéressant d'ajouter que ces *Thamnobryum (T. proboscideum-T. assimile* et *T. fernandesii)* ont une localisation très particulière ils existent tous en deux îles volcaniques qui ont, chacune d'elles, un climat semblable, l'une au Nord, l'autre au Sud (32° N-Madère et 34° S les îles J. Fernandez) et une altitude et physiographie semblables (la Madère 1200-1500 m et îles J. Fernandez de 1000-1700) ; les conditions écologiques doivent être à peu près les mêmes, bien que les références des récoltes soient peu précises. Pour *T. proboscideum* l'échantillon type possède seulement la référence suivante: cascade, où égoutte l'eau, et le type de *T. assimile* la suivant: cascade, près de l'eau. En plus nous avons observé que les échantillons de BERTERO étaient associés à *Fissidens rigidulus* espèce des endroits ruisselants.

Thamnobryum fernandesii colonise les fentes des rochers ruissellants, généralement ombragés, associé à *T. alopecurum, Fissidens taxifoîius* var. *pallidicaulis, Mnium affine, Porella cordeana* et *Porella* sp. et possiblement dans l'association *Homalia subrecta-Thamnium alopercurum* Ges. (HÜBSCHMANN, 1971).

T. fernandesii avait été récoltée en trois localités de Madère à peu près entre 1200 et 1500 m, tout près de Pico Ruivo, en des endroits presque jamais dévassés par l'homme, d'un difficile accès (d'après le renseignement qui nous à été prêté par le Père M. NÓBREGA qui avait herborité les deux meilleures récoites).

Nous pouvons donc conclure que *T. fernandesii* est un élément endémique de l'étage macaronésique supérieur (ROMARIZ, 1953) de caractère hygrophytique de la série Laurisilva de Madère.

Spécimens examinés:

Thamnobryum angustifolium (Holt) Crund. — England, Cresobrooh Dale Derbyshire, 7.6.1978, *Gilbert & S. Furness* (LISU).

Thamnobryum assimile (Broth.) C. Sérg. — Juan Fernandez: Masatiera 6.2.1916, Quebr. Damagniar, vattenfall vid vattnent, 248 m., *Carl. o. Inga Skottsberg* 350 (Isotype, S, sub *Thamnium assimïle* Broth.).

Thamnobryum proboscideum (Broth.) H. Robinson — Juan Fernandez: Masatierra, vattenfaliet; Pangal, St. 6, i rinn, vatten. 250 m s. m., 1.1.1917, *Carl o. Inga Skottsberg* 256 (Isotype, S, sub *Thamnium proboscideum* Broth.).

Thamnobryum rigidum (Mitt.) H. Robinson — Juan Fernandez, 1830 *D. Bertero* (Type, PC, sub *Hypnum? Porotrichum, Thamnium)* ; Juan Fernandez 9.1872, *Ev. Cv. Reed* (PC, sub *Porotrichum rigidum)* ; Juan Fernandez, Challenger Exped, 6.1876, *Mr. Moseley* (PC).

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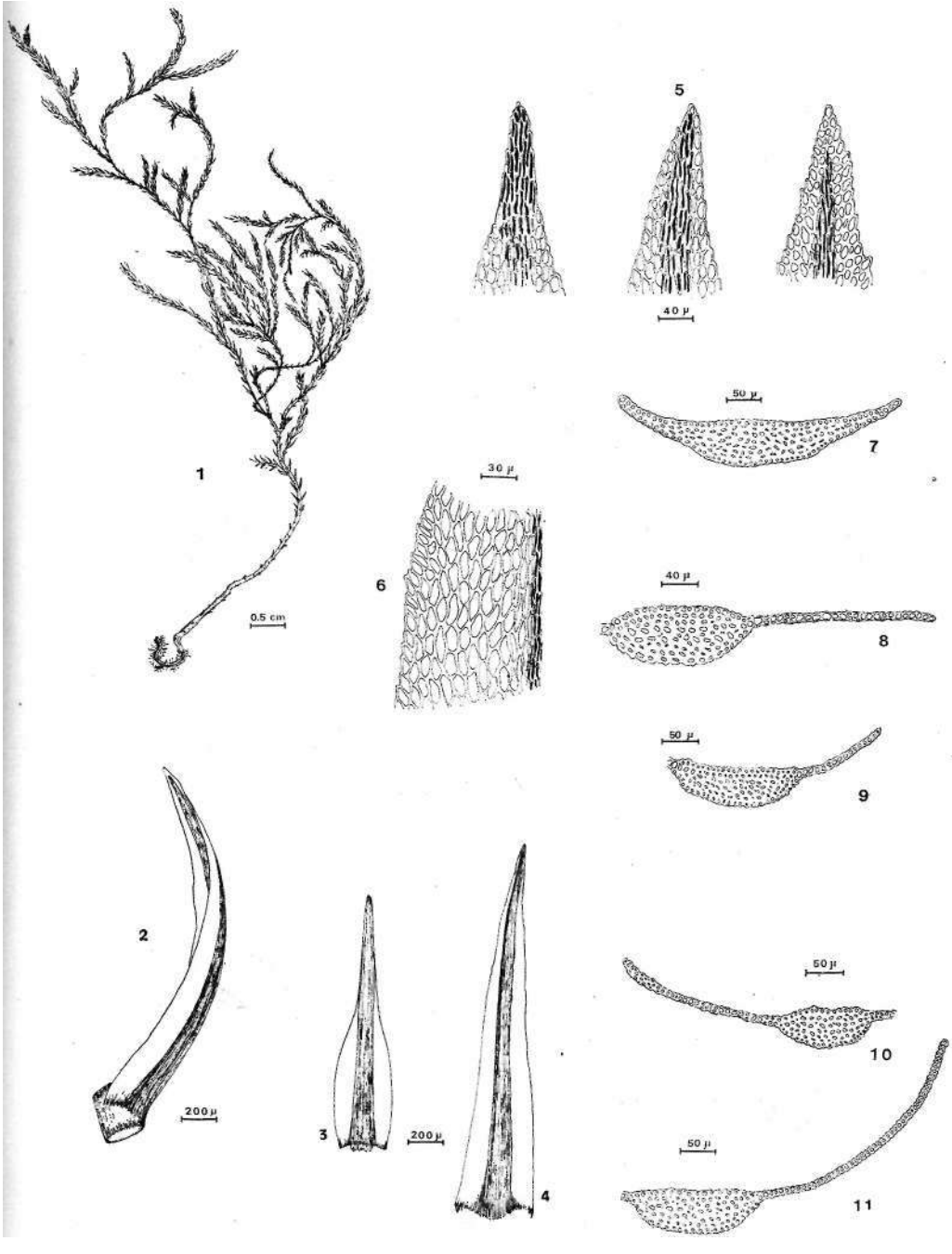
TABULA

TAB. I

Thamnobryum fernandesii C. Sérg. 1 — Habitus. 2 — Folium in caule insertum. 3-4 — Folia ramea-11a. 5 — Apices foliorum. 6 — Cellulae partis mediane folii. 7 — Sectio transversalis basis folii. 8 — Sectio transversalis partis mediane ejusdem. *Thamnobryum assimile* (Broth.) C. Sérg. 9 — Sectio transversalis basis folii. 10 — Sectio transversalis partis medianae ejusdem.
Thamnobryum proboscideum (Broth.) H. Robinson. — Sectio transversalis partis medianae folii.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Bol. Soc. Brot, Sér. 2, 53(2): 1137-1160 (1981).

CONTRIBUTION A L'ETUDE DES FORMATIONS PRË-STEPPIQUES À GENÉVRIERS AU MAROC

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RÉSUMÉ

Les auteurs définissent les caractères écologiques, bioclimatiques et altitudinaux des peuplements clairsemés pré-steppiques à *Juniperus arborescent* du Maroc et en particulier du pourtour du Haut Atlas. Ils précisent leur signification phytosociologique et les rapportent à une classe nouvelle, les *Ephedro-Juniperetea.* Sept associations ou groupements sont définis.

SUMMARY

Authors describe the ecological, bioclimatical and altitudinal signification of the sparse populations of *arborescent Juniperus* in Marocco and specially around the «Haut Atlas». They give their phytosociological value and describe a new classe, the *Ephedro-Juniperectea* and seven new associations.

L´ANALYSE des structures phytosociologiques des formations forestières d'Afrique du Nord et tout spécialement du Maroc, à laquelle nous avons déjà consacré plusieurs publications et en particulier les suivantes: BARBERO, LOISEL et QUEZEL, 1974, ACHHAL *et al.,* 1980, BARBERO, QUEZEL et RIVAS-MARTINEZ, 1980, peut-être actuellement considérée comme bien avancée. Toutefois, la mission que nous avons effectuée durant le printemps 1979 sur le Haut Atlas nous a permis de mieux comprendre et de définir

certains types de végétation d'où les éléments arborescents ne sont pas absents, mais qui posent des problèmes très spéciaux.

En effet, alors qu'en région méditerranéenne, les associations sylvatiques sclérophylles peuvent être rattrachées dans leur quasi-totalité à la classe des *Quercetea Uicis* et à l'ordre des *Quercetalia Uicis* (BARBERO, LOISEL et QUEZEL, 1974, RIVAS-MARTINEZ, 1975, QUEZEL, BARBERO et AKMAN, 1978) et ceci, sur tout le pourtour de la méditerranée, certaines structures de végétation de répartition géographique très précise où dominent en général les Genévriers, les Ephedra, voire localement divers *Quercus*, restent d'interprétation difficile car, pratiquement aucune des caractéristiques des unités citées ci-dessus ne s'observent.

C'est en fait au Proche-Orient que nous avons défini pour la première fois ce type de végétation (ABI-SALËH, BARBERO, NAHAL et QUEZEL, 1976) sous le nom de végétation forestière pré-steppique. En effet, c'est sur les marges des vastes ensembles steppiques du Proche-Orient ou du Maghreb qu'elle s'observent essentiellement.

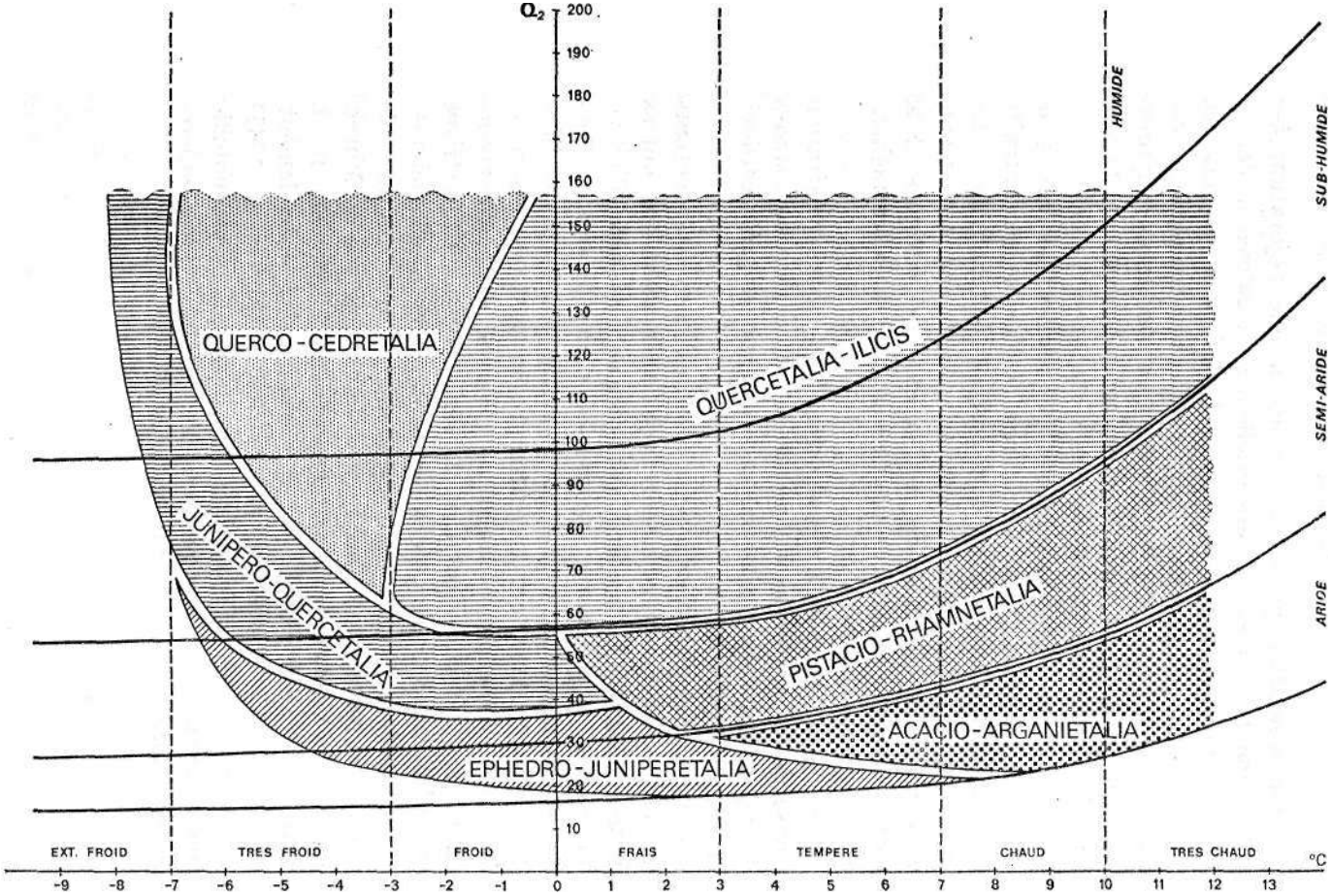
Du point de vue physionomique, ces formations forestières, ou mieux arborées, pré-steppiques, apparaissent le plus souvent constituées par un piqueté arboréen clairsemé, où le recouvrement de la strate arborescente et arbustive ne dépasse généralement pas 40 à 50%, mais qui peut devenir toutefois localement quasi-fermé. Si les représentants du genre *Juniperus* sont ici dominants, comme nous l'avons déjà dit, d'autres genres peuvent être bien représentés et notamment les chênes sclérophylles. En sous-strate, le nombre des espèces phytosociologiquement significatives est réduit, et il apparait alors un cortège souvent important d'éléments se rapportant à la végétation des fruticées et pelouses voisines.

En fait, sur le Haut Atlas marocain où les structures de végétation sont particulièrement bien développées on peut s'apercevoir aisément que cette végétation pré-steppique apparait en fonction de deux critères écologiques essentiels, qui peuvent parfois s'intriquer; péjoration des conditions hydriques, péjoration des conditions thermiques.

La végétation pré-steppique liée à une péjoration des conditions hydriques est essentiellement localisée à l'étage bioclimatique aride, voire semi-aride inférieur (EMBEBGER, 1930) et dans les variantes froide, fraîche et tempérée de cet étage. Elle correspond au Maroc aux structures phytosociologiques décrites ci-dessous et se rapportent à l'ordre des *Ephedro-Juniperetàlia.*

La végétation pré-steppique liée essentiellement à la péjoration des conditions thermiques se localise au contraire en situation bioclimatique semi-aride et sub-humide, voire localement humide et dans les variantes froide, très froide et extrêmement froide de ces types bioclimatiques. Elle correspond aux structures phytosociologiques se rattachant aux *Junipero-Quercetàlia.*

On peut bien sûr se demander si les types de végétation envisagés ici ne correspondent pas en fait aux ultimes stades de dégradation des formations forestières ou préforestières classiques *(Quercetàlia ilicis* et *Pistacio-rhamnetàlia)* de la région. En fait l'absence à peu près totale des représentants de ces unités, mais également l'apparition de divers autres éléments significatifs, rend parfaitement possible l'individualisation de structures phytosociologiques originales. Soulignons également la valeur bioclimatique très particulière de ces formations, qui constituent régionalement de toute évidence, et bien que l'action de l'homme ait souvent contribué à les étendre aux dépends des formations forestières et préforestières, de véritables groupements climaciques. Leur situation bioclimatique par rapport aux structures de végétation forestière et préforestières climaciques au Maroc, apparait d'ailleurs très nettement dans le schéma n° 1. Soulignons qu'en Europe et en Espagne tout spécialement, la classe des *Pino-Juniperetea* (RIVAS-MAETINEZ, 1963) occupe une situation écologique, bioclimatique et altitudinale très comparable à celle que présente au Maroc les *Junipero-Quercetàlia.*



SCHEMA N° 1. — Projection schématique sur le elimagramme d'EMBERGER, des aires occupées au Maroc par les unités phytosociologiques correspondant aux formations climaciques de type forestier, préforestier et présteppique.

! — INTERPRETATION PHYTOSOCIOLOGIQUE

L'ensemble des structures phytosociologiques envisagées ici peut se rattacher à une classe spéciale, celle des *Ephedro-Juniperetea,* classe nouvelle, que les espèces suivantes permettent de caractériser:

Juniperus phoenicea subsp. *phoenicea,*
Juniperus oxycedrus subsp. *rufescens,*
Ephedra major var. *villarsil,*
Ephedra fragilis subsp. *cossonii.*

Cette classe, sans doute exclusivement nord-africaine, peut se subdiviser en deux ordres:

— l'ordre des *Ephedra majoris-Juniperetalia phoeniceae,* ordre nouveau, qui réunit les formations pré-steppiques à déterminisme xérique prédominant est cractérisé par:

Asparagus albus,
Cuprèssus atlántica,
Pistacia atlántica,
Rhus tripartitum,
Rhus pentaphylla,
Polygala balansae,
Warionia saharae.

— l'ordre des *Junípero thuriferae-Quercetàlia rotundifoliae,* ordre nouveau qui regroupe au contraire les formations pré-steppiques à déterminisme thermique prépondérant est caractérisé par:

Berberís hispánica,
Buxus baleárica,
Cotoneaster nummularia,
Crataegus lacimata,
Fraxinus dimorpha,
Juniperus thurifera var. *africana,*
Lonicera arbórea,
Quercus rotundifolía,
Ribes atlanticum,
Rosa sicula.

Le choix des caractéristiques pose bien sûr quelques problèmes, et beaucoup d'entre elles se retrouvent dans d'autres unités phytosociologiques. En fait, cette situation n'est pas étonnante car, si l'on considère le schéma n° 1 il est évident que certaines espèces des *Pistacio-Rhamnetalia* et des *Acacio-Arganietelia* peuvent aisément pénétrer dans l'aire des *Ephedro-Juniperetalia* de la même façon que les espèces des *Pistacio-Rhamnetalia,* des *Quercetalia ilicis,* voire des *Querco-Cedretalia* peuvent apparaître dans les *Junipero-Quercetàlia,* et vice-versa.

En fait, l'ensemble des observations que nous avons maintenant effectuées au Maroc, nous ont convaincu que les espèces citées ci-dessus, présentent bien leur développement optimal tant du point de vue phytosociologique qu'écologique dans les unités phytosociologiques définies ici, du moins au Maghreb, et qu'elles constituent ou participent seulement là à des groupement climaciques, à l'exception toutefois de *Quercus rotundifolia* transgressif ici comme *Asparagus albus* des *Quercetea ilicis* et *Rhus pentaphylla* voire *Rhus tripartitum,* transgressifs localement des *Acacio-Arganietea.*

II - LES GROUPEMENTS SE RAPPORTANT AUX *EPHEDRO--JUNIPERETALIA*

Ils restent encore relativement mal connus, puisque nous n'avons eu l'occasion de les étudier avec une certaine précision que sur le revers septentrional du Haut Atlas central et en particulier dans la vallée du N'Fiss. Aussi les conclusions auxquelles nous arrivons ci-dessous ne peuvent être considérées que comme provisoires.

En fait les groupements des *Ephedro-Juniperetalia* du Haut Atlas semblent exister essentiellement aux étages aride et semi-aride inférieur et dans les variantes tempéré, fraîche et localement froide de ces bioclimats. Ils apparaissent donc sur les marges inférieures de la chaine, mais également dans certaines vallées internes peu arrosées comme en particulier la vallée du N'Fiss. Ils sont essentiellement constitués par des formations plus ou moins clairsemées à *Juniperus phoe-*

nicea, Cupressus atlántica, et plus localement à *Juniperus oxycedrus.*

Dès que les conditions climatiques deviennent plus favorables et tout spécialement lorsque les précipitations augmentent, ces structures de végétation cèdent la place à des peuplements pré-forestiers liés en général aux *Pistacio-Rhamnetalia* et où domine *Tetraclinis articulata,* voire *Pinus halepensis* et *Ceratonia sïliqua* et situés surtout en ambiance bioclimatique semi-aride supérieure, puis au sub-humide, à des groupements forestiers à *Quercus rotundifolia* s'intégrant au *Quercetalia ïlicis.*

Divers groupements et associations peuvent pour l'instant se rattacher aux *Ephedro-Juniperetalia.*

II. 1 — Groupement subrupicole à *Warionia saharae* et *Antirrhinum ramosissimum*

Les rochers et rocailles eruptives à forte inclinaison, hébergent entre 1000 et 1300 m, dans la vallée moyenne du N'Fiss, un groupement spécial dont les 2 relevés ci-dessous permettent de se faire une idée. A côté de *Warionia saharae* toujours dominant l'on peut faire figurer parmi les autres caractéristiques *Antirrhimum ramosissimum* qui trouve ici une de ses stations les plus septentrionales, *Mieromeria hochreuttineri* et saus doute aussi *Hedysarum membranaceum* bien que cette dernière espèce s'observe également sur les éboulis.

L'appartenance de ce groupement aux *Ephedro-Juniperatalia* est certaine comme le montre la composition des relevés ci-dessous:

1 : Vallée du N'Fiss, 5 km au N d'Ijoukak, 1060 m,
2: Vallée du N'Fiss, 3 km au N d'Ijoukak, 1150 m.

Surface :	100	100
Recouvrement :	50	40
Exposition :	WN	W
Inclinaison :	30	40

Espèces caractéristiques et différentielles:

Warionia saharae	3.2	2.2
Antirrhinum ramosissimum	1.2	2.3
Hedysarum membranaceum	1.1	
Micromeria hochreutineri	..	1.3

Caractéristiques dés unités supérieures:

Juniperus phoenicea	1.3	2.3
Ephedra major var. *villarsii*	2.2	1.2
Rhus tripartituni	3.3	
Coronilla ramosissima	+	
Ephedra fragïlis subsp. *cossonii*	..	1.3
Polygala balansae	..	1.2
Rhus pentaphylla	..	2.3

Autres espèces:

Globularia alypum	1.2	1.2
Stipa tenacíssima	1.3	+
Lavandula dentata	2.2	1.2
Phagnalon saxatile	2.1	+
Stipa parviflora	1.2	1.2
Fagonia crética	2.2	1.2
Launaea arborescens	+	1.2
Lavandula maroccana	1.2	
Pallenis spinosa	1.2	
Orizopsis miliacea	1.1	
Launaea acanthoclada	..	1.3
Cymbopogon schoenanthus	..	1.3
Pulicaria mauritanica	..	1.2
Echinops strigosus	..	+
Lavandula tenuisecta	..	1.2

Iî.2 — Association à *Coronilla ramosissima* et *Juniperus phoenica (Coronïllo ramosissimae-Juniperetum phoeniceae* nov. ass.) (tableau n° 1)

Nous décrivons sous ce nom l'ensemble des formations pré-steppiques à Cupressacées situées en ambiance thermophile, sur le revers septentrional du Haut Atlas de Marrakech et dans la vallée du N'Fiss.

Si *Juniperus phoenicea* est pratiquement toujours présent, *Juniperus oxycedrus* subsp. *rufescens* peut s'y associer

Numero des releves	1	2	3	4	5		7	8	9	10	11	12	13	14	15
Surface	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Recouvrement	50	40	50	40	70	60	50	60	50	50	45	40	40	70	50
Exposition	N	N	E	NE	E	SW	N	W	N	E	E	W	N	E	W
Inclinaison	50	50	40	50	25	50	50	60	40	50	45	50	50	80	40
Substrat	Gr	Gr	Sh	Sh	Do	Gr	Gr	Gr	Sh	Gr	Gr	Sh	Sh	Gr	Sh
Altitude x IC	110	130	115	110	190	165	140	160	100	120	110	108	130	125	140
Espèces caractéristiques et différentielles :															
JunipeAtiA pho enicta	3.4		**3.4**	3.2	**2.2**	1.3	2,1	2.3	**3.2**		**3.3**	**2.3**	2.3	1.3	2.2
C.OH-Onitta KamoAiAAima	+			1.1		2.1	1.1	1.2					1.2		1.2
ZupleuAum dumoium	+											1.2	1.3		
Aipa/iagui albuA	+								1.1		1.2	1.2	1.2	**2.3**	
Rhui t/iipatutitim	+								**2.2**		**2.3**	**3.3**		1.3	
CappaKii ApinoAa													1.1		1.2
Cup/teAAuA atlántica	I 2.3		**2.3**	**3.3**	**3.4**	**2.3**	**4.3**	**2.3**							
Espèces caractéristiques des unités supérieures :															
tphedna majon. var. *villaitAii*	+	1.2	2.3	+	1.1	1.1	+	1 3	1.3	**4.2**	1.3	1.3	1 2	3.4.	3
Polygala balamae	1.2	1.2	+	2.2	1.2	1.2	2.3	2 1			+	1.2		1.2	
Viitacia atlántica	2.1	+	1.2	.		1.2					1.2	+	1 2	1.3	1
JunipeMU oxycednai subsp. *M.u£acem*				+	I 2.2	2.2	1.2	3	* I		•	.	Q	**2.3**	
Bpkedfux ^Kagilii subsp. *cononii*				1.2	.	1.2	+					1.2			
WatUonia Aahanae											1.3	1.3			
R/iuò *ptntaphylZa*			1.2	.	.	.	.					+			
Qu.en.cuA KO tundido lia					1.'2										
Vestiges des *Queltcetea iticii* :															
liziphuA lotuA							1.2								
TztKacliniA axticulata	1.2														
Olea eWLopea. subsp. *njlveAtniA*													1.2	1.2	
Ce/iatonia iiliqua													+	1.2	
Espèces des *Ononido-RoAmaHin&tea* :															
Lavandula dentata	2.1	.2	1 2	. '		2.2	2 2	1.2	2.2		2.3	1.2	1.2		
Thymui iatm.eAoid.ei		.2	1 3	3.2	3 2	1.1	2 2	2.3							
Globulaxijx alypum	+	+							+		1.2	+	.	+	
Vhagnalon Aaxatiie	2.2								1.2		1.2	1.2	.	1.3	
BalZota hiipanica			1 2							2 3		.		1.3	2.
Helianthemum vingatum	2.1	.2	1 3												
Stipa tenaciiiima	+											1.2			
Retama daiycanpa	+					+	2 2								
Salvia tataxaciÂolia													+	1.2	l!
Lavandula maxoccana	+								1.2						
CiAtai villoiuA				+				+							
Volycnemon {¡ontaneiii :			1 1												
Stipa niteni						1.3			1.1						
Lavandula multißida										1 2	\	\	'.	\	X.
Autres espèces :															
Patonychia kapela									+						
VactyliA glomenata									1.1	1 1					
Sedum iedi^onme											+				
Btyngium ilLci&olium									1.1	H^	+				
Stipa pativi^loHa												+			
Echinopi itAigoiuA									+						
Hypakfihenia hinta •															
Leacanthemum gayanum															
Lavandula. te.nuZie.cta.											+	1.2			
Onizopiii milLacea															
Kntivaiinum namoiibAimum															
TeucJiium baxbafam												+			
Vulicania mautitanica ...									+			+			
CanJU.no. involucnata.															
Launaea acancho ciada									+	1 3	1.3	**1.2**			
La.una.eA aA.box.ZAce.ni.									+	1 2	1.3	1.2			
Cymbopogon AhoenanthuA ..									2.1		2.3	2.3			
A/Uitida coefiuleAceni ...									1.1		1.3'	1.2			
Ctadanthui anahicuA										**1 1**	.	+			
Senecio {¡lavui												+			
MotoceAoi bi.coh.ne									+			1.2.			

Espèces accidentelles :

tuphonbia nicae&niii 1.2 (6) - *Salvia aeayptiaca* 1.2 *LzyAexa ¿eyAeAoidui* + (11) - Rumex *papilla* 1.2 (12) - *Teuckism coltinum* + (9) - *Bujngium tAiquetnum* •• (7) - *PaMe-ViLi ApinoAa* 1.2 (12).

TABLEAU N» 1

ainsi d'ailleurs que *Cupressu atlántica.* Bn fait, il apparait que la juxtaposition de ces espèces est loin d'être aléatoire, c'est ainsi que *Cupressus atlántica* est liée à la vallée moyenne du N'Fiss qui par sa direction est-ouest offre toutes les caractéristiques des vallées internes chaudes et peu arrosées (ACHHAL, 1979). Il s'agit là de situations tout à fait comparables à celle des Alpes (BRAUN-BLANQUET, 1961, BARBERO, 1979), ou encore des chames pontiques en Anatolie septentrionale (QUEZEL, BARBERO et AKMAN, 1960).

Il est de la sorte possible de distinguer au sein de l'association, diverses sous-unités répondant à des critères géographiques et écologiques précis:

— La sous-association *Ruscetosurn tripartitae* est à peu près strictement liée aux zones atlasiques périphériques, plus ou moins directement offertes aux influences atlantiques, alors que la sous-association *Cupressetosum atlanticae* est au contraire cantonnée en situation interne.

Ces deux sous-associations sont caractérisées chacunes par deux faciès altitudinaux: un faciès inférieur à *Juniperus phoenicea* dominant, représentant l'étage thermoméditerranéen, et un faciès supérieur à *Juniperus oxycedrus* correspondant à l'étage méso-méditerranéen.

Rappelons que nous avons retenu, pour caractériser l'association *Juniperus phoenicea* presque toujours dominant, et deux espèces de souche plutôt macaronésienne et qui par leur présence et leur abondance relative, permettent de différencier aisément cette association des autres groupements ou associations à *Juniperus phoenicea* présents dans des conditions écologiques voisines dans d'autres secteurs du Maghreb. Ce sont *Coronilla ramosissima* et *Bupleurum dumosum.*

— La sous-association *Ruscetosurn* est caractérisée de son côté par *Rhus tripartitum, Asparagus albus* et *Caparis spinosa,* la sous-association *Cupressetosum atlanticae* de son côté, présente comme différentielle exclusive le Cyprès de l'Atlas.

Dans le cortège floristique, apparait un lot important des représentants des unités supérieures et quelques indicatrices des *Quercetea ïlicis,* qui, ne l'oublions pas, sont lar-

gement présentes ailleurs dans cette même région au niveau des groupements forestiers et préforestiers qui ne sont pas étudiés ici. Il faut également signaler un important lot d'espèces compagnes où coexistent divers représentants des *Ononido-Rosmarinetea,* mais également des espèces d'affinité franchement steppique voire sub-saharienne.

Du point de vue écologique, les individus de l'association se localisent sur substrat éruptif, et sur des sols érodés ou très superficiels; du point de vue bioclimatique, c'est essentiellement au semi-aride qu'il convient de rapporter le groupement, quoique, localement dans la vallée du N'Fiss, puissent s'individualiser des îlots arides. Du point de vue des variantes thermiques, schématiquement il est possible de dire de façon schématique que les faciès thermoméditerranéens où *Juniperus phœnicea* existe seul associé souvent à des éléments thermophiles tels que *Launaea acanthoclada, Launaea arborescens, Cymbopogon shoenanthus, Aristida coerulescens, Senecio flavus, Notoceras bicorne* et *Cladanthus arabicus* en particulier, doivent se situer en ambiance thermique tempérée; les faciès méso-méditerranéens à *Juniperus oxycedrus* subsp. *rufescens répondent* plutôt à une ambiance fraîche.

Du point de vue dynamique, il est évident que sous les conditions climatiques actuelles, l'association décrite ici, sous ses divers aspects, représente un véritable climax; son évolution vers des groupements préforestiers des *Pistacio-Rhamnetàlia* et en particulier vers des formations à *Tetraclinis articulata* n'est envisageable qu'en fonction d'une augmentation appréciable des précipitations (semi-aride et supérieur) ou d'une évolution édaphique compensatrice qui **n'est a priori possible** qu'à la faveur de microclimats locaux.

II.3 — Association à *Retama dasycarpa* et *Juniperus phoenicea (Retamo dasycarpae-Juniperetum phœniceae* nov. asso) (tableau n° 2)

Il nous a paru logique d'attribuer le rang d'association à l'unité phytosociologique décrite ici, bien qu'il ait été également possible de la considérer comme une simple sous-

Numero des releves	1	2	3	4	5	6	7
Surface	100	100	100	100	100	100	100
Re c ouvr ement	50	50	40	50	40	40	35
Exposition	NE	W	SW	E	E	NE	W
Inclinaison	25	30	35	30	40	45	45
Substrat	Ma	Ma	Ma	Ma	Gr	Gr	Gr
Altitude x 10	170	200	220	194	130	140	145
Espèces caractéristiques :							
JuntpeAwi pkoenlcea .	3.3	1.2	2.2	**2.3**	1.3	1.1	+
Retama dai.ycah.pa	1.3	2.3	1.1	**2.3**	3.4	2.3	4.4
ChamaeAopi humilié	+		1.2		1.3		1.3
Stipa lagaicae	1.1	1.1	+		+		
Caractéristiques des unités supérieures :							
Ephedfia majofi var. *villa/uii*	1	**2.2**		2.2	+	2.3	1.2
Polygala balamae	2	**2.2**	**3.2**	2.2	1.2	1.2	1.2
Vi&tacia atlántica	1			1.2		2.2	1.2
Junipefi.uA oxycedhiu, subsp. *njx^ej¡ceya¡*			1.1	3.4	1.3	3.2	+
Que/LCUA fco tundido lia			1.1	1.2			
Ç.0K0YÚM.0L lamoòiMima		1.1		1.2			
Ephedfia {¡n.agilij subsp. *coiionii*				1.1			
Ffiaxiwu¡ dimofipha					1.3		
Espèces des *EfiJinacetalia*							
Ofimenii icafiioia	1.2	1.1	**2.2**	1.3	1.3	1.3	1.2
Stipa niteni		1.1	**+**	1.3	1.2	1.2	+
Polycnemon {,ontaneiii	1.1	1.3		+		+	
Salvia tafiaxacifiolia .				1.2	1.3	+	1.1
Cytt&ui balamae	1.1						
Espèces des *Ononido-RoimafLinetea*							
Vhagnalon iaxatile	1.1	1.2					
Globulania alypum	+	2.2		1.2			
Stipa tenaciíiima	2.3			1.3			
Thymuí ¿,atuiteioidoj,				2.3		1.1	
Lavandula mafioccana						1.2	1.1
Autres espèces :							
CafilÁna involu.cfi.ata		1.1					
Echinopi, ¿tfiigoAuj,	+	+					
Lavandula te.nwtie.cXa	+	1.2					
VoKonychia kapela							
Ruta angw&ti{,olÃa							
Thymus palLLdui							
StLpa paAvifjloHA							
Linafiia ventfiicoia				1			
Ley&efia leyieh.oi.det,				1			
Leucanthemum gayanum			2	1			
A/vtitLda co exales cem							
Mélica ciliata					1.1		+
Attemiiia heAba~alba							1.2

Espèces accidentelles :

Cenc.hfi.ui OÍ(¡IOHXU¡ 1.3 (4) ; *Launaea acanthociada* + ¡6) *Teucfiium baA.bafi.um* + (6)

TABLEAU N" 2

association du groupement précédent. En effet, sur le plan purement floristique cette solution est envisageable puisque *Retana dasycarpa, Stipa lagascae* et *Chamaerops humilis* peuvent être retenus comme caractéristiques au moins locales de l'association, mais ce sont les arguments écologiques et dynamiques qui nous ont paru déterminants.

En effet, le *Retano-Juniperetum,* inféodé à des substrats marneux ou éruptifs, se situe à l'étage méditerranéen-supérieur et en ambiance bioclimatique sub-humide froide. Ces particularités écologiques expliquent l'apparition à son niveau d'un lot déjà appréciable d'espèces des *Erinacetalia* et plus spécialement de l´*Ormenion scariose;* de même *Quercus rotundifolia* peut apparaître épisodiquement à côté de *juniperus phoenicea* et surtout de *Juniperus oxycedrus* qui est beaucoup plus abondant que dans l'association précédente.

Le *Retano-Juniperetum* est présent entre 1300 et 1600 m, çà et là sur le Haut Atlas de Marrakech.

Il convient de remarquer que les caractéristiques des unités supérieures sont relativement bien développées alors qu'à l'exception de *Chamaerops,* ne s'observe aucun représentant des *Quercetea ilicis.* Cette association permet d'ailleurs de poser le problème de la signification phytosociologique des formations à Genistées arbustives du Haut Atlas occidental et central, décrites par l'un de nous (QUEZEL, 1957) sous le vocable d'association à *Adenocarpus anagyrifolius* et *Genista florida* var. *maroccana* et intégrées aux *Erinacetalia* et plus spécialement à *YOrmenion scariosae.* En fait, si l'on considère le tableau réalisé pour la description de cette association, mais également les relevés que nous avons pu effectuer en 1978 et en 1979 dans le Haut Atlas de Marrakech, il paraît évident que ce groupement, dont il conviendra sans doute de remodeler les contours, ne possède aucun représentant des *Ephedro-Juniperetea,* et qu'il doit donc bien être considéré comme une formation de fruticée n'intégrant aux *Ononido-Rosmarinetea* et constituant un stade de végétation lié à la dégradation des formations forestières méditerranéennes supérieures, voire montagnardes méditerranéennes à *Quercus rotundifolia.* Il faut encore remarquer que *Retama dasycarpa* peut apparaître dans ce groupement,

mais qu'il y joue en fait un rôle très effacé, l'optimum écologique de cette espèce paraissant se situer au sein de l'association définie ici.

III - LES GROUPEMENTS DES *JUNIPEROQUERCETALIA*

S'ils peuvent épisodiquement apparaître à l'étage méso-méditerranéen c'est en fait essentiellement au méditerranéen supérieur et au montagnard méditerranéen qu'ils sont bien développés.

Sur le Haut Atlas, ils offrent une assez grande complexité tout au long de la chaîne, à la fois en fonction des critères bioclimatiques et édaphiques. Pour l'instant il est possible de leur rapporter les groupements ou associations suivantes classées d'après leurs exigences altitudinales :

— l'association à *Adenocarpus bacquei* et *Buxus baleárica,*
— l'association à *Buxus balearica* et *Quercus rotundifolia,*
— l'association à *Bupleurum spinosum* et *Juniperus phoenicea,*
— l'association à *Berberís hispánica* et *Fraxinus dimorpha,*
— l'association à *Juniperus thurifera* et *Quercus rotundifolia,*
— l'association à *Lonicera arbórea* et *Cedrus atlántica,*
— l'association à *Buxus sempervirens* et *Juniperus thurifera.*

HXl — Association à *Adenocarpus bacquei* et *Buxus baleárica* (*Adenocarpo bacquei-Buxetum balearicae* nov. ass.) (tableau n° 3)

Elle a été observée essentiellement dans la zone des gorges du Dadès, sur le versant méridional du Haut Atlas oriental ,entre 1700 et 1800 m environ. Elle colonise des substrats calcaires et ne se rencontre plus guère que dans des zones de forte pente et d'accès fort difficile à la fois

Numero des releves	1	2	3	4
Surface	100	100	100	100
Recouvrement	50	40	50	30
Exposition	W	SW	E	N
Inclinaison	40	45	35	40
Substrat	Ca	Ca	Ca	Ca
Altitude x 10	170	175	180	180
Caractéristiques de l'association et des unités supérieures				
Bux.ua balzaxica	1.3	3.3	2.1	2.3
Ephíd/ia majoÂ. var. *vilZasuil*	1.1	2.1	2.1	1.1
AdznocasipuA bacquzi	1.2		+	2.3
JunipeAui phoe.ni.cza		+		+
Vnaximu, dimotipka		+		
Rfiomnaò olzoidzi subsp. *atlântica*		+		
Espèces des steppes sud-atlasiques :				
CafithamuA &Kuti.coAu¿	2	3.3	3.2	+
TzucHÁJum malzncontanum	1	1.3	1.2	1.1
AAtzmi&ia atlántica ...	2,	2.3		1.2
AnoAAhinum ^miticoium .	1	2.2		1.2
Czntawiza incana	1	+		
Hzfvtia maAoccana	1			
Linanjjx vzntsitco&a				
V-üthu/uinthoi, ico paxíuí,		+		2.1
Launaza anboKuc&ni ...		1.2		1.2
Cnambz knoLikiÁ.		+		+
Echlum hoKfiidum				
Espèces des *Ononido-'RoimafU.nztza* :				
Stipa pan.vi£lofia	1'. 2	l.1	1.2	2.1
Catananchz coznuZza	H	1.2	+	1.1
Atvtzmi&ía. hzK.ba-al.ba	2, 3	1.2	1.2	
Gzniita tiCOKpiixí,		1.1	1.2	+
Launaza acanthaclada		1.-2	1.2	l.'l
Stipa tznactòiima	n	1.2	.	.
Van.orU.chia. atabica	n	+		
Batlota hiAiuta			1.1	1.1
Stipa lagaicae.			+	1.2
Phagnalon iaxatilz			+	+
Compagnes :				
Galium zphzdxoiÂzi,	1. 2	+	2.1	2.1
ChondJiilZa juncza	1, 1	1.2	1.2	1.1
tÁzLica cÁttata	1, 2	.	+	+
Euphorbia, galeota			+	+
Volycaxpon tzfriaphyllon			+	+
HonXcandia an.vznái&			+	1.1
CappanJj, ¿pinoòa			+	+

Accidentelles :

Onrnzwa, ¿caxioia + *[V,* ; *ThymuA iatWiziodzi* + (2) ; *Volynzmon {,-ontanz&ii.* + (2) ; *Oiizopiii mitiacza* + ¡3) ; *Sidznitii odiAolzuca* + (3) ; *AniitXda coeAuiz&cznil.l* (4)

TABLEAU N° 3

pour l'homme et pour les troupeaux; elle n'est sans doute plus représentée que par quelques populations résiduelles et a cédé presque partout la place à des groupements steppiques dominés par *Carthamus fruticosus, Hertia maroccana, Teucrium malencomanum,* etc.

Les 4 relevés dont nous disposons ne montrent qu'un lot très faible d'espèces significatives parmi lesquelles *Buxus baleárica* et *Adenocarpus bacquei* qui permettent de caractériser l'association. Les unités supérieures sont représentées par *Ephedra major* var. *vïllarsii* toujours abondant et par *Fraxinus dimorpha, Juniperus phoenicea* et *Rhamnus oleoides* subsp. *atlántica* restant beaucoup plus rares.

Ce groupement est certainement endémique du versant méridional du Haut Atlas oriental calcaire. Du point de vue bioclimatique il paraît se situer au contact des zones arides et semi-arides, et également à cheval sur les variantes fraîche et froide de ces dernières.

IIÍ.2 — Association à *Buxus baleárica* et *Quercus rotundifolia (Buxo bàlearicae-Quercetum rotundifoliae,* BARBERO, QUEZEL et RIVAS-MARTINEZ., 1980) (tableau n° 4)

Nous avions déjà étudié cette association au cours de l'été 1978, dans la région de Boulemane sur le versant méridional du Moyen Atlas, en insistant sur sa valeur très particulière et en l'intégrant dans la végétation présteppique. Nous l'avons retrouvée en abondance au cours de l'été 1979 dans tout le Haut Atlas oriental où elle occupe, sur substrats calcaires, marneux voire éruptifs superficiels, une situation tout à fait analogue, entre 1600 et 1800 m d'altitude.

Sur les 9 relevés du Haut Atlas qui sont en notre possession, il est possible de préciser sa structure phytosociologique, et en particulier son appartenance aux unités supérieures définies ici. Les caractéristiques d'association sont *Quercus rotundifolia, Buxus baleárica* et *Juniperus phoenicea.* Il est possible de distinguer 2 sous-associations:

— la sous-association *Quercetosum rotundifoliae* correspond au groupement type ;elle caractérise surtout l'horizon inférieur de l'étage méditerranéen supérieur;

— la sous-association *Fraxinetosum dimorphae* se situe plus en atltitude en ambiance plus humide et surtout à l'horizon supérieur de ce même étage; elle est caractérisée par la présence et l'abondance de *Fraxinus dimorpha.*

Le *Buxo balearicae-Quercetum rotundifoliae* offre un recouvrement important et généralement supérieur à 50%, sa physionomie est très particulière; en effet, le critère majeur est sans nul doute la présence de nappes plus ou moins confluentes de *Buxus baleárica* d'où *Ephedra major* est rarement absent; la strate arborée ne dépassant guère 5 à 6 m de hauteur est constituée par un couvert disjoint où dominent *Quercus rotundifolia, Juniperus phoenicea* et *Juniperus oxycedrus.*

Du point de vue écologique, le *Buxo baïearicae-Quercetum rotundifoliae* se situe essentiellement dans la variante froide du bioclimat semi-aride. Il caractérise électivement dans ce bioclimat et sur le Haut Atlas oriental, l'étage méditerranéen supérieur; en ambiance sub-humide et toujours à cet étage, il cède la place aux associations à *Quercus rotundifolia* s'intégrant au *Balansaeo Quercion* et aux *Quercetalia ilicis* (BaRBErO, QUEZEL et RIVAS-MARTINEZ, 1980).

III.3 — Association à *Bupleurum spinosum* et *Juniperus phoenicea (Bupleuro spinosae-Juniperetum phoeniceae noy.* asso) (tableau n° 5)

Nous avons observé cette association en divers points du Haut Atlas central et sur les crêtes du Sagho, où elle colonise des substrats éruptifs entre 1900 et 2100 m environ. Elle apparaît en général comme un piqueté très lâche de *Juniperus phoenicea* arborescents très dégradés par l'action humaine, et il est pour cette raison souvent bien difficile de réaliser des relevés significatifs.

Le cortège floristique est fort réduit; les caractéristiques sont précisément les deux espèces qui ont servi à nommer le groupement, et assez curieusement *Asparagus horridus* dont la valeur est ici toute locale. Les caractéristiques des

Numero des releves	1	2	3	4	5	6	7	8	9
Surface	100	100	100	100	100	100	100	100	100
Recouvrement	50	70	60	90	80	80	80	70	80
Exposition	E	N	E	N	E	NE	W	E	NE
Inclinaison	20	40	30	45	45	40	40	50	30
Substrat	Ca	Ca	Ca	Col.	Ca	Ca	Ma	Ca	Ca
Altitude	160	170	175	170	190	150	180	150	195
Espèces caractéristiques et différentielles									
QaeAcoó *H-otmndi^otLa*	1.3	3.4	4.3	1.3	2.3	4.5	2.2	4.3	3.2
BUXLU, baleaxlca	3.3	2.3	3.3	4.5	3.4	4.5	4.4	4.4	4.4
JunipeAcu pho enícea	3.3	+	1.1		1.3		2.1	+	
VK.axi.nvjb dimoKpka.'.					1.3	.1.2	2.1	2.2	2.3
Espèces caractéristiques des unités supérieures									
Ja.nA.peMU> oxycedAiu subsp. *fui^eAceni,*	**2.3**	1.3	1.1		2.3	1.3	1.2	+.1	1.1
Ephedra majosi var. *VWLOKÁíÃ*		1.3	1.2	3.4			1.2	+	2.3
Coton&cUsteA nummtxtcuvia.				+			+	+	
BeAbe/tXò *hi&panica*				+			1.2		
Vestiges sylvatiques									
Rabia peAegnlna						+			
Aipanagiu acwtL{¡otiuJ>						1.2			+
Ecita.mae.cL gtabeXAÃma							1.1		1.1
Espèces des *Ononido-Iloimatlnetea.*									
tíeJLLanthemum vÁAgatum	1.3	+	+				+		
fumaria. ¿,pachÃÁ	+	1.3	1.1		+				
GZobuZanÂXL nainti	3.3				**2.3**	**2**	**2** **1.1**		
CoKorulZa mínima			1.2	**.1**					
Teu&Uwn polÂum			1	**.2**	**1.2**	**1**	**1**		
Avena *biomo-ideó*		+							
kístfiagaliu, -incanvu,		1.2		+'					
ScuteZùvUa. demnatenòÁJ,		+							
InuJLa montana		1.1			+				
Catananche. coefwJLejx				+	+				
KoeZeMÁa. i,plendznı¡				+			+		
Thymen Aatiin.eA.odeA					**1.2**	**1**	**1** **.**	**1**	**1**
Autres espèces :									
VactylÁj, gtomeAota ...			1.1	1.1			+	1.1	1.1
BViomai *tacXofium*			+ .	1.1			+	+	
KnanjikLnum{¡tiiutícoAum		1.1	1.2	1.1					
CaucaJLa, bottom,				1.1			1.1	+	
kfitemütia atlántica ..				1.3	1.2				

Accidentelles :

Cytliui balan&ae, 1.3 (1) *Bixpleunum t>pinot>um* 1.2 (1) ; *Stlpa tenacÁA&ima* 1.1 (l) ; *Caxex halleJúana* 1.2 (2) *Otun&níA ¿ccUvLoòa* + (4).

TABLEAU N° 4

Numero des releves	1	2	**3**	4	5	6	**7**		9	10	11	12	13	14	**15**
Surface	100	100	100	100	100	100	100	100	100	**100**	**100**	100	100	100	100
Recouvrement	40	30	40	35	40	30	75	45	50	40	40	40	30	40	**40**
Exposition	S	S	N	NW	N	N	**S**	S	S	SE	**S**	N	S	**S**	**SE**
Inclinaison	40	35	40	45	30	45	30	40	40	40	30	10	40	40	**30**
Substrat	Gr	Gr	Ba	Ba	Ba	Ba	Ca	Ca	Ca	Ca	Col.	Gr	Gr	Ma	**Gr**
Altitude x 10	215	205	200	200	205	210	221	241	230	240	203	170	190	190	197
Espèces caractéristiques et différentielles des associations															
JunipeAui pkoo.YiLc.zcL	3.4	1.3	3.4	3.3	2.3	1.3	2.3	.	.	+	.	.	2.2	1.2	.
BupleuAum ip¿noium	2.3	+	1.2	2.3	2.3	+	.	.	.	.	.	.	.	.	2.2
AipaAagui honAidui	.	.	+	.	+	+	.	.	.	.	.	.	.	.	.
QuL.exc.Lif> fLotu.ncU.fiotia	.	.	.	.	.	.	3.4	3.2	4.3	2.2	4.5	3.4	2.2	3.1	.
JunipeAUi thuAifiCAa	.	.	.	.	.	.	2.3	+	1.1	3.2	+	.	1.2	+	2.1
JunipeAui oxycedAui	.	.	.	.	.	.	2.2	1.2	2.2	2.1	2.3	1.3	+	2.1	1.1
Onmenii ic.cuu.oia	.	.	.	.	.	.	1.2	2.3	3.2	2.2	.	2.2	1.1	.	2.2
Autres caractéristiques des unités supérieures															
EphedAa majon. var. *vittaAiii*					1.2					+	.	.	.	+	
VJaamnui, oto.oi.dej, subsp. *atlántica*					1.2										
CotoneaiteA nummutaAia															
T-Aaxinui (limoupha					1.3										
BeAbeAii hiipanica															
Cnataegui tacJ.yU.ata										**.3**					
Espèces des *tninacetalia* :															
Volycnemon {¡ontaneJ>ii										+					
ScutettoAia demnateniii									1.2	**2.2**					
Agh.opyh.on {,eAtucoidei															
AitAagatui ibhahimianui															
Ohmenii icahioia															
Cytiiui batamae									1.1						
Veituca maiAei									1.1						
Geniita {¡lohida															
Espèces des *Ononido-RoimaAinetea* :															
Catananche co eAulea											+				
Ahtemiiia atlántica			**2**	**2**		**3.2**					1.2				
Bu.ple.uAum ati.anXA.cum	.1														
Gentita icoh.pi.ui															
Alynum atpu>tAz															
lencAium potium	.1														
KoeZeAia iplendem	.1														
Helianthemum cxaceum															
Stipa t&naciiiima															
Avena bhx>moidej>															
AitAagatui incanui															
Euphorbia nicaeemii															
SatuAeÂa neAvoia															
TeucAium chamaedtyi															
Thymui iatuAeioideJ,	.3														
AAtemiiia heAba-atba	.3														
HippocAepii icabAa															
Hetianthemum viAgatum															
Thymui pallidum															
Autres espèces :															
ChondhUta juncea					1.2		+				+				
Vactytu, glomeAatà				1.1		+	1.1				**2.1**				
Scabioia poAieZi			1.2	1.2	**2.2**	**2.2**	**1.2**								
CaAtina involucAata .. .															
Galium ephedhoideA				1.1											
TeucAium matenconianum			1.2			1.2	**1.3**								
Achillea odohata				1.1											
Bhomui hubeni						1.1					+				
Mélica cupani											**1.1**				

Accidentelles

VàAonychia katepa. + (5) - *VeAonica toiea.* 1.1 (5) - *CeAaitium boiAiieAi.* 1,2 (7) - *ScoKzoneAa. pygmaejx* 1.3 (12) -*Thymui zygti* 1.2 (12) *Tumana ipachii* + (12) - *VtitotAichum ipinoium* 1.3 (13) - *Catananche caupitoia* 1.3 (14) '- *Galium mollugo* + (15).

TABLEAU N° 5

unités supérieures sont très mal représentées à l'exception toutefois d'*Ephedra major*.

Le tapis végétal sous-jascent est en fait constitué ici par un cortège plus ou moins dense d'espèces liées aux *Erinacetalia* et aux *Ononido-Rosmarinetea*. Diverses *Artemisia* et *Stipa tenacíssima*, soulignent également le rôle très appréciable joué par les éléments steppiques.

Du point de vue altitudinal, le *Bupleuro-Juniperetum* paraît se situer électivement à l'horizon inférieur de l'étage montagnard méditerranéen, du moins pour les individus d'association provenant du Haut Atlas. Sur le Sagho, l'absence de végétation arborescente aux étages inférieurs rend très délicate l'attribution de ces vestiges ¡forestiers à un étage altitudinal précis.

Du point de vue bioclimatique, ce groupement répond à du semiaride inférieur très froid.

La valeur dynamique de ce groupement est fort réduite ; en effet, les individus d'association que nous avons pu observer répondent à des structures résiduelles fortement agressées par l'homme et ses troupeaux où la régénération des Genévriers est nulle.

III.4— Association à *Berberís hispánica* et *Fraxinus dimorpha (Berberido hispanicae-Fraxinetum dimorphae* nov. ass.) (tableau n° 6)

Elle est très largement répandue essentiellement sur le Haut Atlas oriental, mais aussi en divers points du Haut Atlas central, où elle colonise électivement les sols et substrats érodés ou superficiels, voire certains éboulis fixés sur calcaire mais aussi sur roches eruptives. Elle apparaît entre 1700-1800 et 2000-2200m en moyenne.

Elle succède souvent en altitude à l'association précédente. Sa physionomie est fort différente puisque le Buis fait ici totalement défaut et que les constituants essentiels sont des nanophanérophytes caducifoliés et en particulier *Berberís hispánica, Fraxinus dimorpha, Crataegus laciniata, Ribes atlanticum* qui peuvent d'ailleurs servir pour caractériser l'association. Les éléments arborescents sont épars et

Quercus rotundifolia n'apparait que sur la frange altitudinale inférieure alors que *Juniperus thurifera* est quant à lui lié à l'horizon supérieur. Deux sous-associations peuvent être distinguées:

— la sous-association *Fraxinetosum dimorphae* qui ne dépasse que rarement 2000 m,
—Ja sous-association *Juniperetosum thuriferae,* plus alticole.

Bon nombre de représentants des *Ephedro-Juniperetea (alia)* apparaissent dans le cortège floristique alors que ceux des *Quercetalia iïicis* sont pratiquement absents. Il convient également de souligner la riche représentation en caractéristiques des *Erinacetàlia* (QUEZEL, 1956).

— La sous-association *Fraxinetosum dimorphae* est essentiellement caractérisée par le Frêne dimorphe; elle correspond à du montagnard méditerranéen inférieur, en ambiance bioclimatique sub-humide inférieure très froide, comme semble le confirmer son développement important dans la portion orientale du Haut Atlas oriental, à proximité des zones forestières à *Quercus rotunäifolia* et à C*edrus atlántica.* Il est possible que certains individus d'association puissent encore se rattacher à la frange altitudinale supérieure du Méditerranéen supérieur.

— La sous-association *Juniperetosum thuriferae* est caractérisée de son côté par le Thurifère, mais encore par *Prunus prostrata* et *Rosa sicula*..

Les caractéristiques des *Erinacetàlia* sont ici beaucoup plus abondantes, en raison des exigences altitudinales de cette sous-association qui occupe pour l'essentiel l'horizon supérieur de l'étage montagnard méditerranéen. Sa présence à l'étage oro-méditerranéen n'est pas évidente, puisque comme nous avons pu l'observer, si le Thurifère pénètre dans ce dernier étage, il n'est représenté que par des individus épars, et ne semble pas déterminer l'apparition d'un cortège floristique spécifique, sauf dans de rares cas particuliers et surtout en ambiance bioclimatique sub-humide supérieure. Du point de vue bioclimatique cette sous-association répond

Numéro des relevés	1	2	3	4	5	6	7		9	10	11	12	13	14	15	16	17
Surface	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Recouvrement-	75	90	90	90	90	90	70	80	80	80	70	60	70	90	80	90	80
Exposition	E	E	NW	E	NE	E	N	NE	S	E	S	SE	NE	E	E	N	N
Inclinaison	45	30	15	20	20	40	30	35	40	50	30	25	30	40	25	30	30
Substrat	Ga	Ca	Ca	Ca	Ma	Ma	Ma	Ma	Ga	Ga	Ca	Ca	Ca	Ma	Ga	Ga	Ga
Altitude x 10	200	190	185	140	200	200	200	210	210	200	210	220	220	220	215	210	215

Espèces caractéristiques et différentielles des sous-associations :

BzAbiAii hiipanica	3.3	1.2	1.3	1.2	3,3	4.4	**4.3**	2.2	3.2	**4.4**	1.2		1.2	**4.4**	**4.4**	**4.3**	**2.2**
CfLCLtazgiM laciniata	1.2	2.3	1.3			3.5	**2.3**		1.1		1.2			**3.4**		**2.3**	
VJJoci uva-Ciiipa subsp. *atlántica*	+	.	1.3			1.3		1.3									**1.2**
ïAaxinui dimoApka	2.3	3.4	4.5	5.5	3.2	1.2	+	1.1									
JunipzAui -thuAi&eAa	.	.	.	+	+	2.2	1.3	2.2	2.2	3.4		**2.3**	2.3	2.2	2.2	3.3	
Ro&a iicula	.	.	.	.	1.2			1.2	1.2	1.2			1.2	1.2	1.2		
VAunui pAoitAata	.	.	1.1				1.2		1.3						1.2	1.1	1.2

Espèces caractéristiques des unités supérieures :

EphzdAa majore var. *vilùvtiii*	+							**2.2**		1.2		2.2		1.2	1.2		**2.2**
Junipzfiui oxycidAui subsp. *Au{¡zicznì*	+		1.3	1.2								1.1					
Rkamnui otzoidzi subsp. *atlántica*												2.2			1.2		
OueAcui fio tundido lia	4.4	**1.2**															
CotoncaitiA nummulanÂa	1.1																

Espèces des *EAinacztaZia* :

AAt&miiia atlántica					1,1	1.3	1.1	**2.2**				.1	2	2	1.3	1	2	1.	2.2
Cytiiui balavuae.					1.1	+		**2.2**			**2.3**					1	2		1.1
OAm&nii icoAioia											**2.2**	.1	1	2	1.2				
A&tAagatui ibuahànianui								**2.2**			**2.2**		1	1					**2.2**
VíAonica Aoiea					1.1										2.2			1.	
kAznoAia pu.nge.ni											1.2					1	3		
ScutitloAia aemnatzniiA						**2.2**	1.1								+				
CeAaitium boiiiieAi					1.1														
VtilotAichum ipinoium ..											2.2								**2.2**
Ezituca maiAíi						1.2					1.1								
Kvzna $ili{olia						1.2					1.2								
Bupl&ufium atlanticum ...																			

Espèces des *Ononino-HoimaAinet&a*

EuphûAbia nicae.&niii					1.1	1.1			1.2	1.2				1.2		1.1	
Gzniita icoApim														1.2	1	1	2.3
Teuciium ckamacdAyi, .						1.1											
Thi/mui pattidui																	
AitAagalui incanui ..																	

Autres espèces :

BAomcii tzctoAum	1.3	2.3			2.3												
Vactytù, glomeAata ..									2.1								
GaLLum apaAimtla. ...		1.2	2.3		2.1												
VoteAiam magnoti			2.3														
CaucaLU bi^Aoni		**2.2**	1.3														
SiX-ini infclata										**2**							
LinaAia h&teAophijlZa																	
Scabioia poAíclÁ.										**2**	**2**						

Accidentelles :

Balamaea gtabeAAima 1.1 (2] - *Catamancne. catipitoia* + (6) - *Hctianthomum viAgatum* *COAZX hatlzAiana* + (2) - *VtULoitomon dynii* + (3] *Catananckc coeMilza* + (12) - *CoAcnilZa minima* + (8) - *ChondAilla juncia* 1.1 Í16).

TABLEAU N" 6

à du sub-humide inférieur, voire localement à du semi-aride supérieur, très froid.

Du point de vue altitudinal, le *Berberido-Fraxinetum* se situe essentiellement à l'étage montagnard méditerranéen, quoique certains individus de la sous-association *Fraxinetosum* puissent peut-être encore se rapporter au méditerranéen supérieur.

Du point de vue bioclimatique c'est surtout au sub-humide très froid qu'il convient de rattacher l'association.

La signification dynamique du *Berberido-Fraxinetum* sur le Haut Atlas est intéressante à évoquer. En effet il s'agit là d'une association de type fruticée caducifoliée, de recouvrement important lorsqu'elle est en bon état, qui parait répondre, soit à un stade de dégradation, notamment en clairière ou en lisière, des groupements forestiers à *Quercus rotundifolia* ou localement à *Cedrus atlántica*, soit le plus souvent à un stade d'évolution progressive de la végétation, avec fixation du substrat et constitution d'un sol, dans les zones où l'installation des essences forestières *Juniperus* et *Quercus rotundifolia* surtout est théoriquement possible. En fait, le *Berberido-Fraxinetum* représente le stade de groupement arbustif intermédiaire entre les pelouses écorchées ou les garrigues à xérophytes épineux d'une part et les groupements forestiers d'autre part. Son rôle ne saurait être oublié en particulier dans les tentatives de reforestation sur des sols particulièrement dégradés où à notre avis l'installation artificielle temporaire de ce type de fruticée, permettrait certainement d'améliorer très largement les possibilités de reboisement et aussi leur succès de reprise.

III.5 — Association à *Ormenis scarriosa* et *Quercus rotundifolia* (*Ormeno scariosae-Quercetum rotundifoliae* nov. ass.) (tableau n° 5)

Cette association est très répandue dans toute la portion occidentale du Haut Atlas oriental notamment dans les hautes vallées de l'Ahansal et de ses affluents, des Aït Bouguemez et des Aït Mehammed. Elle s'observe essentiellement sur

des calcaires compacts erodes et fissurés qui apparaissent largement entre les tâches de végétation.

L'aspect du groupement est très caractéristique, puisqu'il est constitué par des peuplements souvent relativement denses (40 à 60%) formés par *Quercus rotundifolia, Juniperus thurifera* et *Juniperus oxycedrus; Juniperus phoenicea* peut même apparaître très localement mais ne joue toutefois qu'un rôle discret. Ces trois premiers arbres ont été retenus pour caractériser l'association, à côté de *Ormenis scariosa* pour des raisons évoquées ci-dessous.

Les caractéristiques des unités supérieures sont très éparses et nous n'avons observé dans nos relevés aucune espèce sylvatique. La végétation qui s'installe sous ces peuplements arborés est constituée essentiellement par des représentants des *Erinacetalia* et des *Ononido-Rosmarinetea.* Remarquons que c'est dans cette région qu'a été défini par Fun de nous l'alliance *Ormenion scariosae* (QUEZEL, 1956) qui appartient à ces unités supérieures et qui avait été considérée comme le plus souvent consécutive à la disparition ou à la dégradation de formation à Chêne vert. Cette opinion est largement confortée par les relevés publiés ici et effectués dans des zones où la couverture arborescente a été conservée. C'est en fait pour souligner ces caractères et insister sur les particularités dynamiques de ces peuplements que nous avons fait figurer *Ormenis scariosa* parmi les caractéristiques de l'association.

l´Ormeno-Quercetum rotundifoliae localisé essentiellement entre 1900 et 2450 m représente un groupement typiquement lié à l'étage montagnard-méditerranéen, comme le souligne la structure des unités de dégradation. Du point de vue bioclimatique, il est situé en ambiance semi-aride très froide avec peut-être localement quelques individus dans le sub-humide inférieur très froid.

Si l´*Ormeno-Quercetum* constitue encore en divers points du Haut Atlas oriental des peuplements appréciables, il convient cependant de souligner que son utilisation abusive par l'homme et les troupeaux tend à en réduire rapidement l'extension et en empêcher à peu près totalement la régénération. Lié à des conditions écologiques extrêmement

sévères, il représente le climax théorique d'une vaste portion du Haut Atlas calcaire (semi-aride très froid).

III.6—Association à *Lonicera arbórea* et C*edrus atlántica (Lonicero arboreae-Cedretum atlanticae)* (BARBERO, QUEZEL et RIVAS-MARTINEZ, 1980)

Cette association définie dans un récent travail n'avait toutefois pas reçu une interprétation phytosociologique satisfaisante. Rangée provisoirement dans les *Quercetea ïlicis*, ces Cédraies de haute altitude doivent en fait être rapportées aux *Querco-Junipsretalia* comme le tableau synthétique ci-dessous le confirme.

Nous ne définirons pas ici à nouveau les caractères écologiques de ces cédraies claires de hautes altitudes (2100-2500 m) présentes à la fois sur le Moyen Atlas et le Haut Atlas orientaux, sur substrat calcaire, à l'horizon inférieur de l'étage oro-méditerranéen et en ambiance subhumide extrêmement froide.

Espèces caractéristiques.,1

Lonicera arbórea	III
Cedrus atlántica	V

Espèces des Ephedro-Juniperetea :

Berberís hispánica	V	*Juniperus thurifera* var. *africana*	I
Juniperus oxycedrus subsp. *rufescens*	IV	*Prunus prostrata*	I
Quercus rotundifolia	IV	*Rosa turetii*	I
Crataegus laciniata	II	*Ribes atlântica*	I
		Buxus balearíaca	*I*

Vestiges sylvatiques:

Crataegus monogyna	I	*Rubia laevis*	I
Calamintha baborensis	I	*Agropyron panormitanum*	I

Espèces des Erinacetalia *et des* Ononido-Rosmarinetea:

Bupleurum spinosum	V	*Ínula montana*	III
Koéleria valïesiaca	V	*Festuca ovina*	II
Cerastium gibraltaricum	V	*Stipa pennata*	II
Avena filifolia	**iv**	*Jurinea humilis*	II
Ptilotrichum spinosum	IV	*Erinacea anthylis*	II
Eryngium bourgati	iii	*Ononis thomsonii*	II
		Artemisia flahaultiana	II

Autres espèces:

Helianthemum rubellum	*IV*	*Rumex tuberosus*	II
Anthémis tuberculata	II	*Calamintha granatensis*	II
Armería allioides	II	*Dactylis glomerata*	II
Cynosurus elegans	II	*Galium lucidum*	II
		Arabis caucásica	*TL*

III.7—Association à *Buxus sempervirens* et *Juniperus thurifera (Buxo sempervirenris-Juniperetum thuriferae,* QUEZEL, 1957)

C'est à l'ensemble d'unités phytosociologiques définies ici qu'il convient de rattacher encore cette association résiduelle et très spéciale décrite par l'un de nous des hautes chaînes du Haut Atlas calcaire (Aïoui, M'Goun), où elle apparait sur les vallées septentrionales entre 2500 et 2700 m. L'interprétation phytosociologique définitive de cette association devient dès lors la suivante:

Espèces caractéristiques de l'association:

Buxus sempervirens	V	*Lonicera arbórea*	V
Juniperus thurifera	V		

Espèces caractéristiques des unités supérieures:

Ribes atlántica	III	*Berberís hispánica*	
Gotoneaster nummularia	I		

Espèces des Erinacetalia *et des* Ononido-Rosmarinetea:

Erinacea pungens	V	*Helianthemum croceum*	II
Thymus pallidus	V	*Jurinea humilis*	**II**
Erysimum bocconei	IV	*ínula montana*	U
Cytisus balansae	IV	*Teucrium polium*	**II**
Ptilotrichum spïnosum	III	*Teucrium chamaedrys*	**II**
Festuca mairei	II	*Festuca hystrix*	II

Autres espèces:

Haynaldia hordeacea	III	*Triserataria flavescens*	II
Pimpinella tragium	III	*Centaurea incana*	II
Arenaria grandifolia	II	*Trifolium humile*	II

Par sa localisation altitudinale, cette association doit se rattacher à l'étage oro-méditerranéen et sans doute du point de vue bioclimatique au sub-humide supérieur, voire à la base de l'humide extrêmement froid, en raison de ses exigences stationnelles (fonds de gorge, pieds de falaises).

L'ensemble des structures phytosociologiques décrites ci-dessus, correspondent au Maroc et en particulier sur les marges du Haut Atlas où nous avons eu l'occasion d'étudier, des types de végétation dont la signification écologique et dynamique avait été totalement sous-estimée ou méconnue. Signalons toutefois que quelques autres avaient déjà prés-senti leur existence et en particulier EMBERGER (1939), PUJOS (1966) et PEYRE (1979).

Il est à peu prêt certain qu'une exploration progressive du Maghreb ,amènera à les rencontrer sur l'ensemble des 3 pays de l'Afrique du Nord, dans des conditions écologiques sensiblement analogues.

En Méditerranée orientale par contre, les formations présteppiques où les Genévriers arborescents également un rôle appréciable, paraissent devoir encore se rattacher aux *Pistacio-Rhammetalia* dont elles constituent, au moins par-tiellement, une alliance particulière, le *Querco calliprini-Juniperion èxcelsae* (BARBERO et QUEZEL, 1979), qui se localise essentiellement sur le Piémont des grands massifs montagneux (Taurus, Liban en particulier) et toujours en ambiance bioclimatique semi-aride.

Localités où les relevés ant été effectués:

Tableau n° 1 — l à 8: Vallée du N'Fiss, près de la route de Marrakech au Tizi n'Test à partir d'Ijoukak; 9 à 11 et 12: Vallée du N'Fiss, en aval d'Ijoukak; 10: Vallée de la Rerarya, partie inférieure des gorges de Moulay Ibrahim; 13: Vallée de la Reraya, partie supérieure des gorges de Moulay Ibrahim; 14 et 15: Vallée du Zate.

Tableau n° 2 — 1 à 4: Versant N. du Tizi n'Test; 5 à 7: Route de Marrakech au Tizi n'Tichka près de Teddert.

Tableau n° 3 — 1 à 4: Gorges du Daddes.

Tableau n° 4 — 1 et 4: Route d'Agoudim à Tounfite, à 10 et 12 km au N d'Agoudim; 2 et 3: Zaouïa Ahansal, partie inférieure de la piste des Aït Mehammed; 5 et 9: Route d'Agoudim à Tounfite, gorges du Masker; 6 et 8: Route de la Zauïa Ahansal à l'Akka n'Igli; 7: Versant S du Masker entre Anemzi et Agoudim.

Tableau n° 5 — 1 et 2: Versant N du Tizi n'Tichka; 3 à 6: Massif du Siroua région d'Oui n'Onssir; 7 à 10: Piste de la Zaouia Ahausal au Tizi n'Ilissi; 11 et 14: Piste d'Agoudim à Mitkane près de la bifurcation sur Tounfite; 12: Vallée de l'Ahansal au-dessus de Tamga; 13 et 15: Versant septentrional du Tizi n'Tichka.

Tableau n° 6 — 1 et 5: 2 km au S de la maison forestière de Tirrhist; 2 et 4: Entre Tirrhist et Anefgou; 3: Gorges du Masker près d'Agoudim; 6 à 8: 5 et 7 km au SW de Tirrhist; 9-10 et 13-17: Piste du plateau des lacs à Tirrhist; 11 et 12: Tizi n´llissi.

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CARYOLOGICAL STUDIES ON BULGARIAN POACEAE

by

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IN this paper the results of a caryological studies of * eleven grass species are presented. These species belong to monotypic genera in Bulgarian flora, and they are as follows: diploids — 2n=14: *Apera spica-venti, Aira capïlaris, Haynaldia villosa;* 2n = 26: *Lepturus cylindricus ;* tetraploids — 2n = 28: *Phalaris bulbosa, Polypogon monspeliensis, Ammophila arenaria, Trisetum flavescens;* 2n = 36: *Danthonixt calycina;* 2n = 40: *Andropogon ischaemum* and *Chrysopogon gryllus.* The chromosomme number of nine of them is reported for the first time on Bulgarian populations.

MATERIALS AND BIETHODS

The specimens studied originate from natural habitats in different parts of the country. Some of them were dug out with soil and reared in pots in greenhouse, some were coillected in seeds and germinated in Petri dishes at room temperature. The method is the same as our previous studies (KOZUHABOV *& at,* 1974).

Voucher specimens are deposited in the Herbarium of Institute of Botany, of the Bulgarian Academy of Sciences (SOM).

RESULTS

1. Andropogon ischaemum L.

2n = 40 (Fig. 1)

(Sofia region, grassy habitats on calcareous ground, near vill. Malo Malovo, distr. Sofia, 18.VII.1975, SK 25557; Struma valley, grassy habitats on silicious ground, near vill. Kresna, distr. Blagoevgrad, 20.IX.1975, SK 25730).

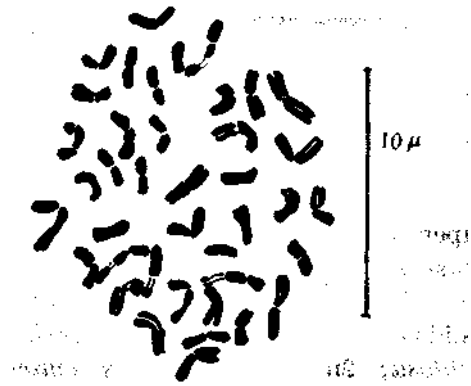

Fig. 1. — *Andropogon ischaemum 2a* = 40.

The species is distributed on dry grassy habitats all over the country (VÂLEV in D. JORDANOV edit., 1963).

Our results follow those of Gould (1956), CELARIER (1957), CHRISTOv & MOSKOVA (1972) and other authors (see FEDOROV edit., 1969). GOULD (1. c.) reported also n = 25 for Texas populations.

The chromomsomes are small being not very distinct morphologycally — mainly of meta- and submetacentric type ad two pairs of SAT.

2. Chrysopogon gryllus (L.) Trin.

2n = 40 (Fig. 2)

(Struma valley, dry grassy and rocky places on the hill «Pcelina» near the railway station General Todorov, distr. Blagoevgrad, 28.VI.1975, SK 25469).

The species is distributed all over the country, mainly in the plains and foothills up to 1300 m a. s. 1. (VÂLEV in D. JORDANOv edit., 1. a).

The chromosome number agrees with the record of AVDULOv (1931) and CELARIER (1959).

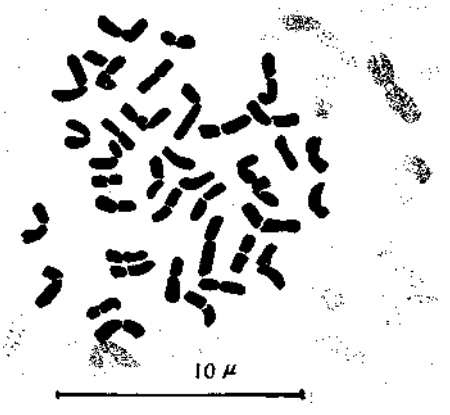

Fig. 2. — *Chrysopogon gryllus* 2n = 40.

The earyotype consists of small chromosomes and as the previous species with not distinct morphology — from meta- and submetacentric type.

3 Phalaris tuberosa L.

2n = 28 (Fig. 3)

(Black sea coast, the salterns near Burgas, 20.VII. 1965, SK 15186; Tundza hilly region, meadows near vill. Meden Kladenec, distr. Jambol, 14.VIIL1975. Sw 25811).

The species is known up to now only from the Southern coast of Black sea (HINKOVA in D. JORDANOV edit., 1. c), sub *P. bulbosa* L.

The chromosome number coincides with this one counted by HANSEN & HILL (1953), AMBASTHA (1956) and others (see FEDOROV edit., 1. a).

The caryotype consists of a pair of long and a pair of shorter metacentric chromosomes, a pair of long, seven

pairs of middle sized and two pairs of short submetacentric chromosomes, a pair of long acrocentric and a pair of middle sized SAT chromosomes of submetacentric type with large satellites. No differences of the caryotype between both investigated populations were found.

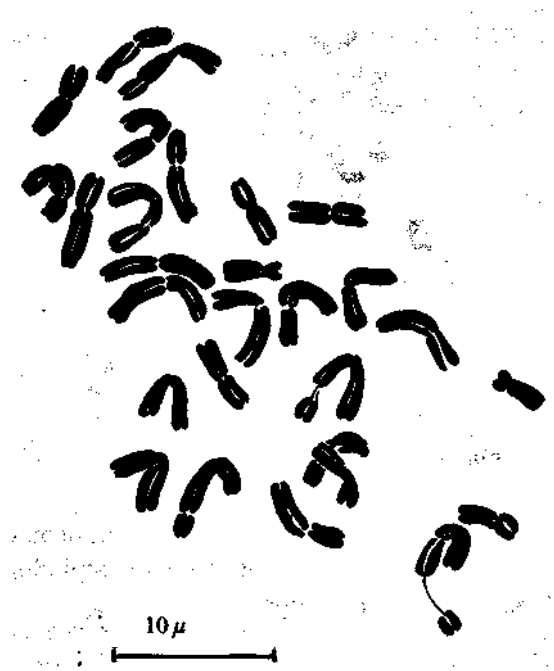

Fig. 3.—*Phalaris tuberosa* 2n = 28.

4. Polypogon monspeliensis (L.) Desf.

2n = 28 (Fig. 4)

(Eastern Rhodope mts., grassy places near vill. Mandrica, distr. Kârdzali, on calcareous ground, 24.VII.1975, SK 25647; Struma valley, eastern slopes of the hill «Kozuha», 30.VII.1977, SK 27294; Black sea coast, grassy habitats, northern of the experimental agricultural station near town Pomorie, 14.VIII.1977, SK 27325).

The species is distributed in the warmer district of this country (PENEV in D. JOKDANOV edit, l. c).

Our results coincide with these of AVDULOV (1931), HEISER & WHITAKER (1948), BOWDEN & SENN (1960), Gould (1968, 1970), GUPTA (1969), FERNANDES & QUEIRÓS (1969), MALIK & TRIPATHI (1970).

The caryotype consists of four pairs of metacentric, eight pairs of submetacentric chromosomes and two pairs of SAT of submetacentric type. No differences in the morphology of the caryotype between the specimens of the three

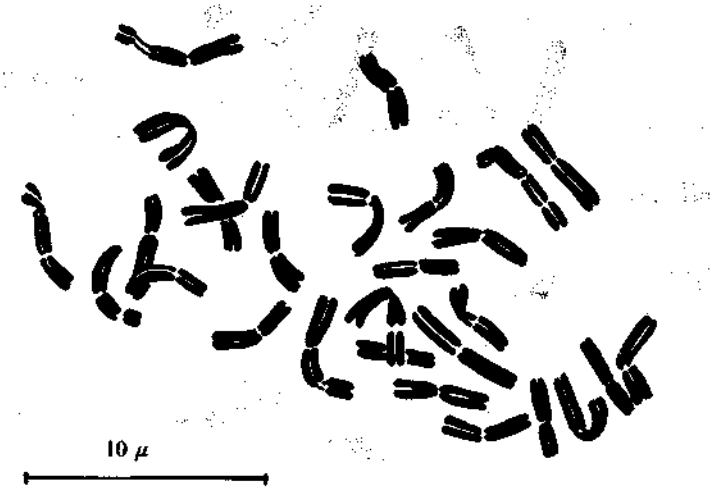

Fig. 4. — *Polypogon monspeliensis* 2n = 28.

investigated populations were found, but our caryotype seems to differ from this one studied by FERNANDES & QUEIRÓS (1. c.) through two pairs of metacentric chromosomes less, and a pair of submetacentric and SAT chromosomes more.

5. Ammophila arenaria (L.) Link

2n = 28 (Fig. 5)

(Black sea coast, on sandy hills along the Ropotamo river, 27.VI.1972, SK 221220; Black sea coast, «Republican beach» near town Sozopol, 4.VIII.1973. SK 23613).

The species is restricted on the coastal sands of the Black sea (GEORGEEV in D. JORDANO v edit., 1. a).

Our results agree with those of TISCHLER (1937), ROHWEDER (1938), LOVE (1954), HEDBERG & HEDBERG (1964),

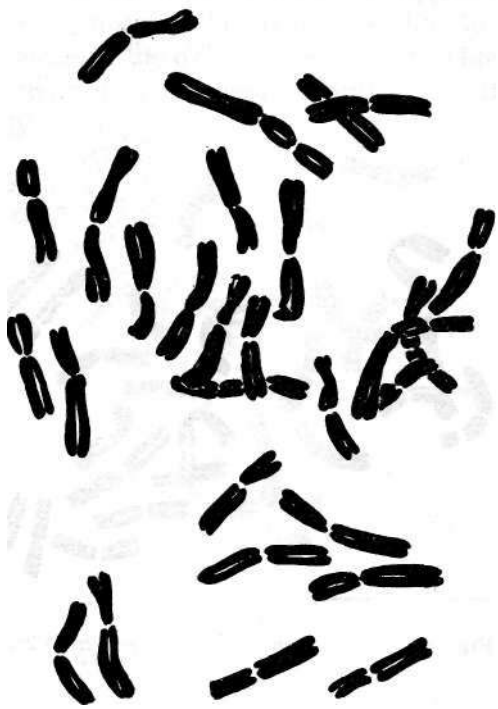

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Fig. 5. — *Ammophila arenaria* **2n = 28.**

KuBiEN (1964, 1965, 1968, 1970), FERNANDES. & QUEIRÓS (1969). SKALINSKA *& al.* (1957) recorded hexaploid chromosome number (2n = 56) for the specimens from Poland.

The caryotype consists of a pair of long and three pairs of shorter metacentric chromosomes. The rest are of submetacentric type ,three pairs of them long, the others shorter and a pair of submetacentric with secondary constriction. No differences in the caryotype between the two

investigated populations were found, as well as between them both from one side and this one studied by FERNANDES & QUEIRÓS (1. c). Most characteristic for the caryotype of the species are the pair of submetacentric chromosomes with secondary constrictions. These chromosomes are supposed by the same authors to be two pairs, but in our caryotype they are one pair as well. The lack of any differences between the Portugal population and the Bulgarian one means a good stability of the caryotype of this species.

6. Apera spica-venti (L.) P. B.

2n = 14 (Fig. 6)

(Black sea coast, on the sands near vill. Acheloi, distr. Burgas, 23.VII.1973, SK 23786).

Fig. 6. — *Apera spica-venti* 2n = 14.

The species is distributed on humid places, meadows, fields and sands all over the country (GEORGIEV in D. JoRDANOV edit, 1. c).

Our result coincides with those of AVDULOV (1931), TiscHlER (1934, 1937), DELAY (1947), GADELA & KLIPHUIS (1971).

The caryotype consists of almost equal in size chromosomes except two longer pairs of metacentric. The others are a pair of metacentric, two pairs of submetacentric and

two pairs of SAT chrmosomes of submetacentric type as **well.**

7, Aira capilaris Host

2n = 14 (Fig. 7)

(Struma valley, dry grassy and rocky places on the hill «Pcelina» near the railway station General Todorov, distr. Blagoevgard, 28.VI.1975, SK 25473; Strandza mt., grassy places on silicious ground, near vill. Slivarovo, distr. Burgas, 7.VIIL1973, SK 23740).

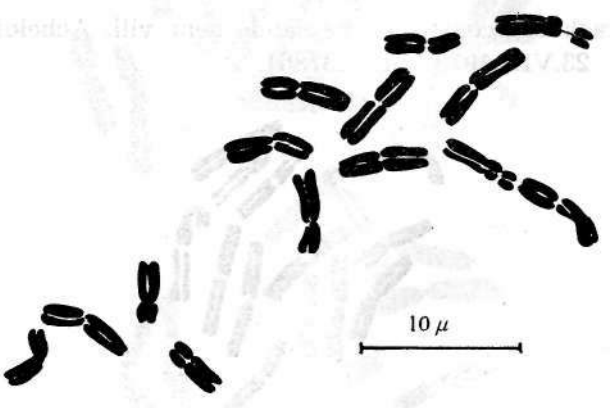

Fig. 7. — *Aira capillaris* 2n = 14

The species is distributed all over the country, on dry and grassy habitats and meadows (GEORGIEV in D. JORDANOv edit., 1. c).

The chromosome number coincides with this one of GOULD (1958, sub *A. elegans* WiIId. ex Gaudin).

The caryotype consists of a pair of metacentric, five pairs of submetacentric and a pair of SAT of submetacentric type chromosomes. No differences in morphology of the caryotype between two investigated populations were found.

8 Trisetum flavescens (L.) P. B.

2n = 28 (Fig. 8)

(Strandza mt., grassy habitats on silicious rocks, near vill. Slivarovo, distr. Burgas, 7.VIII.1973, SK 23744).

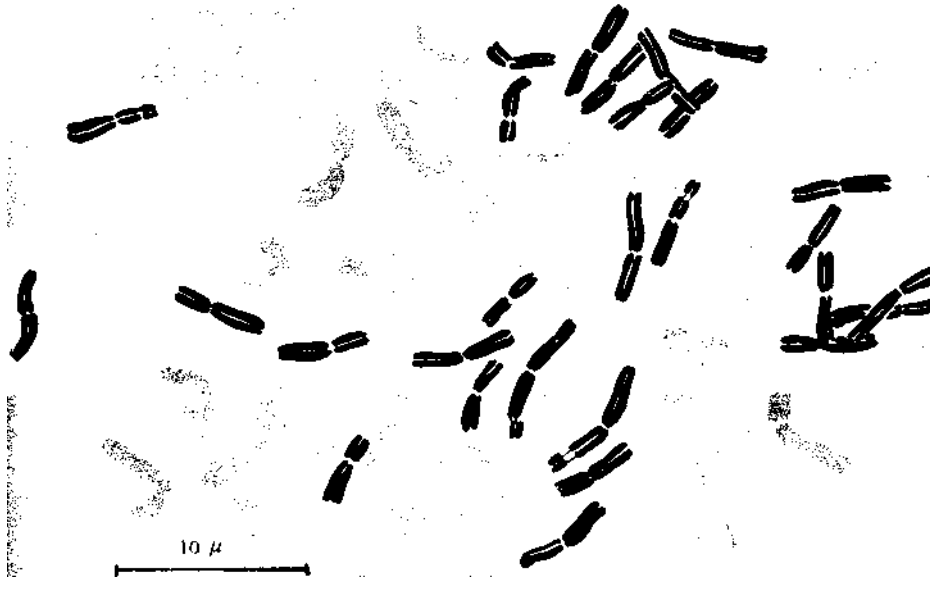

Fig. 8. — *Trisetum flavescens* 2n = 28.

The species is distributed on meadows and shrubs in South Bulgaria (KOZUHAROV in D. JORDANOV edit., l. c). Our result agrees with this of BOWDEN (1960).

The caryotype consists of five pairs of metacentric chromosomes, of which a pair of long, two pairs of middle size and two pairs of short, seven pairs of submetacentric chromosomes — three pairs of long, two pairs of middle size and two pairs of short, and two pairs of submetacentric type SAT chromosomes — a pairs of them short with short little arm and the other pair long with longer short arm.

9. Danthonia calycina (Vill.) Reichenb.

2n = 36 (Fig. 9)

(Sofia region ,grassy habitats on silicious ground, loe. «Pobit kamâk», distr. Sofia, 17.VI.1975, SK 25437).

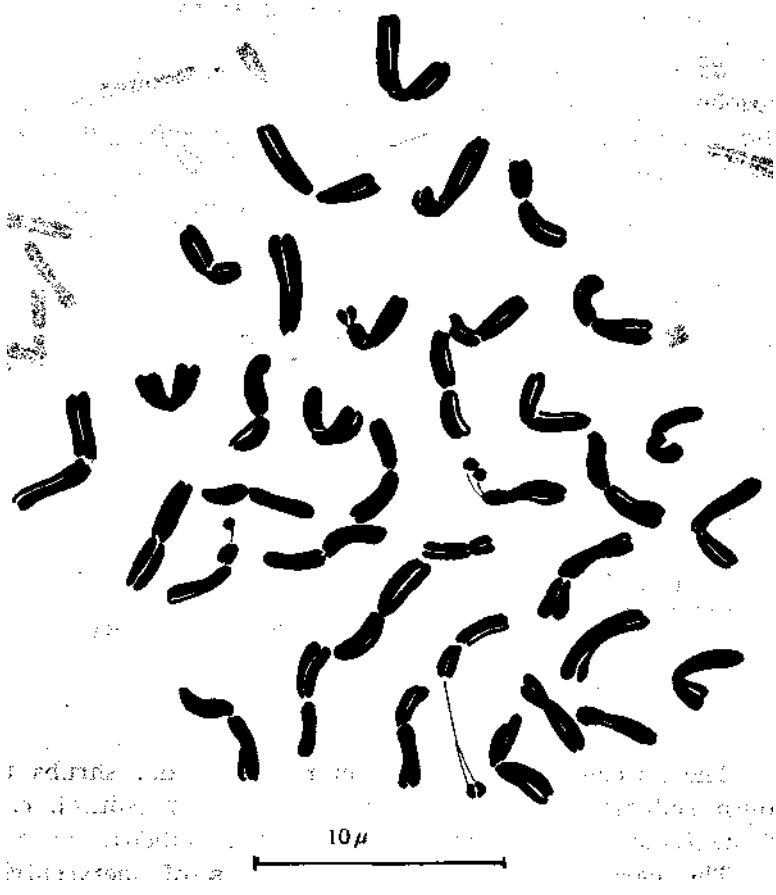

Fig. 9. — *Danthonia calycina* 2n = 36.

The species is sparsely distributed all over the country, except in the northeastern part, till 1500 m a. s. 1. (KOZUHAROv in D. JORDANOv edit., 1. a).

The chromosome number agrees with this one of PACKER (1936 after LOVE & LÖVE, 1961), sub *D. provincialis.*

The caryotype consists of seven pairs of metacentric chromosomes, nine pairs of submetacentric, a pair of them the shortest in the caryotype, and two pairs of SAT chromosomes of submetacentric type.

10. Lepturus cylindricus (Willd.) Trin.

2n - 26 (Fig. 10)

(Black sea coast, grassy and sandy habitats near town Achtopol, distr. Burgas, 5.VIII.1973, SK 23641).

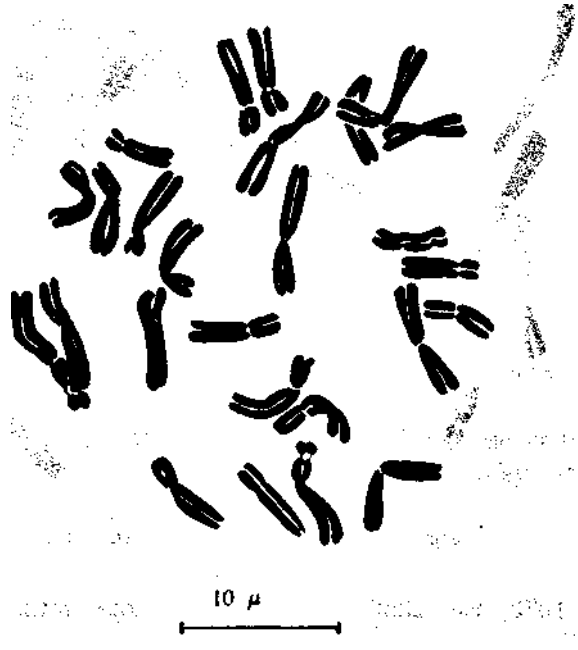

Fig. 10. — *Lepturus cylindricus* 2n = 26.

The species is distributed in Southeastern part of the country (KOCEV in D. JORDANOv edit., 1. c).

Our result coincides with this one of SAURA (1948). HUNTER (1934, see FEDOROV edit., 1. c.) recorded 2n = 52 for this species.

The caryotype consists of a pair of long metacentric chromosomes, two pairs of long, six pairs of middle sized

and three pairs of short submetacentric chromosomes, and a pair of long SAT chromosomes of submetacentric type.

11. Haynaldia villosa (L.) Schur

2n = 14 (Fig. 11, 12)

(Central Stara planina mt., near hut «Hubavec», above town Karlovo, distr. Plovdiv, 31.VII.1969, SK 191716; Znepole region, rocky places on the hill «Cepan», near Dragoman,

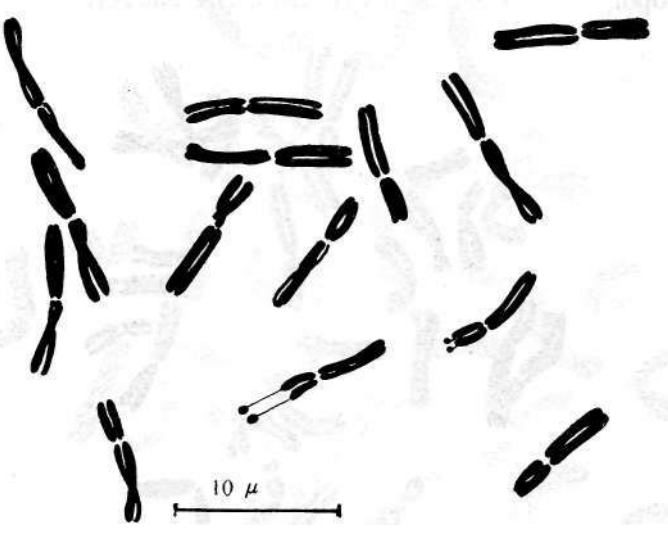

Fig. 11. — *Haynáldia villosa 2n* = 14.

23.VII.1973, SK 23508; Eastern Rhodope mts., eastern of Momcilgrad, 24.VII.1975, SK 25623).

The species is distributed on dry, grassy places all over the country, up to 1000 m a. s. 1. (KITANOV in D. JORDANOV edit., 1. c).

Our results agree with those of AVDÜDOV (1931), SEAES (1948), KOZUHAROV & KUZMANOV (1965) ant others (see FEDOROV edit, 1. c).

The specimens investigated from Stara planina mt. and Eastern Rhodope mts. did not show any defferences in the earyotype. The chromosomes are: a pair of metacentric,

five pairs of submetacentric and a pair of SAT of submetacentric type chromosomes (Fig. 11). The earyotype of the specimen from «Cepan» (Fig. 12) consists only of submetacentric chromosomes, a pair of them SAT. The

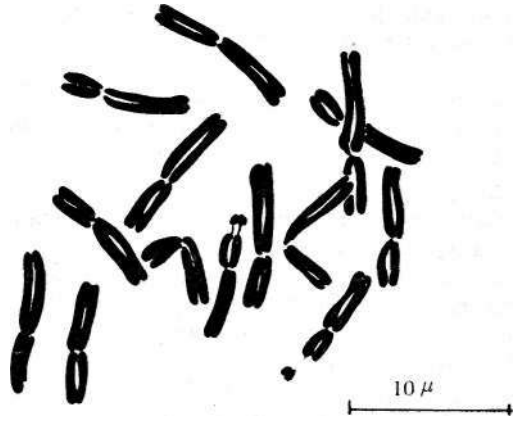

Fig. 12. — *Haynaldia villiosa* 2n = 14.

chromosomes of the species are long with very clear morphology.

KOZUHAROV & KUZMANOV (1. c.) did not describe the caryotype of the specimen from Struma valley studied by them.

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THE CARYOTYPE OF A RELIC GRASS SPECIES AND SOME NOTES ON ITS RELATIONS

by

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THE species of *Brachypodium sanctum* (Jarika) Janka has always been considered by the autorities of the Balcan flora as one of the few relic grasses of the Peninsula.

TURRIL (1929) characterized it as «relatively old type being morphologically very distinct from the other species of the genus». STEFANOV (1943) treated it as «representative of the conservative endemisme (of the Balcan flora) related to *B. macropodum* Hack, from Sierra de Cintra in Portugal».

This species is distributed in North Greece and South West Bulgaria, on calcareous rocks. In Bulgaria it is limited in two opposed localities — the southern slopes of Pirin mountain, and Slavjanka (Alibotush) mountain (KITANOV in D. JORDANOV edit., 1963, sub *Agropyrum sanctum).*

The material for this study was collected from both localities as follows: 1. South Pirin mt., near vill. Delcevo, distr. G. Delcev, 27.IX.1973, SOM-SK 23802. 2. Slavjanka mt, Parilski dol (valley of Paril), 28.IX.1973, SOM-SK 23832. The method is the same as in the previous studies (KOZUHAROV & *al,* 1974).

The caryotype consists of fourteen chromosomes (2n = 14, KOZUHAROV I& PETROVA, 1975, sub *Agropyron sanctum),* partly differentiated only by their size (Fig. 1). All chromosomes according the scheme of KUZMANOV & KOZUHAROV (1967) are submetacentric as follow (Fig. 2) : two pairs of long, the longest in the caryotype (I, II), two

pairs shorter for their short arms (III, IV), and three pairs shortest in the caryotype, their short arms being equal of those of the previous pairs and their long arms — shorter than the previous pairs (V, VT, VII). No SAT chromosomes

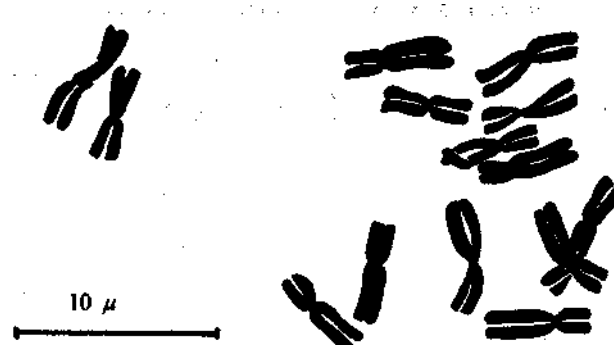

Fig. 1. — Drawing of a metaphase plate of JS. *sanctum.*

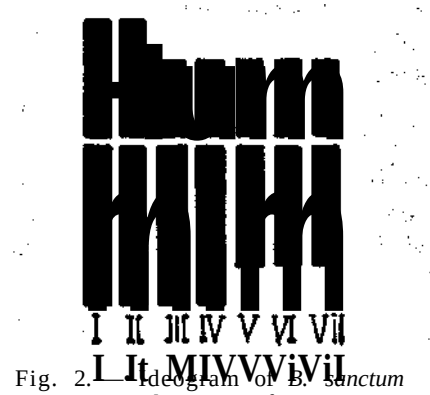

Fig. 2. — Ideogram of *B. sanctum*

Fig. 2. —• Ideogram of *B. sanctum*

were seen in all of the plates studied. Thus, the caryotype seems rather symmetric which coincides with its relic nature.

In the meantime another species of g. *Brachypodium* on the serpentine rocks of S. E. Albania has been twice recognized. And as it turned out — also endemic for the Balcan Peninsula. C. E. HUBBARD was the first who has found it (West Korea near Vaskopoj) and named it *B. serpentina* (Hok. Icon. Plant. 33,1935). His publication evidently has not been easy accessible to the Bulgarian botanists

during the first some years after the second world war, and two of them — B. ACHTAROV and B. KTTANOV, 1948) have recognized it for second time under the name *B. albanicum.* The type specimen[1] originates from Shebenikut. This is almost the same locality recorded later by MARK-

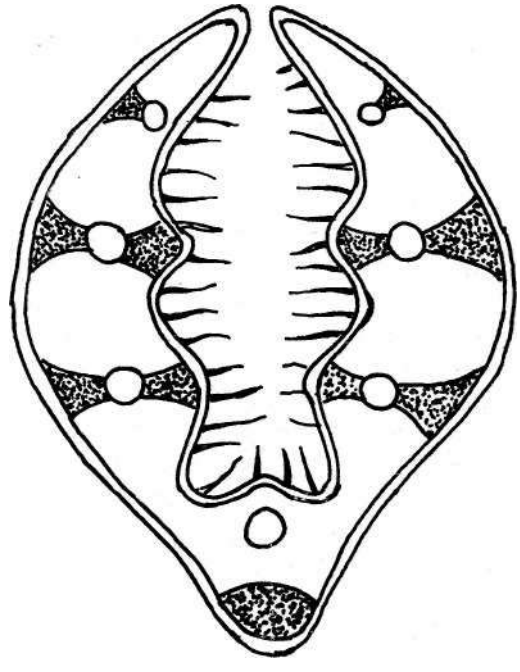

Fig. S. — Leaf section of *B. serpentina.*

GRAF (1949) about one of his gatherings of the plant in 1924.

The taxonomic position of *B. sanctum* and *B. serpentina* seems to be not very sound as yet. *B. sanctum* for instance is often refered to g. *Agropyron* Gaertn., like it was treated in Flora P. R. Bulgaria (KTTANOV in D. JORDANOV edit.,

[1] «In rupestribus subalpinis serpentinicus mt. Shebenikut cca 1970 m s. m., 21.VIII. 1947, leg. *B. Kttanov* — SOM 98001». This specimen has been recorded by B. KUZMANOV (1971) as being doubtfuly kept in Skopje.

l. c). MELDERIS (1978) suggests integrating both species in a separate genus *Festucopsis* based on HUBBARD'S section of g. *Brachypodium.* This integration seems plausible when having in mind the type of the basal sheets of both species.

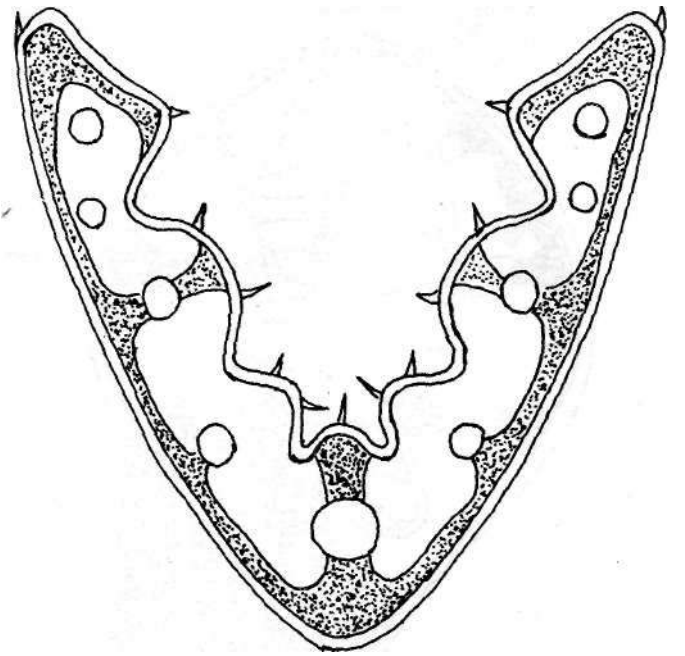

Fig. 4. — Leaf section of *B. sanctum.*

However the type of the basal sheets of *B. serpentina* is far more different from this one of *B. sanctum* than it is possible to keep them in one section or genus. The differences became more considerable taking into account the inflorescens. The inflorescens of *B. sanctum* is more close to this one of some *Agropyron* species from one side and some *Brachypodium* species from the other side.

B. serpentina however seems to be more related to the other species of *Festuca* than either to *Agropyron* or *Brachypodium.* We tryed to compare leave sections of *B. serpentina* (Fig. 3) — (voucher specimen — the type material

of *B. álbanicum* Acht, et Kitan.) and *B. sanctum* (Fig. 4). These species differ rather strongly on the base of disposition of the sclerenchim bands.

The relations between both species from one side and between each of them and *B. macrofodum* on the other side ought to be estimate, and the caryological data will be most important in this estimation.

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SUR L'ORIGINE DES POCHES SÉCRÉTRICES DANS LES FEUILLES *D'HETEROPYXIS* HARV. *

par

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Abstract

One of the anatomic characteristics of the *Heteropyxis* (africain genus with only three species) is the presence of secretory structures in flowers, leaves and juvenile stems. The origin of the glandular cavity of these structures is a controversial matter in some families, among them *Myrtaceae*, where *Heteropyxis* seems should be included. In order to analyse concretely the problem in the species *H. natalensis*, the leaf primordia were isolated for histocytological study in the optical and electron microscopes. The following results are stressed: a. The mesophyll, although of the asymmetric type, is exclusively formed by spongy parenchyma rather heterogenous as far as cell morphology and development of intercellular spaces, b. The secretory structures (one in every mesh of the reticulum) are placed in the parenchyma adjacent to the lower epidermis, showing no syncronization between their differentiation and the foliar development, c. The most juvenile stages observed were small sub-epidermic cell groups which rapidly evolve into spherical masses of polyhaedric cells, structurally very different from the surrounding ones. d. Then, some cell walls of the centrally located cells degrade away (the plasmalemma remaining intact), clearly originating intercellular space. The cavity observed in mature secretory structures has therefore a schizogenic origin.

* Ce travail a été subsidié par l'«Instituto Nacional de Investigação Científica» (I. N. I. C.) (Centre CB2).

INTRODUCTION

DEPUIS que HARVEY (1863) a décrit pour la première fois le genre africain *Heteropyxis,* sa position systématique a été souvent discutée. C'est ainsi que, selon les Auteurs, il pourrait justifier la création d'une famille indépendante *(Heteropyxidaceae),* ou bien il aurait des affinités avec les L*ythraceae, Myrsinaceae, Rhamnaceae, Rutaceae* et *Myrtaceae.* (Pour de la bibliographie et discussion détaillée du problème, voir STERN & BRIZICKY, 1958 et FERNANDES, 1971).

Parmi les caractères qui ont été considérés comme ayant de la valeur taxonomique, figurent les structures sécrétrices présentes dans les fleurs, les feuilles et les jeunes tiges de cette plante arborée ou arbustiva (STERN & BRIZICKY, 1958). Alors, dans le cadre d'une étude en cours sur les aspects ultrastructurales du processus de sécrétion chez les plantes supérieures, nous avons pensé qu'il serait intéressant d'étudier ces structures dans le genre *Heteropyxis,* d'autant plus que, d'une part, leur origine, particulièrement chez les *Rutaceae* et *Myrtaceae* est aussi un sujet controversé et, d'autre part, nous ne connaissons aucun article d'ultrastructure cellulaire concernant le genre en cause.

MATÉRIEL ET MÉTHODES

Pour réaliser ce travail, nous avons profité des cultures d'*Heteropyxis* existant dans l'Institut Botanique de Coimbra, où les plantes ont été obtenues à partir de graines récoltées au Moçambique.

Dans chaque bouton de l'espèce *H. nataïensis* on a isolé à la loupe le méristème apical et les cinq primordes foliares adjacents que, de suite, ont été plongés dans le fixateur (glutaraldhéyde à 2.5% dans le tampon Sorensen 0.025M pH 6-8). Alors, ce matériel et des feuilles adultes ont été découpés, en des petits morceaux, qui ont subi le traitement habituel pour la microscopie électronique, comprenant, en dehors de la fixation, une post- fixation à l'acide osmique (1%), la deshydratation à l'alcool et l'inclusion selon la

méthode de SPÜRE (1969). Les coupes ultrafines ont été contrastées par l'acétate d'uranyle et le citrate de plomb (REYNOLDS, 1963) et étudiées dans un microscope électronique Siemens Elmiskop 101.

Pour les observations au M. O., des coupes semi-minces ont été ramassées sur des lames de microscopie et colorées par le bleu de toluidine (MC-GEE-RUSSEL & SMAtE, 1963).

RÉSULTATS ET DISCUSSION

Dans les jeunes feuilles bifaciales *d'Heteropyxis natalensis* on ne trouve pas un parenchyme chlorophyllien en palissade comme il est courant dans les Dicotyledonae. Malgré ça, le mésophylle est nettement asymétrique, résultant cette asymétrie, d'une part, de l'hétérogénéité de ses cellules en ce qui concerne leur morphologie et dimensions et, d'autre part, du développement et de la distribution des espaces intercellulaires (Pl. I, fig. 3).

En effet, dans les coupes transversales et tangentielles, nous avons constaté que, du côté adaxial, le parenchyme foliaire, par suite d'être constitué par des cellules à peu près sphériques, montre l'aspect d'un tissu homogène à des nombreux et petits espaces intercelulaires (Pl. I, figs. 3 et 4). Par contre, dans la face abaxial, les cellules, beaucoup plus grandes et à morphologie variée, forment un parenchyme spongieux doué de lacunes très développées (Pl. I, figs. 3 et 5). Quant à leur contenu, en dehors du noyau et des grandes vacuoles, ressortent, parmi les organites habituels, des chioroplastes très riches en amidon (Pl. I, figs. 2 et 5). Nous avons constaté qu'il n'y a point de synchronisme entre la différenciation des poches sécrétrices et le développement foliaire. Efectivement, il est fréquent trouver des stades assez jeunes à côté de glandes matures, c'est-à-dire des cavités sécrétrices complètement différenciées (Pl. ET, fig. 1-3). Cependant, on ne voit qu'une de ces structures dans chaque maille du réseau formé par les nervures foliaires.

De même qu'il arrive chez *Eucalyptus* sp. (Myrtacée) (CARR & CARR, 1970), le développement de ces structures

progresse très rapidement, leur étude devenant assez difficile, particulièrement au début de la différenciation.

Les stades les plus jeunes qui nous avons observé comprennent des ensembles cellulaires sous-épidermiques (2-8 cellules) qui, bientôt, évoluent en des massifs plus au moins sphériques, constitués par des cellules polyédriques et parfaitement justaposées (PL II, figs. 1-3).

Au cours de la différenciation de ces massifs cellulaires, les cellules périphériques croissent tangentiellement et s'applatissent par rapport aux cellules centrales (Pl. III, fig. 4). De cette manière, des cellules de deux types se différencient assez tôt: d'une part, des cellules enveloppantes («casing cells») formant une, ou parfois deux assises cellulaires et, d'autre part, des cellules centrales qui vont constituer l'épithélium sécréteur propement dit («epithelial cells») (Pl. IV, fig. 1). Ces cellules, au début allongées radialement, deviennent aussi plus ou moins applaties, au fur et à mesure que le lumen de la glande se développe (Pl. IV, fig. 1). Ã l'exception des parois tangentielles externes des cellules enveloppantes, toutes les parois cellulaires de la structure sécrétrice sont très minces par rapport à celles des cellules du mésophylle (Pl. III, fig. 4 et Pl. IV, fig. I).

On peut dire que la différenciation d'épaississements dans les parois des cellules centrales est le premier signe annonçant l'ouverture de la cavité sécrétrice (Pl. III Fig. 4). Par suite de la dégradation progressive de la lamelle moyenne et des parois primaires, ces épaississements prennent bientôt l'allure d'une sorte de pochettes dans lesquelles on voit souvent un matériel granuleux-fibrillaire (Pl. III, fig. 4).

Il en résulte alors un petit espace intercellulaire dû à l'éloignement des cellules qui ne restent plus liées les unes aux autres aux endroits où il a pris lieu cette dégradation-là (Pl. HT, fig. 5). La continuation du processus dégradatif tout au long des parois radiales permet l'élargissement progressif du lumen de la glande (Pl. III, figs. 5 et 6 et Pl. IV, figs. 1 et 2). On doit remarquer que les surfaces cellulaires limitatives de la cavité sécrétrice conservent le plasmalemme intact, souvent bordé à l'extérieur par une couche d'épaisseur variable, constituée, apparement, par du matériel pariétal

en état plus ou moins avancé de dégradation (Pl. IV, figs. 1 et 2).

Par contre, nous n'avons jamais trouvé des signes inéquivoques de lyse du contenu de ces cellules, même aux stades plus avancés de la différenciation.

Quant à l'ultrastructure, les cellules à sécrétion lipophylique ont été normalement définies par les caractéristiques suivantes: un hyaloplasme assez dense; un reticulum endoplasmique du type «smooth» bien développée et souvent associé aux plastes; des leucoplastes peu structurés et un appareil de Golgi peu développé par rapport à celui des cellules qui sécrètent des polysaccharides (SCHNEPF, 1974; DELL & MCCOMB, 1978).

En dehors d'un cytoplasme très dense par suite de sa richesse en ribosomes et de la faible activité de l'appareil de Golgi, ces caractères ne ressortent pas dans les cellules epitheliales des poches sécrétrices *d'Heteropyxis natalensis.* D'une façon générale, les biomembranes sont peu évidentes (Pl. III, fig. 3) et le matériel osmiophile (couramment identifié avec la sécrétion) est pratiquement absent, tant dans les plastes que dans le cytoplasme. De même pour le lumen de la glande que, le plus souvent, semble, être complètement vide (Pl. IV, fig. 1).

Néanmoins, avant l'ouverture de l'espace schizogène, il apparait parfois dans les vacuoles un matériel electrodense (Pl. III, fig. 4), dont l'origine n'a pas pu être éclaircie.

Bien que nous n'ayons pas employé des techniques spécifiques pour caractériser le produit de la sécrétion, il se peut que, tenant compte des caractéristiques ultrastructurales signalées ci-dessus et, d'accord avec SCHNEPF (1974), l'absence de sécrétion soit réele, c'est-à-dire que nous avons fait la fixation à un moment pendant lequel l'activité sécrétrice de la plante était très faible ou même nulle.

L'origine de la cavité sécrétrice des glandes à l'huille chez les *Myrtaceae* et *Rutaceae* a été très discutée. D'après CARR & CARR (1970) qui ont fait, en microscopie optique, une analyse détaillée du développement de ces structures dans les embryons de quelques espèces *d'Eucalyptus,* cette cavité se forme toujours, schizogènement. Nous avons nous-

-mêmes complementé les observations chez *Heteropyxis natalensis* avec une étude parallèle dans des jeunes feuilles d'*Eucalyptus globulus* (PL IV, figs. 3 et 4) et nous sommes arrivé aussi à la conclusion de que le processus est tout à fait identique dans les deux espèces, c'est-à-dire, les poches sécrétrices sont vraiment schizogènes.

Bien que beaucoup d'autres espèces doivent être étudiées sous ce point de vue, avant d'en tirer des conclusions définitives, d'après ce qu'il est déjà connu au niveau ultrastructurale, on peut peut-être avancer que, vraisemblablement, la schizogénie ne sera pas le processus dominant, et beaucoup moins exclusif, dans le développement des cavités sécrétrices dans les *Rutaceae*. Effectivement, chez cette famille, elles ont été décrites, le plus souvent, comme étant lysogènes (HEINRICH, 1986, PETERSON & col., 1978) ou schizolysogènes (THOMSON & col., 1976; PETERSON & col., 1978). Par contre, dans les *Myrtaceae*, malgré toute l'ancienne controverse, les études de CARR & CARR (1970) indiquent que, chez cette famille, les poches sécrétrices s'ouvrent plutôt par un mécanisme vraiment schizogénique.

STERN & BRIZICKY (1958) et plus tard FERNANDES (1971) ont discuté en détail la position systématique du genre *Heteropyxis* en se basant essentiellement sur des caractères anatomiques (STERN & BRIZICKY, 1958) ou de la morphologie externe et caryologiques (FERNANDES, 1971). Ils sont arrivés à la conclusion que, parmi les familles *Myrsinaceae*, *Rhamnaceae*, *Rutaceae* et *Myrtaceae* (avec lesquelles ont été discutées des eventuelles affinités d'*Heteropyxis*), le genre doit être inclu dans les *Myrtaceae*.

Alors, les donnés ultrastructurales que nous venons de présenter sur l'origine des poches sécrétrices semblent appuier le point de vue de ces Auteurs, selon lequel le genre *Heteropyxis* doit être rangé dans les *Myrtaceae*.

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EXPLICATION DES PLANCHES

1. En dehors des figs. 3 et 4 de la Pl. IV qui concernent des feuilles d'*Eucalyptus globulus*, toutes les autres microphotographies se rapportent à l'espèce *Heteropyxis natalensis*.

2. Méthode de préparation du matériel.

   a. *Microscopie photonique* (PL I, figs. 3-5 et Pl. II, figs. 1-3) : coupes semi-minces du matériel inclu dans une résine (SPURR, 1969) et colorées par le bleu de toluidine (MCGEE-RUSSEL & SMAle, 1963).

   b. *Microscopie électronique* (Pl. I, fig. 2; Pl. III; Pl. IV) : sections ultrafines du matériel fixé au glut/OsO,,, inclu selon la méthode de SPURR (1969) et contrasté par l'acétate d'uranyle suivi du citrate de plomb.

3. Abréviations: a, amidon; c, chloroplaste; m, mitochondrie; n, noyau; nu, nucléole; p, paroi cellulaire; p1, plaste; re, reticulum endoplasmique; v, vacuole.

PLANCHE I

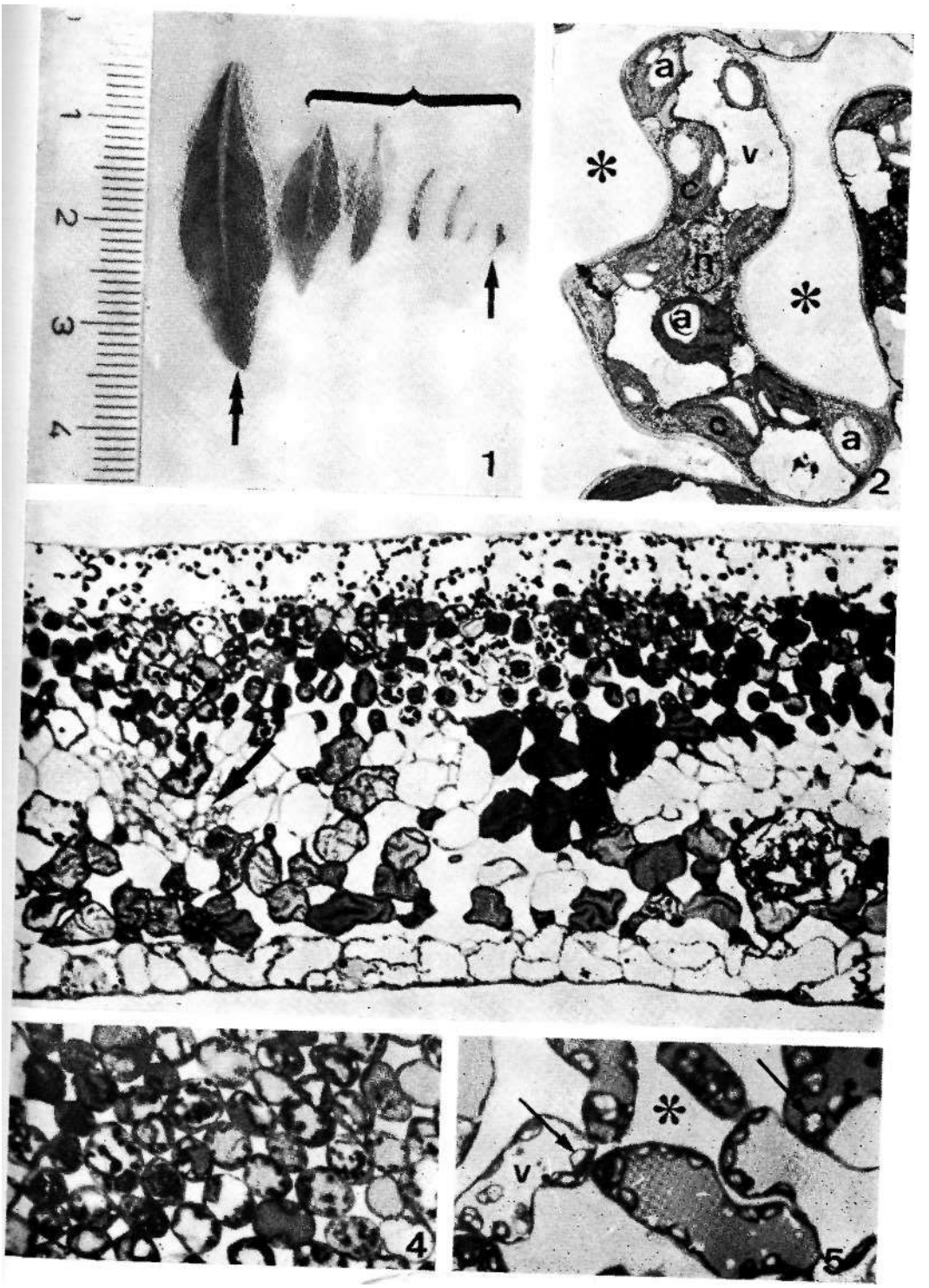
Fig. 1. — Le méristème apical (flèche simple), les cinq jeunes feuilles adjacentes et une feuille adulte (flèche double).

Fig. 2. — Aspect général de l'ultrastructure d'une cellule du parenchyme spongieux: remarquer les chloroplastes riches en amidon (a) et les spaces intercellulaires (lacunas) très développés (*). X 4500.

Fig. 3. — Anatomie du limbe de la feuille: on voit l'épiderme supérieur, le mésophylle asymétrique, l'épiderme inférieur et un faisceau libéro-ligneux (flèche) (voir le texte). X 1250.

Fig. 4. — Détail du mésophylle (côté adaxial): des cellules sphériques forment un parenchyme homogène et relativement compact à des nombreux et petits espaces intercellulaires (méats). X 2400.

Fig. 5. — Détail du mésophylle (côté abaxial) : parenchyme spongieux constitué par des cellules très vacuolisées et contenant des chloroplastes riches en amidon (flèches). Parmi ces cellules ressortent des lacunes très développées (*). X 3000.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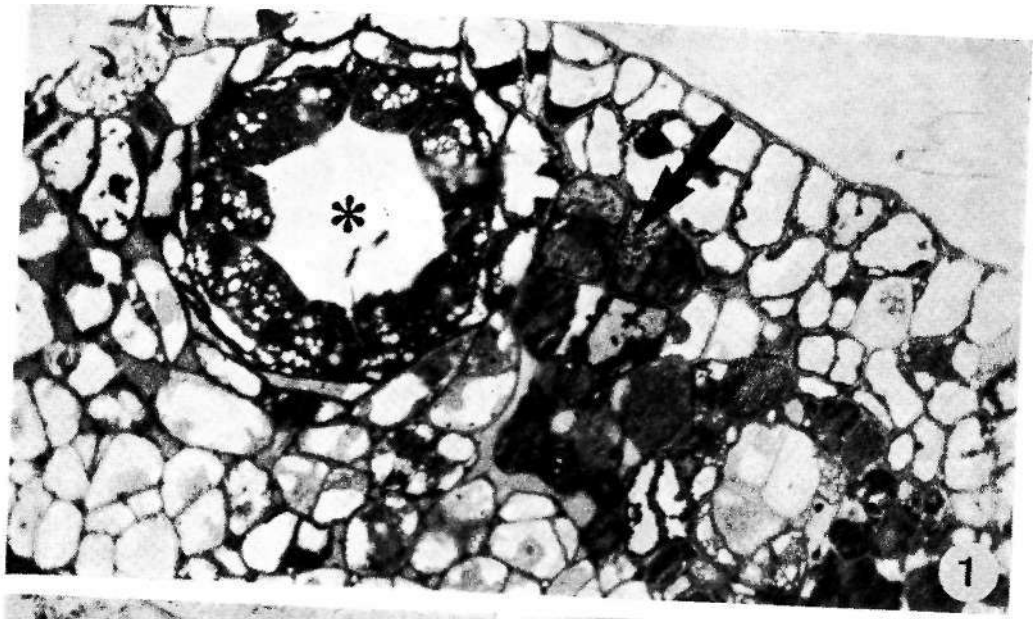
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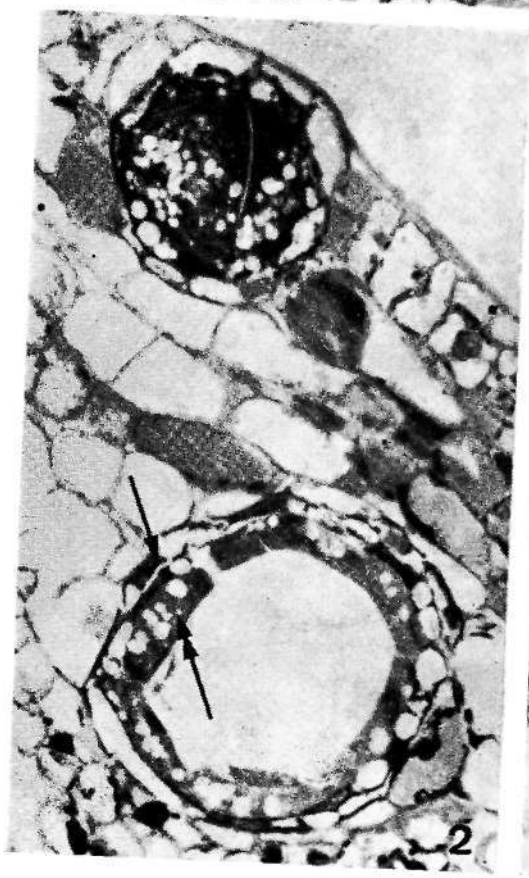
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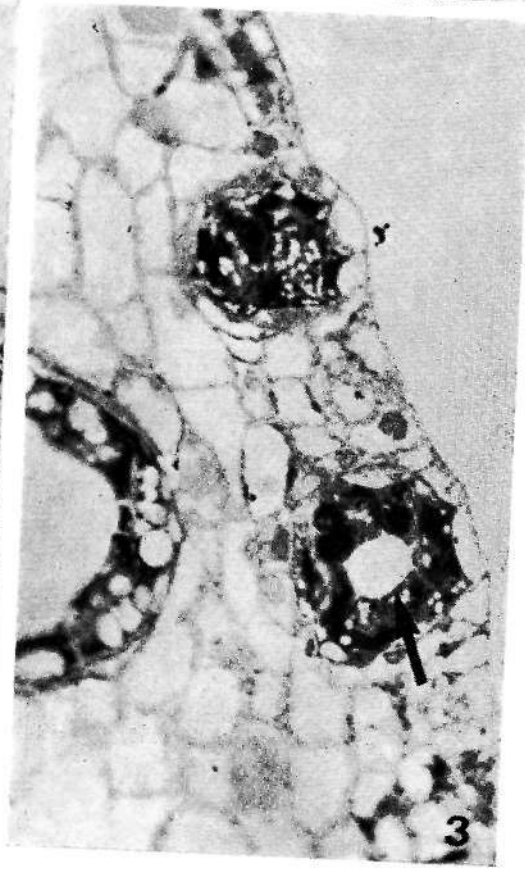
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PLANCHE II

Fig. 1. — Coupe transverrale d'une feuille montrant des stades différents de la différenciation des poches sécrétrices. Remarquer un stade très jeune à quatre cellules (flèche) à côté d'une glande déjà douée d'une cavité bien développée (*). X 2800.

Fig. 2. — Idem. En bas, on voit une poche sécrétrice adulte dans laquelle se distinguent nettement les cellules enveloppantes (flèche simple) et l'assise sécrétrice (flèche double) qui borde la cavité.

Fig. 3. — Idem. En dehors d'une poche localisée profondément (vue partielle), on doit remarquer deux stades jeunes sous-épidermiques, un desquels (flèche) montre déjà un méat précurseur de la cavité sécrétrice. X 2000.

PLANCHE III

Début de la différenciation de la structure sécrétrice. Remarquer la haute densité de ses cellules par rapport aux cellules parenchymateuses voisines. X 6500.

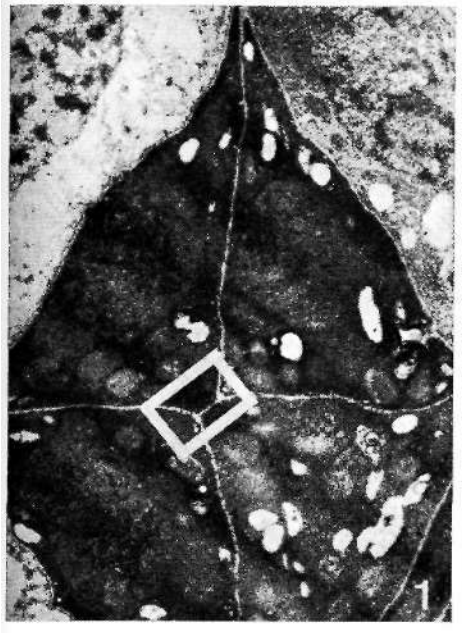
Détail de la fig. 1. Les ribosomes sont extrêmement abondants et les parois assez minces. On voit encore des portions de deux mitochondries (m) et des microtubules (flèches). X 48000.

Les proplastes (pr) des cellules sécrétrices montrent un stroma très dense, tandis que leurs membranes sont peu évidentes. X 30500.

Aspect général de l'ultrastructure de Ia glande avant l'ouverture de sa cavité. Remarquer la minceur des parois (flèche simple) et quelques épaississements de celles-ci à la région centrale de la structure (flèche double). On voit encore du matériel dense dans les vacuoles (voir le texte). X 4500.

Début de la formation de la cavité schizogène. X 3100.

Aspect ultrastructural de la zone de séparation de deux cellules contiglies pendant le développement de la cavité séerétrice (voir le texte). X 36000.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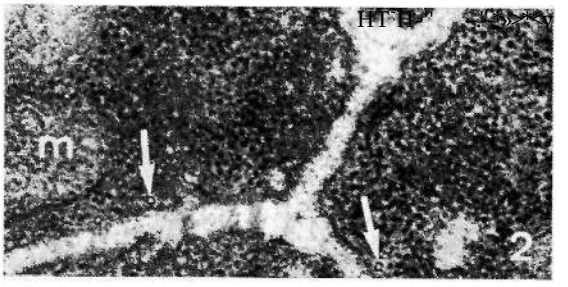
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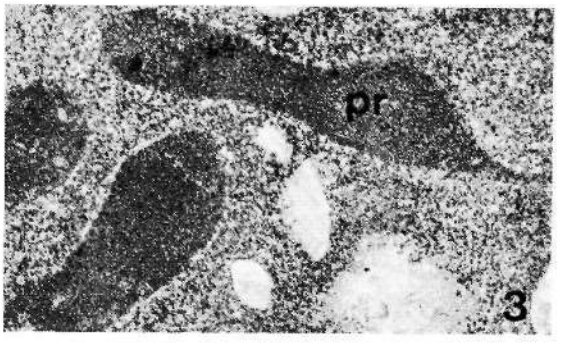
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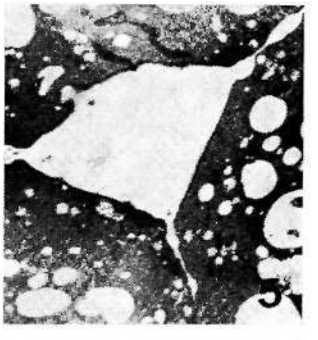
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PLANCHE IV

Fig. 1. — Vue générale d'une poche sécrétrice dans un stade assez avancé de sa différenciation. En dehors des caractéristiques, habituelles, il ressort le début de la dégradation qui a lieu dans les parois radiales des cellules de l'épithelium sécréteur (flèches) (voir le texte). X 6000.

Fig. 2. — Détail de la fig. 1 montrant le plasmalemme intact des cellules sécrétrices (flèches) et des débris provenantes de la dégradation de la paroi. X 48000.

Fig. 3. — Début de l'ouverture du méat central d'une poche sécrétrice dans une feuille *d'Eucalyptus globulus* (comparer avec la fig. 4 de la Pl. III). Remarquer les altérations des parois au niveau de leurs épaississements (flèches). X 5000.

Fig. 4. — Détail de la fig. 3 montrant, à plus fort grossissement, les régions cytoplasmiques adjacentes à une paroi en voie de dégradation. Remarquer l'abondance de ribosomes et le plasmalemme intact (flèche). X 48000.

Bol. Soc. Brot., Sér. 2, 53(2): 1197-1210 (1981).

PYROLIRION AND *ZEPHYRANTHES:* DISTINCT GENERA *

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Wake Forest University and Carolina Biological Supply Company

SUMMARY

Pyolirion differs from *Zephyranthes* morphologically with respect to leaf shape, spathe, perianth form, stigma and filament. In addition, the two taxa have marked differences in chromosome number and type, in the occurrence of numerous acrocentric chromosomes in *Pyrolirion* but not in *Zephyranthes,* and apparently (but perhaps not actually) in basic haploid number.

These differences strongly support the correctness of HERBERT'S oroginal conception of *Pyrolirion* as being distinct from *Zephyranthes,* and indicate that the two should not be treated as one genus.

HERBERT established the genera *Pyrolirion* and *Zephyranthes* in 1821, and the distinction between the two taxa was maintained in his AMARYLLIIDACEAE (1837). Several decades later BENTHAM & HOOKER (1883) submerged all species of *Pyrolirion* in *Zephyranthes.* The BENTHAM & HOOKER opinion has been followed by BAKER (1888), PAX (1888), PAX & HOFMAN (1936), TRAUB (1952 and 1957, but *not* 1963), HUTCHINSON (1934; 1959), and by most other students of the Zephyrantheae. STAPH (1927), SEALY (1937), UPHOF (1947) and TRAUB (1963) have believed, however, that the genera should be accepted as defined by HERBERT. SEALY´S views were based on perceived differences in spathe, perianth, stamen and stigma between

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Pyrolirion and *Zephyranthes.* UPHOF made the cogent statement that «Herbert knew perfectly well the characteristics of the genera he described in 1821».

Since many workers still follow the BENTHAM & HOOKER (1883) treatment, including *Pyrolirion* with *Zephyranthes,* it has seemed desirable to compare the cytological situations in the two taxa. At the same time some review, as well as further study, of the respective morphological characteristics of the taxa could be made. The present work has resulted from these objectives.

EXTENT OF THE GENERA

Several *Zephyranthes* species have been described since SEALY (1937) listed 36 species for the genus. TRAUB (1963) gives 55 as the number of species in *«Zyphyranthes,* Subgenus 1. *Zephyranthes».* Since TRAUB includes *Cooperia,* with 8 species, as «Subgenus 2. *Cooperia»,* of *Zephyranthes,* he places a total of 63 species in the genus. Further, there are at least several still undescribed species of *Zephyranthes* from Mexico, and probably more yet in South America. The various species of this genus are distributed through parts of the southern United States, Mexico, Central America, the West Indies, and South America. They are concentrated in the countries, and states, surrounding the Gulf of Mexico and the Carribbean Sea, but with a number of species occurring rather far south in South America.

When HERBERT (1821) proposed the genus *Pyrolirion,* he admitted and described three species — *P. flammeum, F. aureum* (synonymous with the now cultivated *P. tubiflorum* M. Roem.) and *P. flavum.* Later HERBERT (1837) listed P. *albicans* as a doubtful species. In 1888 BAKER described *Zephyranthes bolivienne* and *Z. xiphopetalum,* which SEALY (1937) moved to *Pyrolirion,* and are now designated as *P. boliviense* (Baker) Sealy, and *P. xiphopetalum* (Baker) Sealy. TRAUB (1963) «tentatively» recognized 10 *Pyrolirion* species. So far as is known all *Pyrolirion* taxa are native to South America, with a concentration in Peru and Bolivia.

MATERIALS AND METHODS

Since we had collected and studied *Zephyranthes,* cytologically and otherwise, for a long period of years, living material of a number of its species — especially from the southern United States, West Indies, and Mexico — were available in our cultures. Bulbs of *Pyrolirion aureum, P. flammeum* and *P. xiphopetalum* were furnished us through the courtesy of several coworkers (Table 2). All three of these species are natives of Peru. Still another accession from Peru which came to us labelled as *Cooperia albicans* appeared much more like a *Pyrolirion* than a *Cooperia.* Further study showed that it conformed with the *P. albicans* described by HERBERT (1837), and possessed the same distinctive characters as the three taxa listed above as *Pyrolirion* species. Accordingly, this fourth taxon has been included in our analysis of *Pyrolirion* species.

Plants of all these were grown in the greenhouse for several years. During this period the chromosome numbers and types of the four taxa considered as *Pyrolirion* species were determined and compared with available data from *Zephyranthes.* Careful comparisons were also made of several different morphological characters.

Chromosome preparations were made from rapidly growing root tips which were removed from the plant and pretreated in .2% colchicine for 2 to 3 hours. Fixation followed in a freshly made mixture of 95% ethanol and glacial acetic acid (3:1). Several hours later cytological squashes were prepared in 1% Gurr's acetic orcein.

CHROMOSOME NUMBER AND TYPE

A number of chromosome studies of *Zephyranthes* have been reported (summaries: FLAGG, 1961; FLORY, **1968, 1977).** With the methods used, the chromosomes of this genus usually range from shorter ones 3 or 4 microns in length, by rather regular steps to long ones 10 to 12 microns — or sometimes more — in length. Usually, the longest and shortest pairs of *Zephyranthes* chromosomes have approximately

median centromeres, while those of intermediate length have either submedian or subterminal centromeres. No telocentric or acrocentric chromosomes have been observed in this genus. Somatic chromosome numbers range in number from 18 to 120, so far as presently known. As might be expected, species with low numbers usually have longer chromosomes, and those with high numbers have a greater proportion of short chromosomes. Most chromosome numbers fall in a euploid series, with a base number of six. Several *Zephyranthes* species and hybrids with aneuploid numbers are known, With these numbers being satisfactorily explainable as tracing from taxa in which x = 6.

In the four species of *Pyrolirion* studied here the numbers and types of chromosomes encountered differ rather markedly from those known to occur in *Zephyranthes.* The

TABLE 1

Somatic chromosome numbers in *Pyrolirion,* with the source of each accession studied

Species	2n	Accession Number	Source
Pyrolirion xiphopetalum	26	15501-62	«La Paz, Bolivia», I. S. Nelson.
Pyrolirion flammeum	34	14835-60	From cultivation, via A. Korsakoff.
Pyrolirion flammeum	34	14882-60*	«Original bulbs from C. Vargas», K. Clint.
Pyrolirion aureum	51	14881-60*	«Originally from Lima, Peru. J. Smith No. 1103», K. Clint.
Pyrolirion aureum	51	14942-60	PERU: Dto. Lima: Mala 75 km s of Lima, Sept. 1960, P. Ravenna s. n.
Pyrolirion albicans	54	14883-60	«Bulbs from Vargas, through F. B. Jones», K. Clint.

* Voucher specimen deposited at the National Herbarium (US).

numbers of chromosomes found in the several accessions of the four *Pyrolirion* species studied are listed in Table 1, along with information on the sources of each accession. It is noted there that the somatic chromosome numbers for the several *Pyrolirion* species vary from 26 in *P. xiphopetalum* (Fig. 1) through 34 in *P. flammeum* (Fig. 2), and 51 in *P. aureum* (Fig. 3), to 54 in *P. albicans* (Fig. 4). [Figures 1 to 4 were used earlier in a paper dealing with family chromosome evolution (FLORY, 1977), but in an entirely different context than is emphasized here].

While chromosomes with median, or near-median, centromeres are prevalent in the complements of *Zyphyranthes* species, comparatively few of this type are found in *Pyrolirion.* Instead, chromosomes with subterminal centromeres are much more common in the latter genus (Figs. 1-4). The chief difference found between chromosomes in the complements of *Pyrolirion* and *Zephyranthes,* however, is in the presence, and comparative frequency, of acrocentric chromosomes in *Pyrolirion.* Acrocentrics, within our knowledge, do not ocur in *Zephyranthes.* In Table 2, the total number of chromosomes in each *Pyrolirion* species studied (as shown in the right-hand column), are divided into the numbers of metacentric and acrocentric ones which occur in each. While there are only two acrocentric, out of 26, chromosomes in *P. xiphopetalum,* approximately half (8/17's, 9/17's and 4/9's, respectively) of the total number of chromosomes in each of the other three species are acrocentrics.

Speculation on the Basic Chromosome Number of *Pyrolirion*

We don't as yet know the true base chromosome number in *Pyrolirion.* The 34 chromosomes in *P. flammeum* and the 51 in *P. aureum* suggest the number 17, as being either a primary or secondary basic number, with one of the species being a diploid, the other a triploid. Also, the 26 chromosomes in *P. xiphopetalum* approximate half the 51 and 54 chromosomes encountered in *P. aureum* and *P. albicans,* respectively. In this there might be some basis for suspecting

13 as a base number, with some aneuploidy occurring in the «tetraploid» forms. A comparison of the numbers of metacentric and acrocentric chromosomes involved, however,

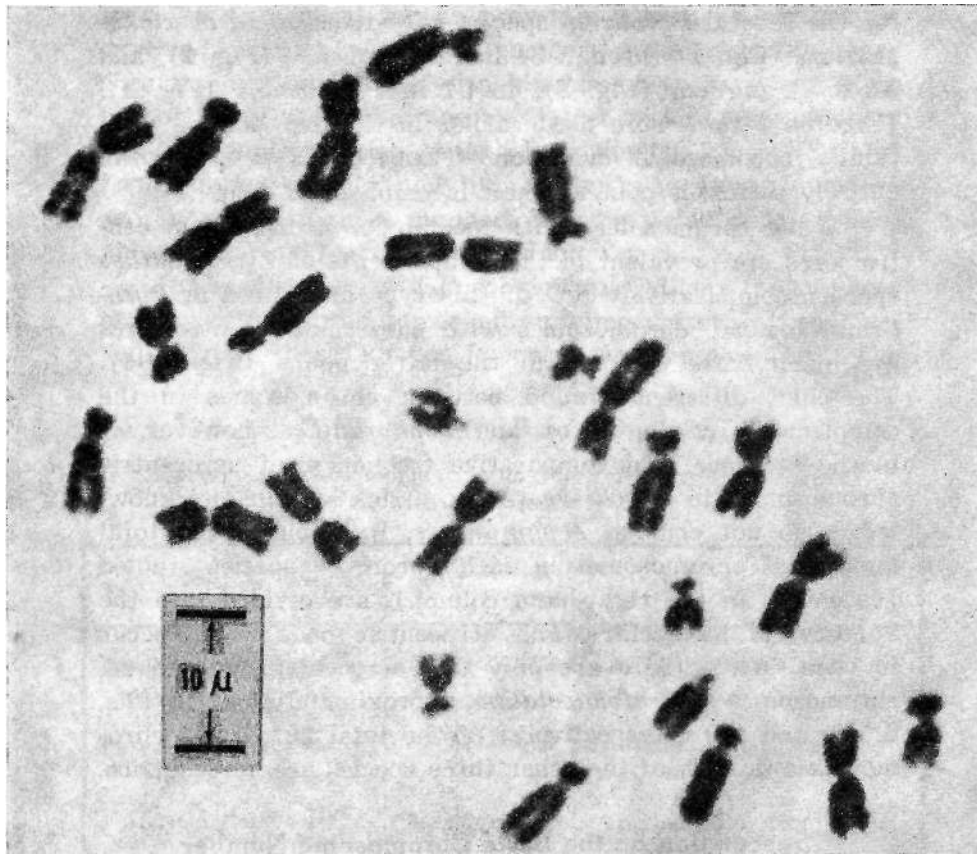

Fig. 1. — *Pyrolirion xiphopetalum* (Baker) Sealy. 2n = 26; 2 acrocentrics.

does not seem encouraging for considering either 13 or 17 to possibly be a true base number here.

It is of interest that the number of metacentric chromosomes (see Table 2) found in each *Pyrolirion* species and accession is evenly divisible by six. All of the non-

metacentrics appear to be true chromosomes, rather than fragments or accessories. None of the acrocentrics appear to be true telocentrics. It doesn't appear that any useful conclusion would be aided by assuming that all the apparent

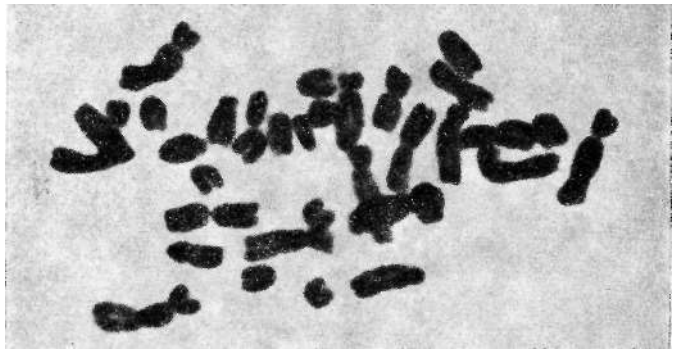

Fig. 2. — *Pyrolirion flammeum* Herbert.
2n — 34; 16 acrocentrics.

acrocentrics are actually telocentrics, each two of which might have resulted from the centric fission of single metacentrics (as with Robinson's Law). Such a situation would mean that, even with a possible potential of each species sometimes having all metacentric chromosomes — with the available chromatin material, the four *Pyrolirion* species would then be expected to have somatic numbers

TABLE 2

Number and types of somatic chromosomes in *Pyrolirion*

Species	Number		
	Metacentric	Acrocentric	2n
P. xiphopetalum (Baker) Sealy	24	2	26
P. flammeum Herbert	18	16	34
P. aureum Herbert	24	27	51
P. albicans Herbert	30	24	54

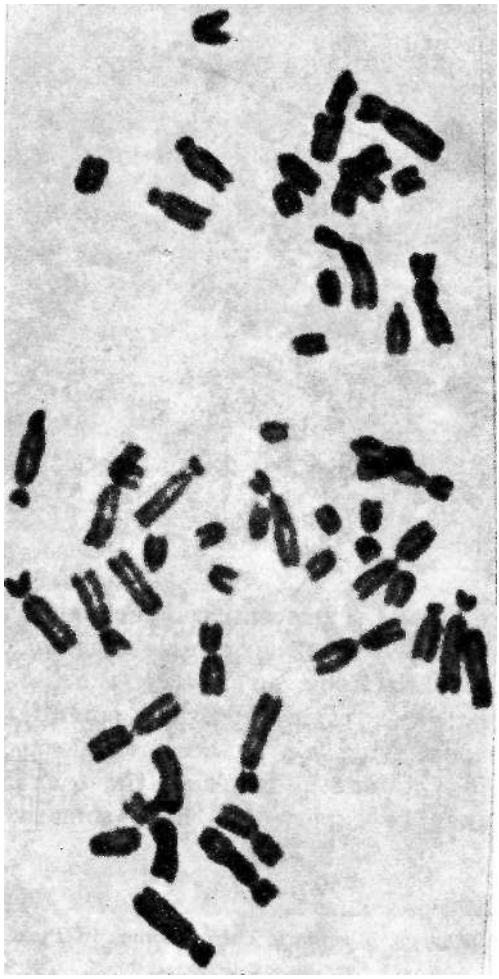

Fig. 3.—*Pyrolirion aureum* Herbert. 2n = 51; 27 acrocentrics.

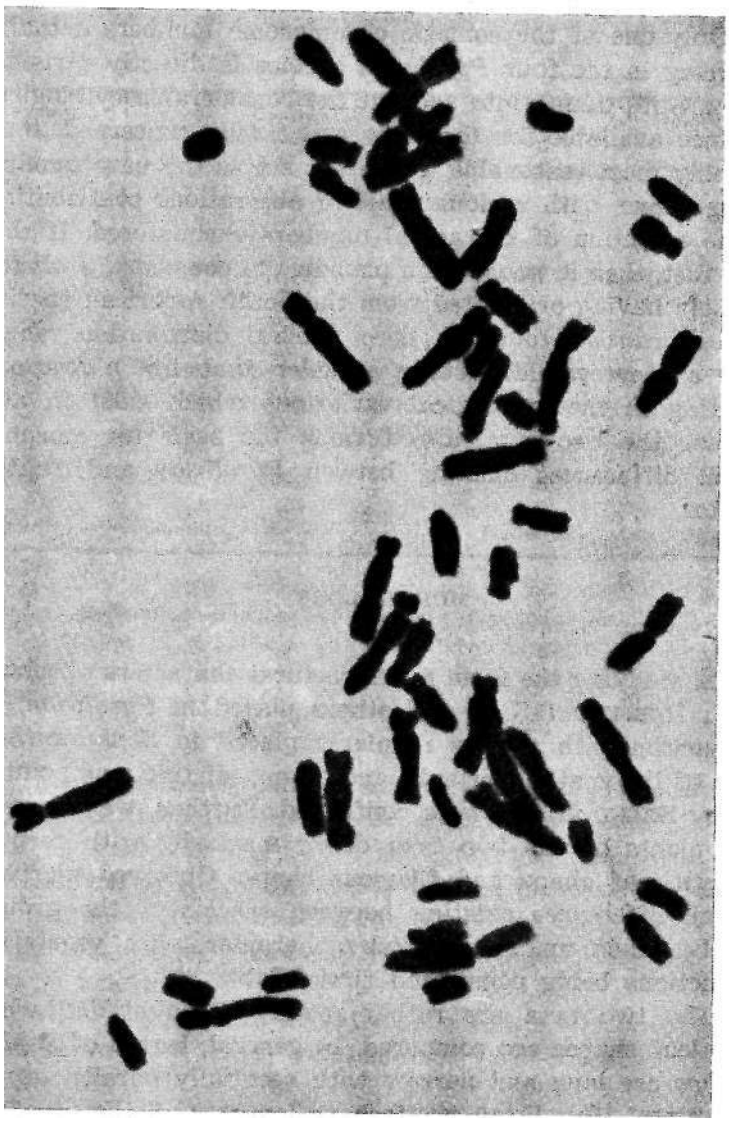

Fig. 4. — *Pyrolirion albicans* Herbert.
2n = 54; 24 acrocentrics.

of 25, 26, 37.5 and 42, respectively. Just one of these numbers is evenly divisible by six.

Only one of the somatic chromosome numbers actually occurring in the four *Pyrolirion* species is directly divisible by six. Still, taking into account the considerable cytological evidence available for the tribe and family concerned, it is probably most reasonable to suspect six as the base number of the genus, with various types of aberrations contributing to the evolution of the actual numbers encountered. If this is correct, then it would seem plausible to consider *Pyrolirion* as likely having originated from the South American species of *Zephyranthes* found in their general distribution range. Such a ,view would further consider that the alterations resulting in the cytological variations which exist in and between the two taxa, also furnish the basis for morphological differences existing betwen *Pyrolirion* and *Zephyranthes.*

MORPHOLOGY

In studying the form and structural characters of plants which HERBERT (1837) and others placed in *Pyrolirion,* in comparison with species regularly placed in *Zephyranthes,* some of the spathe, perianth and stigma distinctions pointed out by SEALY (1937) were confirmed. Further, we observed that plants of the two taxa differ markedly with respect to both, leaf shape and filament shape. Observed morphological differences existing between species of the groups will be taken up, character by character, with vegetative distinctions being considered first (Table 3).

The two taxa are rather readily differentiated when their leaf shapes are compared. In general, leaves of *Zephyranthes* are long and narrow with esentially parallel edges. In contrast, *Pyrolirion* plants have leaves with the broadest part being about midlength, and tapering from this point toward both the tip and the base. Or put in different words, *Pyrolirion* plants have leaves which are, as HERBERT (1837) well stated, «attenuated at both ends». Since the *Pyrolirion*

TABLE 3

Comparison of some morphological characters in *Zephyranthes* and *Pyrolirion*

Characters	***Zephyranthes***	***Pyrolirion***
Leaf edges	Parallel	Not parallel
Spathe	Entire or shallowly bifid	Deeply bifid
Spathe tube	Rarely 2 cm	2 cm, or longer
Perianth	Rotate or funnel-form	Campanulate-cylindric limb
Perianth tube	Short to medium length[1] Widens gradually Erect, sub-erect or declinate	Comparatively long Flares abruptly Erect
Stigmas	Linear or lobad[2]	Spatulate
Filaments	Acicular	Flattened; alate

[1] True of *Zephyranthes* Herbert, but is not true if *Cooperia* Herbert *(Zephyranthes* subgenus *Cooperia* Traub) is included.

[2] Except in *Z. bifolia* (Aublet) Roemer, which Hume (Plant Life 6: 123. 1939) states actually «does not fit into any genus now established».

leaf does not have parallel edges, it is quite distinct in appearance from the linear leaf of *Zephyranthes.*

The spathe of *Pyrolirion* is deeply bifid, above a tube that is 2 cm or longer. In *Zephyranthes,* on the other hand, the spathe is either entire, or shallowly bifid, with the tubular part being usually shorter, and only rarely reaching 2 cm in length.

While the perianth at anthesis is rotate, or funnel-form, in *Zephyranthes,* it is campanulate (bell-shaped) with a cylindrical limb in *Pyrolirion,* with lower parts closely adhering and the upper portions spreading and recurved.

The perianth tube of *Zephyranthes* Herbert (but not of Z. subgenus *Cooperia* Traub) is short to medium in. length; in *Pyrolirion* it is comparatively long. In the latter the tube flares abruptly; in *Zephyranthes* it widens gradually from

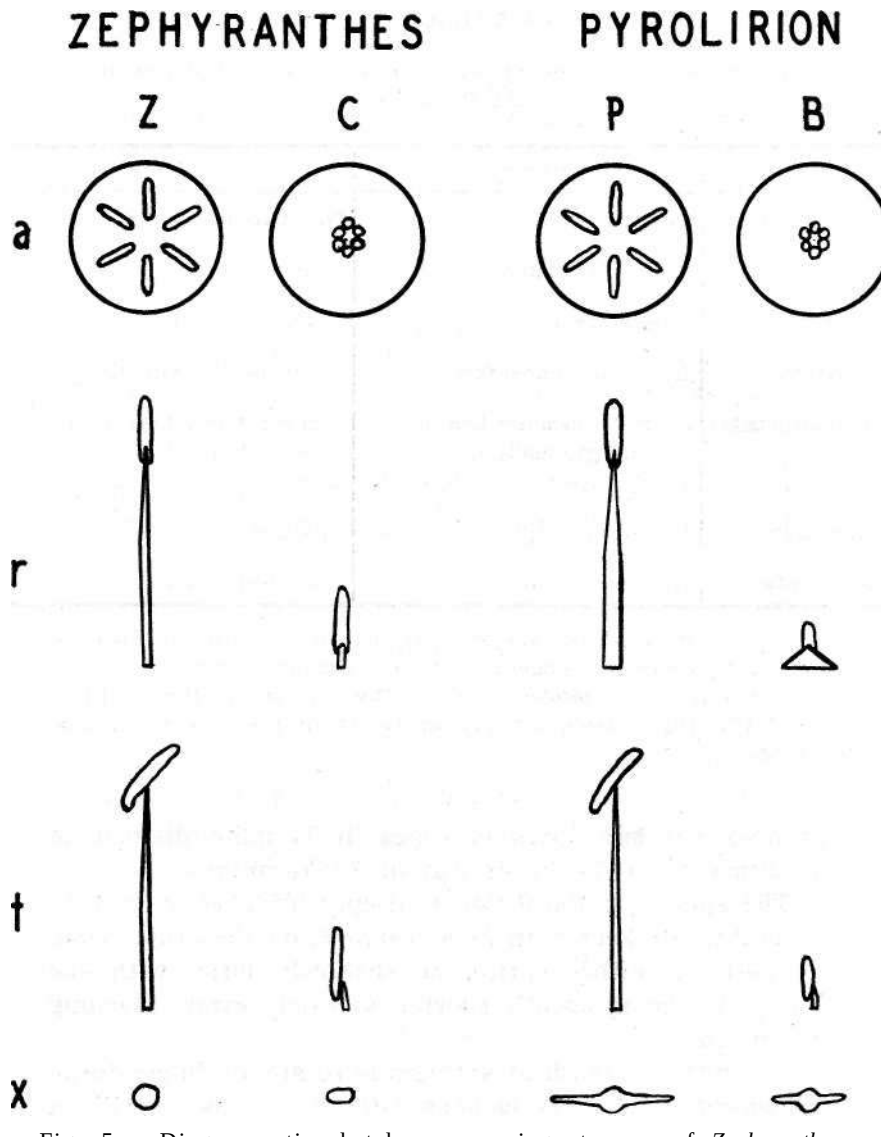

Fig. 5. — Diagrammatic sketches comparing stamens of *Zephyranthes* [including Traub's 1952 subgenera *Zepkyranthes* (Z), and *Cooperia* (C)], with those of *Pyrolirion* [including Traub's 1952 sections Eupyrolirion (P) and Brachylirion (B)]. Letters to the right designate lines comparing: a — views from above anthers showing their relation to perianth; r — radial views of stamens t — «righthand» tangential wiews of stamens; and x — cross-sections of filaments.

the base, distally. The perianth tubes of the four Pyrolirions we have studied are quite erect. This same fact is also true of a number of the *Zephyranthes,* while other species of the latter have tubes which are suberect (as *Z. macrosiphon),* or declínate (examples: *Z. albiella; Z. insularum).*

The stigmas of *Zephyranthes* and of *Pyrolirion* are quite different. Both are trifid. Most *Zephyranthes,* however, have linear or lobed stigmas. The *Pyrolirion* species observed have wider, flatter, spatulate stigmas. Filaments also differ in the two taxa: in *Zephyranthes* they are acicular; in *Pyrolirion* they are flattened and winged, or alate (Fig. 5-x).

On the basis of morphological characters alone there is ample evidence to support HERBERT'S original separation of the two entities being considered.

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NUCLEAR CHANGES ASSOCIATED WITH CALLUS INDUCTION IN *LOBULARIA MARITIMA*

by

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INTRODUCTION

CELLS in mature differentiated leaves of *Lobularia maritima* contain endopolyploid nuclei in the parenchyma cells surrounding the vascular bundles (CATARINO, 1965; 1968). When segments are excised aseptically from full developed leaves and grown on a sterile culture medium, their cells are induced to proliferate and form an undifferentiated tissue mass, the callus. This formation is mostly dependent on a correct balance of auxin-cytokinin in the medium as has been put in evidence by many authors (TOKKEY, 1961; LIBBENGA & TORREY, 1973; D'AMATO, 1975). On the other hand the hormonal balance can also stimulate endopolyploidization or amplification of DNA (NAGL, 1974a and *b*; 1976).

In present plant material, during the course of callus induction, DNA synthesis is stimulated mainly in the cells surrounding the vascular bundles leading to an increase in endopolyploidy. This is followed by cell multiplication in the vascular region. The development of callus proceeds rapidly with formation of undifferentiated nodules wich grow through the leaf parenchymas, finally disrupting the epidermis.

Possible relations between endopolyploidy in cells surrounding leaf vascular bundles and callus induction and development has not yet been investigated in this material.

It is known that in species showing endopolyploidy in their differentiated tissues three possibilities can take place under tissue culture conditions: first, endopolyploid cells can increase the endopolyploid levels, under *in vitro* conditions (DAS *et al.*, 1956); second, the endopolyploid nuclei may undergo mitosis and normal citokynesis (PATAU &. DAS, 1961); finally, endopolyploid nuclei may undergo divisions «via» amitosis (nuclear fragmentation) which can be followed by normal mitosis in both intact and fragmented nuclei (D'AMATO, 1977; 1978).

Here we describe observations on nuclear changes during callus induction and development in *Lobularia maritima* leaf tissue. The present study was undertaken in an attempt to find out some possible role for endopolyploidy that increases in cells close to developing nodules of callus tissue.

MATERIAL AND METHODS

Mature leaves from *Lobularia maritima* L. Desv. were sterilized by imersion for 1 min. in a 0,1'% solution of mercuric chloride and then rinsed in sterile water. Distal half segments of leaf tissue were aseptically cut and cultured on a MURASHIGE and SKOOG agar medium (MURASHIGE and SKOOG, 1962) supplemented with 1 ppm 2,4-dichlorophenoxyacetic acid (2,4-D); 0,1 ppm kinetin and 3000 ppm casein hydrolisate at 27° C under dark conditions.

For microdensitometric measurements the leaf tissues were removed at intervals of 24 h up to 290 h of culture, fixed in Burke's fixative during 24 h and infiltrated in paraffin after dehydration in butyl alcohol series. The staining was made simultaneously in all slides according to Feulgen technique (WARDEN, 1974) and nuclear DNA content, in 50 interphase nuclei per each area taken at random, was measured with a Vickers M 85 eytodensitometer with the scanning spot set at 560 nm. The results were recorded as the mean of two readings. The 2C and 4C levels of DNA were established by measuring mitotic divisions inside the vascular area.

After 12, 24, 36, 48 and 60 hours of culture the expiants were labelled with ^{3}H-Thymidine (spec. act. 28 Ci/mM; concentration 20 uCi/ml; incubation time 12 h). Leaf segments were fixed in 3 % glutaraldehyde during 24 h at 0° C, infiltrated in paraffin after dehydration according to FEDER and O'BRIEN technique (1968), sectioned serially at 6 um and stained with Feulgen. Exposure time after covering the slides with Kodak AR 10 stripping film was from 5 to S days. The frequency of labelled nuclei was estimated by scoring 50 tissue sections from 2 slides at each fixation time.

RESULTS

Callus Growth and Differentiation

The first indication of callus initiation, represented by leaf thickening, usually appears in the main rib in the region of the section. Then callus development progresses to secondary rib regions of the leaf. Leaf enlargement and thickening becomes more evident. In more advanced stages, disruption of leaf parenchymas and epidermis is observed. In later phases undifferentiated mass of cells forming the callus is obtained whereas original leaf tissues are almost necrotic.

In mature leaves main veins exhibit a sheath of large parenchymatous cells packed against vascular bundles (like-bundle sheath cells). Vascular bundles are accompained by collenchyma; between phloem and xylem cambial cells are present (Fig. 1).

cytological analysis of expiants showed that, at about 3 days in culture, cambial cells of the main rib exhibit extended proliferation (Fig. 2).

Accompaning the cambial proliferation there is a marked increase in cell and nuclei volume of the cells surrounding the veins (Fig. 2 and 3). These cells remain in close contact with the first nodules of callus. Through growth of these nodules undifferentiated masses of callus are formed, causing disruption of the bundle sheath (Fig. 4).

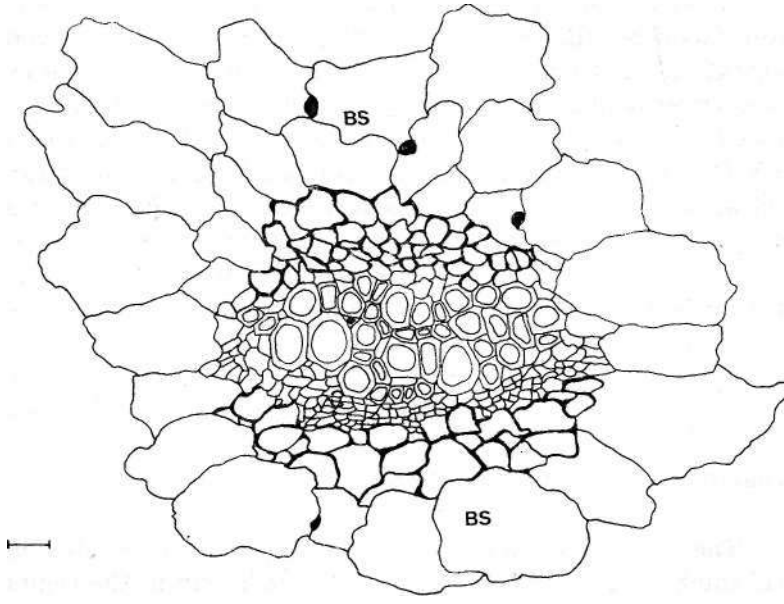


Fig. 1. — Control leaf at day zero. Cross section of the central vascular bundle showing' the like-bundle sheath (BS) and the cambium cells (C). Bar 10 u.m.

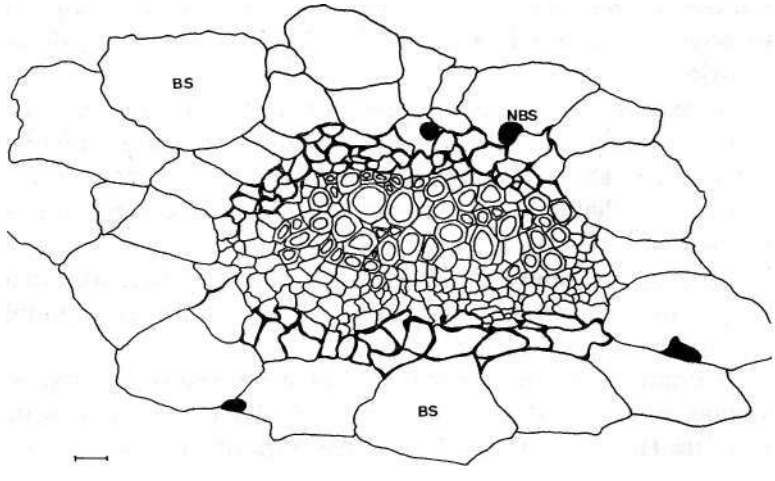
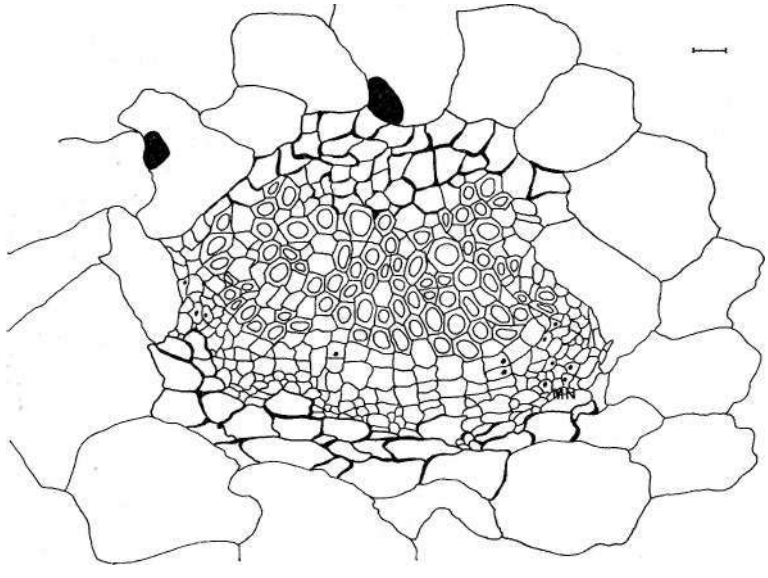


Fig. 2. — Leaf with 3 days in culture showing the proliferation of vascular cells. Note the like-bundle sheath with enlarged cells (BS) and nuclei (NBS). Bar 10 u.m.



Pig. 3. — Leaf with 5 days in culture showing general proliferation of vascular cells with meristematic nodules (MN). Note the intact bundle sheath surrounding the vascular bundle with enlarged nuclei. Bar 10 um.

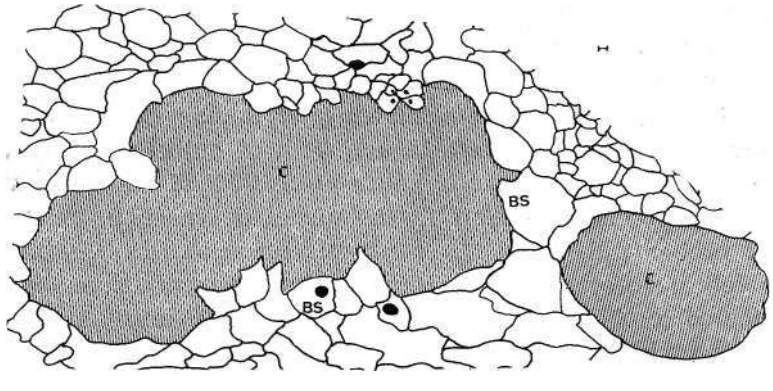


Fig. *i.* — Leaf with 7 days in culture. Note the great proliferation in the two vascular bundles forming already a callus (C). Some intact like-bundle sheath cells still exhibit enlarged nuclei (BS). Bar 10 um.

DNA Content of Leaf **and Callus** Nuclei

Fig. 5 shows DNA values expressed in arbitrary units, as measured in vascular parenchyma, leaf parenchyma, like-bundle sheath and callus cells at different days of culture.

In the control the pattern of distribution of DNA content is slightly different in the three tissues compared: vascular nuclei fell into class 2 to 4C, the parenchyma nuclei into 2 to 8C and the bundle sheath nuclei into 4 to 8C. DNA content seems to increase in the two last tissues as the culture progresses. By the fourth day values higher than 32C can be found in the like-bundle sheath cell nuclei.

After 12 days in culture the original leaf tissues are practically dirupted by the callus. By this reason results presented in Fig. 5 are only referred to callus tissue. DNA in this young callus is distributed into three classes: 2C, 4C and 8C. With 2 months of culture the endopolyploidy of the whole callus seems to increase as cell differentiation is taking place.

DNA Synthesis

The cytophotometric data are confirmed by the results of ^{3}H-Thymidine pulse treatments into leaf material during the first hours in culture. DNA synthesis was detected in vascular, parenchyma and like-bundle sheath cells and the results are summarized in Fig. 6.

The number of nuclei showing thymidine incorporation increases rapidly between 12 and 24h of culture; this rate is less marked in following hours up to 60 h of culture. This trend is specially evident in the nuclei of vascular bundles and probably denots DNA synthesis prior to the beggining of cell proliferation through mitosis. First divisions were detected at 48 h. In like-bundle sheath cells, labell at 12 h, seems to be the highest in all cells analysed. The pattern of incorporation after 24 h of culture in these two types of nuclei is similar. These results can probably be explained assuming that thymidine uptake is related to endoreduplication and mitotic cycles necessary for endopolyploidization

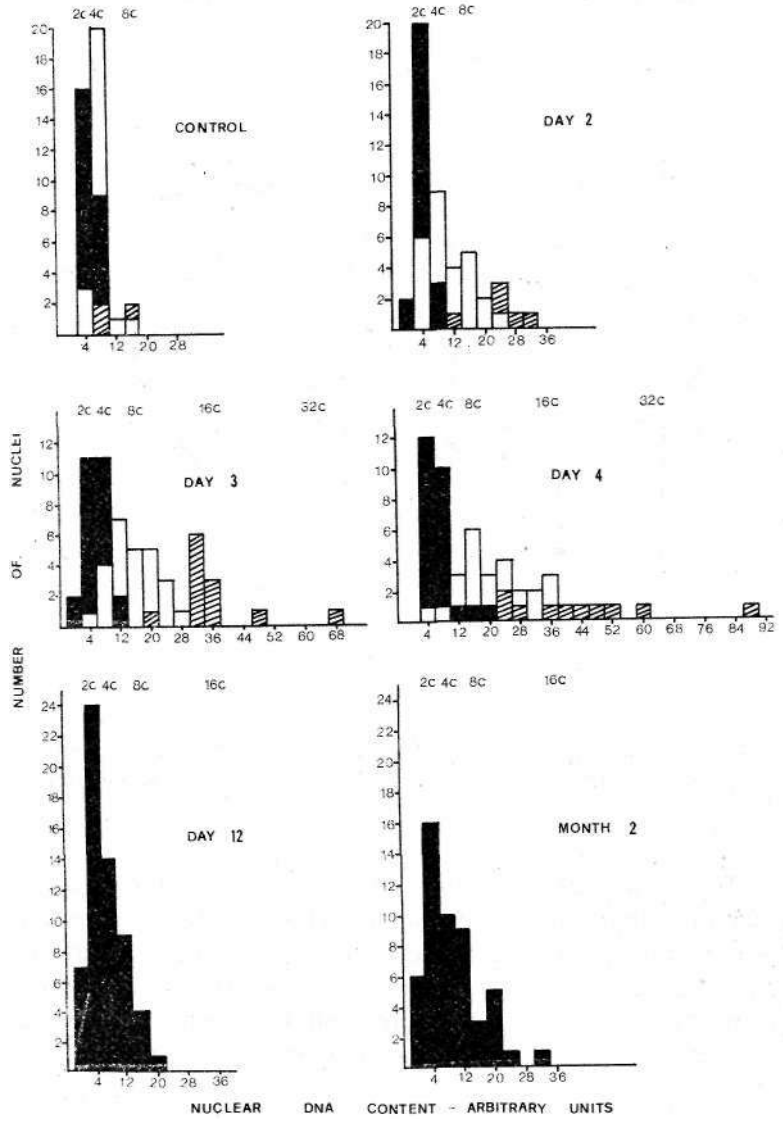


Fig. 5. — Changes of BNA content during callus induction. Black bars — vascular bundles and callus. Open bars — leaf parenchyma. Hatched bars — like-bundle sheath.

and callus proliferation of parenchyma cells close to the bundle.

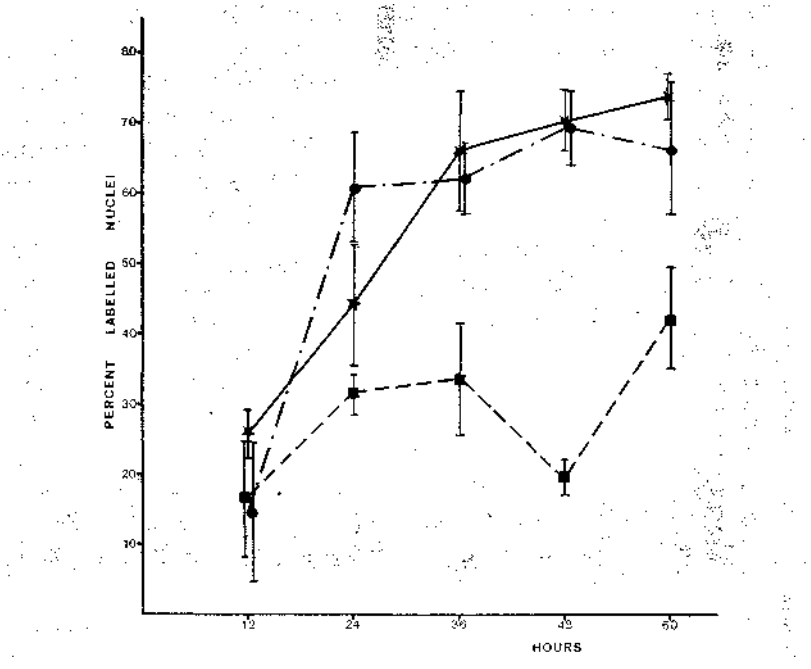


Fig. 6. — Changes of labelling pattern during first 60 hours of culture. Circles — vascular nuclei. Squares — parenchyma nuclei. Stars — bundle sheath nuclei.

In the parenchyma nuclei the increase in incorporation is smaller than the percentage reached in the vein regions. This result is in agreement with the eytophotometric analysis where only a small increase in endopolyploidy during the first days in culture can be detected before the leaf tissues start necrosis due to callus development.

DISCUSSION

The results of the present work have shown that, during the first days in culture of *L. maritima* leaf tissues, callus initiation is only restricted to the vascular meristematic cells of diploid constitution. As a concomitant phenomena,

endoreduplication was found in the parenchyma cells surrounding the veins.

At about the 2nd to 3rd day in culture we can see, by cytophotometric analysis, two processes taking place: a) mitotic cycles of the vascular diploid nuclei leading to callus formation; b) endoreduplicated cycles of the endopolyploid parenchyma cells surrounding the bundles (like--bundle sheath cells) leading to endopolyploid levels of 32C and perhaps more. General endopolyploidy in these cells seems to increase during early stages of callus development.

D'AMATO (1977) has presented evidence that an unbalanced auxin-eytokinin ratio can be causally related to callus induction «via» amitosis (nuclear fragmentation). Several recent reports have described this type of phenomena which is followed by cellularization due to wall deposition between the nuclei (NUTI-RONCHI *et al,* 1970; 1973; MARTINI *&* NUTI-RONCHI, 1974; BENNICI *et al,* 1976; CiONINi *et al,* 1978). These authors have reported regression of endopolyploidy during callus evolution due to nuclear fragmentation. This seems not to occur in our material, as endopolyploidy of the callus increase in culture.

According to our cytophotometric (Fig. 5) and autoradiographic results (Fig. 6) during the first days in culture, DNA synthesis is differently stimulated in vascular, parenchyma and like-bundle sheath cells, in response to the severing of the leaf and the hormonal and nutrient balance of the culture medium. In the vascular tissue the DNA synthesis seems to precede first mitosis leading to cell proliferation and callus formation. In the like-bundle sheat cells the synthesis of DNA seems to be related to endoreduplication cycles that accompanies callus developmemnt. In the rest of leaf parenchyma cells. some endoreduplication can also occur.

On the other hand, during callus growth we found some evidence that endopolyploidy increases (Fig. 5), a result that seems to contradict the hypothesis of nuclear fragmentation. In fact, at least during the first 60 hours of culture our autoradiographic results do not give any

support to the idea of nuclear fragmentation as have been reported in other materials (NUTI-RONCHI *et al,* 1973).

The present results show that endopolyploidy of the developed callus of *L. maritima* leaves arises «de novo», as only cambial diploid cells seem to undergo citokynesis from where callus nodules are originated. The increase of endopolyploidy in cells surrounding the nodules may be related to the production of more master copies in order to meet the heavy demands for RNA production involved in callus induction and formation (ROBERTS, 1976). As we can see in Fig. 6 the ^{3}H-Thymidine incorporation in like-bundle sheath cells at 12 hours of culture seems to be higher than in other tissues. Since no cell division was found in those cells, this probably suggests that the endoreduplication have already started at this time, representing perhaps the first indication for callus formation. We think that these results support the hypothesis that DNA synthesis in these cells may play some role in the control of the development of vascular nodules from which callus are formed.

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TYPE NUMBERS OF THE *H. J. SCHLIEßEN* COLLECTION KNOWN TO EXIST AT LISC, 1980

E. J. MENDES & J. BALSAS
Centro de **Botânica,** Junta de Invesstigações Científicas do **Ultramar**

PTERIDOPHYTA:

Actiniopteris dimorpha Pichi-Serm. —1938.
Trichomanes digitatum Sw.
var. *ulugurense* Reim. — 3028.

ANNONACEAE:

Artàbotrys rupestris Diels —1688.
Lettowianthus stellatus Diels — 5579.
Ophrypetálum odoratum Diels — 5670.
Popowia buchananii (Engl.) Engl. & Diels
var. *tricantha* Diels — 5890.
Popawia dictyoneura Diels —1686.
Uvaria decidua Diels — 5812.
Xylopia collina Diels — 5470.

MENISPERMACEAE:

Desmonema schliebenii Diels — 5838.

CAPPARIDACEAE:

Maerua schliebenii Ch. Gilg— 5266.

FLACOURTIACEAE:

Caloncoba cauliflora Sleumer —1346 & 4254.
Holmalium élegantulum Sleumer—.6111.

Kiggelaria flavo-velutina Sleumer — 3528.
Rawsonia ulugurensis Sleumer — 2795 & 2965.
Scólopia minutiflora Sleumer — 5756.

GUTTIFERAE:

Allanblackia ulugurensis Engl. — 2958.

THEACEAE:

Adinandra schliebenii Melch. — 3175.

STERCULIACEAE:

Cola discoglypremnophylla Brenan & Jones — 5433.
Dombeya trichoclada Mildbr. — 1051A.
Sterculia schliebenii Mildbr. — 5243.

TILIACEAE:

Grewia filipes Burret — 6033.
Grewia meizophylla Burret — 5876.
Grewia schliebenii Burret — 3224.

HUGONIACEAE:

Hugonia arborescens Mildbr. — 5188.

MALPIGHIACEAE:

Triaspis schliebenii Alfons Ernst — 6093.

GERANIACEAE:

Geranium schliebenii R. Knuth — 3486.

BALSAMINACEAE:

Impatiens magnifica G. M. Schulze — 4091.
Impatiens thamnoidea G. M. Schulze — 2951.
Impatiens tricaudata G. M. Schulze — 3554.

RUTACEAE:

Vepris schliebenii Mildbr. — 5759.

OCHNACEAE:

Ochna schliebenii Sleumer — 5777.
Ouratea lutambensis Sleumer — 6110.

DICHAPETALACEAE:

Dichapetalum schliebenii Mildbr. — 5344.

OLACACEAE:

Olax pentandra Sleumer — 5205.

AQUIFOLIACEAE:

Ilex mitis (L.) Radlk.
var. *schliebenii* Loes. — 3529.

VITACEAE:

Ampelocissus schliebenii Werderm. — 6040.
Cissus egestosa Werderm. — 6136.
Cissus macrantha Werderm. — 6216.
Cissus viniferoides Mildbr. —1840.

MELIANTHACEAE:

Bersama gracilipes Mildbr. —1354.

CONNARACEAE:

Vismianthus punctatus Mildbr. — 5757.

LEGUMINOSAE (Caesalpinioideae):

Gopaifera schliebenii Harms — 6123.
Erythrophloeum africanum (Welw.) Harms
var. *stenocarpum* Harms — 6536.
Hoffmanseggia insólita Harms — 5682.

LEGUMINOSAE (Mimosoideae):

Acacia joachimii Harms — 5636.
Acacia schliebenii Harms — 5565.
Xylia schliebenii Harms — 5752.

LEGUMINOSAE (Papilionoideae) :

Afrormosia schliebenii Harms — 5588.
Craibia schliebenii Harms — 3192.
Crotàlaria pterocályx Harms — 5781.
Crotalaria schliebenii Polhill — 2371.
Dalbergia acariaeantha Harms—'5508.
Erythrina schliebenii Harms — 5237.
Indigofera latibracteata Harms — 2458.
Ormocarpum schliebenii Harms — 6038.
Platysepalum inopinatum Harms — 5392.
Rkynchosia calobotrya Harms — 6183.
Rhynchosia ischnoclada Harms — 5614.
Vigna ulugurensis Harms — 3235.

COMBRETACEAE:

Combretum schliebenii Exell & Mildbr. — 6480.
Combretum stenanthoides Mildbr. — 5212.

MYRTACEAE:

Syzygium parvulum Mildbr. — 3922.

MELASTOMATACEAE:

Dissotis schliebenii Markgraf — 6068.
Gravesia riparia A. & R. Fernandes — 3424.
Memecylon lutambense Markgraf — 5688.

PASSIFLORACEAE :

Adenia dolichosiphon Harms — 6001.
Adenia lindiensis Harms — 6066.
Adenia schliebeni Harms — 5975.
Paropsia schliebeniana Sleumer — 5442.

CUCURBITACEAE :

Coccinia ulugurensis Harms — 3643.
Momordica pycnantha Harms — 5932.
Momordica schliebenii Harms — 3660.

BEGONIACEAE:

Begonia stolzii Irmsch. —135.

RUBIACEAE:

Pavetta schiebenii Mildbr. ex Bremek. — 5845.
Pavetta tendagurensis Bremek.
var. *glabrescens* Bridson — 5821.
Psychotria castaneifolia Petit — 3342.
Psychotria cyathicalyx Petit — 4649.
Psychotria megistantha Petit —3414.
Psychotria pseudoplatyphylla Petit — 4362.
Psychotria schiebenii Petit —• 1733.
Rutidea orientalis Bridson —1554.
Tapinopentas ulugurica Verde. — 2730.

CAMPANULACEAE:

Lobelia dealbata E. Wimm. — 2798.
Lobelia giberroa Hemsl.
var. *iringensis* E. Wimm. — 1400.
Lobelia saliensis E. Wimm. — 2033.
Lobelia usambarensis Engl.
var. *hispidella* E. Wimm. — 2921.
Lobelia unamata E. Wimm. — 2934.

PRIMULACEAE:

Anagallis schliebenii R. Knuth & Mildbr. —1420.

MYRSINACEAE:

Rapanea gracilior Mildbr. — 3921.
Rapanea schliebenii Mildbr. — 3591.

SAPOTACEAE:

Mimusops acutifolia Mildbr. — 6102.
Mimusops aedificatoria Mildbr. —• 3896.
Mimusops schliebenii Mildbr. & Schulze — 2520.

OLEACBAE:

Jasminum ellipticum Knobl. — 5991.
Jasminum stolzeanum Knobl. — 5955.
Jasminum tomentosum Knobl.
var. *lutambense* Knobl. — 5558.
Olea kilimandscharica Knobl. — 5065.
Olea schliebenii Knobl. — 3553.

ASCLEPIADACEAE:

Tylophora gracillima Markgraf — 3067.

STRYCHNACEAE:

Strychnos angolensis Gilg
var. *tanganykae* Duvign. —1932.

GENTIANACEAE:

Urogentias ulugurensis E. & Ch. Gilg — 2786.

CONVOLVULACEAE:

Ipomoea heterocalyx Schulze-Menz — 3250.
Ipomoea lutambensis Schulze-Menz — 6181.
Ipomoea microcalyx Schulze-Menz —1947.
Ipomoea trinervia Schulze-Menz — 6285.

SOLANACEAE:

Solanum inaequiradians Werderm. — 2707.
Solanum lignosum Werderm. — 3150.
Solanum schliebenii Werderm. — 3415.

BUDDLEJACEAE:

Adenoplusia ulugurensis Melch. — 2756.

SCHROPHULARIACEAE:

Gradería iringensis Melch. —1414.

GESNERIACEAE:

Didymocarpus stolzii Engl.
var. *minor* Mansf. — 3421.
Linnaeopsis subscandens B. L. Burtt — 2936.
Saintpaulia inconspicua B. L. Burtt — 3068.
Streptocarpus bambuseti B. L. Burtt — 4094.
Streptocarpus glandulosissimus Engl.
var. *longiflorus* Mansf. — 4094.
Streptocarpus bullatus Mansf. — 3586.
Streptocarpus minutiflorus Mansf. — 3585.

ACANTHACEAE:

Chlamydostachya spectabilis Mildbr. — 3755.
C*rabbea longipes* Mildbr. — 6004.
Dicliptera insignis Mildbr. — 5240.
Dicliptera olitoria Mildbr. — 2295[1].
Dischistocalyx pubescens Lindau
var. *longipilosus* Mildbr. — 3568.
Isoglossa oreacanthoides Mildbr. — 2983.
Isoglossa schliebenii Mildbr. — 4102.
Justicia dolichopoda Mildbr. — 5847.
Justicia psammophila Mildbr. — 3309.
Pseuderanthemum campylosiphon Mildbr. — 2810.
Pseudoblepharis insignis Mildbr. — 5871.
Schliebenia secunda Mildbr. — 5394.

VERBENACEAE:

Clerodendrum formicarum Gurcke
var. *sulcatum* Thomas — 3217.
Clerodendrum johnstonii Oliv.
var. *sulcatum* Thomas — 4130.

[1] In its protologue, *Notizvl. Bot. Gart. Mus. Berl* 11: 1085 (1935), only one unnumbered and undated gathering is quoted: «Bez. Mahenge: Ngombe, ca. 400 m.ü.M., Parklandschaft. Kraut, meist kriechend. Blüten lila. Auf Kipogoro: l u m b u t s c h u l u . Blätter werden als Gemüse gegessen». The DISC specimen is labelled as follows: «Mahenge-Bezirk c. 500 m.ü.M. Ngombe, Parklandschaft, Kraut c. 40-60[1] cm, häufig, Bl. lila. 9.6.1932. H. J. Schlieben 2295».

AMARANTHACEAE :

Cyatula divulsa Suesseng. — 2245.

HYDROSTACHYACEAE :

Hydrostachys insignis Mildbr. & Reim. — 111OA.

THYMELAEACEAE:

Peddiea subcordata Domke — 3092.

EUPHORBIACEAE:

Omphalea mansfeldiana Mildbr. — 5289 & 6206.
Ricinodendron gracilior Mildbr. — 5669.
Ricinodendron viticoides Mildbr. — 5590.

BUXACEAE:

Notobuxus obtusifolius Mildbr. — 5818.

ORCHIDACEAE:

Aërangis schliebenii Mansf. ex Schlieben — 6419.
Liparis latialata Mansf. — 3091.
Microstylis schliebenii Mansf. —1848.
Polystachya convallarioidea Mildbr. — 2743.

ZINGIBERACEAE:

Aframomum laxiflorum Loesener ex Lock — 3094.

IRIDACEAE:

Gladiolus trichophyllus Diels — 1136A.

LILIACEAE:

Antherieum collinum v. Poellnitz — 3183.

COMMELINACEAE:

Coleotrype brueokeriana Mildbr. —1910.

CYPERACEAE:

Cladium flexuosum (Boeck.) C. B. Clarke
var. *polyanthemum* Kukenth. — 6139.

C-RAMINEAE:

Andropogon lindiensis Pilger — 6290.
var. *hirsutissima* Pilger — 6447.
Andropogon chirensis Höchst. — 6075.
Andropogon spanianthus Pilger —1015.
Aristida cumingiana Trin. & Rupr.
var. *reducta* Pilger—-2468.
Aristida schliebenii Henr. — 2317.
Beckera scabra Pilger — 999.
Brachiaria coronifera Pilger — 439; 768 & 1031.
Digitaria comifera Pilger — 6151.
Digitaria gymnostackys Pilger — 6267.
Digitaria ulugurensis Pilger — 3640.
Eragrostis adenocoleos Pilger—6381.
Eragrostis blastocaulos Pilger — 1029.
Eragrostis lukwangulensis Pilger — 3545.
Eragrostis muerensis Pilger — 6289.
Eragrostis setulifera Pilger — 2318.
Hyparrhenia iringensis Pilger — 828.
Melinis inamoena Pilger — 736.
Panicum adenophylluni Pilger — 6064.
Panicum issongense Pilger — 2130.
Panicum, lindiense Pilger—-6243.
Panicum lukwangulense Pilger — 3520.
Panicum- maximum Jacq.
var. *pübiglume* K. Schum. — 3737.
Panicum microlemma Pilger — 6231.
Perotis leptopus Pilger — 2316.
Sckizackyrium inspersum Pilger —1025.
Stenotapkrum diplotapkrum Pilger —6545.

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THE STRANGE HISTORY OF *HERMAS PILLANSII*

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ABSTRACT

It is argued that *Scabiosa Janata* Hill (1763) is based wholly on the illustration published by J. BURMAN (1739) as *Scabiosa hirsuta, foliis nervosis rubrotundis, floribus proliferis.* It is further argued that the plant originally illustrated was *Hermas pïllansii* Norman (Umbelliferae). The validity of Hill's name is questioned.

THERE are three components to this story. The first is an illustration made at the Cape of Good Hope about 1685 and reproduced by BURMAN in 1739: the second is *HILL'S* description and plate of *Scabiosa Janata* (1763) : the third is *Hernias pillansii* C. Norman (1928). I shall argue that these three components all refer to a single species. The eclipse of this species between 1763 and 1928 supplies one of the strange elements in the story: the relation between Hill's account and that of BURMAN supplies the other.

The illustration was one of those drawn by HENDKIK CLAUDIUS for NICHOLAAS WIESEN of Amsterdam and it came to form part of the collection known as the *Codex Witsenii.* T have not seen the original; the information I use here comes from BURMAN'S reproduction *(Rar. Afr. Pl. dec.* 8, tab. 72, fig. 3; 1739) and his comments, some of them quotations from the original notes in *Codex Witsenii.*

In the *Codex* this illustration (cf. fig. 1) was labelled *Angelica africana, Montana, odorata, floribus viridibus.* That is, it had been recognized as a member of Umbelliferae, was aromatic, and had green flowers: a perfectly possible plant.

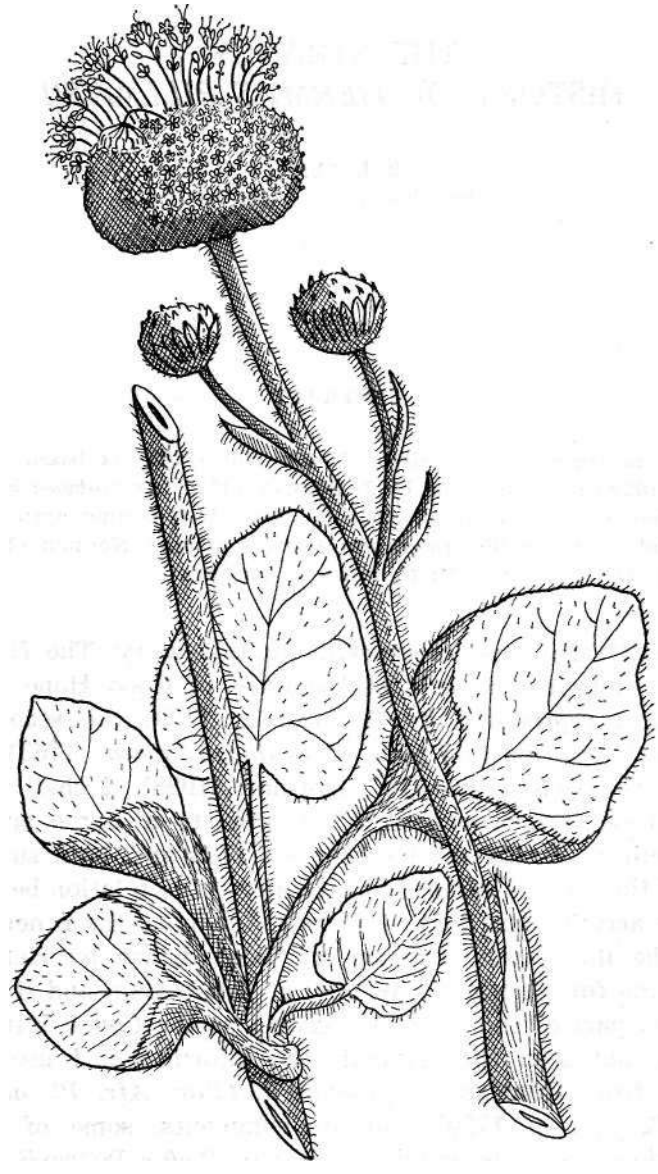

Fig. 1. — *Scabiosa hirsuta, foliis nervosis subrotundis floribus proliferis;* copied from BURMAN, Rar. Afr. Pl. dec. 8, tab. 72, f. 3 (1739).

Admittedly the woolly cordate entire leaves may have seemed odd for Umbelliferae, though, except for the indumentum, both the leaves and the tight inflorescence might have evoked some faint memory of *Eryngium alpinum*, with a touch of *Astrantia* about the head. Be that as it may, BURMAN decided that the plant could not belong to Umbelliferae. He consulted GARCIN and between them they decided it must be a scabious: *Scabiosa hirsuta, foliis nervosis subrotundis, floribus proliferis* was the name BUKMAN gave it. It may be mentioned that a search in all likely places in the BURMAN herbarium at Geneva failed to produce any specimen of the plant; but this was not unexpected as, at the time of writing, BURMAN clearly had none and was working entirely from the plate and accompanying notes.

In 1763 JOHN HILL published volume five of his *Vegetable System*, and this volume includes scabiouses. Here we find (tab. 33) *Scábiosa lanata*, a name not previously published. HILL gave an account of this plant, and said that BURMAN alone of earlier botanists had known it. He does not give a precise reference, but the plant we have just been discussing was the only *Scabiosa* described by BURMAN, so there is no doubt about this. If there had been, a comparison of the plates would soon dispel it, for there are some remarkable likenesses (cf. fig. 2 with fig. 1). The resemblances in the leaves are so striking that HILL'S look very much as though they were copied from BURMAN. The heads on HILL'S plate are flatter and look more like those of a scabious; however it is remarkable that where the plates diverge HILL'S follows not BURMAN'S plate but BURMAN'S text comments. BURMAN said the plant was a scabious, and HILL'S illustration looks in general like one: BURMAN said the flowers were green and HILL'S plate shows green flowers. It is difficult to believe that Hill's plate is not a copy based on BURMAN'S but modified to fit BUKMAN'S text. Thus one must ask, did HILL ever have a specimen of this plant? His description admittedly includes much circumstantial detail, such as reference to the springiness of the indumentum on the stem, but when it has been carefully studied one can only conclude that it really adds nothing to the information given

Fig. 2. — *Scabiosa lanata* Hill; based on the plate in *HWL,* Vegetable System (folio ed.) 5: t. **33 (1763), X c % .**

by BURMAN. Hill, said that his plant, unlike BURMAN'S, was not proliferous. Nevertheless he shows a detached head, scabious-like as are the others, but proliferous. I therefore consider that HLLI/S illustration and description of *Scabiosa Janata* was based solely on BURMAN'S plate and description, not on a plant or specimen in his own possession.

This suggestion is not so outrageous as it may seem. Writing of another work by HILL, *Eden or a compleat body of gardening,* HENREY (1975, 2: 97-98) notes that «a number of the figures have been copied from the Hortus Floridus of Crispian van de Paase the Younger». For *Eden,* as for the *Vegetable System,* the drawings were advertised as being «all made from nature by Dr. HILL». That this one plate in the *Vegetable System* should have been based on a previously published illustration, rather than on a specimen, is therefore not unthinkable.

Further evidence that HILL had no specimen of *Scabiosa lanata* is that the plate lacks a drawing of a single flower removed from the head: this is present on almost all the other plates of *Scabiosa.* It is clear, from the introductory discussion of volume five, and from plate 1 (showing the details of the flower head), that HILL understood the structure of a scabious quite well. Had he seen a specimen of this plant he would have quickly realized that it was wrongly placed in *Scabiosa.*

The real test of this argument clearly lies in finding the plant that these plates are intended to portray. There is no known *Scabiosa* that is strongly aromatic, or has thick woolly entire cordate basal leaves or has green flowers, either in South Africa or anywhere else. However, as recently as 1928 the late CECIL NORMAN described a 'new' species of the umbelliferous genus *Hermas, H. pillansii,* from the Cape Peninsula. It bears a very fair resemblance to CLAUDIUS' illustration as reproduced by BURMAN. This plate shows several leaves with the margins infolded towards the base, but there is one at the back of the figure which is flat and cordate: this is the typical leaf-base of *Hermas pillansii.* The flower head in *H. pillansii* is not so dense as in BURMAN'S plate, but that may be because it is not proliferous.

Hermas pillansii grows on mountain cliffs and has roots smelling strongly of carrots. BURMAN, quoting from the notes in *Codex Witsenii,* gives the habitat as «scarcely approachable rocks» and says the smell is so strong that it will last in a room for a week after the plant has been removed. *Hermas pillansii* has yellowish green flowers; BURMAN writes 'floribus viridibus' in his phrase name, but quotes 'ex luteo virentes' in the text.

H. pillansii is distinguished from the other species by the umbels being round-topped (ADAMSON & SALTER, 1950, p. 615). CLAUDIUS' illustration shows a round-topped head. I believe there can be no doubt that the plant drawn by ClAUDius about 1685 is *Hermas pillansii,,* described by NORMAN in 1928. *Scábiosa lanata* Hill is the same species: the différences in his plate, that make the plant look more like a scabious, were not taken from a specimen but from his own imaginative interpretation of BURMAN'S comments and name.

It must be mentioned that BURMAN'S illustration is cited by SONDER (in HARVEY & SONDER, 1862, 567) under *Hermas ciliata,* without comment. However, it cannot be that species, which differs, amongst other things, in having glabrous stems. The only other possibility, apart from *Hermas pillansii,* is *H. gigantea* L. f.; however this species has a much longer, oblong leaf-blade which is white-woolly on both sides; BURMAN clearly says his plant has leaves green above white-woolly below. *Hermas pillansii* has leaves sparsely villous above (and thus appearing green) and white-woolly below. This seems to be the only possible identification for the plant.

These conclusions lead to a very awkward nomenclatural situation. Must HILL'S epithet *lanata* now be adopted in *Hermas* to replace *H. pillansii?* First, what is the type of HILL'S name? On the evidence given above it can only be CLAUDIUS' illustration. Neither BURMAN nor HILL added anything more to that illustration than we could add looking at it today. It is an illustration without dissections: of itself it could not validate a name. Why then should *8. lanata* Hill be valid because HILL wrote down what he saw by looking

at the plate? My only hesitation about rejecting Hill's name as invalid, is the uncertainty whether the principle could be unduly extended and cause chaos. Certainly a description that only records vegetative features has to be accepted in principle: in practice it may well have to be rejected if no type specimen is extant and especially if it has been placed under the wrong genus. However it would then be *nomen dubium* not *nomen invalidum.*

Nevertheless my hesitation about outright rejection does not permit me to go to the other extreme and accept Hill's name as valid. At the moment I throw it into the arena tagged *'nomen invalidum?:* let us see what the nomenclatural lions make of it.

Thus we have the following situation:

Hermas pillansii C. Norman in Journ. of Bot. 66: 195 (1928) ; Adamson & Salter, Fl. Cape Penins. 616 (1950).

Syn. : *[Angelica africana, montana, odorata, floribus viridibus* Codex Witsenii (n. v.)].

[Scabiosa hirsuta, foliis nervosis, subrotundis, florïbus proliferis Burman, Rar. Afr. Pl. dec. 8, 199, tab. 72, fig. 3 (1739)].

[Scabiosa *lanata* Hill, Syst. Veg. (folio) 5: 46, tab. 33 (1763), (ed. 2) 5: 46, tab. 33 (1772); (quarto) 5: 62, tab. 33 (1763); (octavo) 5: 86, tab. 33 (1763)—nomen invalidum?].

It will be noted that I have quoted HILL'S Vegetable System in fólio, quarto and octavo editions. The folio and octavo are described both in HENREY (1975, 3: 56-59) and in STAFLEU & COWAN (1979: 202-203, 1979). The quarto is not recorded in either of these works, but there is a copy in the library of the Royal Botanic Garben, Edinburgh, and another in that of the University of Edinburgh. Mr. M. V. MATHEW (librarian at the Royal Botanic Garden, Edinburgh) will publish a bibliographical account of this edition shortly.

I am indebted to Miss R. M. SMITH for the preparation of the figures.

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CHROMOSOME STUDIES ON *TRILLIUM KAMTSCHATICUM* PALL.

XXXI. ON THE LIPID AS ONE OF THE CHROMOSOME CONSTITUENTS

by

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DURING the last decade much attention has been paid by a number of cytologists to the problems surrounding DNA which is the main element of chromosome constitution. On the other hand, however, it has generally been suggested that lipids may play an important role as a constituent of protoplast, through so many works on the so-called «liposomes». On this basis, I attempted to demonstrate first the existence of lipids within DNA or chromosomes and secondly to invoke a role for them as binding substances. Up to the present, I am not aware of such an experiment as shown in the present paper, though a few workers demonstrated histochemically the presence of lipids in chromosomes, and WILKINS and his associates (cf. BUSH, 65, p. 105) suggested that sphingomyelin or other lipids are present in deoxyribonucleoprotein preparations from their X-ray diffraction studies.

MATERIAL AND METHODS

Some scores of corms of *Trillium kamtschaticum* grown in our experimental garden were collected on late November

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1965 for materials of the present work. The PMCs at this date proved to he at leptotene to zygotene of the first meiotic division. When whole anthers are put on water-wet blotting paper in petri-dishes, meiosis in their PMCs proceeds in normal fashion and attain to tetrad stage about one week later.

With the object of pulling lipid out of chromosomes, these dishes were put in a vacuum dessicator whose strength of diminution in air pressure is 14 mm Hg. by the exertion for 4 minutes. Then they are reverted to usual atmosphere condition for about half an hour, and further they are again subject to the same procedure. In the present work, this repeating was carried out in four classes, 3, 5, 8 and 12 times. For PMCs of each class, were made acetocarmine preparations pretreated with water (MATSUURA '38) and observations were done under a phase-contrast microscope. Besides PMCs, the tapetum cells and Plasmodium were also supplementally observed.

OBSERVATIONS

Myelin formes

The preparation made immediately after the diminished pressure procedure are always characterized by the occurrence of myelin forms which lie in the medium surrounding PMCs or tapetum Plasmodium (Text-figs. 1-3). As already described by many workers (cf. YASUI '38), their forms are various, thin thready, headed thready, spherical, bladder-like, rodshaped, spirally coiled, etc. They are instantly changing their forms, being swollen, adsorbing water and about two days lather they fuse together making a very thin film on the surface of the medium (Text-fig. 2).

The myelin makes a strong contrast to the lipid in chromosomes in that the former is easily pulled out of the cells, whereas in the latter stronger exertion of the procedure is required for pulling it out of chromosomes, thus suggesting that the former exists as free drops, while the latter is strongly combined with chromosome structure. In

the present work, it was shown that at least 12 times exertion of the procedure is necessary for this purpose.

The decomposition oí nuclear envelope, nucleolus and cell-wall

One of the most striking features of the malignancy is the absence of nuclear envelope, nucleolus and cell-wall as well (Plate I and others). These malignant features

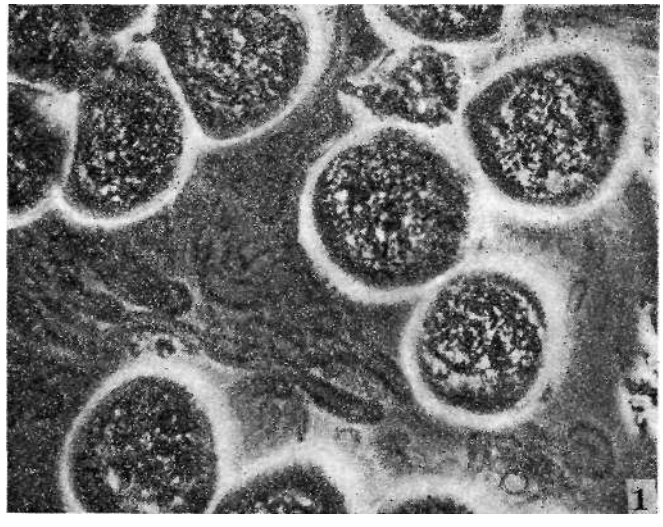
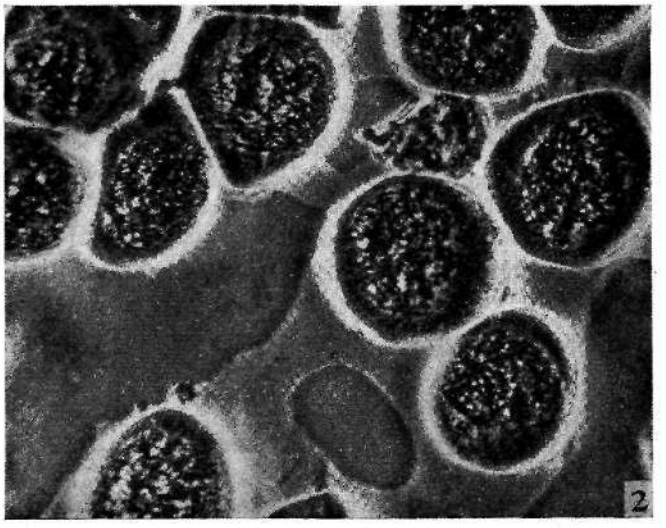
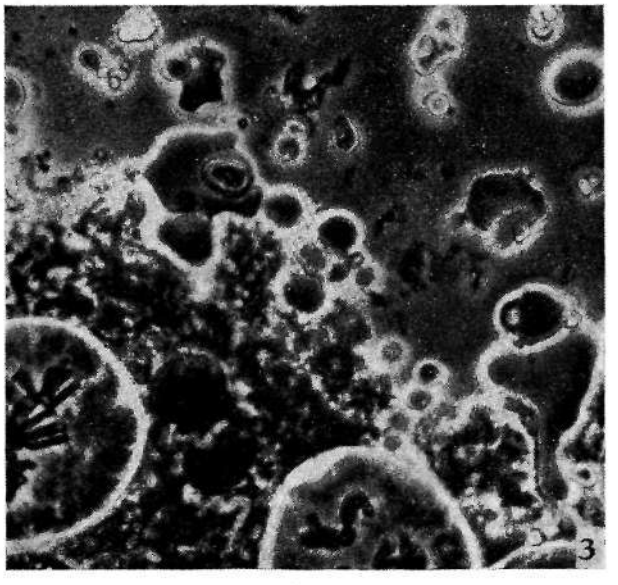


Fig. 1. — Myelin forms in acetocarmine medium surrounding PMCs at meiotic prophase. The same scale as figs. 2 and 3.

continue during the whole cycle of meiosis and the following mitosis, so indicating that these structures or cell organs are to be considered as those holding identity, as do the chromosomes and the other organelles. Also it is conceivably suggested that these malignancies are so related one another in function by the absence of their existence and hence their function. Furthermore they may have common effects on the other cellular activities. Thus the appearance of myelin forms as mentioned above may owe to lipid drops streamed out of these structures. It is also interesting to note, in





Figs. 2 & 3. — 2, the same region as 1, 50 hrs later. 3, represents myelin forms originated from tapetum Plasmodium. Calibration bar equals 50 $_{u}$m.
All to same scale.

such naked nuclei as well naked cells how are deformed the mode of cell division and of chromosome structure and its behavior.

«Lipidated» chromosomes

The «lipidation» of meiotic chromosomes is recognizable as the eruptions all over the chromosomes. They appear either as blight whitish patches or coats covering the chromosome surface or as larger globular clusters at arm ends, both distal and proximal (viz. the kinetochore region). Such a specified localization of lipid gatherings seems to give direct evidence for that these are intrinsic ones of chromosomes, not the additives of external ones; that is, these lipid substances which are most probably lipoproteins are regarded as one of chromosome constituents.

Such «lipidated» chromosomes act like hitherto known sticky ones of a mutationally arisen individual, being similar in that they give rise to sticky adhesions between two or more chromosomes and to the formation of «sticky bridges» and fragments at anaphase when the chromosomes or chromatids associated in such adhesions move to opposite spindle poles. Here, however, the degree of stickiness is much greater than that in naturally found cases. Very frequently several chromosomes adhere together in complex fashion, so making impossible to analyze the chromosome identity.

In the preesnt case, the adhesions usually occur between an arm end and another end, between an end and an interstitial part and between an end and a kinetokore region, as diagrammatically represented in Text-fig. 4.

These types of adhesions give rise to corresponding types of fragments which are categorized as free fragments *(abbr.* f), a fragment attached to chromosome end (fe), a fragment attached to interstitial chromosome part (fi), and fragment attached to kinetochore (fk). Besides these, there are more complicated adhesions, such as multiple fusion involving more than three arms at one point, fusion along entire arms, adhesives covering an entire bivatent, etc. Moreover the fragmentation may occasionally involve entire

arms, breakage of the chromosome occurring at a locus close to the kinetochre (F). Thus F fragmentations may distinguish correspondingly to the f ones as F, Fe, Fi and Fk.

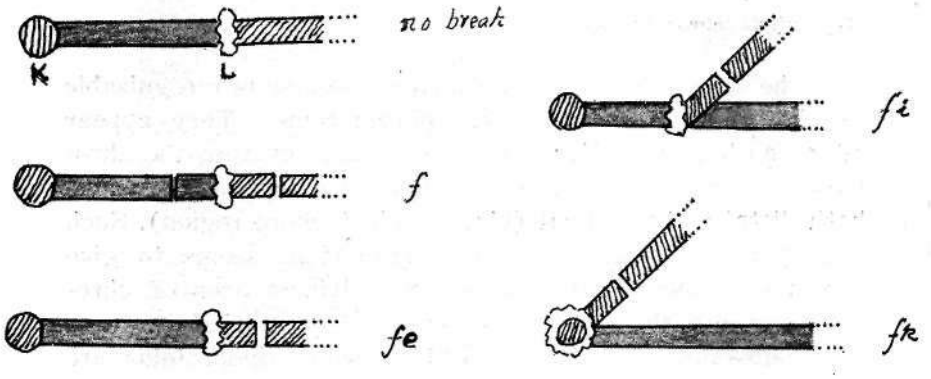


Fig. 4. — Diagrammatic representation of the origin of f, fe, fi and fk types in «lipidated» chromosomes. K stands for kinetochore '.<•', and L for lipid cluster.

The frequency of these fragments was recorded in the total 50 cells in which each member of the complement was analyzable (Table I).

By the way, the frequency of primary chiasmata (cf. MATSUURA, '41) in those 50 cells was only 18, that is 3.8 % per cell indicating much lower frequency of chiasma formation as compared with that in the control material.

TABLE I

Frequency of various types of fragments at MI

Total cell no.	f	fe	f;	(k	F	Fe	Fi	Fk	Total
50	**2**	**4**	**1**	**1**	**3**	**1**	**—**	**2**	**14 (28%)**

Next, concerning the deficiencies in nucleokinesis at Meta-and Anaphase I, the following facts are noteworthy, (i) Variable modes of chromosome spiralization (Plate I, 2-11). In most cells the spiralization is distinctly much larger in dimension as compared with that of the control. In some

cells, however, they are much thiner and more elongated. Such variable effects on the chromosome structure seem to be due to depend on somewhat decrease »in viscosity of chromosome matrix owing to the deff iciency of lipid materials resulting in larger chromonema spirals as well as somewhat decrease in chromosome matrix formation itself yielding smaller spirals, (ii) Scattering location of the chromosomes over the equational plate (Plate I, 9, 10). This is regarded to be due to the absence of the nuclear envelope and also to the discrepancy in structure and function of atractoplasm. (iii) Non-disjunction of bivalents, seemingly depending upon the same above mentioned reasons (Plate I, 12, 13). (iv) Asynchronization of nucleokinesis between different cells as in different chromosomes within the same cell (Plate I, 8). This lack of unification in núcleo- or chromosome-kinesis is regarded as also due to the lack of nuclear envelope. Lastly (v) Apparently rare occurrence of bridges and fragments at anaphase I (Text-fig. 5). As described above, the breakage of chromosome at the adhesion regions is of rather meagre occurrence in spite of numerous adhesions and too the frequency of fragments at anaphase is very much smaller than that expected. Being different from the usual breaks followed by exchanges between homologuous chromosomes, here the break points are masked by lipid clumps at metaphase, being not easily distinguishable as breaks, and at anaphase such fragments may go to the pole together with the attached chromosomes, instead remaining on the equatorial plate as usual (Plate III). This is the reason why in sticky chromosomes breaks and fragments are scored as less frequent ones.

«Pollen Plasmodium» and crystallization of chromatin

At anaphase I, some half-bivalents tend to aggultinate together as shown in Plate I, 8. Such a tendency is more clealy distinguished at further stages, where the chromosomes enter individually into interphase, making several chromatin masses of different sizes (Plate I, 14, 15). These deformed nuclei have no nuclear envelope and no nucleolus

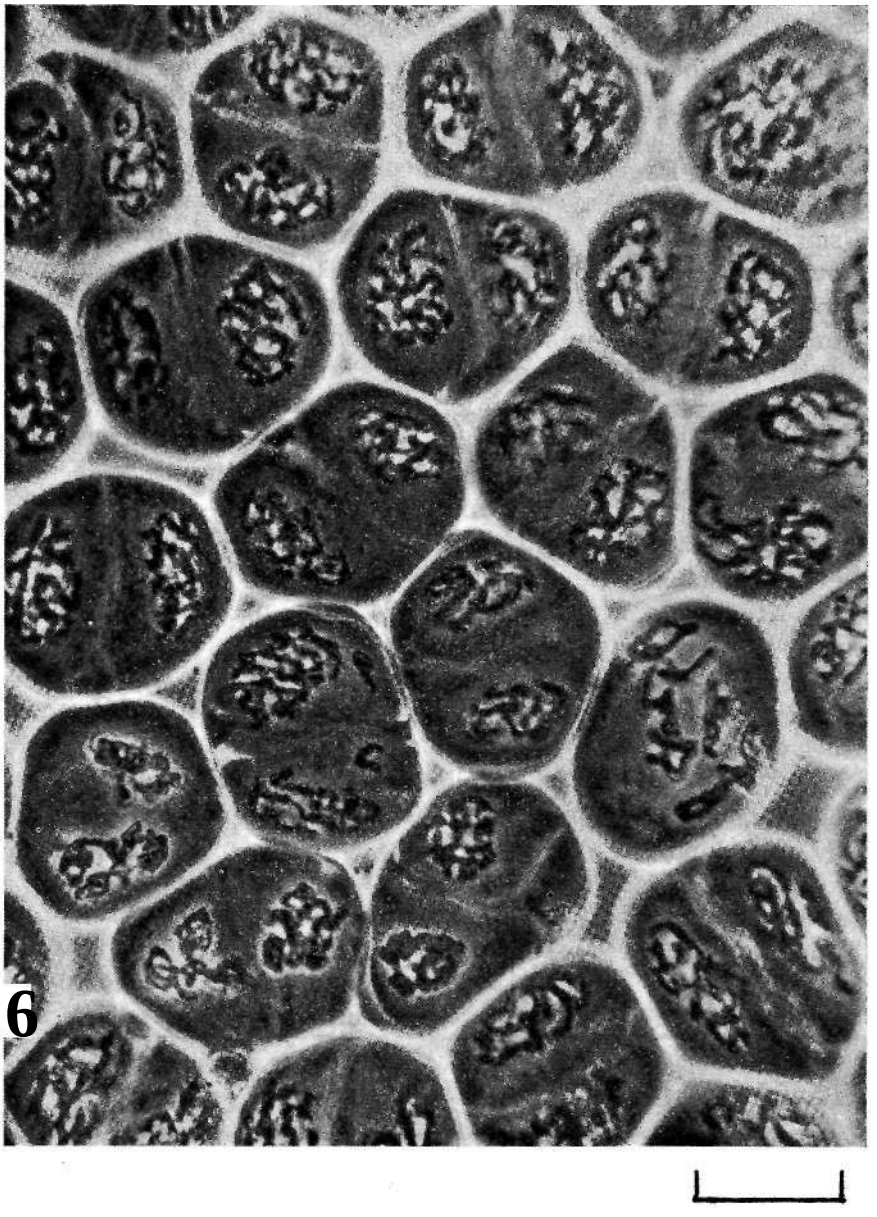


Fig. 5. — A group of PMCs at anaphase I in this material, indicating how bridge and fragment formation is of rather rare occurrence in spite of sticky configurations occurring at metaphase I. Calibration bar equals 50 urn.

as well. Furthermore at these stages the formation of cell wall and callose is severely deficient, so inducing the Plasmodium instead of the tetrads. Such «pollen Plasmodium» becomes bigger by fusing together with neighboring pollen Plasmodium. The chromatin aggultination proceeds further and gives rise to definite conical bodies, each of which contains much compressed chromatin mass at its center and is enveloped by thick lipid rim (Plate IV, 3). Later on, these bodies are subject to strong elongation and the inside chromatin comes to change into a number of microfibrils which are arrayed in the bundle (Plate IV, 6). Since chromatin deprived of lipid is considered to be nucleoprotein, these phenomena are assumed to be its crystallization and growth. In fact, many true crystals of various sizes are observable within the pollen Plasmodium. These crystals are always covered by thin lipid film («Plate *TV*, 6-8). The bundle of these microfibrils are broken down transversally at nearly equal intervals (Plate IV, 5). Such breakage seems to proceed further and lastly severe pulverization takes place untill pulverized particles become so small as they can not be caught by a light microscope (Plate IV 9).

In comparison of these series of events in the pollen plsmodium were studied with those in the tapetum Plasmodium. The results indicated that the both were entirely identical with each other (Plate V).

COMMENTS AND SUMMARY

By means of putting the anther of *Trillium kamtschaticum* under strongly diminished air pressure, many malignant symptoms were found to occur in cellular activities in general, nucleokinesis and chromosome structure and its behavior. The symptoms are related to the lack or defficiency of lipid substances. They are listed as follows:

i) The appearance of myelin forms,
ii) The decomposition of nuclear envelope, nucleolus and cell-wall.

iii) The occurrence of nuclear extrusion[1].
iv) The formation of «lipidated» chromosomes,
v) The formation of «pollen» plasmodium.
vi) The crystallization of chromatin substances and their severe pulverization.

Based on a chain of these events, it was concluded that the lipid is an important element of chromosome constituents, and play an important role for the maintenance of chromosome organization.

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[1] This topic was not be dealt with in the present paper. The concerning data will be published in another paper of this series.

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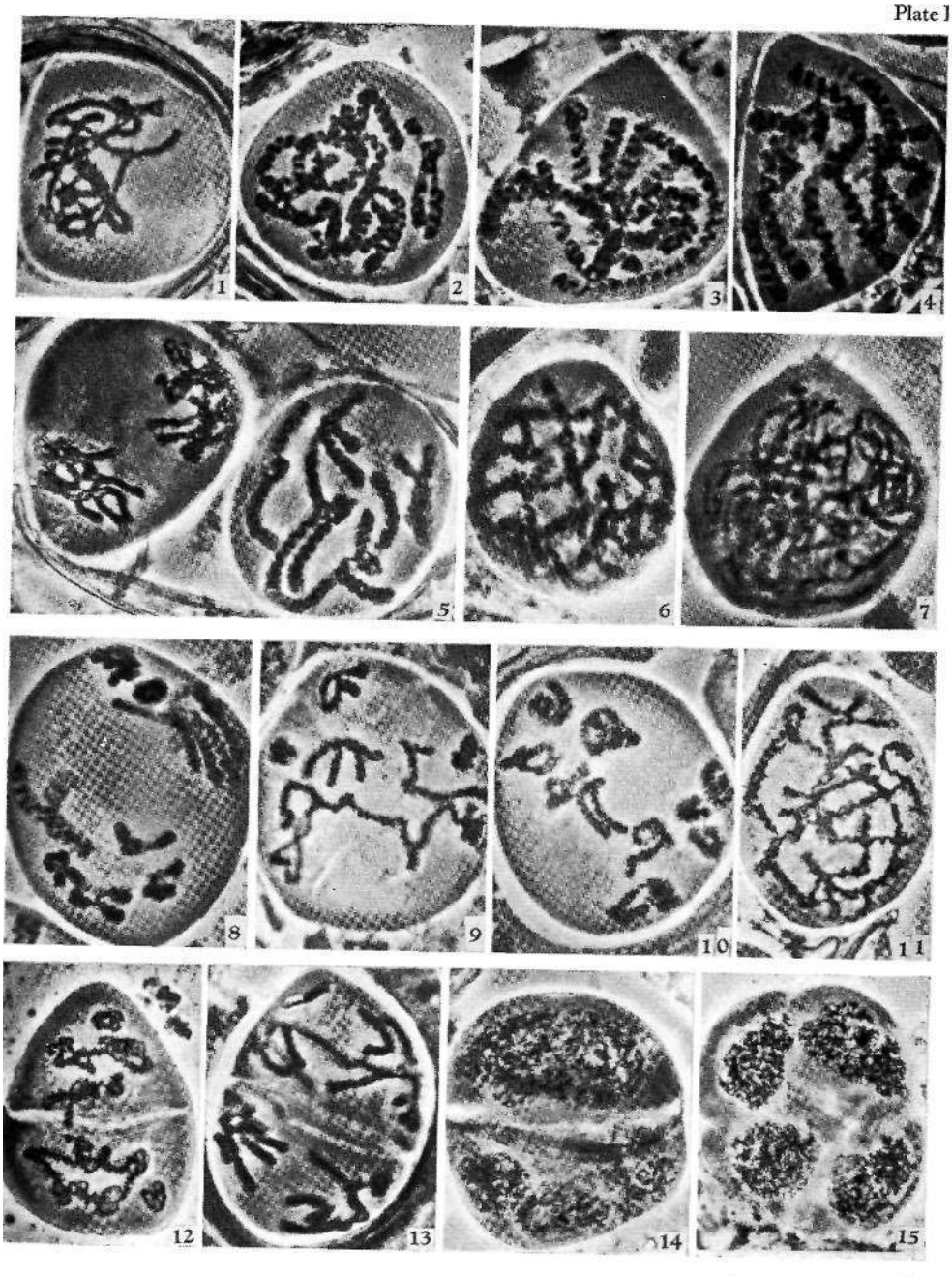
PLATES

PLATE I

Demonstrating how PMCs deprived of lipids are maligrant in structure, function and movement of the chromosomes in metosis I. 1, A cell at diakinesis I. Note the non-formation of nuclear envelope, this situation being the same in all other figures. 2-4, 6, 7 and 11, Cells at metaphase I, showing variant chromonema spirallization. 5, Showing asynchrony in division, left cell being at anaphase, while right one at metaphase. 8-10, 12 and 13, Cells at anaphase. Asynchrony in chromosome kinesis (8), bridge formation (9), rampant orientation of each half-bivalents over the cell (10), chromosomes clumping together due to severe «lipidation» (12), abnormal formation of cell plate independent of chromosome movement (12, 13). 14, 15, Cells at further stage. Formation of interphase nuclei of various sizes (14). Pseudo-tetrad formation (15).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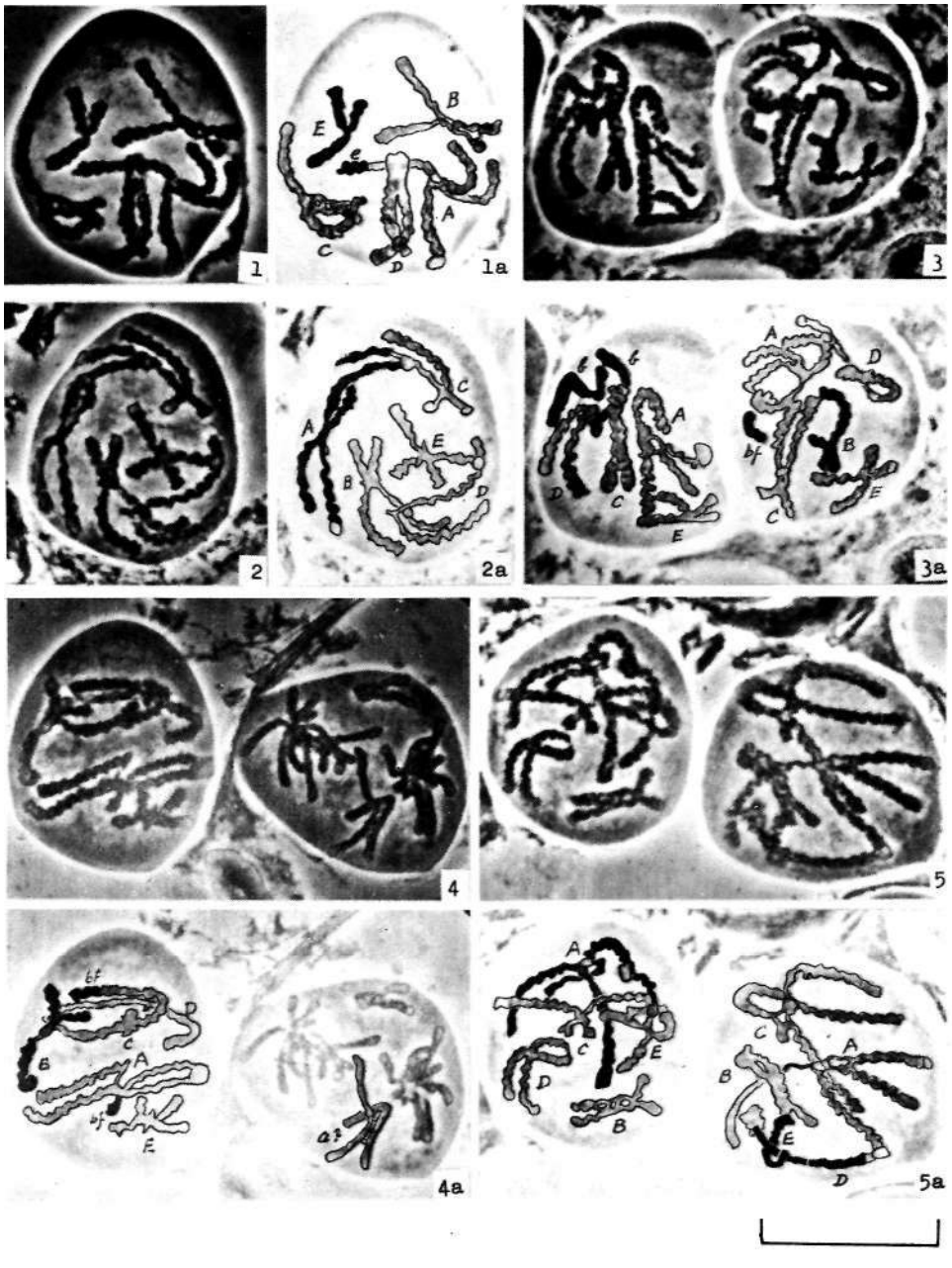
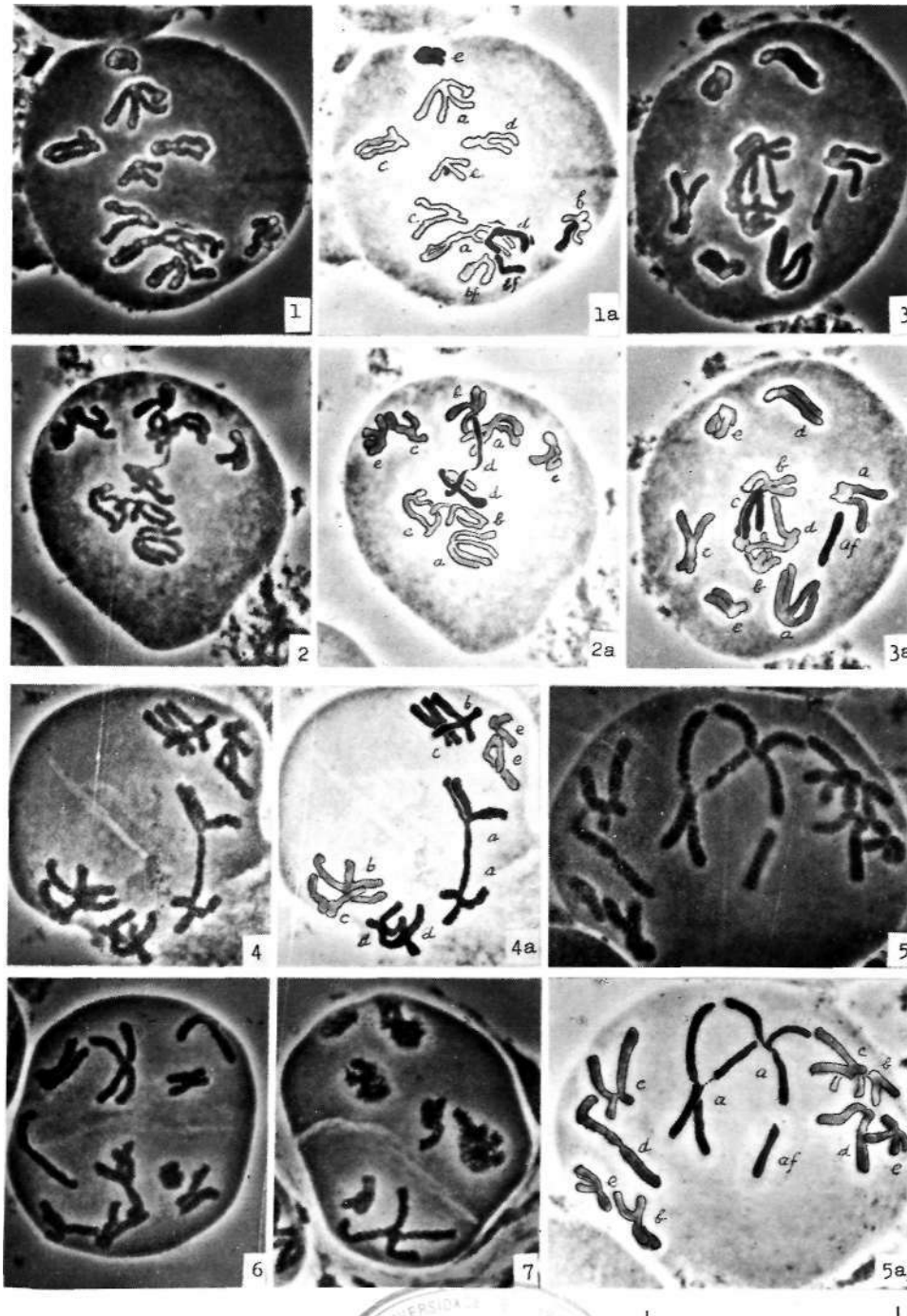

1
1a
3
2
2a
3a
4
5
4a
5a

PLATE II

PMCs at metaphase I (1-5 appended with respective explanatory sketches, la-5a), showing «lipidation» and adhesions of the chromosomes. The same designation of the chromosomes of a complement as in the other papers of this series by me has been adopted in the present paper; capital letters, A-E, designate the five chromosome (bivalent) types of a complements, small letters, a-e, stand for univalents, or half-bivalents and letters suffixed by f indicate fragment chromosomes. Configurations in right cell in 4 are too complicated to be analyzed.

PLATE III

Abnormal behaviors of half-bivalents at anaphase I, demonstrating the status of adhesions of chromosomes giving rise to the formation of bridges and fragments (1-5) and non--disjuction of paired half-bivalents (6, 7).



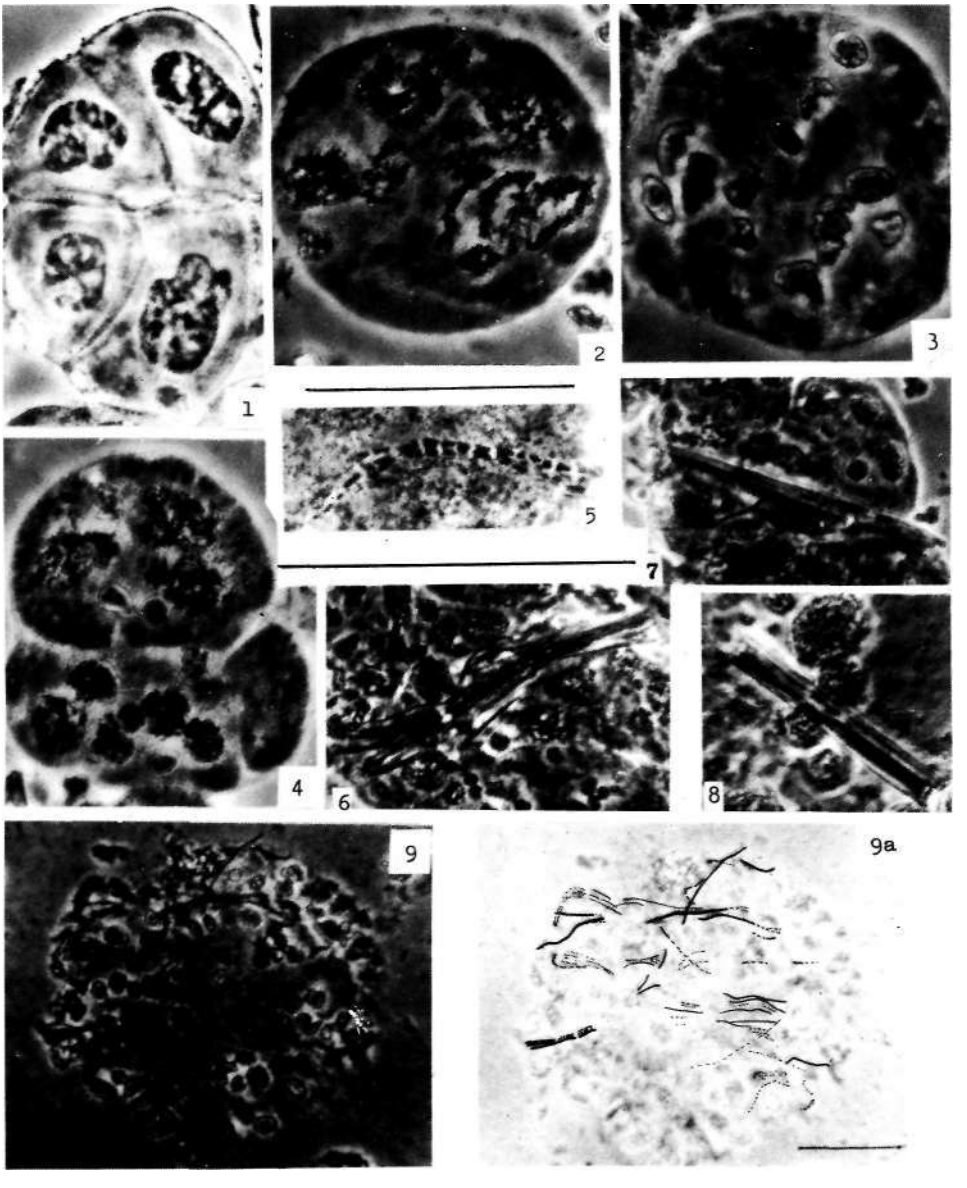
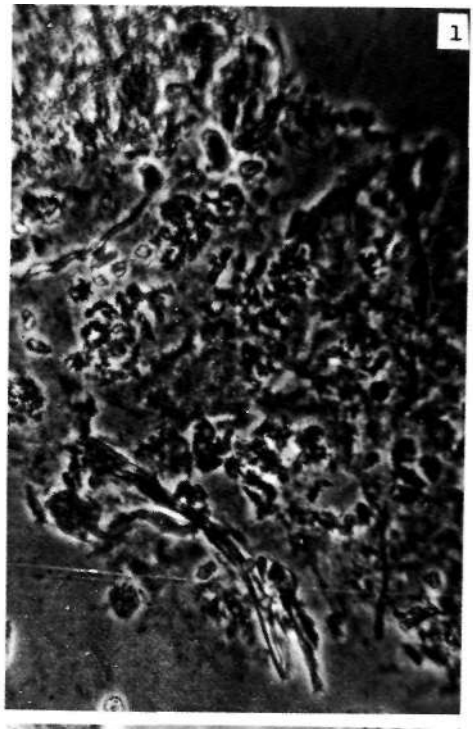

1
2
3
4
5
6
7
8
9
9a

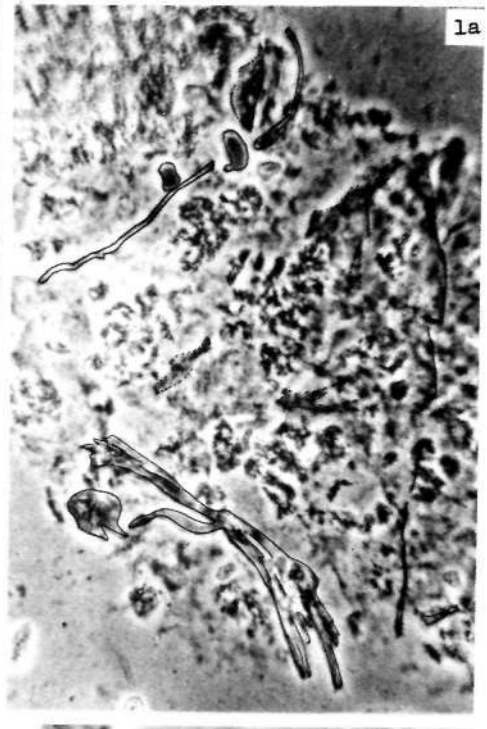
PLATE IV

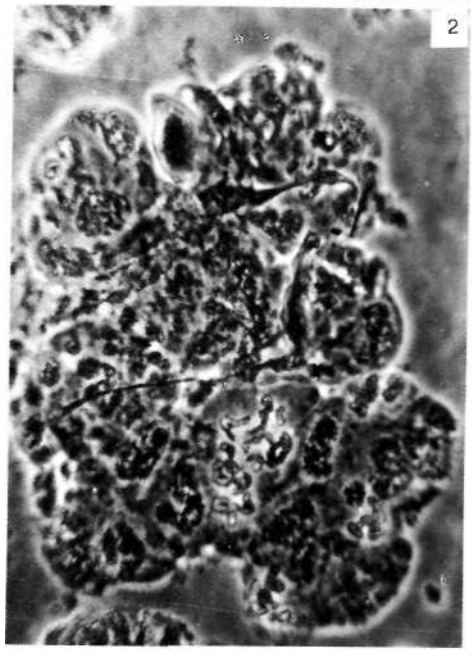
Demonstrating the severe destruction of chromosome organization at the end stages of meiosis. Refer to text, p. 1249. 1. only one case of apparently normal tetrad formation. 2-4, the formation of «pollen» Plasmodium. 6, initiation of pulverization of elongated microfibrils bundle. 6, a bundle of microfibrils coated by lipid layer. 7, 8, crystals of ribonuclear proteins also coated by lipid layer. 9 and its explanatory figure, 9a, showing extreme pulverization of chromatin microfibrils.

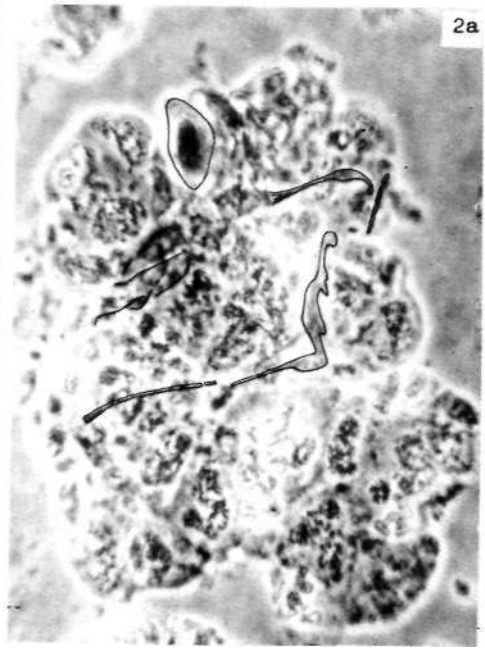
PLATE V

Comparison of autolysis of chromatin mass between tapetum Plasmodium (1, la) and pollen Plasmodium (2, 2a). Note that the both are quite similar with each other.


1


1a


2


2a

Bol. Soc. Brot., Sér. 2, 53(2): 1257-1286 (1981).

CORRELATION OF CYTOLOGY AND PHYTOCHEMICAL CONSTITUENTS IN LABIATAE

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INTRODUCTION

THE group Labiatae is a dicotyledonous family of angiosperms. The position and relationship of some of the taxa within the family have formed subjects of considerable debate. BENTHAM & HOOKER (1873) included the family in their Gamopetalae, which was incorporated in between Polypetalae and Monochlamydeae. In ENGLER & PRANTL'S (1897) system, it was superseded by four other groups as far as the advanced status was concerned. HUTCHINSON (1969) placed the family at the top of his Herbaceae, a group of fundamentally herbaceous plants. However, in the present review, BRIQUET'S system of classification as published in ENGLER & PRANTI/S Die Natürlichen Pflansenfamilien (1877) has been followed. This system, besides being a phylogenetic one, deals with the delimitations of several genera which are subdivided into different subgenera and/or sections. These detailed delimitations have enabled the present authors to ascertain their status and affinity, while dealing with cytological and phytochemical findings. The principal objective has been to determine correlations, if any, between the chromosomal complements on the one hand and the phytochemical constituents on the other. A brief account of the BRIQUET'S system (1897) is given here:

The entire family is divided into eight subfamilies, namely Ajugoideae, Prostantheroideae, Prasioideae, Scutellarioideae, Lavanduloideae, Stachydoideae, Ocimoideae and Catopherioideae. Of these, Ajugoideae, Stachydoideae and Ocimoideae have beem further split into different tribes (ending with *-eae)* and subtribes (ending with *-nae).* Ajugoideae has been divided into two tribes Ajugeae and Rosmarineae and Stachydoideae into twelve tribes and subtribes (such as, Marrubiae, Perilomieae, Nepetae, Brunellinae, Melittinae, Lamiinae, Glechoneae, Salvieae, Meriandreae, Monardeae, Hormineae, Lepechinieae, Melissinae, Hyssopinae, Thyminae, Menthinae, Perillinae and Pogostemoneae) and Ocimoideae into subtribes Hyptidinae, Plectranthinae and Moschosminae. All the genera included in this review, except *Rosmarinus, Glechoma, Anísameles* and *Origanum,* have been further subdivided into different subgenera and/or sections, each comprising of species related to each other.

In the present review, in addition to considering the correlation of cytology and phytochemistry of the Labiatae, the status and affinity of these plants in BRIQUET'S system have also been taken into account. Such a correlated approach to the principal genera of the mint family through evidences from cytology, phytochemistry and taxonomy was deemed desirable to solve the problems of taxonomic dispute in this family. While dealing with the chromosome number, only recent records have been mentioned.

Cytology and phytochemistry of the species — a correlated analysis

In the subfamily Ajugoideae, three genera — *Ajuga* and *Teucrium* of tribe Ajugeae and *Rosmarinus* of Rosmarineae — have been included in this review. *Ajuga reptans* L. having n = 16 chromosomes (MAJOVSKY *et al.,* 1974) contains harpazide and harpazide acetate (KOMISSARENKO *et al.,* 1976), which are not found in any member of the subfamily Ajugoideae. In *Teucrium,* the compound is different — teucrin P (POPA *et al.,* 1977) and a diterpenoid picropoline (BRIESKORN & THOMAS, 1966). Their chromosome numbers,

being n = 13 (MARKOVA & THU, 1974) and 26 (UECH, 1974a, b), are *also different.*

The position of *Rosmarinus* has been a debated one. It has been kept under Ajugoideae by BRIQUET (1897) and separated and elevated to a subfamily level (Rosmarinoideae) by MELCHIOR (1964). The chromosome number of *R. officinalis* L., the only species both cytologically and phytochemically studied, is 2n = 24 (NATARAJAN, 1978), whereas in *Ajuga* it is 32 and in *Teucrium* 26 and 52. Though the chromosome number itself can not be considered as the determining character between the two genera of the subfamily, the difference in chromosome number is manifested clearly. But, the chemical components noted in *R. officinalis* are a-and b-pinenes, satinene, camphene, u-phellandrene, p-cymene, eucalyptol, camphor, terpineol, borneol, linalool, borneol acetate, geraniol (KARAWYA *et al.*, 1970), royleanone and cryptotanshinone (BRIESKORN & BUCHBERGER, 1973), none of which is found in other genera of Ajugoideae. This may, to some extent, support the separation of the genus from Ajugoideae and elevation to a subfamily Rosmarinoideae, as done by MELCHIOR (1964). However, more confirmatory evidences are needed from other species of Ajugeae and Rosmarineae to substantiate their separation and elevation.

In *Scutellaria*, belonging to a separate subfamily Scutellarioideae, varying compounds, including sterols, flavones and alkaloids are found. None of the intermediates of the flavone biosynthetic pathway has yet been reported in this genus. However, the final flavoné products of their derivatives invariably occur. Scutellarin (scutellarein-5-glucuronide), which is a glucoside derivative of scutellarein (5:6:7:4' — tetrahydroxyflavone) is found only in *8. columnae* All. (MARSH, 1955) having polyploid forms with 2n=32, 34 chromosomes (BAKSAY, 1958; MARKOVA & THU, 1974). The parent compound scutellarein is found in the diploids — *8. Orientalis* L. (BEKIROV *et al*, 1974) with 2n = 16 chromosomes (Quezel, 1957) as well as in several other species, such as, *8. polybdon* (BANDYUKOVA & BOIKOVA, 1969), *8. prze-*

walskei (DENIKEEVA *et al,* 1970) etc., whose chromosome numbers are not reported.

It is remarkable that carthamidin and isocarthamidin are found only in *S. baicalensis* Georgi (TAKIDO *et al,* 1976). This species has a chromosome number 2n — 18 (SUZUKA, 1953), a number unknown for the genus. Even though baicalin is also found in other species, it appears that the alteration in the haploid chromosome number (n = 9) may be associated with the change in the phytochemical component. Such alterations might have occurred following gene mutation, resulting in the evolution of new compounds.

Baicalin (baiealein-6-glucuronide), a glucoside of flavone baicalein, is present in several species, such as, in *S. altíssima* L. (BESHKO *et al.,* 1975), *S. baicalensis* (KHNYKENA, 1962), *S. orientalis* (BEKIROV *et al.,* 1974), etc. having both n = 8 (MORTON, 1973) and 9 chromosomes (MARKOVA & IVANOVA, 1974) as haploid numbers. But the parent compound baicalein is found only in species having haploid chromosome numbers either as 8 or its derivatives as in *S. altíssima* (BESHKO *et al,* 1975) with 2n = 28 and 34, S. *orientalis* (BEKIROV *et al,* 1974) with 2n = 16 chromosomes, etc.

In general, the species of *Scutellaria* are homogeneous in their contents of different compounds, such as, baicalein, baicalin, scutellarein, scutellarin, etc. Cytologically also, different species of this genus show homogeneity in having n=8, 10, 11, 16, 17, 18 and its derivatives. The chromosome numbers of three species of *Scutellaria* have been reported for the first time by the authors — *S. adenostegia* with 2n=20 (SINGH, 1979), *S. intermedia* having 2n=22 (SINGH, 1979) and *S. haematochlora* with 2n = 20 chromosomes (personal communication, in press). None of these species have been studied from the phytochemical aspect. Both aneuploid and polyploid series are seen and the base number appears to be 8, as believed by DARLINGTON & WYLIE (1955) also. This basic number may have given rise to other numbers through duplication and polyploidy. As a few species, such as S. *galericulata* L. contain cytotypes, it will be worthwhile to find out the correlation of such cytotypes with chemical contents.

Of the four sections of the genus *Lavandula* under the subfamily Lavanduloideae, only some of the members of the section Stoechas have been studied both eytologieally and phytochemically. In Stoechas, the chromosome numbers reported are n = 15 each. *L. stoechas* L. contains 2n = 30 chromosomes (VON BOTHMER, 1970), *L. pedunculata* Cáv. 2n = 30 (GARCIA, 1942) and *L. dentata* L. 2n = 45 chromosomes (NESTERENKO, 1939). Cineole and camphor are the two compounds common to all the three (DE PASCAUL TERESA *et al.*, 1976), whereas feuchone is seen only in the diploid species, *L. stoechas* (GRANGER *et al.*, 1973) and *L. pedunculate* (DE PASCAUL TERESA *et al.*, 1976). The uniformity in chemical content, correlated with homogeneity in chromosome number, related to a slight variation due to genotypic differences is quite remarkable. In *L. officinalis* Ch., the chromosome numbers reported are 2n = 36 (LAWS, 1930) 42 and 48 (BUYUKLI, 1970), 50 (MAKING, 1951) and 54 chromosomes (DELAY, 1947) and this species has not been listed under any section of the genus. A variety of compounds has been found in this species, including cineole, borneol and camphor (TooRE & CARMEN, 1974). With the range of cytotypes so far reported, it would be worthwhile to correlate their genetic constitution with the chemical constitution. In general, *Lavandula*, as a genus, is quite remarkable in having camphor as its component in all the species worked out so far. In view of the similarity in chemical contents of the three species as well as that of *L. officinalis*, which also resembles other species in chemical constitution, it is likely that the genus has a common basic number from which the other forms have arisen through polyploidy, aneuploidy and hybridization. The homogeneity of the genus as a whole appears unquestionable.

The subfamily Stachydoideae is wide in scope and has a number of taxa within its orbit (namely, Marrubiaeae, Pevilomiiae, Nepetae, Brunellinae, Mellillinae, Lamiinae, Glechoneae, Salvieae, Meriandreae, Monardeae, Hormineae, Lepechinieae, Melissinae, Hyssopinae, Thyminae, Menthinae, Perillinae and Pogostemoneae). Though 8 or 9 chromosomes are the deep-seated numbers, yet aneuploidy is not very

uncommon. In the genus *Marrubium* of Marrubiaeae, the chromosome numbers reported so far are 32, 34 and 54, the chemical constituents being peregrinine and its derivatives, marrubiin and stachydrine. Though species differ with regard to the content of some specific derivatives, yet a general uniformity in the chemical constituents is noted — *Marrubium catariifolium* containing peregrinol (POPA & SALEI, 1973), *M. leonuroides* having dihydroperegrinin (POPA & SALEI, 1973), *M. peregrinum* L. containing peregrinol (SALEI *et al.,* 1966; POPA & SALEI, 1973), peregrinin (POPA & SAXEI, 1973) and peregrinine (SAISI *et al.,* 1966) and *M. praecox* with peregrinol (POPA fe SALEI, 1973). The occurrence of 2n = 34 (STRID, 1971) and 54 (PODLECH & BAKER, 1974) chromosomes in *M. alternidens* Rech. f. and 32 (MARKOV A & IVANOVA, 1971) and 34 (MAJOVSKY *et al.,* 1970a, b) in *M. peregrinum* L. indicates intraspecific variations. The number 2n — 34 may be a derivative of 32 or 36 in *M. vulgare* L. (HEISER & WHITAKER, 1948) through duplication of one or more chromosomes or an aneuploid cross between x = 8 and x = 9 chromosomes.

In the allied genus *Sideritis,* under the same tribe, though chemical constituents have been analysed in 9 species (namely, *8. angustifolia* Lag., *S. canariensis, S. candicans, S. catillaris, S. grandiflora, S. lagascana, S. montana* L., *S. pusïlla* (Lge.) Pan and S. *valverdii),* cytological observations have been made on only three, namely S. *angustifolia* with 2n = 24 (FERNANDEZ-PERÁLTA *et al.,* 1978), S. *montana* having 2n = 32 (FERNANDEZ-PERALTA *et al.,* 1978) and S. *pusilla* showing 2n = 22 chromosomes (FERNANDEZ--PERÁLTA *et al.,* 1978). The chemical compositions of the different species of the genus show qualitative differences. The above three species, so far cytologically studied, show differences in chromosome number as well as in the chemical constituents. Pusillatriol and its derivatives are the principal components in S. *pusïlla* (GARCIA DE QUESADA *et al.,* 1974), jativatriol in *S. angustifolia* (VON CARSTENN-LICHTERÍBLDE *et al.,* 1974), several alkaloids, tannins and saponins in S. *montana* (DZHUMAZHANOVA, 1968) and kaurene and beyerene in S. *lagascana* and S. *valverdii* (DE QUESADA *et al.,*

1974). In absence of any further data on chemical constituents in the genus *Sideritis,* it is difficult to comment on the homogeneity of the genus. In this genus, however, x = 8 chromosomes may represent the basic set from which others have been derived.

In Nepetae, two genera *(Glechoma* and *Nepeta)* have so far been studied, both from cytological and chemical standpoints. Extensive investigations have been carried out in the latter. In the genus *Glechoma* having only one species *(G. hirsuta* Waldst. *et* Kit.) with 2n = 36 chromosomes (MARKOVA & THU, 1974), the principal chemical constituents are phytol, b-sitosterol and betulin (POPA & PASECHNIK, 1974). In absence of further data on cytology and phytochemistry, it would not be proper to comment on the genus. However, the presence of some of the triterpenoids (betulin and aleanolic acid) and b-sitosterol in *Glechoma hirsuta* and some of the species of *Nepeta* [betulin in *N. aragonensis* (VON CARSTENN-LICHTERMM)E *et al.,* 1973), oleanolic acid in *N. teydea* (BRETÓN *et al.,* 1970) and b-sitosterol in N. *industana* Hains (SESHADRI & SHARMA, 1973) and *N. teydea* (BRETÓN *et al.,* 1970)], together with the basic set of x = 9 chromosomes in both the genera, evidently bring them together to be closetted under a single tribe Nepetae. In the genus *Nepeta?* a large number of terpenoids has been recorded in different species, namely, in *N. cataria* L. (RÉGNIER *et al.,* 1967), *N. citriodora* (RÉGNIER *et al.,* 1967), *N. leucophyïïa* Benth. (GUPTA, 1973) and *N. mussini* (RÉGNIER *et al.,* 1967). In addition to these, nepetalactone and epinepetalactone in *N. cataria* L., *N. citriodora* and *N. mussini* (RÉGNIER *et al.,* 1967) and nepetrin and nepetin in *N. hindustana* Hains (SESHADRI & SHARMA, 1973) and their derivatives are also common in all the species. Sterols (b-sitosterol) too, are found in a few. Even in the absence of certain derivatives of a parent compound, a general uniformity in the phytochemistry is noticeable. The chromosome number too, is mostly n = 9 [as in *N. cataria* L. (LEE, 1969; PODLBCH & DIETERLE, 1969), *N. hindustana* (Vu & KASHYAP, 1976), *N. leucophyïïa* (Gill, 1969), *N. mussini* (VAKAR & LESHUKOVA, 1970)] with occasional polyploids and aneuploids,

such as, in *N. cataria* with 2n = 30 (MAJOVSKY *et al, 1970a,b),* 32 (BUSHNELL, 1936), 34 (MARKOVA & THTJ, 1974) and 36 chromosomes (LEE, 1969; FODLECH & DIETEELE, 1969). The genus is marked by uniform chromosome number with common chemical constituents.

The tribe Lamiinae presents certain interesting features from the phytochemical standpoint. Though flavones, flavanols, sterols and alkaloids have been found in different genera of the group, one can establish a pattern of their presence correlated with their genotypic constitution. In the genus *Lamium,* the common constituents of all the species are quercitrin or its derivative quercimeritrin, eaffeic acid and to some extent kaempferol (DUCHNOWSKA & BORKOWSKI, 1964; KRITIKOS & HARVALA, 1970). The chromosome numbers reported for the species of the genus are 2n — 18 in *L. amplexicaule* L. (MARKOVA & THU, 1974) and *L. purpureum* L. (MARKOVA & THU, 1974); 2n = 16 (MARCHAL, 1920), 18 (BHATTACHARYA, 1976) and n = 9 (MHATTACHARYA, 1976) and n = 9 + 1B chromosomes (GILL, 1970) in *L. album* L., 2n — 18 (MAJOVÍSKY *et al.,* 1974) and n = 9 chromosomes (KLIPHUIS & WIEFFERING, 1972) in *L. maculatum* L.

The species *L. album,* can serve as a good material for the analysis of the effect of B-chromosomes on the phytochemical constituents. In *Lagochilus,* three species (L. *inebrians* Bunge, *L. pubescens* Vved. and *L. setulosus)* have so far been analysed for the chemical contents and the alkaloid stachydrine has been found to be common to all (PULATOVA, 1969). Of these, *L. inebrians* and *L. pubescens* have been cytologically studied, both showing 2n — 34 chromosomes (ZAKHARYEVA & ASTANOVA, 1968). The chromosome number is quite different from those of *Lamium* spp. and possibly derived from a cross between species having n = 8 and 9 chromosomes, followed by doubling of the complement. The presence of certain similar constituents in all the three species, along with identical and high chromosome numbers in at least both the species, is significant.

In *Galeopsis,* two species, namely, *G. ladanum* L. and *G. speciosa* Mill., have been phytochemically and cytolo-

gically analysed, indicating the presence of flavonoids, alkaloids and saponins (HRYTSENKO & ZINCHENKO, 1967). The chromosome number, being n — 8 (LÖVE & LOVE, 1956), is also identical in both, unlike *Lagochilus* and *Lamium.* Added to the prevalent deep-seated chromosome number of n — 8 or 9 in the Lamiinae, two more numbers have been found to occur, namely n = 10 in *Anisomeles ovata* R. Br. (BIR & SIDHU, 1974) and n = 11 in *Ballota nigra* L. (VAN LOON & DE JONG, 1978), the chemical constituents being ovatodiolide in the former (HoDAC *et al.,* 1963) and ballonigrin in the latter (SAVONA *et al.,* 1976).

In Lamiinae, therefore, each genus so far studied from both cytological and phytochemical standpoints, has distinct phytochemical characteristics associated with distinct chromosome numbers. However, the chromosome numbers x=8, 9, 10, 11 and 17 are quite likely derivatives from a basic set of 8 or 9, as evidenced especially by intraspecific variation in some of the species, for example, *Lamium album,* having 2n = 16 and 18 chromosomes.

In *Salvia* (tribe Salvieae) there are series of chromosome numbers in different species, such as, n = 6, 7, 8, 9, 11, 12, 15, 16, etc. Intraspecific polyploids [such as, in *S. carduacea* Benth. having 2n = 24 (STEWART, 1939) and 32 chromosomes (EPLING *et al.,* 1962), *S. virgata* Ait. with 2n — 16 (AFZAL-RAFII, 1971) and 32 chromosomes (BENOIST, 1937)] and aneuploids [such as, *S. nemorosa* L. with 2n = 12 (MAJOVSKY *et al.,* 1970a, 6) and 14 chromosomes (MARKOVA & IVANOVA, 1974) ; *S. officinalis* L. with 2n = 14 (nx, 1971a) and 16 chromosomes (SCHEEL, 1931) and *S. verbenaca* L. with 2n = 42 (KRAMER *et al.,* 1972), 54 (BHATTACHARYA *et al.,* 1971), 60 (DAHLGREN, 1971) and 64 chromosomes (VAN LOON *et al.,* 1971) are also common in the genus. However, of all the numbers, n = 8 chromosomes appear to be more prevalent as compared to those of the rest of species of *Salvia.*

Phytochemical studies have been carried out in nearly twenty species. Majority of them are characterized by having terpenoids like *a*- and u-piñenes (MÜLLER & MÜLLER, 1964; PETRI VERZAR & MARIA, 1974; IVANIC & SAVIN, **1976) ;**

cineole (MULLER & MULLER, 1984; MÜLLER, 1965; IvANiC & SAVIN, 1976), borneol (PETRI VERZAR & MARIA, 1974; IVANIC & SAVIN, 1976) ; linalool (SHEVCHENKO & TIKHOMIROVA, 1973; IVANIC & SAVIN, 1976); geraniol (SHEVCHENKO & TIKHOMIROVA, 1973), camphor (MÜLLER & MULLER, 1964; MULLE, 1965; PETRI VERZAR & MARIA, 1974) etc., sapogenin, like aleonolic acid (BRIESKORN *et al.*, 1961; PETTIT *et al.*, 1986; GONZALEZ *et al.*, 1975) and sterols, such as ^-sitosterol and others (BRIESKORN *et al.*, 1961; GUSAKOVA *et al.*, 1968; GONZALEZ *et al.*, 1975). Flavonoids (like apigenin, luteolin, kaempferol, kaempferide, gengkwanin, chrysoeriol, ayanin, etc.) and their derivatives are also very common in the species of *Salvia* (GELLA & PROKOSHERA, 1970; WOLLENWEBER, 1974). Though, several of the compounds and their derivatives are also common among the species, a clear correlation as in Lamiinae could not be established between species with distinct chromosome numbers and their phytochemical constituents. Moreover, inspite of the wide diversity in chromosome number, intraspecific variation clearly indicates that they are all indirect or direct derivatives of a common basic set (SINGH & SHARMA, unpublished 1980). The gametic number being 8 or its multiple, it is quite likely that this number is the basic number for this genus also.

In the genus *Hyssopus* of subtribe Hyssopinae (Staehydoideae), phytochemical investigation has been carried out on three species only, namely, *H. ferganensis*, *H. officinalis* L. and *H. seravschanicus* (Dul.) Pazij. Of these, the report of chromosome number is available for the last two only—-both having 2n = 12 chromosomes (REESE, 19526; MATVEEVA *et al.*, 1968b; MARKOVA & THU, 1974). Cytosterine, betulin, oleanic and ursolic acids are the important constituents in *H. ferganensis* and *H. seravschanicus* (ZkXPOV & KHARANOViCH, 1975) and *a*- and ß- piñenes, a-terpinine,, pinocampheol, cadalene (SHARMA *et al.*, 1963; JOULAIN, 1976), pinic, pinonic and myrtenic acids (JOULAIN & RAGAULT, 1976) in *H. officinalis.* The genus is quite homogeneous from both cytological and phytochemical standpoints.

However, the chromosome number is rather unusual, in having 6 as the haploid number.

Of the subtribe Thyminae (Stachydoideae), only two genera *(Origanum* and *Thymus)* have been studied from phytochemical and cytological points of view. The chromosome numbers are remarkably uniform, being n = 15 and 14, the former a deep-seated number both in *Origanum* and *Thymus.* The interspecific variations noted in several species of *Thymus,* including *T. vulgaris* L. with 2n = 30 (NATARAJAN, 1978) and 56 chromosomes (BONNET, 1966) suggest that the two chromosome numbers (n = 15 and 14) may be derived one from the other. Only in *T. serpyllum* L., 2n = 20 (ROHWEDER, 1937) and 24 chromosomes (JALAS & POHJO, 1985a, *b)* are recorded, though the phytochemical constituents are mostly common to other species of *Thymus.* The way through which it has been derived is yet to be investigated. Out of the 24 species, phytochemically analysed, 15 — *T. caucasicus* Willd., *T. collins* Bieb. with 2n = 30 chromosomes, *T. dagestanicus* with 2n = 28 chromosomes (Gogina & SVETOZATOVA, 1972), *T. desyatoviae* Ronn., *T. forminii* Klock et Shost., *T. karamarianicus* Klock *et* Shost., *T. marschallianus* Willd., having 2n = 28 chromosomes (TRELA-SAWICKA, 1970, 1972), *T. soshawskyi* Grossh. with 2n = 60 chromosomes (GoGINA & SVETOZATOVA, 1972), *T. tuflisiensis* Klok. *et* Schost. showing 2n = 56 chromosomes (MATVEEVA & TIKHONOVA, 1968a, 6), *T. trautvetteri* Klok. *et* Short, and *T. ziaratinus* Klok. *et* Shost., *T. pastoralis* Hjin., *T. pseudonummularis* with 2n — 30 chromosomes (JALAS & KALEVA, 1967) and *T. rariflorus* C. Koch. — contain caffeic acid, 1-caffeylglucose, 6-caffeylglucose, rosemarinic acid and garashangin (SIMONYAN *et al.,* 1972). This remarkable uniformity indicates that *Thymus* is a very natural assemblage. Therefore, both cytological and phytochemical studies confirm the homogeneity of the genus. At the same time, the presence of the same basic chromosome number (x = 15) and the common occurrence of some of the terpenoids (such as carvacrol and thymol) and a sapogenin in both *Origanum* and *Thymus* species strongly

suggest the inclusion of both the genera under the same subtribe Thyminae.

The genus *Mentha,* belonging to subtribe Menthinae (Stachydoideae), is one of the most important taxa in Labiatae, both from commercial and medicinal standpoint. It is widely cultivated for the content of menthol, its derivatives and other important chemical principles. The species, which have been studied both from cytological and phytochemical standpoints, belong to different sections, namely, Eupulegia containing *Mentha pulegium* L., Audiberliae having *M. arvensis* L., Capitatae including *M. aquática* L. and Spicatae with *M. viridis* L., *M. longifólia* (L.) Huds. and *M. spicata* L. The chromosome number in *M. pulegium* ranges from 10 to 46 (NAGAO, 1941; PÓLYA, 1950; MORTON, 1956; SOBTI, 1965; VON BOTHMER, 1970; MAJOWSKY *et al.,* 1970; NATARAJAN, 1978). Majority of them are multiples of 5. Such low chromosome numbers have not been found in any other sections of *Mentha,* so far cytologically studied (except, a few scattered reports in some of the species).

Phytochemical studies have shown that pulegone is the principal constituent in *M. pulegium* (MURRAY *et al.,* 1971) in addition to methone (CHOPRA *et al.,* 1964; ZWAVING & SMITH, 1971; FRAZÃO *et al.,* 1974; ALPMEN, 1975), cadenene, piperitenol, isopulegone and pulegone (CHOPRA *et al.,* -964), menthol (FRAZÃO *et al.,* 1974; ALPMEN, 1975), and limonene (ZWAVING I& SMITH, 1971). Pulegone is the immediate precursor of menthofuran (FUJITA, 1960), which occurs widely in all other species of *Mentha.* The precursor of both pulegone and menthofuran, that is, linalool, also occurs profusely in all other species. Except in *M. pulegium,* the chromosome numbers in all other species of *Mentha* are multiples of either 8 or 9. It appears, therefore, that *M. pulegium* with its principal constituent pulegone and chromosome number of multiples of mostly 5 or 10 is rightly placed in a separate subgenus Eupulegia under the genus *Mentha.* The chromosome number noted for the species during the investigation carried out by the authors is 2n = 20 (SINGH & SHARMA, unpublished 1980). A large number of cytotypes of *M. pulegium* is also on record. It is

of interest that reproduction of the species is practically vegetative which has also been selected for commercial propagation. Such large scale vegetative propagation has undoubtedly helped in the survival of the cytotypes in nature.

The species *Mentha aquática,* on the other hand, studied in this laboratory (SINGH & SHARMA, unpublished, 1980), has 96 chromosomes in its somatic cells indicating a high polyploid constitution. Phytochemical studies have revealed a wide range of compounds — menthofuran being the principal constituent (MURRAY *et al.,* 1971). The others worth mentioning are apigenin, acacetin, luteolin and their glycosides (BURZANSKA-HERMANN *et al.,* 1977), caryophyllene, limonene, germacrene D, bicyclogermacrine, viridiflerol, menthone, menthol (MULINGRE & MAARSE, 1974), *a-* and ß-pinenes, sabinene, myrcene, cineole, cis- and trans-ocimenes, y-terpinene, linalool, sesquiterpene — KW, humulene (HEFENDEHL, 1967), £-cadinene and isopino-eamphone (SHIMIZU *et al.,* 1966).

A wide range of cytotypes, like *M. pulegium,* has been recorded in *M. arvensis* also, their chromosome numbers ranging from 2n = 24 (OUWENEEL, 1968) to 132 (MORTON, 1956) with polyploids (NAGAO, 1941; OLSSON, 1967; OUWENEEL, 1968; TAYLOR & MULLIGAN, 1968; BELYAEVA & KOVINEVA, 1972) and aneuploids (WOLF, 1929; NAGAO, 1941; TAYLOR & MULLIGAN, 1968). *M. longifolia* as well has many cytotypes with chromosome number between 2n = 18 (HEIMANS, 1938) to 84 (NAGAO, 1941). As compared to *M. pulegium,* in all other species studied by the authors (SINGH & SHARMA, unpublished, 1980), the chromosome numbers are, in general higher than those of the former. The lowest chromosome number in *M. pulegium* with pulegone as its principal constituent and comparatively higher chromosome number in other species having menthofuran (an oxidation product of pulegone) as the dominating phytochemical constituent indicates a correlation between cytological features and phytochemistry. Though it is prematuro to state precisely the chromosomes involved in causing this difference, without a correct estimation of the phytochemistry of each cytotype, the remarkable distinction of the

subgenus Pulegium from the rest (both in cytology and phytochemistry) is evident.

In the subfamily Ocimoideae, two genera — *Coleus* and *Ocimum* of subtribes Plectranthinae and Moschosminae respectively — have been subjected to phytochemical and cytological analysis. In *Coleus*, ß-sitosterol-jß-D-glueoside (MiSRA *et al*, 1976) is the principal constituent. The chromosome numbers, so far reported in two species of *Coleus* — *C. aromaticus* Benth. with 2n = 32 chromosomes (MORTON, 1982) and *C. blumei* Benth. having 2n = 24 (MORTON, 1962), 48 and 72 chromosomes (MORTON, 1962) — show mostly a multiple of 8 with numerous cytotypes in *C. blumei*. In view of a large number of chemical constituents in the species as well as the occurrence of several cytotypes, it would be necessary to have a correlated approach of the chromosome complements on one hand, and the phytochemical constituents on the other, of the individual cytotypes.

The genus *Ocimum* is one of the most important taxa in the family Labiatae from a phytochemical standpoint. The entire genus is extremely uniform and homogeneous with characteristic eugenol content (DUTT, 1939; RAKSHIT, 1939; PING-HSIEN YEH, 1960; MANITTO *et al*, 1974; ROVESTI, 1975). Camphor is also one of the constituents in several species of the genus, such as, *O. basilicum* L. (SOBTI *et al.*, 1976); *O. canum* Sims. (PUSHPANGADAN *et al*, 1975) and *O. Mlimanãscharicum* Guerke (CHOWDHRI & HAKSAR, 1964; MOOKHERJEA, 1973). Cinnamic acid (Au & SHAMSUZZAMAN, 1968a, *b;* NIGAM & RAO, 1968), a precursor of eugenol (MANNITTO *et al*, 1973; 1974) as well as camphene (NiGAM *et al*, 1965; POGANY *et al*, 1968), geraniol (NIGAM *et al*, 1970; GUPTA *et al.*, 1971), ocimene (PING-HSIEN YEH, 1960; NIGAM & RAO, 1968; GUPTA *et al*, 1971), eucalyptol (GUPTA *et al*, 1971), thymol (TÁLALAJ, 1964; SOFOWARA, 1970; SOBTI *et al*, 1977) and several other constituents of essential oil characterize this genus.

In all the species, wherever extensive investigations have been carried out, several cytotypes have been revealed, the predominant chromosome number being x = 8. For instance, the diploid chromosome number range in *O. basilicum* is 16

(SZ.-BORSOS, 1970) to 48 (MEHRA & GILL, 1972), in *O. canum* 24 (PUSHPANGADAN *et al,* 1975; SINGH & SHARMA, 1978) to 26 (BHATTACHARYA, 1978; SINGH & SHARMA, 1978), in *O. gratissimum* L. 40 (SINGH & SHARMA, 1981) to 64 (GOLUBINSKI, 1936), in *0. suave* WMd. 32 (DE WET, 1958; MORTON, 1962), in *0. viride* Willd. 38 (SINGH, 1978) to 64 (SOBTI, personal contact), in *O. sanctum* L. 32 (MEHRA & GILL, 1972; SINGH & SHARMA, 1978) to 64 (GOLUBINSKI, 1936), in *O. americanum* L. 72 (SINGH & SHARMA, 1981; PUSHPANGADAN *et al.,* 1975) to 84 (SINGH & SHARMA, 1981) and in *O. kilirnandcharicum,.* it is 76 (KUMAR *et al.,* 1957; SINGH, 1978). In the investigation carried out by the authors, several populations of *Ocimum* species have been studied, showing the chromosome numbers as 2n = 24 and 26 in *O. canum* (SINGH & SHARMA, 1981), 2n = 72 (SINGH & SHARMA, 1981) and 84 in *O. americanum* (SINGH & SHARMA, 1981), 2n=40 in *O. gratíssima,* 2n = 38 in *O. viride* (SINGH, 1978), 2n = 32, 34 and 36 (SINGH & SHARMA, 1981) in *O. sactum,* 2n = 48 in *0. carnosum* Link et Otto. (SINGH, 1978) and 2n = 76 in *O. kilimandscharicum.*

The species *O. americanum-,* with 2n = 72 chromosomes, has been assumed by PUSHPANGADAN *et al.* (1975) to be derived from two different parents — *O. canum,* (2n = 24 chromosomes) and *O. basilicum* (2n = 48 chromosomes) containing citral (DWIVEDI *et al.,* 1963) and methyl chavicol (NIGAM *et al.,* 1970; MANITTO *et al.,* 1974; NIGAM and RAO, 1968), respectively. PUSHPANGADAN *et al.* (1975) reported the presence of both the components in *O. americanum.*

The nature of nucleolar chromosomes in two chemotypes of *O. americanum* (chemotypes 'methyl chavicol' having 6 or two types and 'citral' with 8 such chromosomes of three types) is different (SINGH & SHARMA, 1981). It is likely that such structural changes in the nature of these chromosomes might have involved the genes for methyl chavicol and citral.

PUSHPANGADAN *et al.* (1975) recorded that in *O. canum,* camphor is found in 2n = 26-chromosomed type, whereas in 2n = 24-chromosomed type, the principal constituent is linalool. During the present work, a sample of seeds with

reported content of linalool (PUSHPANGADAN *et al.*, 1975) has also showed 26 chromosomes in somatic cells. It is likely that both the components (linalool and camphor) are present in the type with 2n = 26 chromosomes. This fact is supported by SOBTI *et al.* (1978). It indicates that the additional chromosomes in this chemotype (2n = 26) are responsible for bringing about certain desirable phytochemical qualities. *O. americanum*, on the basis of phytochemical constituents and chromosome numbers, has been treated as species distinct from *O. canum* (SINGH & SHARMA, 1981). The two species are sympatric in distribution.

The species *O. gratissimum* and *O. viride* of the subgenus Gratissima, show certain interesting features during the authors' investigation. The former species (where two populations have been studied, both showing 2n = 40 chromosomes) contains ß-sitosterol (ALI & SHAMSÜZZAMAN, 1868a *b)*, while the latter (having 2n = 38 chromosomes only) is not reported to contain this chemical constituent at all. The presence of the parent intermediate compounds, such as, geraniol, geranyl acetate (SOBTI *et al.*, 1978), farnesene (NIGAM *et al.*, 1970), squalene (NICHOLAS, 1962), and ß-sitosterol, of sterol-biosynthesis in several species of *Ocimum* suggests that sterol-biosynthesis does exist in the genus, which probably *O. viride* lacks. This deficiency of a particular compound indicates its correlated connection with the lack of a pair of small chromosomes in the latter species (SINGH & SHARMA, 1981), *O. viride*, studied here, is deficient in containing a pair of chromosomes with very short arms. The other terpenoids, such as, thymol, are common in both the species (TALALAJ, 1964; SOTOWORA, 1970). However, it is to be recorded that SOBTI (personal communication) has found 2n — 40 chromosomes in *O. viride*. It would be worthwhile to find out the chemical constituent in that population, which may provide a definite evidence of the correlation as suggested.

The species *O. kilimandscharicum*, having considerable commercial importance due to its camphor content, has not been included in any of the sections of the genus in BRIQUET'S (1897) system. This species has an unusual number of

2n = 76 chromosomes (SINGH & SHARMA, 1981). The phytochemical constituents found in the species are camphene, mycene, ß-phellandrene, terpinene, p-cymene, borneol, selinene (NIGAM *et al.*, 1955), ß-sitosterol, oleanolic acid and ursolic acid (MOOKHERJEA, 1973) with very high camphor content (MOOKHERJEA, 1973). The meiotic analysis also shows the evidence of duplication of chromosomes with high multivalent formation (SINGH & SHARMA,, 1981). However, the segregation is quite regular as seed formation is profuse and no other aneuploids have been found in this species. In any case, populations from Calcutta (altitude 7 metres), of temperate as well as from Gureghar (altitude 1392 metres) of subtemperate areas, show the same chromosome number (SINGH & SHARMA, 1981). The extent to which this unusual chromosome number, noted in this species, is correlated with its high camphor content, indicates the need for a through analysis of other populations as well. It is remarkable here that only the 26-chromosomed *O. canum* and this species (both having additional chromosomes in their genetic complements — 2 in the former and 4 in the latter) have camphor as their constituent.

The basic number for the genus has been suggested as x = 8 chromosomes (VAARAMA, 1947; DARLINGTON & WYLIE, 1955). However, for the Basilieum group (including sections Ocimodon and Gymnocimum of BRIQUET) of the genus, x = 12 has been considered as the base number by MORTON (1962) and others. In BRIQUET'S (1897) system of classification, *O. gratíssima*, *O. suave* and *O. viride* (all belonging to the same group Gratíssima) have been isolated from *O. sanctum*, to be included with *O. basilieum* and *O. canum* under the section Ocimodon.

In the first three species, the basic chromosome number appears to be eight. Added to this, the characteristic chemical constituent eugenol is same in all these four species. Hence, the separation of these three species from the *Ocimodon* and their incorporation in *Hierocimum* parallel to *O. sanctum* is suggested. The status of *O. selloi* and *O. nudicaule*, whose chromosome number is not known and phytochemical study also is insufficient, can not be ques-

tioned. The base number for the rest of species *(0. americanum, 0. basilicum, 0. canum, 0. kilimandicharicum).* may be suggested as 12, which might have arisen, from x = 8 chromosomes. The occurrence of 2n = 84 chromosomes in one population of *0. americanum* is an added evidence in favour of 12 as the base number in this species. In view of the presence of the extensive number of cytotypes present in most of the species, it is difficult to indicate precisely a particular chromosome number to be the basic one. However, on the basis of secondary association of bivalents during meiosis, an initial base number of x = 4 chromosomes has been suggested by the present authors in *O. sanctum* (SINGH & SHARMA, 1981) and BHATTACHARYA (1978) in *O. canum* with 2n = 26 chromosomes (a material identified as *O. americanum).*

CONCLUSIONS

The following conclusions may be arrived at on the basis of the analysis of available data on cytology and phytochemistry:

The separation of the genus *Rosmarinus* from Ajugoideae and elevating it to a separate family Rosmarinoideae, as done by MELCHIOR, is supported.

The species of *Scutellaria* of the subfamily Scutellarioideae are homogeneous in their contents of different compounds, such as baicalin, scutellarin and derivatives. Their homogeneity is also reflected in their cytology, though a clear cut correlation between the two is yet to be worked out. In *Lavandula,* the uniformity in chemical content may be associated with homogeneity in chromosome number. All the species are characterized by their camphor content. A common basic chromosome number for the genus has been suggested.

The genus *Marrubium* of Stachydoideae is uniform in its chemical constituents, whereas in *Sideritis* the species differ markedly. The differences in their chromosome numbers are also quite marked. In the tribe *Nepetae,* the genera *Nepeta* and *Glechoma* are allied in both the phyto-

chemistry and chromosome number. In Laminae, each genus has distinct phytochemical characteristics, associated with distinct chromosome numbers. In *Salvia* (tribe Salvieae), intraspecific variations are quite common. The species are characterized by having terpenoids, flavonoids and their derivatives. A clear correlation between phytochemistry and cytology could not be established and x = 8 appears to be the basic chromosome number for the genus. The genus *Hyssopus* is quite uniform in its cytology and phytochemistry, with x = 6 chromosomes representing the basic number. The presence of the same basic chromosome number, that is, x = 15 and the common occurrence of some terpenoids and a sapogenin in both *Origanum* and *Thymus*, strongly suggest their homogeneity and justify their inclusion under the same subtribe Thyminae.

In the genus *Mentha* (Stachydoideae), a clear correlation has been shown between the two different basic chromosome numbers, that is, x = 5 and 6 and their chemical contents. The species, with x = 5 as the base number, have a different dominating chemical content (pulegone), in contrast to species showing x = 6 chromosomes and having mentho furan as the principal constituent. The position of different species of *Mentha* under different subgenera of the genus in BRIQUET'S system is supported on the basis of their cytology and phytochemistry.

Ocimum, of the subfamily Ocimoideae, besides having a correlation between different cytotypes and phytochemistry, shows a relationship between the number and structure of nucleolar chromosomes. The chemical contents of the cytotypes show a general homogeneity as well.

It has been suggested that changes in the number and structure of nucleolar chromosomes may simultaneously involve genes for different chemical constituents, as in *O. americanum.* The two chemotypes of this species contain methyl chavicol and citral separately. Its origin from a cross between *O. basilicum* and *O. canum* has been endorsed on the basis of their phytochemistry and cytology.

On the same grounds, *O. canum* has been considered as a species distinct from *O. americanum.* The separation

of *0. gratissimum*, *0. suave* and *0. viride* from the section Ocimodon and their incorporation in Heirocimum of the genus *Ocimum* parallel to *0. sanctum* has been suggested.

Though, x= 8 and 12 have be considered as the basic chromosome numbers, on the basis of secondary association of the bivalents met with in some of the species, a lower base number of 4 has been suggested for the genus as a whole.

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REGULATION OF CELL DIVISION IN MERISTEMS

I. MITOSIS

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SUMMARY

The dependence relationship between macromolecular synthesis before and during mitosis and some of the mitotic morphogenetic processes have been analyzed in *Allium cepa* L. meristems.

Initiation of mitosis depends on protein(s) synthesized in early G_2 while is independent of any G.-synthesized RNA.

This G, protein diffuses from advanced nuclei throughout cytoplasm and anticipates mitotic entrance in lagging nuclei sharing a common cytoplasm. Apparently this protein has to do with the mitotic synchronization observed by metaphase in plurinucleate cells.

Progression of the chromosome condensation cycle in prophase depends on protein synthesized in early prophase, while the breaking down of nuclear envelope (which marks the end of the prophase) depends on RNA synthesized in mid-prophase.

In anaphase, centromere migration, but not division, depends on a simultaneous high energy supply. Finally, chromosome decondensation in telophase accelerates when simultaneous protein synthesis is interrupted. Simultaneously with this acceleration, there is advancement of nucleologenesis, a morphogenetic process which takes place in telophase and whose completion depends on a short period of RNA synthesis at the very start of nuclear transcription in telophase. This newly synthesized RNA acts as a trigger of the nucleologenesis assembly process.

Lastly, the reinitiation of a new cell cycle seems to depend on proteins synthesized during telophase, stressing the close integration between cellular functioning in interphase and mitosis.

VIRCHOW'S aforism «omnis cellula e cellula» was but a scientific prediction in 1855, since cell division was still unknown. BALBIANI (1876) described how nuclei became transformed in a number of «bâtonnets étroits» which first coalesced and afterwards divided in two groups. Improvement of fixing and staining conditions as well as the development of immersion objectives increased the accuracy of the cellular observations.

In 1879, FLEMMING, SCHLEICHER & STRASBURGER, independently, described the process of nuclear division after their observation on living material. The two former scientists observed embryonic cells of salamander, STRASBURGER those of *Tradescantia* staminal hairs. The described nuclear division was called mitosis by FLEMMING and karyocinesis by SCHLEICHER.

The similarity in the process for both plant and animal cells proved to extend to its biological basis as well as to the compartmentation in phases, to their sequence and, probably, to the regulatory mechanisms which control cellular proliferation as well.

The present work will cover the regulatory points operating on nuclear division control, i. e. the mechanisms implicated in the precise distribution of the hereditary material. It is mainly intended to be a revision of a portion of the experiments carried out in root meristems by our group in Madrid.

1. Premitotic requirements

The nucleus is that portion of the cell which possesses the hereditary information of the organism. Hence, the importance of its unaltered permanence and its duplication. The doubling of the nucleus is the cellular way of preparing its equivalent partition into the two descendant nuclei. Doubling and division are the two main stages of the cell division cycle (Fig. 1), this cell cycle being the basic process Which provides the whole organism with all its structural and physiological units. Doubling of cellular material takes place in interphase, the duplication of genome being confined

to S period, while both nuclear and cytoplasm division occurs later on.

The cell cycle is a gene programmed event in the cellular life, as gene analysis in lower eukaryotes have brightly shown (HÄRTWELL, 1971; HOWELL, 1974; FRANKEL & DE BAULT, 1976). Hence, gene information is sequentially expressed throughout the cell cycle allowing its progression. For this, we may consider that cells in a proliferative tissue are indeed differentiated to proliferate.

There may be controls operating in the initiation of replication, in the initiation of division, in its termination probably. The study of the sequential triggers operating in cycle progression is a main stream in the area of cell proliferation since its knowledge is the perequisite for handling it.

In relation to the initiation of nuclear division it could be thought that the mere termination of replication might be the trigger. However endoreduplication (D'AMATO, 1964; NUTI-RONCHI, AVANZI ¡& D'AMATO, 1965; TSCHEKMAK-WOESS, 1971 & NAGL, 1974) and polytenia (AVANZI, BRUNORI & *D'*AMATO, 1969; BRADY & CLUTTER, 1974) are two processes which positively prove that this is not the case. The existence of 4C cells in temporarily quiescent meristems (SANS &. DE LA TORRE, 1979) as well as the fact that when deprived from aminoacids they stop in G_2 (VAN't HOF, HOPPIN & YAGI, 1973) reinforce these facts.

In 1974 GONZALEZ-FERNANDEZ, GIMENEZ-MARTIN, FERNANDEZ-GOMEZ & DE LA TORRE studied the protein requirements in the premitotic period, G_2. For this, they used a synchronous cell population labelled as binucleate in the meristem. Fig. 2 shows how binucleate cells are formed by a short treatment with caffeine, which labels those spontaneously synchronous cells traversing telophase. The binucleate cells formed by the caffeine treatment immediately initiates their interphase. This binucleate cell population is easily distinguished from the mononucleate cells which form the meristem. They can be followed throughout their cycle and the duration of the different cell compartments directly measured. The timing of the different cycle com-

partments under control conditions were previously known (LOPEZ-SAEZ, GIMENEZ-MARTIN & GONZALEZ-FERNANDEZ, 1966; GONZALEZ-FERNANDEZ, GIMENEZ-MARTIN & DE LA TORRE, 1971a). In order to test whether any protein synthesized in G_2 was involved in the entrance onto mitosis the experimental scheme used is shown in Fig. 3. Sequential treatments with a protein synthesis inhibitor (anisomycin) proved to affect entrance into mitosis in a differential way. Only those treatments covering the 19-20th. interval of the interphase prevented cells from entering into prophase. Accordingly, the last protein required for nuclear division to take place was synthesized in the early G_2.

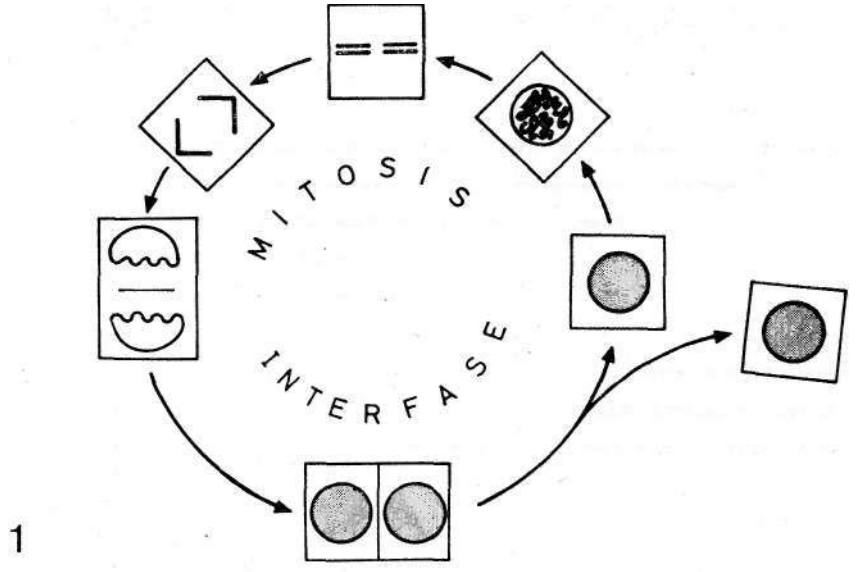
This regulatory step located in G_2 was further analyzed by using a different cell system: homokaryotic plurinucleate cells formed by caffeine inhibition of two sequential cytokineses (Fig. 4).

Fig. 5 shows such a 2n-2n-2n-2n-2n plurinucleate cell, whose two central nuclei often fuse in a tetraploid nucleus (Fig. 6), so that a 2n-4n-2n cell is formed. Less frequently fusion takes places between terminal nuclei and its corresponding adjacent nucleus so that a 4n-4n binucleate cell is formed. Though plurinucleate cells initiates prophase asynchronously, there is a strict synchrony in metaphase (Fig. 7) and anaphase (Fig. 8).

The development of the cell cycle in the nuclei of these cells Was followed so that nucleus-cytoplasm relationships were made evident (Fig. 9). There Was a clear asynchron'y in the development of replication in the different nuclei which shared the common cytoplasm. This asynchron'y,

Pig. 1. — Diagrammatic representation of cell cycle. It starts when cell in interphase is ready to divide. Leftwards, prophase, metaphase, ana- and telophase follow, with the result of formation of two cells Which qualitatively are identical to the premitotic cell, while quantitatively have half its content.

Fig. 2. — When cells progressing through their telophase are treated with caffeine, they are unable to form the cytokinetie plate, so that a binucleate cells is formed. This binucleate cell starts its new cycle immediately after its formation.



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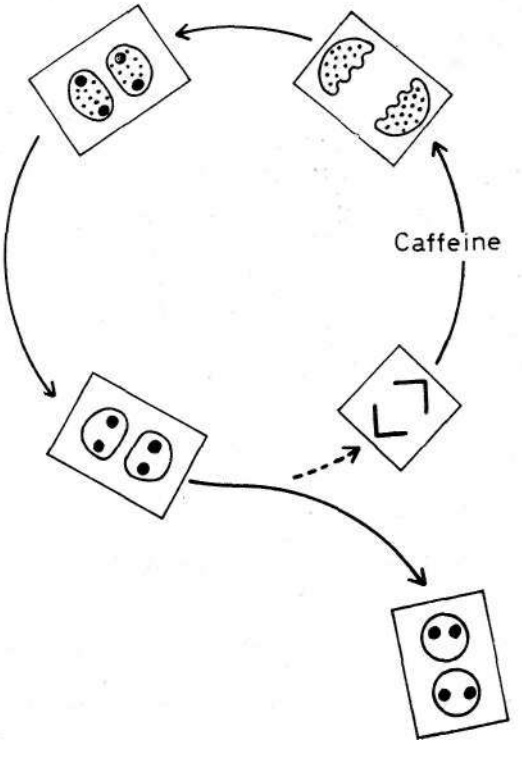

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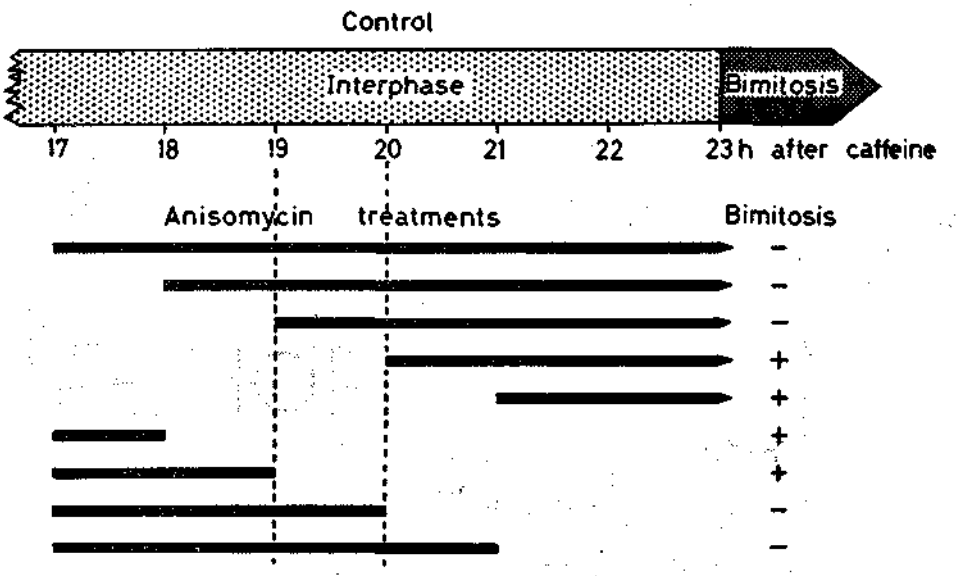


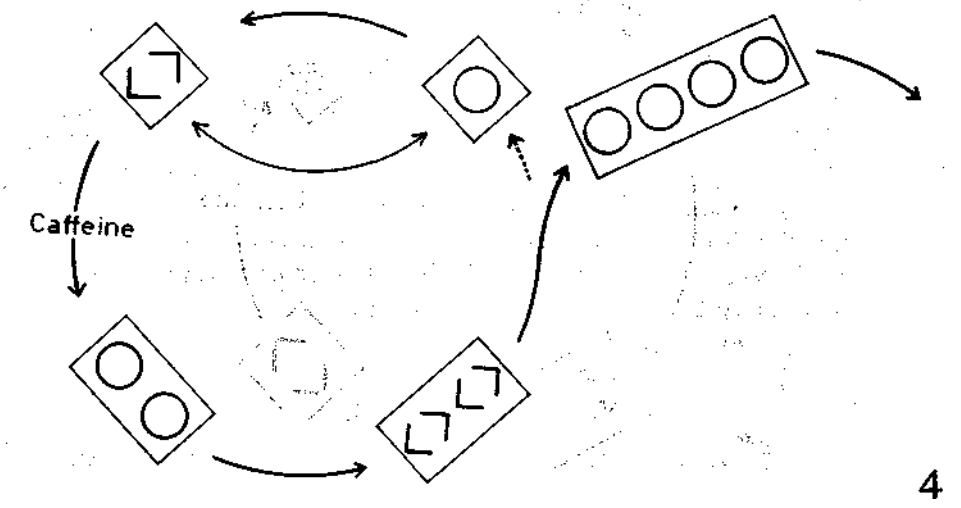


Fig. 3. — Sequential treatments with a protein synthesis inhibitor (anisomycin) during last portion of interphase of binucleate cells. These cells start their G_5 around the 19th hour of their interphase. The presence of prophases in this population at the 23rd. hour was checked, when they Were observed in normal untreated meristems. It is seen how those treatments covering the 19th-20th hour prevent entrance into mitosis.

Fig. 4. — Formation of 8n plurinuçleate cells by a double treatment with caffeine, so that during the first one telophases give rise to binucleate cells. These binucleate cells when in their next telophase (bitelophase) if treated again by a second caffeine-treatment produce the tetranucleate cells which immediately start their next interphase.

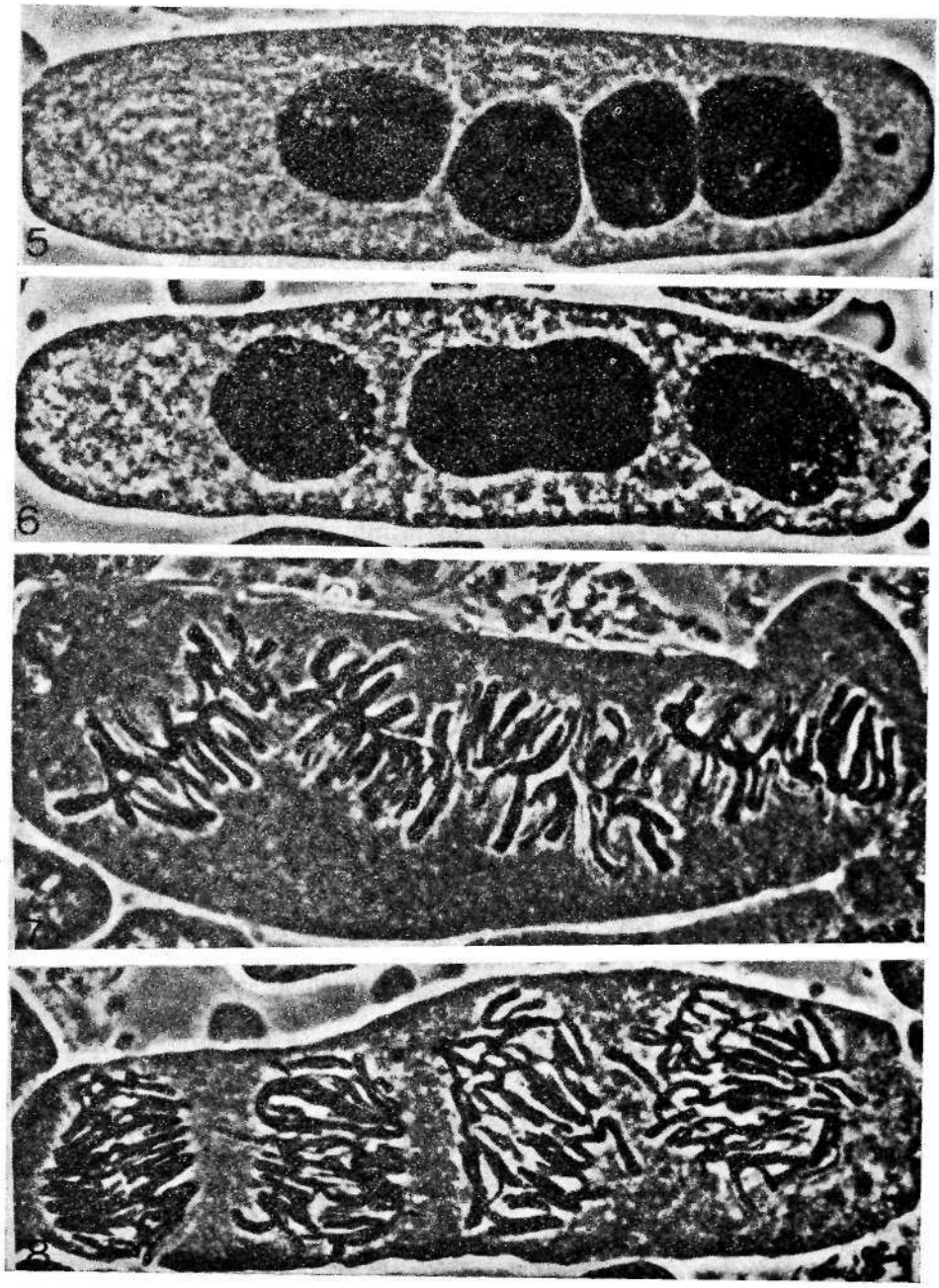


Fig-. 5.—Plurinucleate cell (2n-2n-2n-2n) formed by inhibition of two sequential telophases in meristem cells of *Allium cepa* L. meristems.

Fig. 6. — Plurinucleate cell (2n-4n-2n) formed, as in Fig. 5, but whose two central nuclei spontaneously fused by their physical proximity.

Fig. 7. — Plurinucleate cell whose 4 nuclei are synchronously traversing metaphase. Synchrony from metaphase onwards is the rule in these plurinucleate cells, though they enter prophase asynchronously.

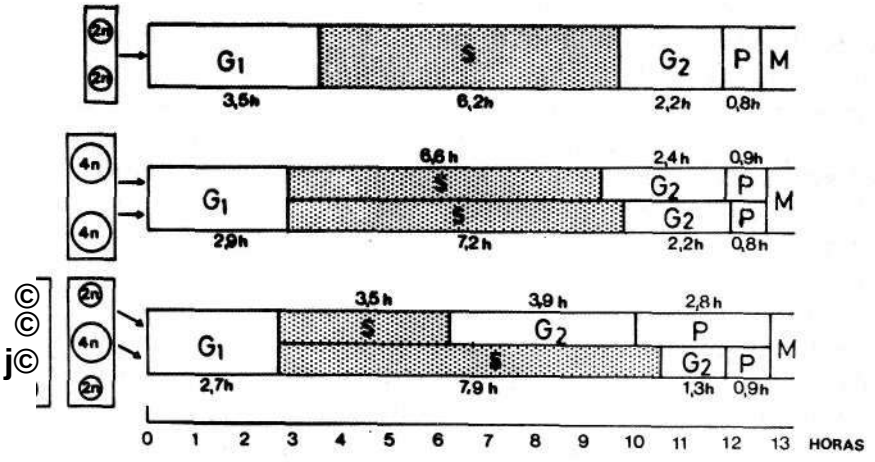
Fig. 8. — Synchronous plurinucleate cell in anaphase.

measured as différent length of the replication period (S period) was partially compensated by the shortening and lengthening of the respective G_2 and prophase, so that all nuclei were again synchronous by metaphase (GONZALEZ-FERNANDEZ, GIMÉNEZ-MARTÍN, DIEZ, DE LA TORRE & LOFEZ-SAEZ, 1971b; DE LA TORRE & GIMENEZ-MARTIN, 1977),

It was theoretically suggested a role of that protein synthesized in G_2 in the synchronization of homokaryotic nuclei sharing a common cytoplasm. Nuclei having first ended the S period reached first the G_2 control point and, accordingly, they started the synthesis of that regulatory protein(s). The protein synthesized diffused throughout cytoplasm so that the level of the stimulatory protein enough to trigger mitosis in the advanced nucleus was reached later than in a mononucleate cell. On the other hand, the lagging nucleus got a similar cytoplasm level of the mitotic stimulus, even early than its enterting into G_2. For this reason, the lagging nucleus will have a shorter G_2.

Fig. 9. — Relative duration of the different cycle comportments in 2n-2n and 4n-4n binucleate cells (upper and mid bars respectively) as well as in 8n plurinucleate cells (lower bar). We can see how ine nuclei of the plurinucleate calls replicate their DNA in times which essentially differs from those of the 2n-2n binucleate cells-Some of the nuclei in the plurinucleate cell have a very short S period, while other show longer S. However all nuclei in the plurinucleate cell reach metaphase in a total synchrony. The synchronization process involves a compensation of the S period by the duration of G_2 + prophase.

Fig. 10. — Analysis of the effect of protein synthesis inhibition on G_2 of plurinucleate cells. Anisomycin was used as a protein synthesis inhibitor in *Allium cepa* L. meristems. All the sequential treatment covering from the 6th. hour up to the 12th. hour of interphase were accomplished. For the sake of simplicity, we have only represented those two treatments covering early G_2 in fastly replicated nuclei as well as in their late G_2. Only when these plurinucleate cells are treated on early G_2 of fast nucleus there is a lengthening of G_2 both in «fast» and «slow» nuclei. Moreover, both nuclei start prophase synchronously. This advancement in reaching cellular synchronization points out the possible involvement of the G_2-protein in this process.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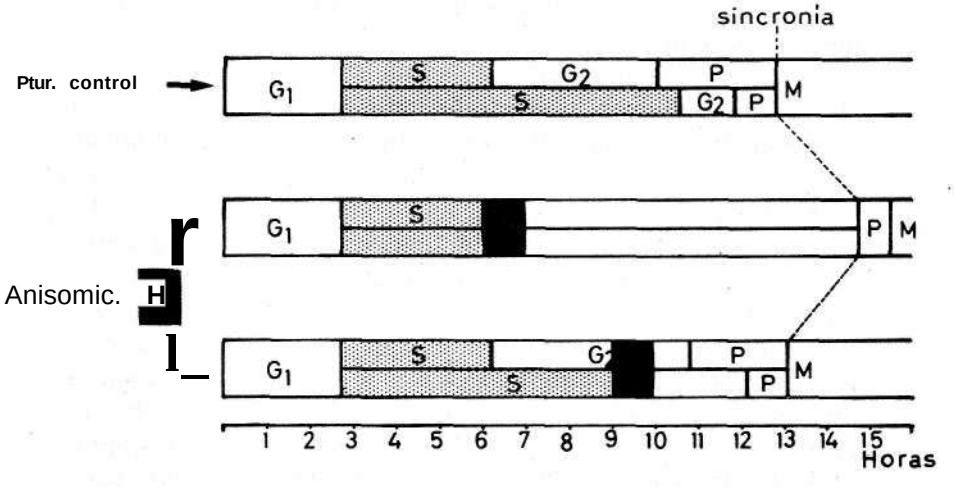
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This working hypothesis allowed the planning of new-experiments to know whether inhibition of protein synthesis in the G_2 control point of the advanced nucleus affected the entrance into mitosis of the lagging nucleus. The experimental scheme used was that of Fig. 10. As we see inhibition of synthesis of that protein in G_2 affects mitotic initiation. The data also suggest that the synthesis of such protein always take place at the same distance from the end of the replication period.

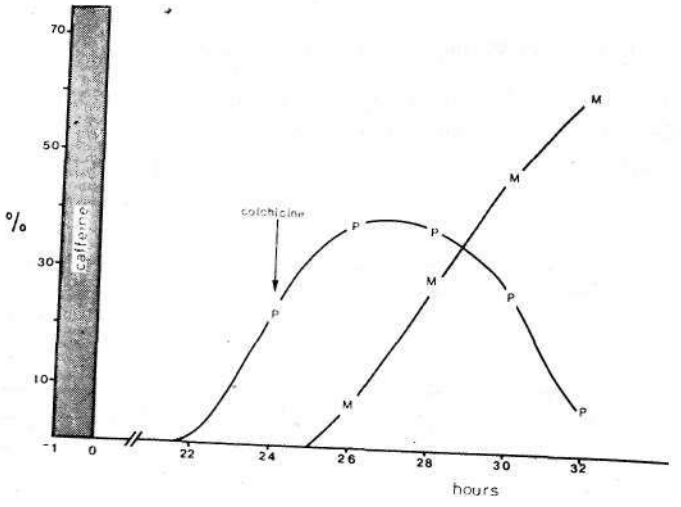
When looking to the role of RNA synthesis as a pre-mitotic requirement we can tell that similar experiments show that there is not any apparent role for the RNA'S synthesized in G_2 in relation to mitotic entrance.

2. Requirements in prophase

Nuclei that are ready and stimulated in G_2 start their prophase. Morphologically, prophase is easily recognizable by the increase in chromatin condensation which leads to a gradual discrimination of individual chromosomes as well as for the nucleolar desorganization. Prophase ends when nuclear envelope breaks down.

It was systematically accepted that a cell which had started prophase was inevitably commited to complete it. However, this is not the case. In 1974 GARCIA-HERDUGO, FERNANDEZ-GOMEZ, HIDALGO & LOPEZ-SAEZ (1974) wanted to know whether there were some controls regulating the development of prophase. For this, they took the synchronous cell population labelled as binucleate and they studied the kinetics of its mitotic development (bimitosis) (Fig. 11). When protein synthesis was inhibited in such population around mitotic entrance, the kinetics of entering into prophase was unaltered (Fig. 12). However, metaphases hardly appeared. This was interpreted as a revision of the prophase back to interphase. The experiments showed that some protein(s) synthesized in prophase regulates the normal progression of prophase itself towards metaphase.

In 1979 and 1980, similar experiments are being carried out in homokaryotic plurinucleate cells as a way of deter-



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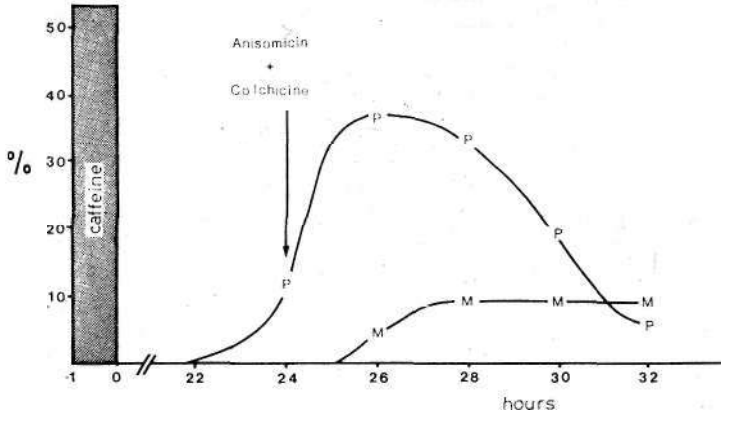

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Fig. 11. — Analysis of mitotic entrance into mitosis in binueleate cells. Colchicine is given from the 24th. hour onwards. A vawe of prophases is recorded, followed by an accumulation line for metaphases (colchicine effect).

Fig. 12. — Analysis of mitotic entrance into mitosis in the binueleate cells when protein synthesis is inhibited by anisomycin from the 24th. hour onwards, when colchicine is also used. The wave of prophases is similar to that in control conditions (Fig. 11). However metaphases do not accumulate, suggesting reversion of prophase under the protein synthesis inhibition in prophase.

mining the role of the prophase protein in nuclear synchronization by metaphase (DEL CAMPO, 1980). Fig. 13 shows the experimental scheme used as well as the duration of

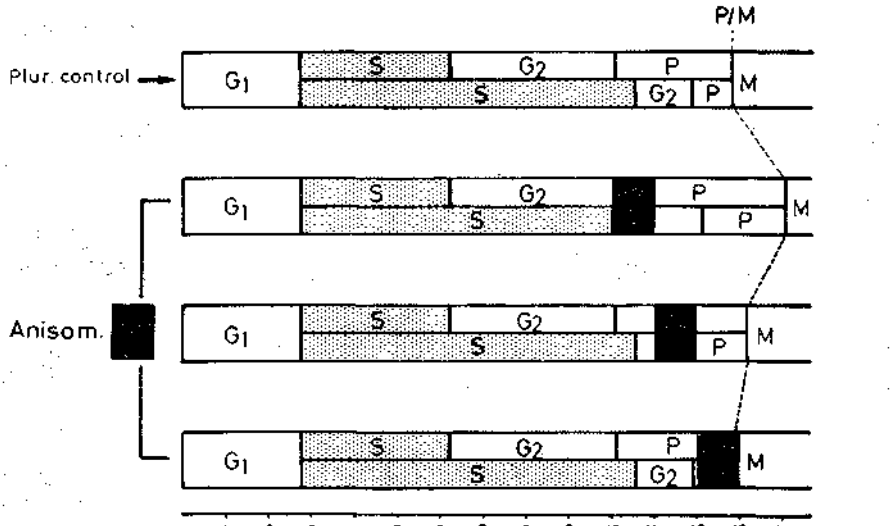

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Fig. 13. — Analysis of the effect of protein Synthesis inhibition during prophase in plurinucleate cells. Inhibition during early prophase of «fast nuclei» produces lengthening of prophase in both «fast» and «slow» nuclei (second row), while it does not affect prophase progression when used in late prophase (last row). This experiments show the position in early prophase of this protein-controlling point.

the different phases for both advanced and lagging nucleus in these cells,

The results show that inhibition of protein synthesis in the start of the prophase in the advanced nuclei lengthens prophase both in advanced and lagging nuclei, while if applied farther into prophase does not alter both kinetics.

In relation to the role of RNA synthesis in prophase, GONZALEZ-FERNANDEZ, FERNANDEZ-GOMEZ, STOCKERT & LOPEZ-SAEZ, 1970a; GONZALEZ-FERNANDEZ, GIMENEZ-MARTIN & LOPEZ-SAEZ, 19706 had already shown that when a cell

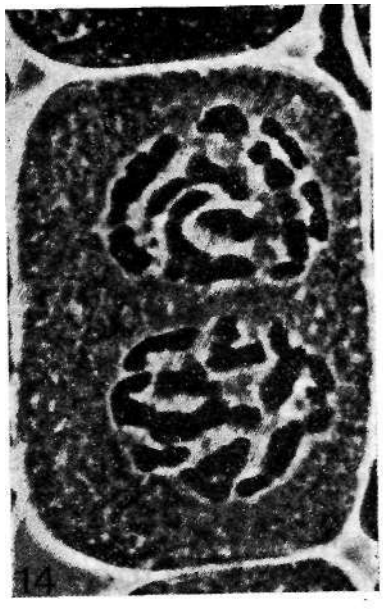
population in prophase is treated with such inhibitors the breaking down of nuclear envelope is prevented. Apparently the rupture of nuclear envelope was an RNA-dependent event. Those prophase cells under inhibition of RNA synthesis initiated an endomitotic process (GIMENEZ-MARTIN, GONZALEZ- FERNANDEZ, DE LA TORRE & FERNANDEZ-GOMEZ, 1971), since chromosome cycle went on in the interior of an intact nuclear envelope (Fig. 14, 15). Sequential treatments with an RNA synthesis inhibitor during prophase of plurinucleate cells showed that lengthening only occurred when applied in mid prophase (Fig. 16).

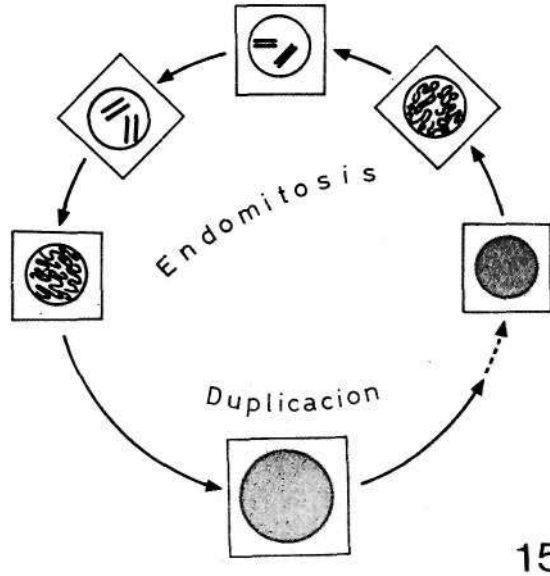
GARCIA-HERDUGO, FERNANDEZ-GOMEZ, HIDALGO & LOPEZ-SAEZ (1974) determined the relative position of both RNA and protein synthesis into prophase, by modifying the time of initiation of the inhibitory treatment. The results (Figs. 17, 18) showed that the prophase zone where synthesis of regulatory proteins took place was located earlier than the zone of synthesis of RNA required for the rupture of nuclear envelope. The nature of this regulatory RNA is difficult to discern. It must not be a messenger since there is not sensitivity to protein synthesis between the time of its synthesis and action. Moreover, although mitosis is a stage characterized by genome silence, the «in situ» assay for the activity of endogenous RNA polymerase has shown how transcription takes place in nucleoli up to the very instant of nuclear envelope breaking down, when all nucleolar remnants disappear (Fig. 21) (MORCILLO, DE LA TORRE & GIMENEZ-MARTIN, 1976).

3. Metaphase requirements

After nuclear envelope breaks down metaphase starts. Chromosomes migrate towards the cell equator; very longitudinal half chromosome ends its dehelicoidization while remaining united by the centromere.

The breaking down of nuclear envelope allows the direct contact between nuclear and cytoplasmic components and, as a result, there is an almost instantaneous connexion between centromeres and centrioles, or the atraction poles





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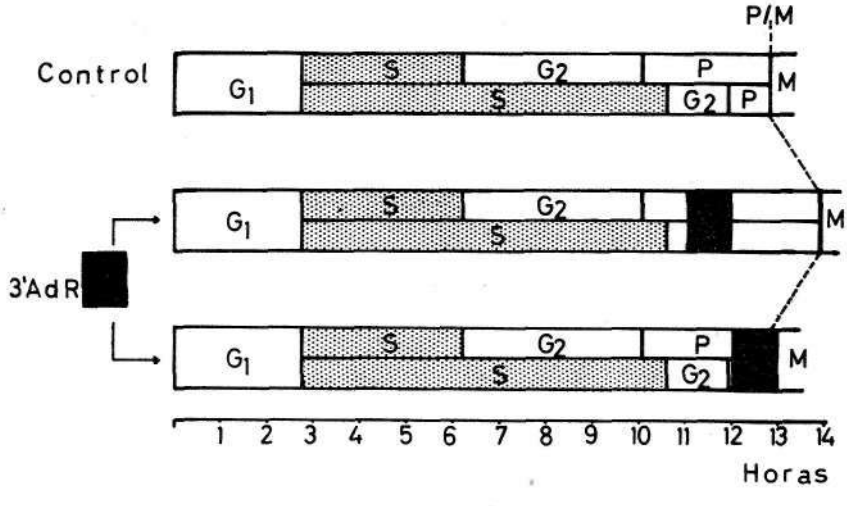

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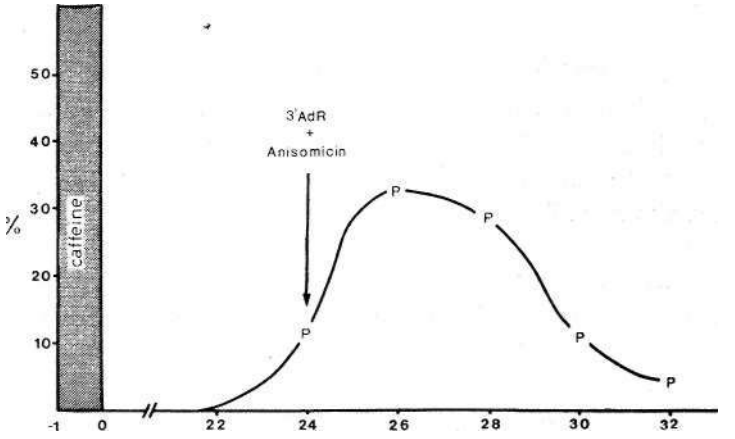
Fig-. 14. — Binucleate cell which has been treated with an RNA-synthesis inhibitor (3'-deoxyadenosine) during prophase. Breaking[1] down of nuclear envelope does not take place (as confirmed by electron microscopy). On the other hand, chromosome condensation goes on, so that highly condensed chromosomes are seen in the interior of the nuclei. The process resembles initiation of endomitosis.

Fig. 15. — Schematic representation of endomitosis, process which spontaneously occurs in some plant tissues. A cell ready to divide reaches prophase. Nuclear envelope rupture does not occur and divided chromatids stay and decondense. This nucleus Which possesses 4n chromosomes (tetraploid) initiates a new cycle.

Fig. 16. — Effect of an RNA synthesis inhibitor (3'-deoxyadenoslne) when applied in the prophase of 8n plurinueleate cells. Inhibition at mid prophase but not on other times lengthens prophase in both «fast» and «slow» nuclei.



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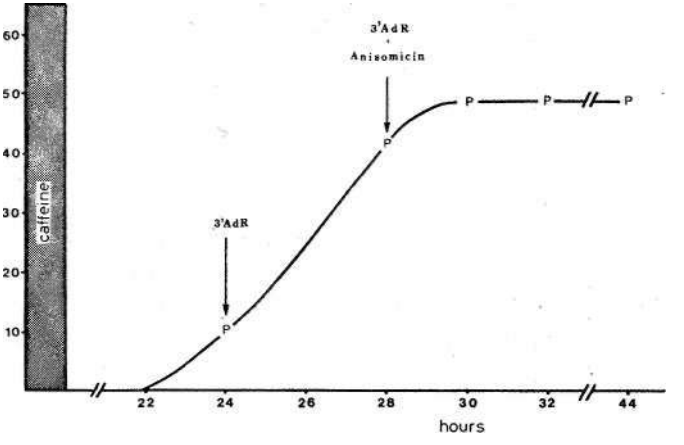

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Fig. 17. — Analysis of prophase wave in the binucleate cell population, when both RNA and protein synthesis are simultaneously blocked from the 24th. hour of interphase onwards. The wave resembles that obtained when inhibiting protein synthesis alone (Fig. 12). This suggests that the prophase protein synthesis step is located earlier in prophase than the RNA-synthesis step.

Fig. 18. — Analysis of the prophase wave in the binucleate cell population. The experimental scheme involves the inhibition of RNA synthesis (by 3AdR) from the 24th. hour onwards, and simultaneous inhibition of RNA and protein syntheses from the 28th. hour of interphase. The curve shows how the cells are permanently blocked in prophase, confirming the sequence of protein and RNA synthesis control points in prophase.

when centrioles are absent, as in the plant cell. That cennection is mediated by microtubules. Centromeres, centrioles and microtubules form the achromatic spindle which constitutes the so-called mitotic apparatus. In fact this apparatus is responsible of the proper migration of the two similar halves of the cellular genetic material, earlier duplicated.

The formation of this mitotic apparatus in the cell is a mere assembly process since it occurs «in vitro» where added microtubules spontaneously assembly on centromere of isolated chromosome (TEELZER, Moss & ROSENBAUM, 1975).

Drugs which block polymerization of the microtubule monomeres stop «in vivo» the formation of the mitotic apparatus. They are called c-mitotic drugs and mitotic poisons. The most well-known of these drugs are colchicine, vinblastine, podofiline, gammahexane, etc.. As a consequence of the inhibition by these drugs of the microtubule assembly in the mitotic spindle, chrommosomes remain dispersed in the cytoplasm, centromeres do not divide and chromosomes adopt an X shape, with their chromatid highly condensed. Chromosomes are unable to migrate, and after a period of time, chromosomes start decondensing, a nuclear envelope reforms and a polyploid cell is formed. Colchicine was the first drug used for producting polyploidy after having determined its action (BLAKESLEE & AVERY, 1937).

In a normal mitosis, metaphase is characterized by the equilibrium in the position of its chromosomes, equilibrium which breaks down when migration of chromosomes starts. Division of centromeres marks the end of metaphase. However between the division of centromeres and the migration of chromatids it is possible to distinguish another conditioning factor, the simultaneous availability of energy. Hence, under deprived cellular 0_2-concentration (8-oxiquinoline-treatment, TJIO & LEVAN, 1951; hypoxia, GIMENEZ-MARTIN & LOPEZ-SAEZ, 1982) this chromosomal migration towards cellular poles either does not take place, or takes place very slowly (Fig. 19, 20). The situation was reversed at once when' O_2 was supplied (GIMENEZ-MARTIN & LOPEZ-SAEZ, 1982). Metaphase chromosomes under hypoxia show

separated centromeres while telomeres remain closed (Fig. 20).

4. Anaphase. The regulation of chromatid migration

Anaphase starts with migration of each chromatid toward opposite poles as direct by centromere pulling. Anaphase is the real stage of cell cycle where physical distribution of gene material is accomplished.

Two different kinds of microtubules are observed: those continuous microtubules (MTs) i. e., giving from pole to pole, and on the other hand, those microtubules which go from each centromere to its opponent pole. They follow different kinetics in anaphase for as those continuous MTs seems to lengthen, those connecting with centromeres shorten while chromatids migrate (BAJER & MOLE-BAJER, 1972).

There are many different theories trying to explain the dynamic relationships between the different component of the mitotic spindle. However there is not any fully accepted unitary theory for such complex relationships in anaphase. UV-irradiation by microbeam has allowed to detect some link between half-chromosomes migrating towards opposite poles, since irrediation in front but nor behind centromeres interrupts migration of the other sister chromatid towards opposite pole (BAJER & MOLE-BAJER, 1979).

There is a number of substance which alter the physiological migration of anaphase chromatids. C-mitotic substances modify the number of atraction poles. MAZIA, HARRIS & BIBRING, 1960; GIMENEZ-MARTIN & LOPEZ-SAEZ, 1980; HERVAS, FERNANDEZ-GOMEZ & GIMENEZ-MARTIN, 1974) was able to induce the formation of 3 or 4 chromosomes poles and, as a consequence of observing qualitative and quantitative unbalanced gene distribution.

5. Telophase

The arrival to poles of both groups of quali- and quantitative similar chromatids originates two nuclei qualitatively identical to that which initiated mitosis, but quantitatively

with only half its content. This process allows the physiological activity of nucleus to be resumed, both in relation to its structural and functional reorganization. However, when multipolar cells are formed by unequal distribution of chromosomal material, nuclei are unbalanced in the sense that they are genetically different to the initial, this difference producing changes in their activity.

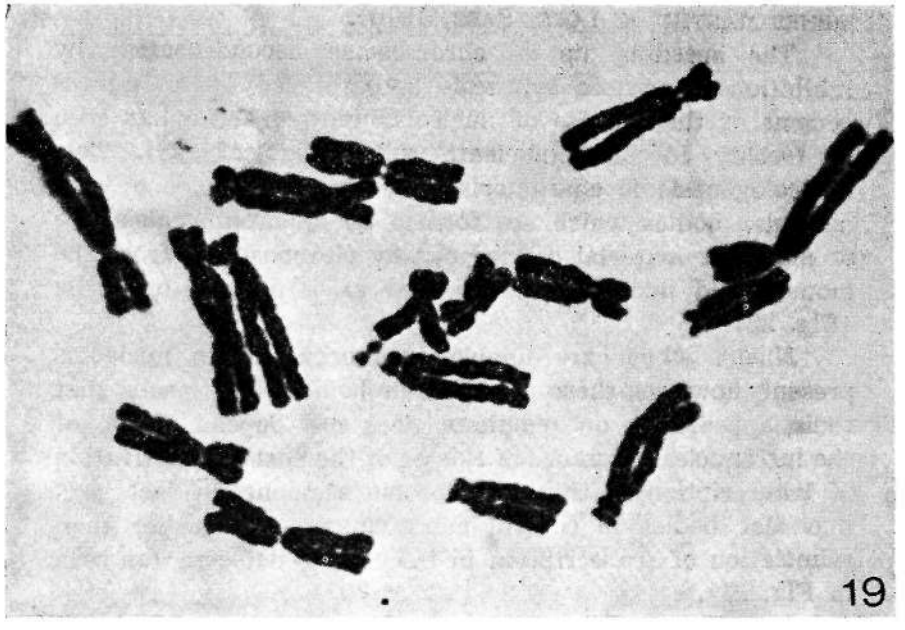
The first sympton of telophase is the nucleus/cytoplasm compartmentation, for a nuclear envelope is reformed around of each chromosomal group. This reformation occurs idependently of the chromosome content and of the functions which those chromosomes are able to assume. Under the electron microscope it is seen how telomeres present remnants of old nuclear envelope, which probably act as the assembly nucleus of endoplasmic reticulum to constitute the new nuclear envelope.

In telophase, chromosomes start decondensing. This process of chromosome decondensation is accelerated by simultaneous inhibition of protein synthesis (MORCILLO & DE LA TORRE, 1979a). This suggests that chromosome condensation actively depends on protein synthesized during mitosis itself, both in prophase (as earlier commented) and in telophase.

Simultaneously to the chromosome decondensation nucleolar reformation talkes place. This nucleologenesis is always linked to the intranuclear presence of a NOR, as it has been shown in aneuploid nuclei produced by unbalanced

Fig. 19. —- *AUiUm cepa L.* chromosomes from meristems treated by 8-oxiquinoline. The diploid chromosome number 2n = 16 is confirmed: The pair of satellized chromosomes is easily distinguished the degree of extension of their secondary constriction being different for each chromosome of the pair. Chromosomes are fully condensed. Most chromatids have lost their relational coils. Centromeres remain undivided and no anaphases are observed during treatment.

Fig. 20'. — *Allium cepa* L. chromosomes from meristems under hypoxia conditions. Chromatids remain parallel while some centromeres are already divided. Migration of chromatids is not observed under hypoxia, so that chromosomes remain dispersed throughout cytoplasm.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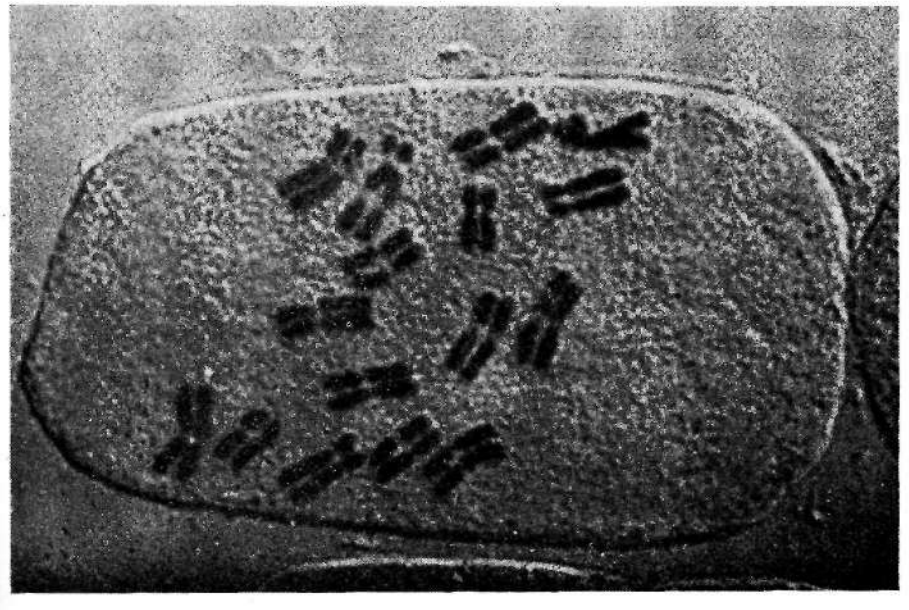


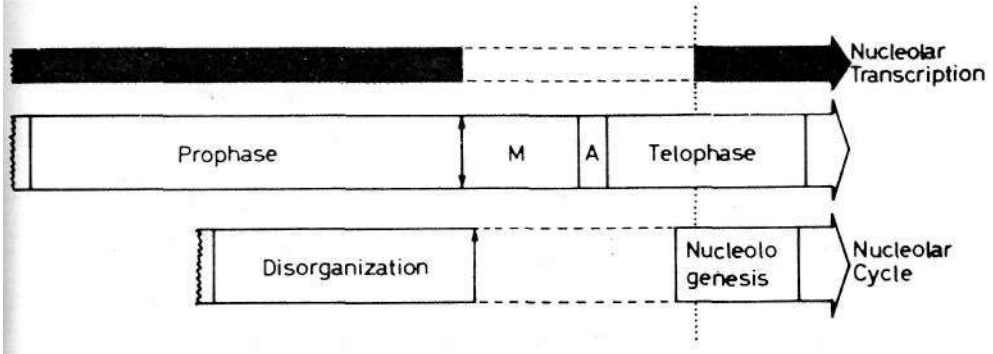
chromosome migration (STOCKERT, FERNANDEZ-GOMEZ, GIMENEZ-MARTIN & LOPEZ-SAEZ, 1970).

The speeding up of chromosome decondensation by inhibition of protein synthesis is paralleled by an advancement of the process of nucleolar reorganization, as seen in freshly formed binucleate cells (Fig. 22, 23). This nucleologenesis is characterized by the appearance of prenucleolar bodies which are formed by apparent coalescence of nucleolar material carried out by chromosomes from the moment old nucleolus disappeared as a defined organelle (Fig. 22).

Nuclei which are unable to reorganize its nucleolus present, however, these prenucleolar bodies. This means that their appearance on telophase does not depend either of the intranuclear presence of NOR or of the normal reactivation of transcription in this chromosome segment. In fact, prenucleolar bodies in control meristems appear earlier than reinitiation of transcription in the NOR is detected (as seen in Fig. 21).

Nucleologenesis appears regulated by chromosome condensation in the time while it depends on the existence of a NOR, whose reactivation produces as a first effect the coalescence of prenucleolar bodies on it. Hence, inhibitor of protein synthesis advanced nucleologenesis, while tetraploid nuclei induced by c-mitotic drugs which produced an increased chromosome condensation show a lengthened nucleologenesis time.

Inhibitors of RNA synthesis given at the time of nucleologenesis prevents NOR activity to reinitiate. Simultaneously we can observe how prenucleolar bodies remain and new nucleolus is not formed. Experiments with sequential inhibition of RNA-synthesis throughout telophase (Fig. 24) have shown that the reinitiation of RNA synthesis 'which is enough to allow nucleologenesis to complete covers, in fact, a very limited zone of telophase. This new RNA synthesized ihi telophase behaves as an initiator or trigger of nucleologenesis process which from this time onwards seem to be E:. mere assembly of the dispersed nucleolar material (MORCILLO & DE LA TORRE, 1979b).



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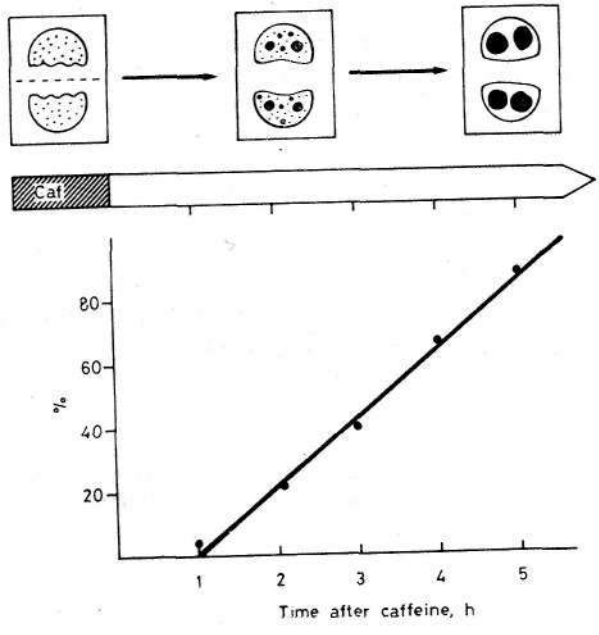

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Fig. 21. — Matching of nuclear transcription with mitotic phases and with phases of the nuclear cycle, as measured in the total meristem population of *Allium cepa* L. meristems. The diagram shows how nucleolar transcription stops at the very end of prophase as it restarts by midtelophase, after the presence of prenucleolar bodies which characterizes nucleologenesis is detected.

Fig. 22. — The use of binucleate cells for studying nucleologenesis rate. A freshly formed binucleate cell, formed by caffeine-inhibition of cytokinesis, is characterized by the presence of prenucleolar bodies (middle figure in upper row). Binucleate cell with fully organized nucleoli is depicted at the right. The graph shows how the recording of the frequency of binucleate cells with fully organized nucleoli at different times after the binucleate cells production gives us a way of estimating the minimum and the medium and maximum nucleologenesis time in these cells.

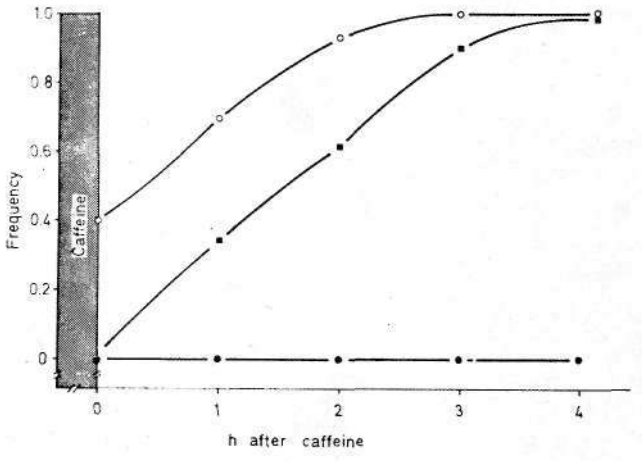
In the same way there is independence between replication and nuclear division (as it is shown in endoreduplication and polytenia) nuclear and cytoplasm division are not always sequentially linked, as it occurs in hepatocytes where spontaneous binucleate cells can be formed.

Experimentally it is also possible to dissociate both nuclear and cytoplasmic division, allowing either only mitosis to take place (caffeine-mediated effect) or only cytoplasmic division to occur (ethidium bromide effect). In this last example the development of the cytokinetic plate occurs at the expected time when the nucleus still remains in prophase. The growth of this cytokinetic plate ends by dividing the nuclear content with the final result of an unbalanced share of its content (Fig. 25, 26).

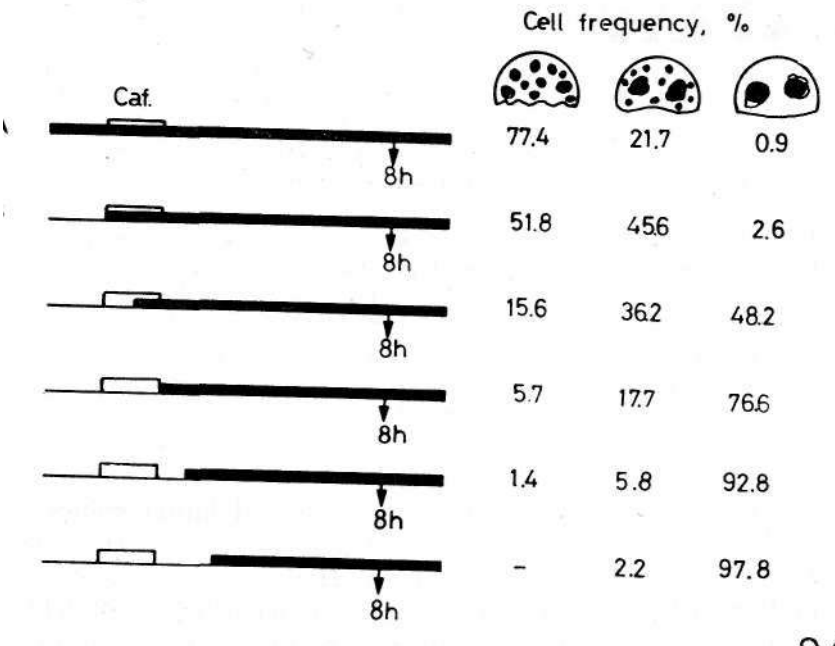
Finally, anisomycin given to cells which are completing telophase, produces an important lengthening of G_1, as measured by the delay in entering S period. At the same time 50 '% of the population is unable to enter in replication (SELMAN, in preparation). These experiments suggest that proteins synthesized at the end of telophase might be related to the post-mitotic decision of continuing or abandoning proliferation.

Fig. 23. — Kinetics of nucleologenesis in binucleate cells, both in control conditions (lower line) and under simultaneous inhibition of protein synthesis. We can see how this inhibition induces an advancement of the nucleologenesis period in relation to the cell cycle.

Fig. 24. — Schematic representation of the experiments carried out to detect the role of reinitiation of RNA synthesis in the nucleologenesis process. Binucleate cells were used in all cases. The treatment With an RNA synthesis inhibitor (3'deoxyadenosine or ethidium bromide) was progressively displaced in relation to the caffeine treatment. 8h after the end of this treatment, the frequency of nuclei with fully organized nucleoli (last column) was recorded. We can see how inhibition of RNA synthesis from 1 hour after caffeine onwards does not affect nucleologenesis, though as we saw in Fig. 22 at this time only very few cells showed fully organized nucleoli. The experiments suggest nucleologenesis depends on RNA synthesized during telophase and the first hour of interphase, but not on later synthesis.


1.0
0.8
0.6
0.4
0.2
0
Frequency
Caffeine
0
1
2
3
4
h after caffeine

23


Cell frequency, %
Caf.
8h
8h
8h
8h
8h
8h
77.4
21.7
0.9
51.8
45.6
2.6
15.6
36.2
48.2
5.7
17.7
76.6
1.4
5.8
92.8
–
2.2
97.8

24

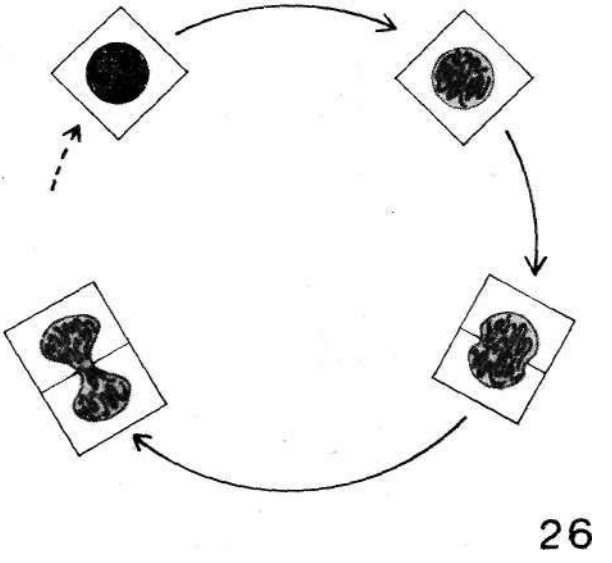


Fig. 25, — Mononucleate cell treated with an RNA synthesis inhibitor (ethidium bromide) during its prophase. Cell division has taken place while unequal and uncomplete nuclear distribution of undivided chromosomes is observed.

Fig. 26. — Diagram showing the occurrence of cytoplasmic division in a nucleus whose nuclear envelope did not break down and whose chromosomes appear as in early or mid prophase. The growing of the cytokinetic plate finally gets the physical unequal division of the undivided chromosomes, in a process similar to the described amitosis, with the end result of the formation of two genetically unbalanced nuclei.

On the other hand, RNA synthesis inhibition shows a similar effect on the delay in initiating replication the cells treated during its late telophase. However, the frequency of them which reach replication is not modified at all. This RNA synthesis in mitosis might well be related to the protein synthesized much later in the cycle, in the initiation of G_2, protein which controls the entering into mitosis of the cells.

CONCLUSIONS

These earlier commented data suggest that:

1. The stage of nuclear division — or mitosis — is not an independent stage in the general picture of the cycle. Synthesis of protein in G_2 of interphase conditions the start of nuclear division and protein(s) synthesized in late telophase condition the development of the following interphase. Moreover proteins synthesized before telophase is completed, apparently, are responsible for the decision of the cell to going on cycling.

2. Synthesis of protein in early prophase controls the progression of prophase itself, since its inhibition make the cell to reverse towards interphase.

3. RNA synthesized towards midprophase acts on the breaking down of nuclear envelope while does not affect the chromosomal condensation cycle. Its inhibition induces an endomitotic process.

Nucleolar transcription occurs throughout all prophase though nucleoli are in process of disorganizing.

4. Synthesis of microtubular proteins and their assembly are required for the formation of the mitotic spindle and, secondarily, for the emigration of the divided chromosomes.

5. The start of anaphase, after depending of all previous steps, is also dependent of a simultaneous energy supply which is much higher than the one required in other mitotic stages. Lack of this energy stop cells in metaphase and, after a new energy supply, chromosomes fastly reinitiate their polewards movements.

6. Gene silence is broken again towards mid telophase.

7. The reinitiation of transcriptional activity in the nucleus is immediately followed by the reformation of nucleolus. Inhibitors of RNA synthesis do not prevent the appearance of prenucleolar bodies but prevents their coalescence in the new nucleolus.

8. Inhibitors of protein synthesis facilitate the decondensing of chromosomal material and, simultaneously, advance of nucleolar reorganization.

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REGULATION OF CELL DIVISION IN MERISTEMS

II. CYTOKINESIS

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ABSTRACT

Cytokinesis occurs generally by cell plate formation in meristem cells. The cell plate arises in the mid region of the cell and is «spun out» toward the edges. The new plasma membrane cannot simply be an extension of the preexisting plasmalemma as it could be in cytokinesis by cleavage. The position of the cell plate is determined by the preprophase microtubule band and the cytoplasm division has only a «chance» to divide in each cell cycle, since the inhibition of one cytokinesis gives rise to a permanent binucleate cell.

Plant cytokinesis can be considered as a topographycally organized secretion process, where the cell plate is formed by the coalescence in the equational plane of small vesicles produced by Golgi bodies.

The membranes of these vesicles make up the new plasma membrane and the contents of the vesicles gives rise to the amorphous matrix of the new wall. Therefore, origin, translocation and fusion of the small Golgi vesicles are the physiological processes involved in plant cytokinesis. Meanwhile, the production, accumulation, arrangement and fusion of Golgi vesicles are the morphological phases of cell plate formation.

Experimental analysis with selective inhibitors has demonstrated the essential role of Golgi apparatus in the small vesicle production, of the microtubules in vesicle translocation, and of the membrane fusion reaction for vesicle arrangement and coalescence. Lastly, calcium and magnesium appear to be cytokinesis requirements by afecting membrane fusion.

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FOLLOWING *karyokinesis* or *mitosis*, which separates sister chromatids from one another to each pole, *cytokinesis* eventually takes place, and this cytoplasmic division may be considered as the last event of one cell cycle.

Cytokinesis (from greek: cytoplasmic movement) occurs generally by furrowing in animal and by cell plate formation in plant cells. But this statement is, of course, an oversimplification, for not only in animal cells cytokinesis takes place by furrowing, but also in algae, fungi and most meiotic divisions in vascular plants, while centrifugal formation of the cell plate is ubiquitous in vascular plants. Generally, cytokinesis overlaps the last third of mitosis and represents a small fraction of the whole cycle time (2-4%), taking place only in a few minutes in most species. This formation of a new cell wall takes place by the so-called *phragmoplast* (from greek, phragma = separation), which appears as a plasma body in the equatorial region of the mitotic apparatus during anaphase and telophase. Inside the phragmoplast small droplets form a plane which is perpendicular to the spindle axis and equidistant from the two anaphase poles. This plate arises in the mid region of the cell and is «spun out» toward the edges. The new plasma membrane cannot simply be an extension of the preexisting plasmalemma as it could be in cytokinesis by cleavage. Apparently, the position of the future cell plate is simultaneously determined with the location of the mitotic apparatus during prophase and it has been demonstrated that the *preprophase microtubule band* determines metaphase plane and cell plate position in normal mitosis and cytokinesis as well as in highly asymetrical ones (PICKETT-HEAPS, 1969).

STRASBUBGER'S studies (1875) of cell division *in vivo* in stamen hair cells of *Tradescantia* already described the phragmoplast as a filamentous texture and the cell plate formation as the fusion of granules («Köperchen»). Later BELAR (1929) & BECKER (1938) studied the earlier stages of cell plate development, showing in a series of elegant experiments that this structure is double with a soft consistency in its interior.

At anaphase, the first indication of cell plate formation was the appearance of stainable nodules in the equatorial plane and these nodules were originally believed as thickened spindle fibres but BECKER (1935) showed that the small droplets were able be vitally stained with vacuome dyes, such as neutral red or cresyl blue. In fixed preparations, the small granules apparently agglutinated with the spindle fibers of the phragmoplast, whilst on observation in living and unflattened state in *Tradescantia* stamen hairs, they are identified as a semi-liquid region.

Eventually, the small droplets coalesce to form the cell plate across the mother cell. This plate, which starts near the center of the equatorial plane, grows outward to the lateral walls, in a similar way to the openning of an iris diaphragm. This plate can be stained vitally with basic dyes, such as ruthenium red or methylene blue, suggesting the presence of acidic compounds like pectin precursors and other uronides.

Now, it is well known that the phragmoplast initiates from remnants of the achromatid spindle, but later the cytoplasm bordering the spindle region also contributes. «Phragmoplast filaments» are composed of microtubules bundles, and the particles which appear in the phragmoplast are Golgi bodies.

The fibrillar component of the phragmoplast has a transport function. The dimensions of particles entering the phragmoplast suggest that they correspond to Golgi bodies. BAJER (1965) & BAJER & ALLEN (1966) have clearly demonstrated with interference microscopy that the transport of a considerable amount of organic matter toward the cell plate is an important process involved in plant cytokinesis, and they have proposed that some kind of co-operation between particles and «phragmoplast filaments» is responsible for this mass transport.

PHASES OF CYTOKINESIS

As many others biological processes, cytokinesis appears to be a continuous one in which it is difficult to separate the various mechanisms that operate as one harmonious whole or equally difficult to dissect the process into phases.

However, electron microscopy has revealed the ultrastructural stages of plant cytokinesis. Former studies, with permanganate fixation, were able to find the membrane behaviour during cell plate formation (PORTER & MACHADO, 1960; PORTER & CAUFIELD, 1960; WHALEY & MOLLENHAUER, 1963; FREY-WYSSLING, LOFEZ-SAEZ & MUHLETHALER, 1964; RISUEÑO, GIMENEZ-MARTIN & LOPEZ-SAEZ, 1968; MOLLENHAUER & MOLLENHAUER, 1978) and latter approaches, with glutaraldehyde fixation, have confirmed the basic findings and suggested an important role for microtubules in the process (ESAU & GILL, 1965; PICKETT-HEAPS & NORTHCOTE, 1966; HEPLER & NEWCOMB, 1967).

The ultrastructural study of normal cytokinesis allows to distinguish several phases, which overlap each other like tiles on a roof (Pig. 1).

Fig-. **1.** — Schematic diagram of plant cytokinesis showg the different phases.

I — Production of Golgi vesicles. During anaphase and telophase many dyctiosomes are accumulated on the outer surface of the interzonal spindle and the small vesicles appear uniformly distributed throughout the cytoplasm.

II — Accumulation of Golgi vesicles. During early telophase Golgi vesicles accumulate in the middle of the equatorial region forming as a cloud between the two telophasic nuclei.

III — Arrangement of Golgi vesicles. During telophase, the small vesicles become arranged in the equatorial plane. This phase, as the vesicle accumulation, begins in the central region and proceeds centrifugally.

IV — Fusion of Golgi vesicles. The coalescence of the vesicles begins in the inner part of the forming cell plate during middle telophase. When the growing cell plate reaches the longitudinal walls of the mother cell cytokinesis is complete and two daughter cells have become independent.

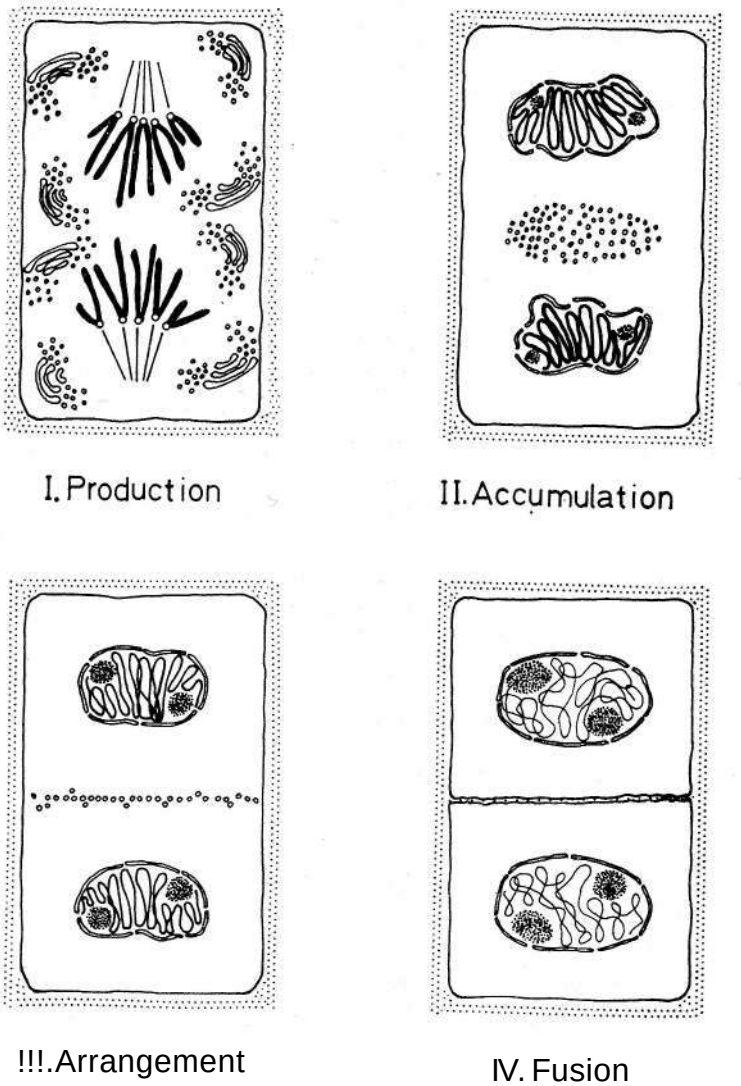
Plant cytokinesis
I. Production
II. Accumulation
!!!. Arrangement
IV. Fusion

Fig. 1.

In order to understand in an easy way the characteristic features of cytokinesis it should be born in mind that cell plate formation begins in the central area of the equatorial plane, growing outward throughout this plane toward peripheral walls of the mother cell. That is to say, the process is not a synchronous one in the whole plane but it begins in the middle region moving centrifugally as a circular wave arised by a stone falling into water.

1) Production of Golgi vesicles

This stage involves the formation, in large quantities, of small vesicles from Golgi bodies. The dyctiosomes marshall the precursors of the cell plate. It is difficult to point out exactly the moment at Which this phase begins and ends during mitosis. However, likely the period of the highest production rate lasts from metaphase. During anaphase and telophase many dyctiosomes are accumulated on the border of the phragmoplast, on the outer surface of the interzonal spindle. The small vesicles, uniformly distributed throughout the cytoplasm during prophase and metaphase, congregate at the center of the equatorial region, inside the interzonal spindle.

2) Accumulation of Golgi vesicles

In the middle of the equatorial region, this stage is initiated with anaphase and reaches its highest point at early telophase. Golgi vesicles accumulate forming as a cloud, and the continuous supply of new ones facilitates the centrifugal spreading of the accumulation cloud to the greater part of the equatorial region of the mother cell.

Glutaraldehyde-fixed, osmium tetroxide-post-fixed material has revealed the involvement of the microtubular system in this stage of cell plate formation (ESAU & GILL, 1965; PICKETT-HEAPS & NORTHCOTE, 1966; HEPLER & NEWCOMB, **1967).**

During accumulation of Golgi vesicles, a great density of microtubules occurs through the central part of the

mitotic cell, where microtubules run parallel to one another and perpendicular to the prospective plane of the plate. As the accumulation cloud expandes and the small vesicles are superseded by continuous portions of cell plate, the microtubules are absent from the central region but abundant at the cell plate edges, from which they run obliquely back toward the two anaphase poles (KEPLER & NEWOOMB, 1967).

In longitudinal sections, vesicles are commonly observed between the microtubules in long chains apparently attached to one another and flowing into the plate (ESAU & GILL, 1965; KEPLER & JACKSON, 1968).

The great evidence accumulated involving microtubules in cytoplasmic streamings makes logical to assume that interzonal spindle is the system that accumulates small vesicles by producing cytoplasmic flows coming from each daughter nucleus to rest in a «zone of equilibrium» in the equatorial region. As proposed by PORTER (1966) microtubules may determine the channels along vesicles move, and this idea is consistent with observations by ESAU & GILL (1965) and KEPLER & NEWOOMB (1967) on microtubular system during cytokinesis.

Microtubules appear concerned with vesicle accumulation in the equatorial region and they act probably to determine the vesicle pathway. KEPLER & NEWOOMB (1967) have proposed that the vesicles must move quickly through the microtubule region, since the vesicle density is apparently high in the vecinity of dictyosomes and again in the plate edges, but this vesicle density is low in the intervening region.

3) Arrangement oí Golgi vesicles

During telophase the small vesicles become arranged in the equatorial plane, so that a row of Golgi vesicles can be observed along this plane in longitudinal sections. As the vesicle accumulation, this phase begins in the central region and proceeds centrifugally.

The expanding plate is always rimmed by clusters of Golgi apparatus producing small vesicles, and large numbers

of Golgi vesicles continue to be added to the cell plate during late telophase.

In relatively advanced stages of plate formation vesicles of two different sizes, both the smaller vesicles and the larger ones can be identified (LOPEZ-SAEZ, RISUEÑO & GIMENEZ-MARTIN, 1966; WHALEY, DAUWALDER & KEPHART, 1966). The smaller ones, measuring about 50-100 nm in diameter, appear to be the direct product from the Golgi bodies, while larger vesicles, which are observed only in or near the zone of the cell plate, appear to arise from fusion of the smaller vesicles (HEPLER & NEWCOMB, 1967).

4} Coalescence of Golgi vesicles

The fusion of Golgi vesicles starts in the inner part of the equatorial plane. At the beginning, during middle telophase coalescence, arrangement and congregation can be observed respectively in the inner, intermediate, and marginal parts of the forming cell plate. This kind of observation clearly makes evident the course of the overlapping phases which make up cytokinesis.

The study of transverse sections from telophase cells has confirmed the centrifugal growth of the cell plate and revealed that microtubules almost entirely disappeared from the region of vesicle fusion, being observed in large numbers near the plate edge, where an earlier stage of development is observed (HEPLER & NEWCOMB, 1967).

While this stage of coalescence proceeds many vesicles fuse between the clusters of microtubules in the established plane of the growing plate. The origin of plasmodesmata from entrapped elements of the endoplasmic reticulum is generally accepted to take place during this phase, when the continued growth of the cell plate constrics cytoplasmic strands and eventually catchs some tubules of endoplasmic reticulum (FREY-WYSSLING, LOPEZ-SAEZ I& MUHLETHALER, 1964). Finally, the growing cell plate reaches the longitudinal walls of the mother cell. Cytokinesis is complete and two daugher cells have become independent.

Later development transforms the cell plate into the middle lamella of the mature cell wall.

EXPERIMENTAL ANALYSIS OF PLANT CYTOKINESIS

The induction of binucleate cells in a proliferative population by any treatment constitutes a very good test for screening of cytokinesis inhibitors. Thus, RISUEÑO, GIMENEZ-MARTIN & LOPEZ-SAEZ (1968) selected thermal shock, colchicine and lindane, caffeine and high hydrostatic pressure as cytokinesis inhibitors. In the screening, a lot of difefrent chemical drugs and physical agents were tested, bearing in mind that the more different the nature of a binucleating agent the more probability of a particular mechanism of action.

In relation to the cytokinesis phase preferentially blocked we know nowadays three groups of treatments: 1) Inhibitors of Golgi vesicle production, 2) inhibitors of vesicle accumulation and 3) inhibitors of arrangement and fusion of Golgi vesicles.

1) Vesicle **production blockage**

When meristem cells are submitted to a sublethal thermal shock for one hour at 40-42° C, during recovery in culture conditions many binucleate cells can be detected in the meristem population. Light microscopy shows the absence of phragmoplast in telophases inmediately after shock, but no apparent distortion of the interzonal spindle. Under the electron microscope, any cell structure but Golgi bodies appears morphologically well preserved. However, dyctiosomes characterized in these cells as piles of flatened sacs are not more apparent. Likely, some thermolabil factor is essential to preserve the Golgi body structure and after this treatment small and flat sacules can be observed stattered throughout the ground cytoplasm. These structures do not accumulate wall precursors or segregate small vesicles in their edges. As a consequence, telophases show a very low number of Golgi vesicles and the accumulation and

following phases for cell plate formation are missed. Logically, blockage of the first stage in a chain of events disturbs the whole process inhibiting cell plate formation.

Recently, MEYER & HERTH (1978) has described 2,6-dichlorobenzonitrile as an effective inhibitor of cell plate formation, without affecting nuclear division. This herbicide was introduced as a cellulose-synthesis inhibitor in higher plants (HOGETSU, SHIBAOKA & SHMOKORIYAMA, 1974) and it seems to be a chemical candidate for blocking Golgi vesicle production, although there is no direct evidence supporting this hypothesis for the moment.

2) Vesicle accumulation blockage

The c-mitotic substances, colchicine and lindane, are characterized essentially by their effects on the microtubule depolimerization. Any interference in this system affects cytokinesis by completely or partially inhibiting the formation of the cell plate.

The characteristic effect of colchicine is to produce polyploid cells. We observe that after more than 3 h treatment the chromosomes of the dividing cells are dispersed throughout the cytoplasm, the Golgi apparatuses are producing vesicles in apparently normal quantities, and the number of these vesicles in the cytoplasm is increasing. When the chromosomes are enclosed within the nuclear envelope, a polyploid nucleus is formed, while the small vesicles remain dispersed about the cytoplasm. In this case, both the distribution of the nuclear material and the division are observed to have been inhibited.

Fig. 2. — Schematic diagram of cytokinesis inhibition by caffeine or high hydrostatic pressure.

In both cases the I (production of Golgi vesicles) and the II (accumulation) phases of cytokinesis were observed to prõoceed normally.

III — The cloud of vesicles spreads on the equatorial region without arrangement.

IV — Finally the vesicles disperse throughout the cytoplasm without form the cell plate. The result is a binucelate cell.

Cytokinesis inhibition by caffeine

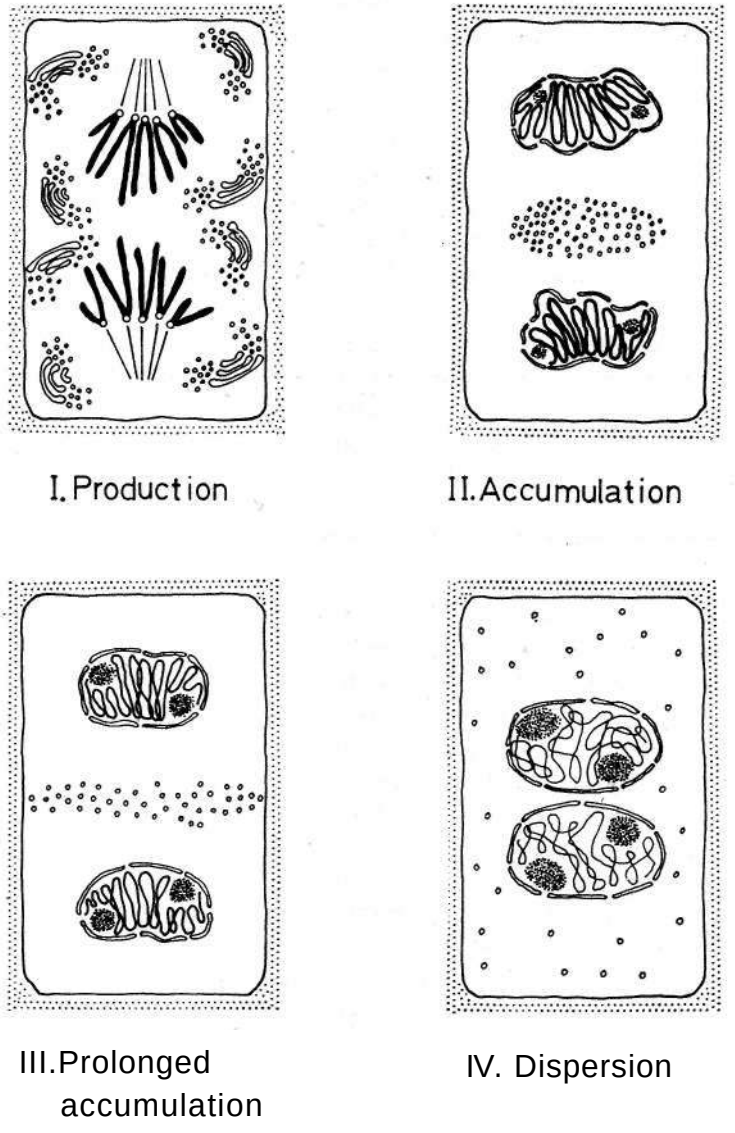

Fig. 2.

When the incubation with colchicine affects anaphase cells, where chromosome distribution took place but cell plate formation is at the very beginning, the treatment induces binucleate cells by inhibiting cytokinesis. In these cells, there is apparently a normal production of small vesicles in the Golgi bodies and these vesicles appear evenly distributed throughout the cytoplasm without any accumulation in the central region of the cell. As a matter of fact, colchicine precludes the arrangement and fusion of vesicles apparently by preventing the accumulation phase (WHALEY, DAUWALDER & KEPHART, 1966; PICKETT-HEAPS, 1967; RISUEÑO, GIMENEZ-MARTIN & LOPEZ-SAEZ, 1968).

In short, these observations suggest that c-mitotic substances do not inhibit the production of small vesicles, but they block cytokinesis insofar as they prevent the vesicles from accumulating in the equatorial region, whether the nuclear material is distributed or not.

3) Vesicle arrangement and fusion blockage

Cells going through their division cycle under hydrostatic pressure of 400 atmospheres or caffeine show normal development of the various phases of mitosis. For caffeine could be shown that the treatment did not prolong mitosis, this is, the mitotic apparatus apparently functions normally (PICKETT-HEAPS, 1969; JUNIPER & LAWTON, 1979). In both cases the first and the second phase of cytokinesis were observed to proceed normally, the whole process being blocked by failure of the small vesicles to arranged and fuse (DEYSSON & BENBADIS, 1966; LOPEZ-SAEZ, RISUEÑO & GIMENEZ-MARTIN, 1966). Therefore, we have postulated the blockage of cytokinesis by caffeine and high hydrostatic pressure at level of the arrangement of vesicles in the equatorial plane (Fig. 2).

Certainly, none of the treatments which have been used to inhibit cytokinesis has proved capable of blocking only the fusion of the small vesicles with a certain degree of selectively: that is, once the arrangement took place the

process led, through coalescence, to the formation of the new wall.

CALCIUM AND MAGNESIUM REQUIREMENTS

PAUL & GOFF (1973) described an apparently normal aggregation and organization of vesicles in caffeine-treated telophases, but the fusion of vesicles was insufficient to form a cell plate. And when these authors also studied the cytokinesis inhibition in the case of calcium deficiency they described a cytological picture similar to that found in caffeine treated cells. Thus, these authors suggested that methyxanthines interfere with cytokinesis by releasing calcium from the vesicle membranes and/or by inhibiting the membrane-associated ATPase, both of which appear to be required for membrane fusion. This hypothesis is consistent with POSTE and ALLISONS suggestion about the requirement of ATP and Ca^{L} ', $Mg+^{L}$ activated ATPase for membrane fusion (POSTE & ALLISON, 1971).

Recently, BECERRA (1977) and BECERRA & LOPEZ-SAEZ (1978) have demonstrated the action of calcium and magnesium on caffeine cytokinesis inhibition and proposed that caffeine interferes with plant cytokinesis involving some aspect of membrane fusion, where calcium and magnesium are essential requirements for cytokinesis.

CONCLUSIONS

1. The cytoplasm has the «chance» to divide only once in each cell cycle. So that the inhibition of cytokinesis at this time gives rise to a permanent binucleate cell.

2. Plant cytokinesis is a topographically organized secretion process. The cell plate is formed by the coalescence in the equatorial plane of small vesicles produced by Golgi bodies. The membranes of these vesicles make up the plasma membrane of the new cell surfaces; and the contents of these vesicles gives rise to the amorphous matrix of the new wall. Therefore, origin, translocation and fusion of these

vesicles are the physiological processes involved in plant cytokinesis.

3. The production, accumulation, arrangement and fusion of Golgi vesicles may be considered the morphological phases of cell plate formation.

4. C-mitotic drugs inhibit the accumulation phase of cytokinesis by destroying the fibrillar component of the phragmoplast, hence the mierotubular system appears to be essential for vesicle translocation.

5. Caffeine and high hydrostatic pressure are efficient inhibitors of the vesicle arrangement and coalescence. Apparently, arrangement and coalescence of Golgi vesicles are dependent of membrane fusion and likely a certain degree of fusion of small vesicles is required for a narmal arrangement.

6. Calcium and magnesium are essential requirements for cytokinesis by affecting likely the membrane fusion reaction.

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NOTAS SOBRE BORAGINACEAS ESPAÑOLAS

I. *LITHODORA PROSTRATA* (LOISEL.) GRISEB. Y *L. DIFFUSA* (LAG.) I. M. JOHNSTON.

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RESUMEN

Tras un estudio de material español de *Lithodora* y del tipo de *Lithospermum diffusum* Lag., se ha llegado a la conclusión de que *Lithodora prostrata* (Loisel.) Griseb. y *L. diffusa* (Lag.) I. M. Johnston deben separarse a nivel específico. Se propone la nueva conbinación: *L. prostrata* subsp. *lusitanica* (Samp.) Valdês, comb. nov.

SUMMARY

As a result of the study of Spanish material of *Lithodora* and the type of *Lithospermum diffusum* Lag., the separation of *Lithodora prostrata* (Loisel.) Griseb. and *L. diffusa* (Lag.) I. M. Johnston is made. The following new combination is proposed: *Lithodora prostrata* subsp. *lusitanica* (Samp.) Valdês.

LAGASCA (1805: 39), describió una nueva especie de *Lithospermum* con el nombre de *L. diffusum.* Un año más tarde, LOISELEUR-DESLONGCHAMPS (1806: 105), describió *Lithospermum prostratum,* basándose en material procedente de Bayona (Bajos Pirineos, Francia). Al separar GRISEBACH (1844: 85) el género *Lithodora,* incluyó en él *Lithospermum prostratum* Loisel., estableciendo por tanto la combinación *Lithodora prostrata* (Loisel.) Griseb.

DE CANDOLLE (1846: 81) consideró que *Lithospermum diffusum* Lag. y *Lithospermum prostratum* Loisel. consti-

tuían una sola especie, para la que adoptó el nombre de LOISEIEUR-DESILONGCHAMPS, ya que creyó que *L. diffusum* había sido descrito por LAGASCA en 1816.

COSSON (1849: 42) separó como *Lithospermum prostratum* var. *erectum* unas plantas del S de España (Alcalá de los Gazules, Cádiz). Dicha variedad se encuentra también en Portugal y fue elevada a categoría de especie por SAMPAIO (1913: 123) con el nombre de *Lithospermum lusitanicum.*

Al aceptar la separación de *Lithodora* del antiguo género *Lithospermum,* JOHNSTON (1924: 56) establece la nueva combinación *Lithodora diffusa* (Lag.) I. M. Johnston, incluyendo como sinónimos *Lithospermum diffusum* Lag., *Lithospermum prostratum* Loisel. y *Lithodora prostrata* Griseb. Adoptó por tanto el criterio de DE CANDOLLE, seguido hasta ahora por la mayoría de los autores, de reconocer una sola especie, aunque indicó más tarde (JOHNSTON, 1953: 267) que se trataba de una especie muy variable que necesitaba un estudio detallado. Siguiendo esta indicación, PINTO DA SILVA & ROZEIRA (1964: 170) consideraron que las plantas del centro y sur de Portugal, SW de España y N de Marruecos, constituían una subespécie independiente: *Lithodora diffusa* subsp. *lusitanica* (Samp.) P. Silva & Rozeira (= *Lithospermum prostratum* var. *diffusum* Cosson).

La mayoría de los autores están de acuerdo en aceptar la existencia de una sola especie, que ocupa las regiones atlánticas y subatlánticas comprendidas entre Finisterre (Francia) y el NW de Marruecos, penetrando por el sur de España hasta la provincia de Málaga. Para esta especie se han utilizado más frecuentemente los nombres *Lithospermum diffusum* Lag., *Lithospermum prostratum* Loisel. y *Lithodora diffusa* (Lag.) I. M. Johnston. A partir de PINTO DA SilVA & ROZEIRA (1. c), se reconoce la existencia de dos subespécies: la típica, que ocupa toda la parte norte del área de distribución de la especie (desde Finisterre hasta la cuenca del Tajo), y la subsp. *lusitanica* (Samp.) P. Silva & Rozeira, que ocupa la parte sur, desde la cuenca del Tajo, hasta el NW de Marruecos, con algunas localidades aisladas en el N de Portugal.

Dentro del género *Lithodora,* lo que se ha venido llamando hasta ahora *L. diffusa* presenta una disposición del androceo muy notable. En todas las especies conocidad de este género, los cinco estambres se insertan a la misma altura en el tubo de la corola o en la garganta. Sin embargo, en esta especie los estambres se insertan a distintas alturas, lo que confiere a este taxón una sitúción tan peculiar dentro del género, que JOHNSTON (1953: 267) formó con dicha especie la sección *Lasioglottis.* Esta situación es semejante a la que dentro del género *Macrotomia* DC. persenta *M. echioides* (L.) Boiss. que es igualmente la única especie de este género con estambres insertos a distintas alturas (HUYNH, 1971).

Con motivo de unos estudios sobre Boraginàceas españolas, el autor de esta nota ha podido comprobar que dentro de lo que se ha venido llamando *Lithodora diffusa, Lithospermum prostratum* o *L. diffusum,* se encuentran dos grupos de plantas muy différentes, sobre todo en cuanto a la posición del androceo se refiere.

Un grupo está formado por plantas que presentan estambres insertos a distintas alturas obre el tubo de la corola, con anteras de 0,6 a 1,3 (-1,8) mm. Tienen además inflorescencias con (3-) 6-14 flores, y núculas densa y diminutamente tuberculadas. Las plantas que presentam estos caracteres se extienden desde Finisterre (Francia) hasta Marruecos.

Otro grupo está formado por plantas que presentan estambres insertos aproximadamente a la misma altura, ya sea hacia la parte media del tubo de la corola o en la garganta, con anteras de 1,2 a 1,5 mm. Tienen además inflorescencias con menos flores: 2 a 6 (-10), y núculas lisas. Las plantas que presentan estos caracteres se encuentran restringidas a diversas localidades de los montes de Léon y Cordillera Cantábrica, descendiendo en Santander hasta algunas localidades costeras, como San Vicente de la Barquera, Suances y Santoña.

Por los datos cariológicos de que se dispone hasta el momento, ambos grupos presentan distinto número cromosómico. FEKNANDES & LEITÃO (1972: 390) indicaron 2n = 32 para material de S. Paulo de Frades, Coimbra, perteneciente

al primer grupo, mientras que KÜPPER (1974: 34) indicó 2n = 16 para material de Peña Olvidada (Picos de Europa, Santander), recuento que hay que referir al segundo grupo, como se ha podido comprobar estudiando el material utilizado por este autor (NEU 00009).

Dada la importancia que la morfología floral tiene en la taxonomía de Boragináceas, no se duda en separar ambos grupos con categoría específica.

Se identifica el primer grupo, con estambres insertos a distintas alturas, con *Lithospermum prostratum* Loisel. y dentro del género *Lithodora* debe por tanto denominarse *Lithodora prostrata* (Loisel.) Griseb.

En cuanto al segundo grupo, con estambres insertos a la misma altura, se identifica con *Lithospermum diffusum* Lag., por las razones que se indican más adelante, y ha de denominarse por lo tanto *Lithodora diffusa* (Lag.) I. M. Johnston.

Se indican a continuación los nombres correctos, sinónimos, caracteres diferenciales y distribución de ambas especies, así como de las categorias infraespecíficas reconocidas.

Lithodora prostrata (Loisel.) Griseb., *Spicil. Fl. Rom.* 2: 85 (1844).

Lithospermum prostratum Loisel., *Fl. GaU.* 1: 105 (1806).

Subarbusto de ramas decumbentes, ascendentes o erectas, con hojas seríceas, o estrigosas e hirsutas. Inflorescencias con (3-) 6-14 flores, alargándose en la maduración hasta 40 (-70) mm. Corola con garganta y tubo ± densamente pelosos interiormente. Estambres insertos a distintos niveles por encima de la mitad del tubo o hacia la garganta, con anteras de 0,6-1,3 (1,8) mm. Núculas 1-3 maduras por flor, de 2-3,5 X 1,3-2 mm., densa y diminutamente tuberculadas.

Numero cromosómico. 2n = 32 [FERNANDES & LEITÃO, 1972: 390, sub *L. diffusa* (Lag.) I. M. Johnston].

Distribución. Región atlántica de Europa y África, desde Finisterre (Francia) hasta el NW de Marruecos. En el S de España, este taxon penetra hasta la provincia de Málaga.

JOHNSTON (1953: 267) describió esta especie como monomórfica, aunque indicó haber encontrado diferencias en la longitud de los estilos y tamaño de las anteras. El estudio de abundante material de sus dos subespécies, permite asegurar que en ambas se presenta una clara distilia, situación que se encuentra en todas las especies de *Lithodora* de la Peninsula Ibérica. *L. fruticosa* (L.) Griseb. presenta, sin embargo, distilia imperfecta, ya que los estambres se encuentran siempre insertos en la parte superior del tubo de la corola, variando solamente la longitud del estilo, que es largo en las plantas longistilas, de manera que sobrepasa las anteras, y corto, alcanzando aproximadamente la mitad de la longitud del tubo de la corola, en las plantas brevistilas.

Subsp. prostrata

Decumbente. Hojas planas o de margen ligeramente recurvo, patentes, seríceas, con pelos de las hojas viejas de base pustulada. Corola con tubo de (8-) 10-12 mm. y limbo (9-) 10-13 mm., con garganta densa y largamente pelosa. Anteras 1-1,3 mm. Núculas 2-2,5 (-3,5) X 1,3-2 mm., ovoideas.

Distribución. Región Atlántica europea, desde Finisterre (Francia) hasta la cuenca del Tajo (España y Portugal).

Presenta distilia, con dos formas bien distintivas. Las plantas longistilas presentan los estambre insertos en 3 niveles por encima de la mitad del tubo o hacia la garganta, y estilo largo, de maneira que el estigma queda por encima del anillo de pelos de la garganta, aproximadamente hacia la mitad del limbo de la corola. Las plantas brevistilas presentan los estambres insertos en la garganta a tres niveles en un espacio corto, con filamentos de 0,5 a 1,5 mm., y estilo corto, que no llega a la mitad del tubo, quedando aproximadamente a la altura que ocupa el estambre mas inferior de las flores longistilas.

Subsp. lusitanica (Samp.) Valdês, comb. nov.

Lithospermum lusitanicum Samp., *Lista Herb. Port.:* 123 (1913).

Lithodora diffusa subsp. *lusitanica* (Samp.) P. Silva & Rozeira, *Agron. Lusit.* 24: 170 (1964).

Lithospermum fruticosum Brot., *Fl. Lusit.* 1: 292 (1804), non L. (1753).

Lithospermum prostratum var. *erectum* Cosson, *Not. Pl. Midi Esp.* 1: 42 (1849).

Lithospermum diffusum var. *erectum* (Cosson) Rouy, *Fl. Fr.* 10: 314 (1908).

Lithospermum fruticosum subsp. *diffusum* Maire, in Jahand. & Maire, *Cat. Pl. Maroc* 3: 602 (1934).

Ascendente o erecta. Hojas fuertemente recurvas, adpresas o patentes, con indumento doble: strigoso de pelos largos de base pustulada, sobre todo en el haz, e hispido, de pelos cortos, más abundantes en el envés. Corola con tubo de 8-9,5 mm. y limbo de 8-9 mm., con garganta y parte superior del tubo escasamente pelosa, con 5 bandas longitudinales de pelos largos, a casi glabra. Anteras 0,6-1,3 (-1,8) mm. Nuculas 3-3,5 X 1,8-2 mm., oblongas.

Distribución. Desde la cuenca del Tajo hasta el NW de Marruecos, con algunas localidades aisladas en el N de Portugal (PINTO DA SILVA & ROZEIRA, 1964: 171), penetrando en el S de España hasta la provincia de Málaga.

Presenta distilia. Las formas longistilas tienen 5 bandas de pelos en la garganta y estambres insertos a tres alturas por encima de la mitad del tubo, o cerca de la garganta, con filamentos de 0,5 a 1,2 mm. De ellos, generalmente 2 están algo mas altos, y otros dos, alternando con los anteriores, algo mas bajos; el quinto estambre se inserta un poco por debajo de los demás. El estilo es largo, situándose el estigma hacia la garganta o por encima de la misma. Las plantas brevistilas presentan garganta casi glabra y estambres a distintas alturas. Generalmente, tres de ellos se

insertan hacia la garganta y tienen filamentos de 1 a 1,3 (-1,8) mm., y los otros dos, con filamentos de 0,4 a 0,6 mm, están insertos por encima de la mitad del tubo. El estilo es corto, alcanzando aproximadamente la mitad del tubo de la corola.

Lithodora diffusa (Lag.) I. M. Johnston, *Contr. Gray Herb. Harvard Univ., new ser.* 73: 56 (1924), excl. syn. *Lithospermum prostratum* Loisel. et *Lithodora prostrata* Griseb.

Lithospermum diffusum Lag., *Var. Ci.* 4 (19) : 39 (1805).

Subarbusto con ramas decumbentes, despues ascendentes, con hojas seríceas ó hirsutas. Inflorescencias con 2-6 (-10) flores, alargándose en la maduración hasta 30 (-50) mm. Corola con garganta y tubo provistos interiormente de abundantes pelos largos. Estambres insertos a la misma altura hacia la parte media del tubo o en la garganta, con anteras de 1,2-1,5 mm. 1-2 (-4) núculas maduras por flor, de 2,5-3 X X 2 mm., lisas.

Numero cromosómico. 2n = 16 (KÜPFER, 1974: 34).

Tipo. Asturias (Arvas, VII-VIII, *Lagasca* (MA 96526, lectotipo).

Distribución. Montes de León, Cordillera Cantábrica y zonas bajas de la provincia de Santander.

LAGASCA (1805: 39) no indicó ninguna localidad al describir *Lithospermum diffusum.* Sin embargo, años más tarde (LAGASCA, 1816: 10) repitió literalmente la descripción original de esta especie, sin hacer referencia al trabajo de 1805, añadiendo como localidades para la misma: «in dumetis prope Arvas, Pajares et in perquampluribus alus Principatus Asturicensis plagis».

El autor de esta nota está de acuerdo con COLMEIRO (1858: 192) en que el trabajo de 1805 fue un anticipo del

de 1816, ya que el mismo LAGASCA escribió «doy unicamente el extracto de mis observaciones... reservando para una obra posterior, que espero publicar muy pronto, hablar con la extensión debida» (LAGASCA, 1805: 34). La obra a que se refería era sin duda su *Genera et Species Plantarum* (1816), que es la más extensa de este autor, ya que su proyectada Flora Española no llegó a publicarse (véase LAGASCA, 1924: 2).

Por ello, aunque en 1805 no indicó material alguno, puede asegurarse que utilizó para describir *Lithospermum diffusum* el indicado en 1816.

En el herbario del Jardín Botánico de Madrid (MA), se conservan dos pliegos de *Lithospermum diffusum* recolectados por LAGASCA; uno (MA 154902) en León y otro (MA 96526) en Arvas (= Colegiata de Arbas, Puerto de Pajares, León, que a principios de siglo XIX se escribía con «v», como indica LAÍNZ, 1975: 66). Este último corresponde a la primera de las localidades específicamente citadas por LAGASCA (1816: 10). Se puede afirmar por las noticias históricas que se tienen, que LAGASCA recolectó en León y Asturias, y muy concretamente en Arbas (= Arvas) en 1803 (LAGASCA, 1805: 38; COLMEIRO, 1858: 192; PEREZ DE CASTRO, 1971: 5; CASASECA, 1976: 194), por lo que no hay duda de que este es parte del material a que LAGASCA (1816: 10) hizo referencia, por lo que se toma el ejemplar contenido en el pliego procedente de Arbas (= Arvas, MA 96526) como tipo de *Lithospermum diffusum* Lag.

Estudiado con detalle este ejemplar, se ha comprobado que se trata de una planta brevistila que presenta los estambres insertos en la parte superior del tubo, con anteras largas situadas todas a la misma altura.

La morfología floral corresponde por lo tanto a la que presenta este taxón, por lo que no hay duda de que el primer nombre aplicado al mismo es *Lithospermum diffusum* Lag. y por tanto, el nombre de este taxón en el género *Lithodora* es *Lithodora diffusa* (Lag.) I. M. Johnston, a pesar de que JOHNSTON aplicó este nombre a las plantas con estambres

insertos a distintas alturas, o sea, a *Lithodora prostrata* (Loisel.) Griseb.

Lithodora diffusa (Lag.) I. M. Johnston es una especie distila. Las plantas longistilas presentan estambres insertos por encima de la mitad del tubo, con filamentos de apenas 0,5 mm., y estilo largo, que casi alcanza la base de los lóbulos de la corola. Las plantas brevistilas presentan los estambres insertos en la base de la garganta, con filamentos de c. 2 mm., de manera de que las anteras alcanzan la base de los lóbulos de la corola, y el estilo corto, quedando el estigma un poco por encima de la mitad del tubo de la corola.

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LA PRAIRIE MONTAGNARDE DES MONTS LOMA (SIERRA LEONE)

par

PAUL JAEGER et JACQUES-GEORGES ADAM f

SOMMAIRE

RÉSUMÉ

Enclave herbacée dans l'étage montagnard forestier, la prairie d'altitude du Loma doit son origine et sa pérennité, non pas au climat, mais au feu. Elle s'est substituée à une forêt basse — maquis à *Dissotis leonensis* — trouée de clairières édaphiques où, depuis des âges reculés, se sont perpétués des orophytes non forestiers. En refoulant cette forêt vers les hauts sommets rocheux, le feu a favorisé l'extension de la prairie et, partant, la savanisation de l'étage culminai.

I — LA DORSALE LOMA-MAN

Aspects physiques et biogéographiques

A quelques 250 km de la côte du golfe de Guinée, et parallèlement à elle, une ligne de hauts reliefs s'étire d'une façon subcontinue en direction NW-SE, depuis les contreforts orientaux du Fouta-Djalon jusqu'aux hauteurs de Man en Côte d'Ivoire, soit une distance de 350 à 400 km. Il s'agit de la dorsale Loma-Man ou chaîne guinéenne (44). Elle est formée d'une succession de plateaux et de massifs sans direction orographique prédominante où «au premier

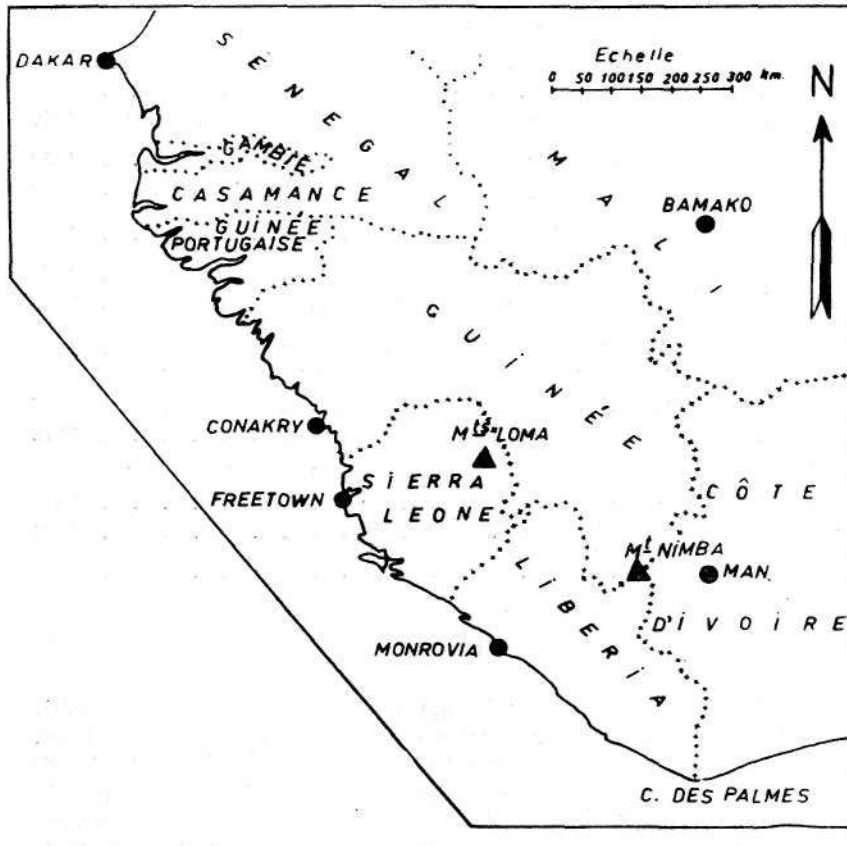


Fig. I. — Les monts Loma dans le cadre géographique ouest africain.

abord l'observateur ressent une impression de confusion et de désordre» (83).

Cette chaîne s'inscrit à l'intérieur du bouclier libérien d'âge précambrien; elle constitue une unité orographique qui par A. AUBRÉVILLE fut définie comme le sous-domaine afro-montagnard occidental (12). Caractérisés, sur le plan floristique par un important fond commun, chacun des massifs de cette chaîne — Loma, Fon, Simandou, Ziama, Nimba, Dans — se distingue néanmoins, sur le plan physique et biogéographique, par une «personnalité» qui lui est propre.

En raison de l'inclinaison NW-SE de l'axe de la dorsale, ses deux extrémités se trouvent décalées quant à leur latitude : alors que le Loma et le Nimba s'étirent respectivement entre 9°00 à 9°17' et 7°25' à 7°45 LN, la localité de Man se situe par 7°24' LN. Il en résulte de l'Est à l'Ouest un amenuisement progressif de la pluviosité et, partant, un appauvrissement de la flore en espèces ombrophiles. La dorsale Loma-Man culmine en Sierra Leone au Pic Bintumane (monts Loma) à 1924 m[1] qui, de ce fait, est le sommet le plus élevé de l'Afrique occidentale à l'ouest de la chaîne camerounaise. Le Nimba, à cheval sur la Guinée, la Côte d'Ivoire et le Libéria, plafonne au mont Richard-Molard à 1752 m ; grâce aux travaux de R. SCHNELL et de J. G. ADAM (119; 2), il est actuellement le massif ouest africain le mieux connu quant à sa flore et sa végétation. Le Fon-Simandou atteint 1656 m et le Ziama culmine à 1350 m au mont Ghali. Près de Man, en Côte d'Ivoire, le massif des Dans est constitué par tout un ensemble de dômes rocheux étudiés successivement par A. CHEVALIER et par A. AUBRÉVILLE: le mont Dou (1370 m), le Tonkoui (1190 m), le mont Momy (1180 m). Le Nimba, affecté par des plissements antécambriens est formé, essentiellement, de schistes et de quartzites redressés, le tout fortement ferruginisé. D'un bout à l'autre ce massif est dominé par une crête étroite taillée en «lame de

[1] Valeur obtenus à la suite de 9 lectures du point d'ébullition de l'eau bidistillée effectuées à plusieurs jours d'intervalle, en oct--nov. 1944, au sommet du Pic Bintumane.

couteau ébréchée» limitée, de part et d'autre, par d'abrupts versants herbeux (119; 78).

Orienté parallèlement par rapport au Nimba mais situé, contrairement à celui-ci, à latitude moins basse et au nord

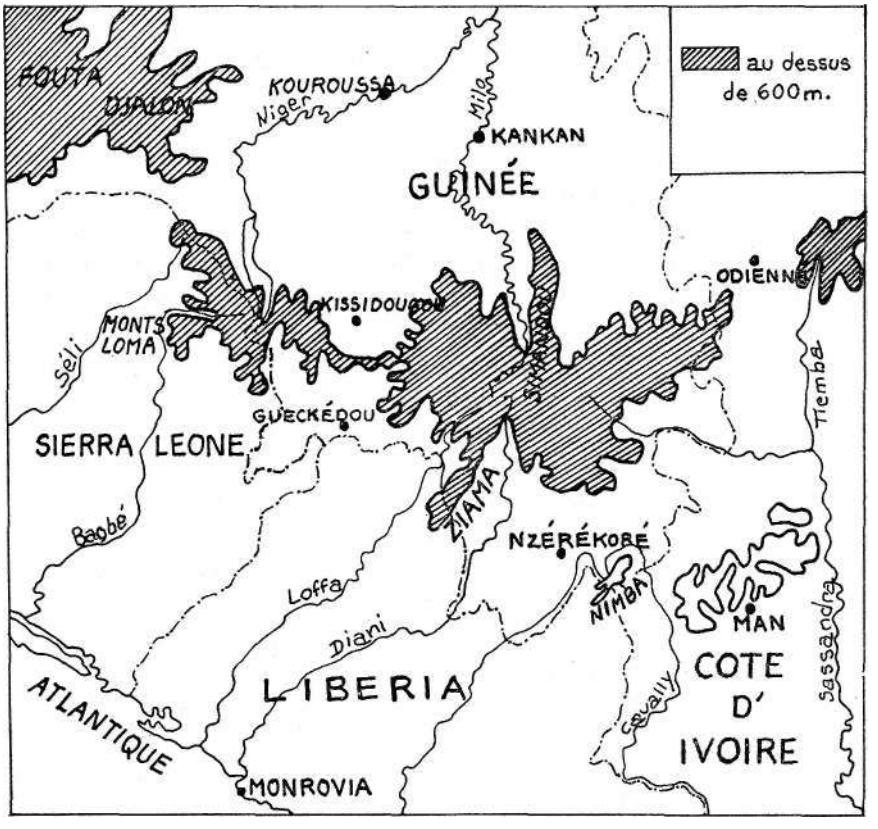


Fig. 2. — La dorsale Loma-Man et la partie orientale du Fouta-Djalon (d'après P. JAEGER, M. LAMOTTE et R. ROY).

de la limite actuelle de la forêt dense, le massif du Loma, quelques venues doléritiques mises à part, est d'une ossature essentiellement granitique. Il est constitué par la juxtaposition de quatre blocs montagneux alignés du nord au sud, séparés les uns des autres par des cassures, souvent vives, drainées par des cours d'eau torrentiels (v. fig. 3).

Le bloc septentrional, le plus élevé, correspond à la puissante pyramide du Pic Bintumane; elle est couronnée par un minuscule plateau sommital limité au Sud, à l'Ouest et au Nord par d'abruptes coulées doléritiques.

Au Sud d'une cassure drainée en sens inverse par les eaux du Kongbundu et du Neji, s'étale une vaste étendue d'allure quadrangulaire (4 à 5 X 5 à 6 km), le Plateau. Il est limité à l'Est et à l'Ouest par des pentes raides et drainé, dans le sens nord-sud, par toute une série de ruisselets à cours grossièrement parallèles, le Miramira étant le plus proche du rebord oriental (v. fig. 5).

Au Plateau qui est par excellence le domaine de la prairie d'altitude fait suite, vers le Sud, la région la plus accidentée du massif où se dressent les dômes granitiques du Serelen-Koriko (1480 m) et du Sarabaldou (1330 m), ainsi que les crêtes rocheuses en dents de scie du Da-Oulen (1470 m) et du Fuen-Koli (1400 m) (v. fig. 5).

Enfin, le massif s'achève au Sud, au-delà de la vallée du Wuliko, par un haut plateau orienté SW-NE qui, vers son extrémité orientale, est dominé par la coupole granitique du Peran-Koriko (860 m).

A. AUBRÉVILLE (6; 12) et R. SCHNELL (119) ont fait remarquer que dans les massifs montagneux de la dorsale Loma-Man la végétation climacique «tant sur les crêtes que dans les régions basses» est de nature forestière. L'individualisation au-dessus de 1000 m-1200 m de forêts montagnardes à *Parinari excelsa* permet à ces auteurs de définir, dans ces montagnes, deux étages de végétation: un étage guinéo-équatorial inférieur occupé par le forêt dense humide de basse altitude et un étage guinéo-équatorial supérieur ou étage à *Parinari excelsa,* parfois désigné sous le nom d'étage culminai, occupé à la fois par la forêt montagnarde (71) et par la prairie montagnarde elle même dominée, au Loma, par quelques hauts sommets rocheux.

Vers sa limite supérieure la forêt montagnarde s'effiloche en une série généralement divergente de trainees arborées, les galeries forestières d'altitude; le plus souvent disposées en éventail, elles jalonnent le thalweg humide des vallées et des ravins, escaladent les pentes herbeuses les plus

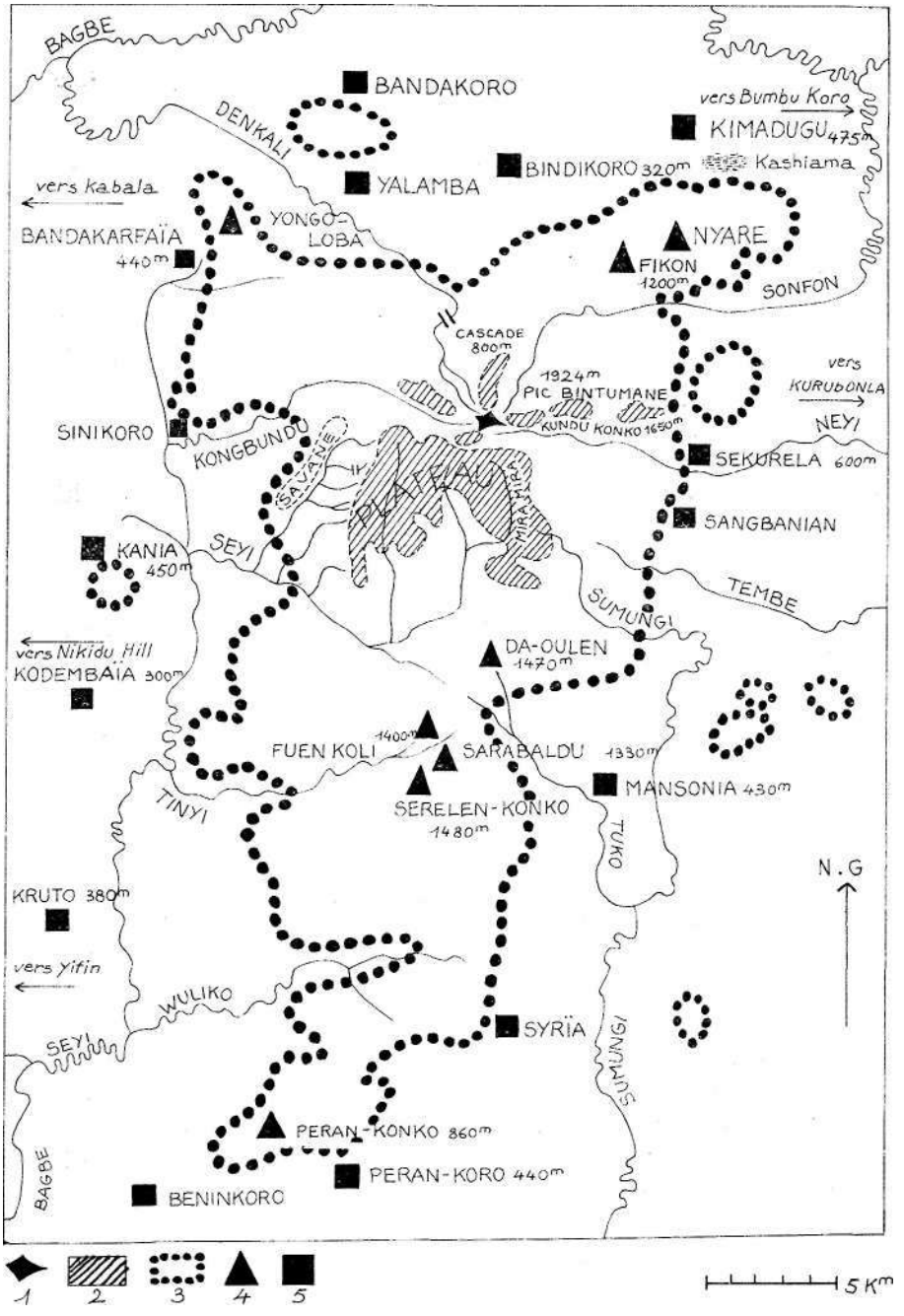


Fig. 3. — Le Massif des Monts Loma (carte semi-schématique d'après S. DAVEAU; modifié).

1 — Pic Bintumane; 2 — Eperons du Pic Bintumane et Plateau; 3 — Limites du Massif; 4 — Sommets; 5 — Villages.

raides pour, finalement, se dissoudre dans la prairie montagnarde bien avant d'avoir atteint les sommets (v. fig. 4).

En raison de son orientation subméridienne et, partant, de son exposition aux vents dominants, mousson et harmattan, on n'est nullement suppris de constater qu'au Loma la forêt couvre d'un seul tenant non seulement l'ensemble des versants ouest et sud-ouest, mais aussi une grande partie du massif au Sud du Plateau, à l'exception toutefois des hauteurs qui ceinturent le Serelen-Konko, secteur soumis au vent d'Est.

Il en va différemment du versant oriental qui reçoit l'harmattan de plein fouet; là le manteau forestier a été morcelé en lambeaux d'étendue variable, séparés les uns des autres par des couloirs herbeux, tantôt larges, tantôt étroits, occupés jusque vers 1000 m par la savane guinéenne banale à *Lophira lanceolata, Parkia biglobosa, Pterocarpus erinaceus, Cussonia barteri...* et, au-delà, par la savane submontagnarde à *Kotschya lutea* avant de déboucher sur la prairie d'altitude; ce sont là autant de voies qu'empruntent les feux pour accéder à l'étage culminai et même jusqu'au sommet du Pic Bintoumane (v. fig. 6).

La présente note est consacrée à l'étude de l'étage supérieur du Loma et, plus spécialement, à celle de la prairie montagnarde et des sommets rocheux qui l'encadrent, la forêt ayant fait l'objet d'un travail antérieur (71). De plus, en cours d'étude une certaine importance est accordée aux orophytes, à ces végétaux qui trouvent en altitude les conditions optimales à leur survie; on tâchera de répondre aux problèmes que posent leur répartition, leur origine, leur signification biogéographique.

H — LES HAUTS SOMMETS ROCHEUX DOMINANT LA PRAIRIE D'ALTITUDE

Une des originalités du Loma réside en la présence autour de la Prairie d'altitude de quelques hauts sommets rocheux ayant donné asile à des orophytes, espèces résiduelles ou endémiques. Comme au cours des époques révolues des échanges ont dû se faire entre ces sommets et la prairie située en contre-bas, la signification biogéographique de

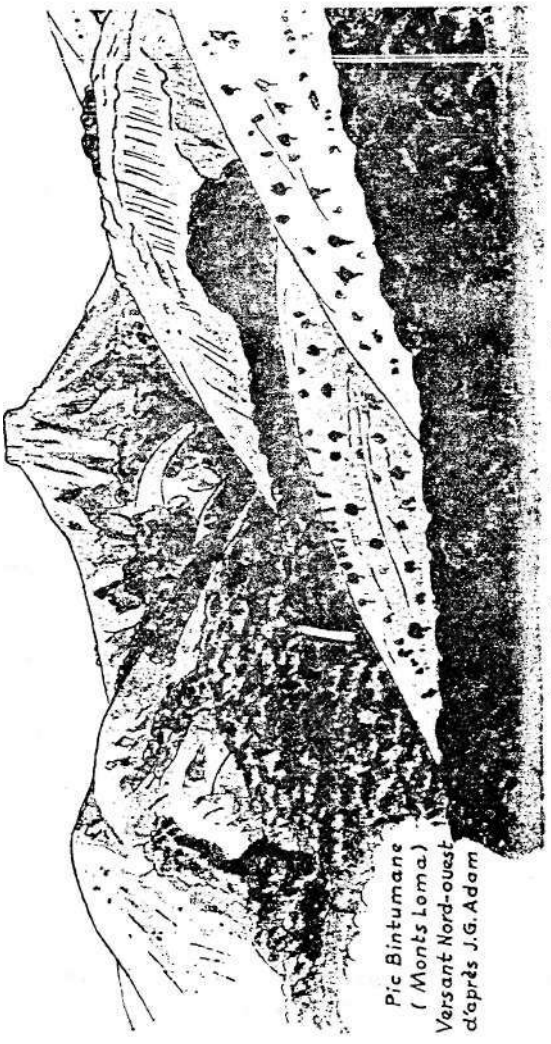


Fig. 4.—Versant NW du Pic Bintumane (au fond); bassin de reception du Denkali avec cascade vers 80© m (J. G. ADAM dess. oct. 1944).

celle-ci n'apparaîtra en pleine lumière que grâce aux données fournies par les hauts somemts qui l'encadrent.

Parmi ceux-ci nous distinguerons d'une part les abruptes doléritiques qui ceinturent le Plateau sommital du Pic Bintumane et, d'autre part, les dômes granitiques du Serelen-Konko, du Sarabaldou et les crêtes déchiquetées, également de nature granitique, du Da-Oulen et du Fuen KoIi.

A) Les à-pics doléritiques du Bintumane

Dans tout le massif du Loma il n'y a pas d'affleurement doléritique aussi spectaculaire que celui des falaises qui limitent le plateau sommital du Pic Bintumane; particulièrement développés face à l'Ouest, au Sud et au Sud-ouest, ces abrupts sont soumis à un. mode d'usure qui se traduit à la fois par le débit dans le sens vertical et dans le sens transversal des colonnes basaltiques. Il en résulte, principalement vers le sommet de ces colonnes, une superposition de blocs rocheux dont certains semblent en équilibre instable (70) (v. photo 9).

Ainsi se créent, tant dans le sens vertical que dans les sens horizontal, toute une série de fentes, de crevasses, d'anf ractuosités ; certaines, en s'élargissant, forment des cavités ombragées dont les parois suintantes témoignent d'un microclimat frais et humide; elles se garnissent d'un placage d'Hépatiques *(Plagiochasma* sp.) et de coussinets de Mousses *(Polytrichum* sp.) ; ce milieu s'avère éminemment favorable aux Fougères: *Dryopteris manniana, D. pentheri, D. athamantica;*,on y remarque aussi des herbacées comme: *Arthraxon lancifolius, Utricularia* sp... des orophytes à aire disjointe comme *Cyperus mannii, Streptocarpus elongatus, Pïlea tetraphylla, Pouzolzia, parasitica, Lobelia heyneana* var. *inconspicua...* dans ce cortège se range aussi le *Cheilanthes farinosa,* une Fougère pantropicale, réviviscente, aux frondes subtriangulaires étalées par temps humide et recroquevillées sur elles-mêmes par temps sec ; à ce moment devient visible sa face inférieure enduite d'une sécrétion cireuse blanchâtre.

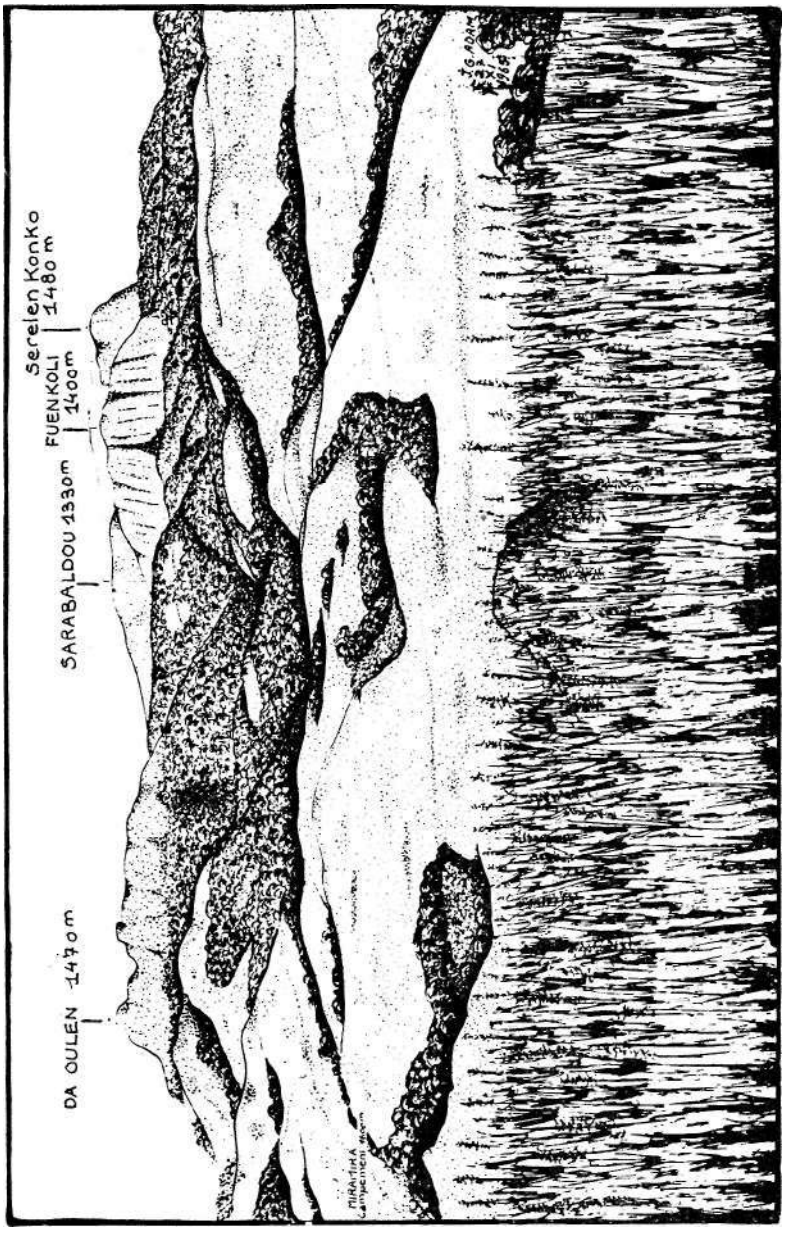


Fig. 5. — Vue prise de la pente Sud du Pic Bintumane sur la fraction orientale du Plateau et sur les massifs du centre des monts Loraa (J. G. ADAM dess. nov. 1965).

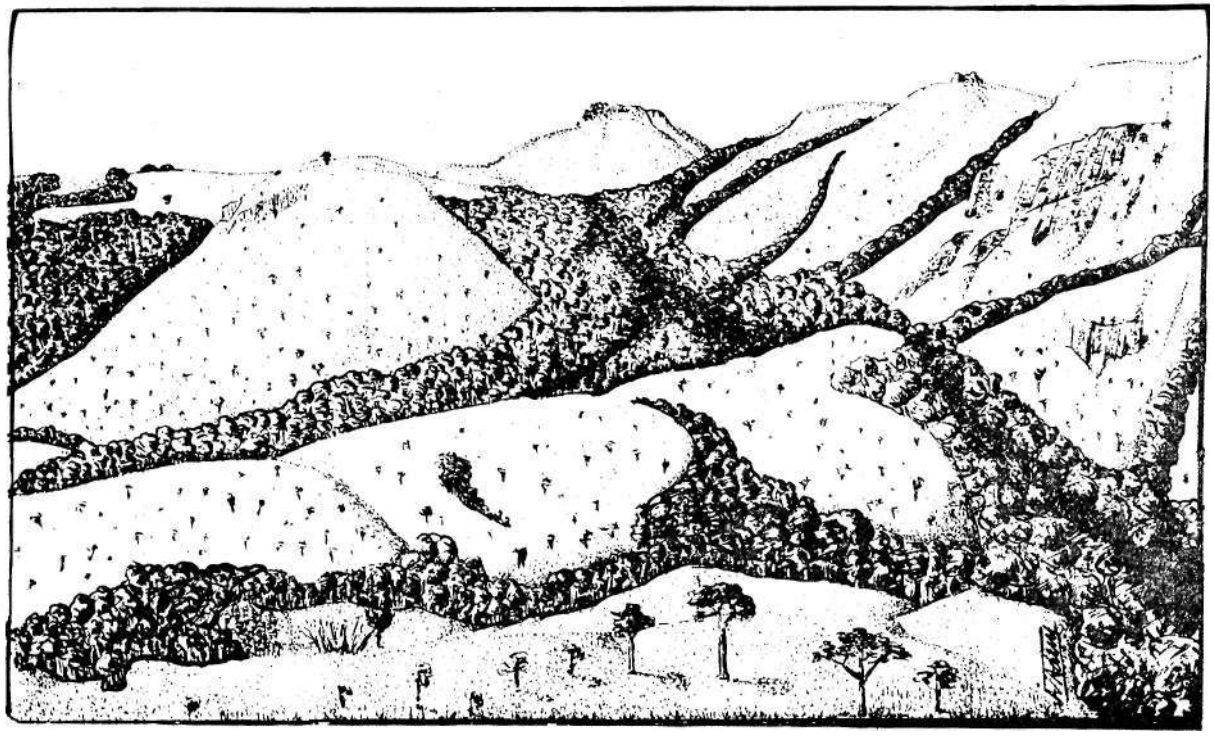


Fig. 6. — Vallée du Neji: vue des abords de Sekurela (piedmont B vers 600 m). Remarquer les galeries forestières d'altitude escaladant le versant Sud du Kundu-Konko; au fond la table doléritique du Pic Bintumane (J. G. ADAM dess. nov. 1965).

La formation, à divers niveaux de la falaise, de rebords subhorizontaux est propice à l'installation de saxicoles héliophiles comme *Afrotrilepis pilosa, Gladiolus aequinoctialis* var. *aequinoctialis* et d'autres orophytes comme G*ynura miniata, Conyza gigantea, Anisopappus africanus, Homalocheilos ramosissimus...*

Enfin, la falaise peut être interrompue et céder la place à des corridors herbeux à forte pente reliant le pied des escarpements à la prairie du plateau sommital; là on remarque, les plantes banales de la prairie montagnarde mises à part, des orophytes aussi significatives quant à leur répartition géographique que: *Pennisetum monostigma, Rhytachne glabra, Andropogon mannii, Tripogon major* subsp. *jaegerianus, Nerophïla gentianoides...*

B) Le Bush Montagnard à Dissotis leonensis

Les hauts sommets à soubassement granitique abritent une des plus étonnantes originalités du Loma: *le Bush montagnard* à *Dissotis leonensis.* Cette Mélastomatacée se présente sous la forme d'un arbrisseau ramifié dès la base; sa couronne, quand elle se développe sans entraves, prend l'aspect d'une boule hémisphérique de 2 à 3 m de diamètre, posée à même la dalle; elle rappelle en cela les «'Kugelschirmbäume», de C. TROLL (130). En peuplement, ces couronnes, serrées côte à côte, sont à l'origine de cet aspect moutonnant connu des forêts à *Parinari,* mais qui, dans le cas présent, est rendu plus frappant encore par la couleur cendrée du feuillage. En saison sèche (déc.-janv.) les feuilles, toutes insérées à la périphérie de la couronne, se colorent en un rouge vineux avant de tomber, pour être remplacées pour peu de temps, par de grandes fleurs (3 à 3,5 cm diam.) inodores, d'un rouge rosé. La richesse en epiphytes (Mousses, Usnées et autres Lichens) est une réplique à l'humidité excessive qui, en saison pluvieuse, règne sur ces hauteurs (v. photo 12).

Sur les hauts sommets au sud du Plateau, le *Dissotis leonensis* forme des fourrés à allure de maquis. Son extrême sensibilité au feu se traduit fréquemment par une dislocation

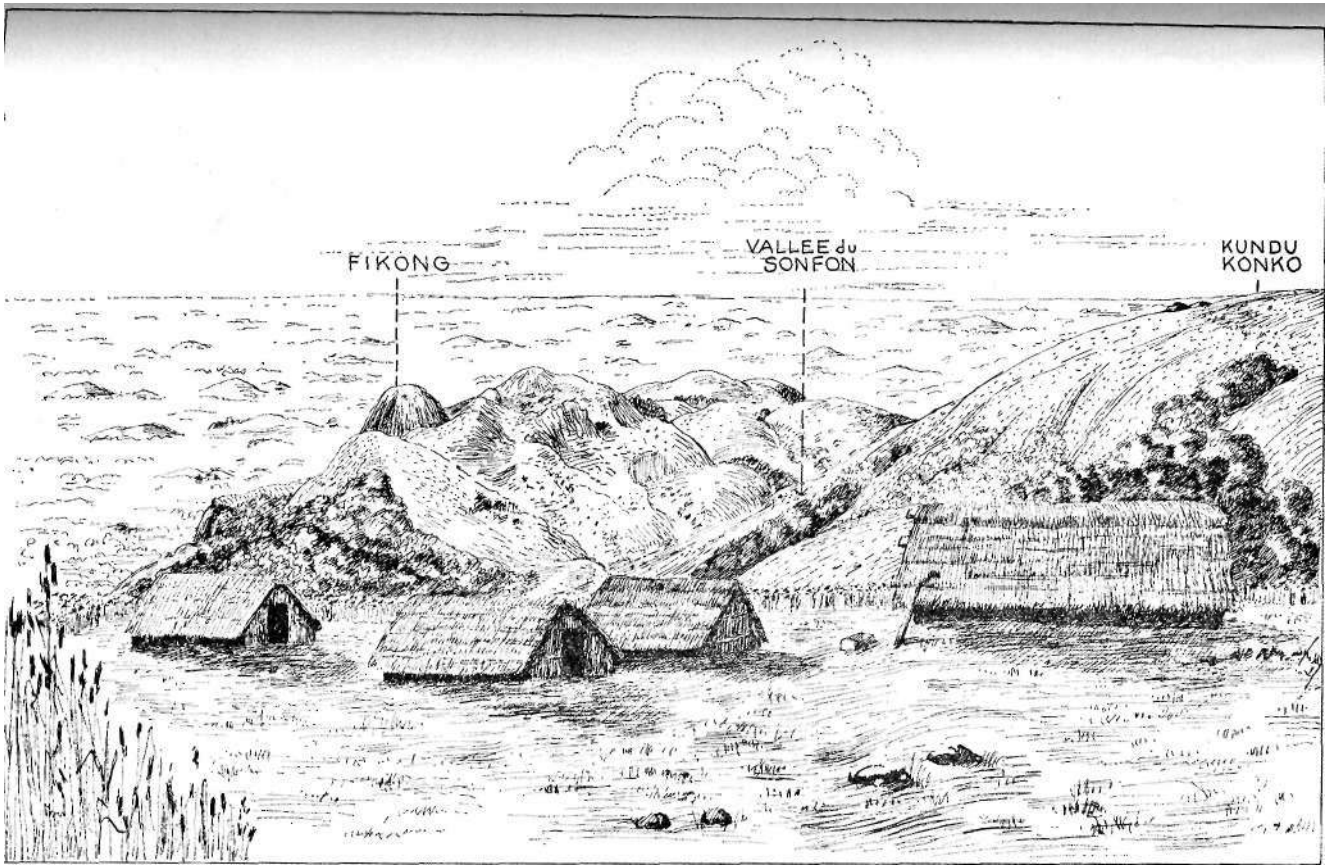


Fig. 7. — Vue de la pente herbeuse à exp. NE du Pic Bintumane (1500 m) sur une fraction de la façade N du Loma dominée par le dôme en pain de sucre du Fikong (1200 m). L'extrémité E du Kundu-Konko est séparée de la façade N par la vallée du Sonfon dont les eaux proviennent du plateau sommital du Pic Bintumane (J. G. ADAM dess. oct. 1944).

en lambeaux de ces fourrés, parfois aussi par la destruction totale de tout ou d'une partie d'un peuplement (Da-Oulen). Il arrive que cet arbrisseau ne forme que des îlots exigus, réduits à quelques individus implantés sur les blocs et affleurements rocheux en prairie d'altitude ou même en savane submontagnarde bien en-deçà de la limite supérieure de la forêt.

Installée sur un sol arénacé pauvre ou encastrée dans les fentes et crevasses du substrat rocheux, cette Mélastomatacée vit dans un milieu où les conditions climatiques s'avèrent extrêmes. En saison sèche ces hauteurs subissent les rigueurs de l'harmattan; la plante se défend en se débarrassant de ses feuilles; en saison humide une fraction importante de l'eau pluviale ruisselle, sans être retenue, sur la dalle nue souvent à forte déclivité; cependant les Mousses et la tourbe édifiée par les *Afrotrilepis (A. pilosa, A. jaegeri)* sont néanmoins responsables du stockage d'une certaine quantité d'eau.

Réduit à une seule strate arbustive, ce bush montagnard, souvent difficilement penetrable, comprend divers autres ligneux, petits arbres, arbustes ou buissons dont certains sont des orophytes endémiques de l'ouest africain comme: *Peddiea fischeri, Eugenia leonensis, E. pobeguinii, Memecylon fasciculare, Leocus pobeguinii, Pavetta lasioclada;* d'autres comme: *Ficus eriobotryoides, F. leprieuri, Gaertnera longevaginalis, Psychotria calva, Vincentella passargei...* se retrouvent dans les massifs forestiers guinéo-occidental et guinéo-congolais. La Vitacée *Cyphostemma rubrosetosum* qui montre une nette prédilection pour les milieux rocheux en altitude, s'installe dans les fentes et crevasses du granite; ses tiges herbacées et glanduleuses s'étalent en guirlande sur la couronne des buissons et des arbustes. Dans la strate herbacée, toujours ouverte, on remarque des saxicoles comme *Mesanthemum jaegeri, Impatiens jacquesii, Kalanchoe crenata, Utricularia* sp. *Nephrolepis undulata,* des Mousses (v. photo 18). Dans les clairières ou à la périphérie du maquis, la roche nue donne asile à une végétation pionnière à base de Lichens *(Parmelia pseudotinctorum, Usnea pulvinata...),* de Mousses

(Bryum argenteum, B. petrophilum, Canphylopus chevàlieri, C. introflexus, Leucoloma sericeum, Rhodobryum staudtii...)[1].

Souvent la microtopographie du substrat rocheux est mise en valeur par un minuscule gazon essentiellement graminéen, haut de 5 à 15 cm à peine où les orophytes abondent : *Panicum pusillum, Schizachyrium djalonicum, Loudetia jaegeriana, Trichopteryx glanvillei, Tripogon major* subsp. *jaegeranus, Bulbostylis congolensis, B. densa, Nerophüa gentianoides, Olderilandia echinulosa...*

Pionnier par excellence, *l'Afrotrilepis pilosa* colonise les surfaces rocheuses quel que soit le degré de leur déclivité ; après le passage du feu ses touradons noircis et d'aspect coralloïde confèrent au paysage un aspect étrange. Edificatrice d'humus, cette espèce recouvre la dalle d'une pellicule de sol tourbeux, détrempé en saison pluvieuse où s'installent des espèces comme *Mesanthemum prescottianum, Utricularia* sp., *Bulbostylis* sp., *Nephrolepis undulata... Polystachya microbambusa* aux fleurs jaunes épanouies en saison pluvieuse, vit en epiphyte sur les touradons de la Cypéracée (v. photos 5 et 6).

Alors que l'*Afrotrilepis pilosa* dont Faire s'étale sur toute l'Afrique occidentale humide est une saxicole très répandue au Loma quelle que soit la nature du soubassement, granite ou dolérite, les mottes de l'*Afrotrilepis jaegeri,* remarquablement ajustée au cycle saisonnier et au substrat granitique, n'ont été observées jusqu'à ce jour au Loma que sur la crête du Da-Oulen et sur les pentes du Serelen-Konko (70). Cet orophyte a été récolté par la suite aux Tingi Hills par J. K. MORTON, seules stations actuellement connues (v. photo 15).

III — LA PRAIRIE *V*ALTITUDE. SES ENCLAVES

Dépourvue d'arbres et d'arbustes, la prairie d'altitude du Loma se présente comme une vaste étendue herbeuse,

[1] M. H. DES ABBAYES s'est chargé de la détermination des Lichens et M. M. BlZOT de celle des Mousses de mon herbier Loma; je les en remercie très vivement.

formée essentiellement d'espèces vivaces, sufifrutescentes et herbacées où les Graminées jouent la note dominante. Ces étendues sont cependant loin d'être d'une seule pièce; abstraction faite des trainees arborées à *Parinari excelsa* qui jalonnent les cours d'eau du Plateau, le tapis prairial est disloqué par de nombreuses enclaves, rocheuses ou marécageuses.

A) Les enclaves rocheuses

D'imposants blocs granitiques, isolés ou groupés, sont éparpillés à travers la prairie altimontane; et souvent le tapis végétal est dilaceré par l'affleurement de dalles rocheuses.

Les blocs granitiques, habituellement fracturés par des cassures verticales ou horizontales, donnent asile à des touffes arborées formées de buissons ou de petits arbres où, à côté de *Nuxia congesta, Hymenodictyon florïbundum, Clausena anisata...* nous retrouvons de nombreux représentants du bush montagnard des sommets granitiques, à savoir : *Dissotis leonensis, Memecylon fasciculare, Eugenia leonensis, E. pobeguinii, Pavetta lasioclada...* Ces îlots, fragments minuscules du maquis à *Dissotis leonensis,* témoignent des échanges qui ont dû se faire à certaines époques, entre le «réservoir» sommital et la prairie située en contre-bas.

Blocs et dalles rocheuses donnent asile à une végétation pionnière où nous retrouvons les saxicoles héliophiles, Lichens et Mousses déjà inventoriées sur les sommets granitiques. Sur la tourbe à *Afrotrilepis pilosa,* Cypéracée tout aussi fréquente ici que sur les crêtes, on remarque la présence du *Gladiolus leonensis,* une endémique du Loma dont les fleurs blanches et penchées s'épanouissent dès les premières ondées d'avril (photo 16). Dans ce même milieu a élu domicile l'*Oxyrachis gracillima* subsp. *occidentalis,* Graminée aux feuilles capillaires et d'un vert cendré; cet orophyte, connu de l'Est africain et de Madagascar, atteint au Loma la limite occidentale de son aire de répartition.

A même le roc, et parfois sur ses parois verticales, dans un milieu d'une sécheresse extrême, s'étalent les racines du

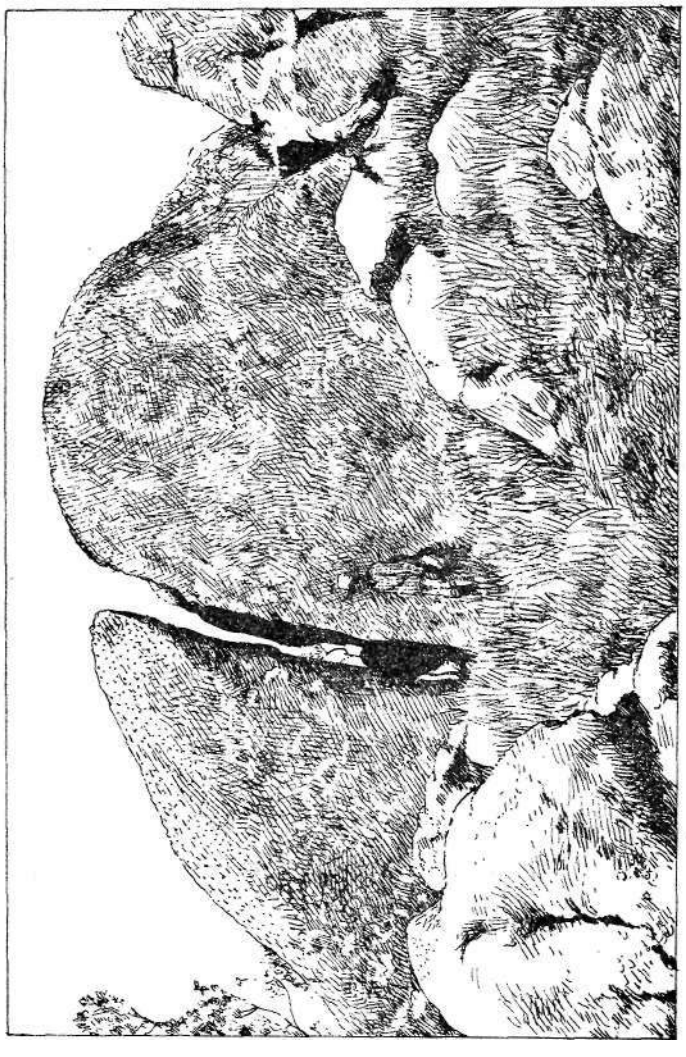


Fig. 8. — Bloc granitique fracturé de part en part. Prairie d'altitude vers 1600 m — Mts. Loma (K. WATRE dess. d'apres photo P. JAEGER).

Pólystachya dalzielli, dont les fleurs lilacées, très voyantes, se montrent au plus fort de la saison sèche; d'autres Orchidées comme *Bulbophyllum bifarium, B. scariosum...* ont un comportement analogue; il est vrai que pour affronter les rigueurs de la saison sèche, ces plantes disposent des réserves hydriques stockée dans leurs pseudobulbes.

Le *Gladiolus aequinoctialis* var. *aequinoctialis* profite des moindres fissures du roc pour abriter son bulbe, et il en est de même du *Pólystachya bequaertii,* une endémique ouest africaine. Les fleurs très voyantes, blanches, striées de rouge, penchées et inodores de l'ridacée sont épanouis au plus fort de la saison pluvieuse; la reproduction sexuée par graines est complétée utilement par une active multiplication végétative (bulbiles) (68) (v. fig. 12).

Les cassures horizontales de blocs rocheux (70), véritables abris ombragés et humides sont le lieu d'élection de diverses espèces à tempérament sciaphile; on y remarque des Ptéridophytes comme *Dryopteris athamantica, Nephrolepis undulata, Notholaena inaequalis* une fougère révivis-cente, des *Sélaginelles;* parfois, à l'entrée même de ces crevasses se dressent, alignés en une rangée fleurie (novembre), des pieds de *Kalanchoe crenata,* l'unique Crassuîaeée de notre massif.

B) Les enclaves marécageuses

Parmi les dalles granitiques afleurantes il y en a qui sont suintantes; d'autres, légèrement inclinées, sont couvertes d'un film d'eau courante; d'autres enfin, à pente plus raide, sont à l'origine de cascades ou de cascadelles aux eaux torrentielles. Les dalles à forte déclivité et, partant, à courant rapide, sont favorables à l'implantation d'une Lentibulariacée remarquablement adaptée au milieu: *Utricularia tetraloba.* Cette herbe aquatique de taille minuscule (0,5 à 1 cm) est solidement amarrée au substrat rocheux par l'intermédiaire de crampons; les feuilles, toutes submergées, sont pourvues d'utricules et les fleurs, aériennes, d'un blanc crème, petites et odorantes, sont entomophiles; les graines se gélifient au contact de l'eau en une masse mucilagineuse qui adhère au

substrat. Cette Utriculaire forme de vastes colonies herbacées orientées dans le sens du courant; elles sont repérables au loin au moment de la floraison. Cette espèce endémique de l'ouest africain est proche de l'*U. rígida,* observée par nous dans les cascades du massif de Kita (Mali).

Rappelons qu'au Loma les cascades sont favorables à l'implantation des Podostémonacées. Ainsi, le *Tristicha trifaria* fut observé vers 1280 m au sommet de la grande cascade du versant ouest dans un microgazon muscinal léché par les eaux agitées, et le *Ledermanniella jaegeri* s'installe dans le lit du Miramira vers 1400 m sur des blocs granitiques temporairement exondes. Quand la déclivité de la dalle va en s'amenuisant, des coussinets de Mousses s'implantent; ils sont séparés les uns des autres par une pellicule de sol noirâtre et détrempé, véritable marécage temporaire à sec en dehors de Ia saison pluvieuse. Là on remarque, associé à des Algues: *Utricularia subulata, U. pubescens, Genlisea africana, Eriocaulon pulchellum, Mesanthemum auratum, Xyris festucifolia, Sebaea luteo-alba, Nemum bulbostylidoides, Panicum pusillum...* L'amenuisement de la pente se poursuivant, les coussinets muscinaux entrent en coalescence pour former un gazon continu, toujours humide, où prolifèrent des espèces hygrophiles comme: *PoIygala lecardii, Swertia mannii, Utricularia* sp., *Bulbostylis densa, Eragrostis cenolepis, Schizacfayrium brevifolium, Anadelphia leptocoma, Lycopodium cernuum, Osmunda regalis, Smithia ochreata .;* de là on passe, suivant les cas, à la prairie montagnarde ou à un chaos de blocs rocheux donnant asile à: *Afrotrilepis pilosa, Nerophïla gentianoides, Dissotis leonensis, Leocus pobeguinii, Eugenia leonensis, Nuooia congesta, Hymenodictyon florïbundum...*

Particulièrement intéressante s'avère l'étude d'une enclave marécageuse située vers l'extrémité supérieure de la galerie forestière du Miramira (1650 m). L'emplacement est marqué par la présence d'un ensemble de touradons herbeux, verdâtres, subcylindriques hauts de 50 à 75 cm, séparés les uns des autres par un intervalle subhorizontal (de 0,5 à 2m) drainé par des filets d'eau claire et limpide;

le sol vaseux, détrempé et de couleur noire est épais de quelques centimètres à peine.

Le sommet des touradons qui représente la partie la moins détrempée est occupé par des herbacées caractéristiques des biotopes humides comme : *Pycretis nuerensis, Eragrostis cenolepis, Lipocarpha chinensis, Xyris decipiens...* Les parois subverticales des touradons de plus en plus humides à mesure qu'on s'approche de leur base et surtout la zone intermédiaire subhorizontale et fortement marécageuse, est caractérisée par la présence d'espèces héliophiles comme: *Drosera pilosa, Genlisea africana, Utricularia subulata, U. pubescens, Eriocaulon pulchellum, Xyris* sp.... ; de plus, les tiges flasques du *Lobelia rubescens* (proche de *L. kamerunensis),* herbe couchée-ascendante, serpentent d'un touradon à l'autre à la surface des sols spongieux et humides.

Il en est de même de celles, couleur rouille, du *Laurembergia tetrandra;* enfin, les tiges dressées aux sommets penchés du *Lycopodium cernuum* se montrent dans les espaces séparant les touradons.

Le marécage du Sonfon

Le plateau sommital du Pic Bintumane où le Sonfon prend sa source, est légèrement creusé en auge; ses pentes, très douces, sont drainées par des filets d'eau qui convergent en un thalweg médian occupé en partie par un marécage d'où s'échappent les eaux du Sonfon. Avant de dévaler les pentes herbeuses à exposition NE du Pic Bintumane, elles passent par un déversoir rocailleux marqué par la présence de : *Conyza gigantea, Otomeria cameronica, Dissotis sessïlis...*

Dans le bassin sommital on voit affleurer, par endroits, des dalles doléritiques d'allure polygonale (50 à 100 cm) ; formant dallage, elles sont séparées les unes des autres par d'étroits sillons où s'est constitué un sol squelettique formé essentiellement de fragments doléritiques en voie d'altération, reconnaissables à leur coloration brunâtre en pain d'épice.

La surface des dalles exempte de toute altération chimique donne asile à une flore pionnière à base de Lichens,

de Mousses, de touradons *d'Afrotrilepis pilosa...* Sur les coussinets de Mousses on voit s'installer: *Cyanotis rupicola, Solenostemon* sp…; *Nerophila gentianoides* est loin d'être rare (v. photo 8).

A la fin des pluies (oet.) le marécage du Sonfon présente un sol noir détrempé, recouvert, par endroits, d'une mince pellicule d'eau. Ce groupement, encore richement fleuri à cette époque de l'année, est repérable au loin grâce à un peuplement de Cypéracées à allure joncoïde, et aux épillets d'un brun-noir luisant: *Pycreus atrorubidus, Nemum spadiceum, N. bulbostylidoides* une endémique de la dorsale Loma-Man. Au ras du sol on observe des plantes minuscules: *Eriocaulon pulchellum* (1 à 5 cm) à rosette de feuilles basales appliquées à même le sol et parfois couvertes d'un film d'eau; *Utricularia pubescens* aux fleurs d'un mauve violacé, *U. micropetala* var. *macrocheilos* aux fleurs jaunes; *Panicum* pusillum, une Graminée exiguë haute de 5 à 10 cm, *Xyris festucifolia* (10-15 cm) ; sont de taille plus importante: *Mesanthemum prescottianum, Scleria dieterlinii, Habenaria* sp. de la sect. *Bilábrella, Schisachyrium lomaense,* une endémique de la dorsale; à la périphérie en milieu moins humide, on note: *Swertia mannii, Cyanotis rupicola, Nerophila gentianoides, Afrotrilepis pilosa...*

Pendant les heures chaudes de la journée, le sol noir de cette cuvette marécageuse s'échauffe rapidement par rapport au milieu ambiant; cet échauffement est surtout appréciable aux emplacements où la végétation est clairsemée ou absente. Des mesures ont été réalisées le 6 Octobre 1966 par temps clair et ensoleillé, l'atmosphère étant agitée par un léger vent d'Est. La température de l'air a été mesurée au moyen du thermomètre fronde.

Heure	Température de l'air	Température du sol
11 OO	18°8	22°4
12 45	19°4	30°6

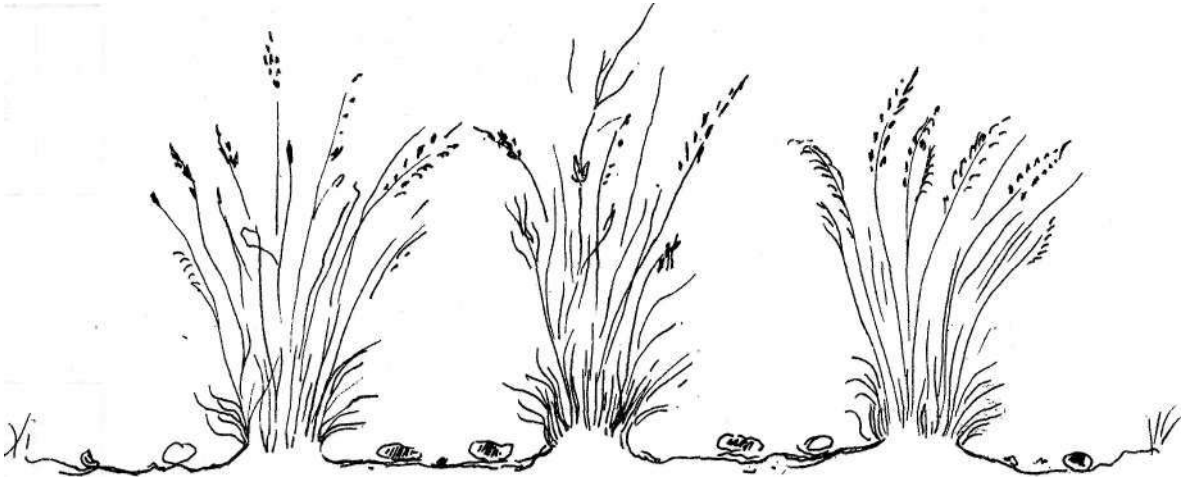


Fig. 9. — Végétation graminéenne en touffes cespiteuses séparées les unes des autres par le sol nu persemé de cailloux ferrugineux. Prairie d'altitude du Plateau vers 1600m (Mts. Loma).

IV — LA PRAIRIE D'ALTITUDE: DUALITÉ ÊDAPHIQUE ET FLORISTIQUE

Le tapis herbacé, essentiellement graminéen de la prairie d'altitude du Loma, abstraction faite des enclaves arborées, rocheuses ou marécageuses, est loin d'être homogène. En divers points du Plateau on remarque la présence de vastes «lambeaux cuirassés» affleurants ou subaffleurants. Nullement comparables quant à leur origine, aux cuirasses des bowé soudaniens, ces «pseudocuirasses» sont moyennement indurées et de couleur rouille; elles sont de structure alvéolaire et scoriacée avec de nombreux grains de quartz et quelques éléments ferromagnésiens, le tout amalgamé par des tramées ferrugineuses; en bref, l'ensemble se présente comme une arène granitique concrétisée par des venues ferrugineuses.

La «pseudocuirasse» et le sol squelettique chargé de concrétions qui en dérive, porte une prairie basse (50 à 70 cm), peu dense à *Loudetia Kagerensis;* les orophytes y abondent: *Cyperus nduru, Digitaria minutiflora, Panicum ecklonii, Rhytachne glabra, Protea occidentalis, Vernonia nimbaensis, Thesium tenuissimum, Sopubia mannii* var. *tenuifolia, Coreopsis camporum, Aristea angolensis, Helichrysum nudifolium* var. *leiopodium, H. mechovianum, Brachycorythis paucifolia,„..;* ainsi que trois des neufs endémiques appartenant en propre au Loma: *Digitaria minutiflora, D. phaeotricha* var. *patens, Loxodera strigosa* (v. fig. 11).

Côte à côte avec ces îlots cuirassés s'étalent de vastes étendues dépourvues d'horizons carapaces; les sols y sont plus profonds et pratiquement sans concrétions; la texture est sableuse (sables grossiers et fins: 65%; limons 18,9%, argile 9,5%), et la valeur basse du C/N (7,3) parle en faveur d'une minéralisation active.

La strate herbacée, haute de 1,50 à 1,75 m (octobre), est constituée esenfiellement de Graminées communes dans les savanes de piedmont; indifférentes à l'altitude, elles couvrent une aire souvent très vaste s'étendant ou dépassant parfois l'Afrique tropicale; citons: *Anadélphia afzeliana, Andropogon africanus, A. gayanus, A. schirensis, Beokeropsis*

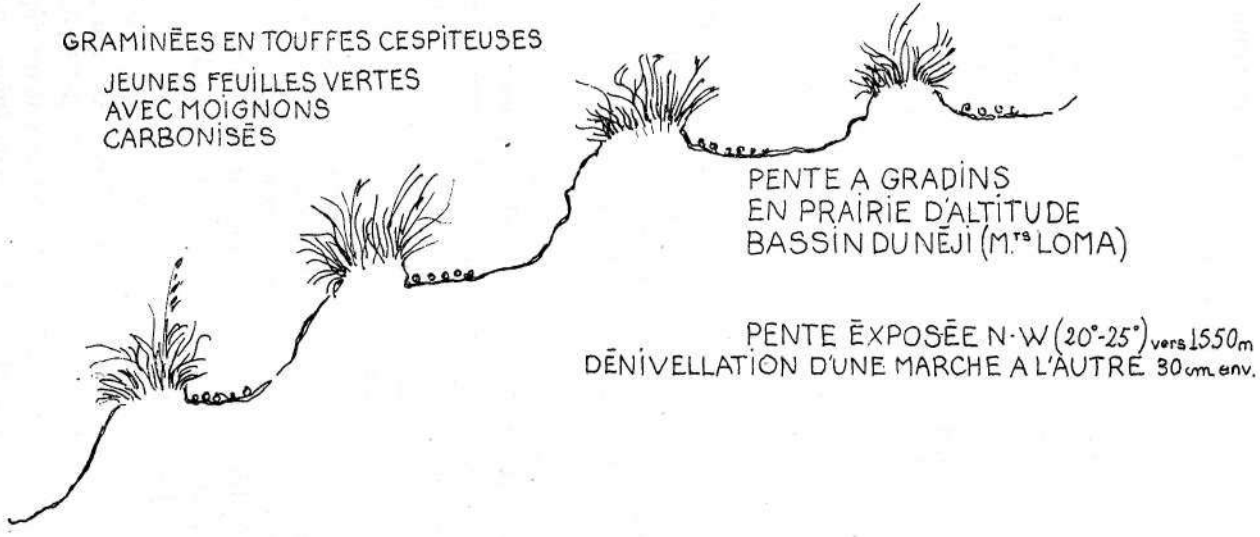


Fig. 10. — Pente a gradins en prairie d'altitude; bassin du Neji vers 1550 m (monts Loma).

uniseta, Digitaria diagonális var. *hirsuta, Elymandra androphila, Hyparrhenia diplandra, H. rufa, Panicum praealtum...* Les orophytes sans être absents de ce cortège essentiellement graminéen ont, en grande partie, succombé dans la lutte contre la poussée envahissante de ces herbacées très compétitives et grandement favorisées dans leur progression par la vague ignée.

V - L A PRAIRÏE D' ALTITUDE

Origine et signification biogéographique

Pour ce qui est de ces étendues à sol profond, rien ne s'oppose, *a priori,* à l'installation de la forêt. Aussi la présence de ces vastes surfaces herbeuses, véritables enclaves dans l'étage forestier submontagnard, soulève-t-elle le problème de leur origine et de leur signification biogéographique.

Dans les monts Loma, comme dans les autres massifs de la dorsale guinéenne, la limite supérieure de la forêt se situe à une altitude inférieure à celle qu'elle occupe dans d'autres massifs montagneux plus élevés. Aussi est-il permis de penser que si les massifs de la chaîne guinéenne étaient plus hauts, la limite supérieure de leur forêt serait, elle aussi, portée à une altitude plus élevée.

Il y a cependant lieu de faire remarquer que vers leur extrémité supérieure les galeries forestières du Loma présentent une physionomie qui n'est pas sans rappeler celle d'autres forêts tropicales arrivées à la limite de leur extension altitudinale. Ainsi, la similitude entre une tête de galerie faisant irruption dans la prairie montagnarde du Loma et celle, située vers 2000 m dans les monts Uluguru (Est africain), ne peut pas passer inaperçue. Dans les deux cas on passe brusquement, sans transition, de la forêt à la prairie et les arbres, surchargés d'épiphytes, aux couronnes hémisphériques et au feuillage périphérique toujours vert, constituent un type biologique — immergrüne Kugelschirmbäume de C. TROLL — qui se correspond d'une région à l'autre. Cette coïncidence des types biologiques et du modelé de la forêt montagnarde parvenue à sa limite supérieure, nous

amène à supposer l'entrée en action de tout un ensemble de facteurs qui en auraient la responsabilité; ceux qui s'imposent avec le plus d'évidence seraient, à première vue, les agents climatiques exacerbés à proximité de la crête et des sommets. Au Loma, en raison de l'orientation de la chaîne, en raison aussi de son insularité, cette exacerbation semble se traduire avec un maximum d'acuité (71).

Cependant en altitude, où, en saison sèche, l'harmattan souffle avec impétuosité, ni le *Parinari excelsa,* ni la *Syzygium staudtii* n'adoptent de faciès vexillaire; mieux, contrairement à ce que l'on remarque en savane de piedmont où la plupart des essences sont défeuillées en saison sèche, ces deux arbres étonnent par la persistance de leur feuillage.

Il est communément admis que la végétation des savanes de l'Afrique intertropicale est d'origine secondaire. La végétation primitive, essentiellement forestière correspondant au *climax,* a été détruite par le feu et les défrichements c'est-à-dire par l'homme. La vague ignée en déferlant périodiquement durant des millénaires sur ces vastes étendues, a exercé une véritable sélection. N'ont survécu au traumatisme que les espèces préadaptées ; parmi les ligneux, celles dont l'écorce était protégée par une épaisse couche de liège *(Karité, Bombax, Spondias...);* parmi les herbacées celles dont le cycle reproducteur était ajusté au rythme des feux, les graines étant déjà disséminées au moment de l'irruption de la vague ignée ou celles, comme les Graminées céspiteuses, dont les jeunes innovations sont protégées par un feutrage de gaines sèches; et il en est de même des bulbes, tubercules et rhizomes qui sont à l'abri sous une certaine épaisseur de sol (70).

Les non-adaptées, por contre, ont succombé à la lutte ou se sont réfugiées dans des stations inaccessibles aux feux; c'est le cas, entre autres, des peuplements de Kololo *Gilletiodendron glandulosum)* qui se sont maintenus dans les buttes **gréseuses du Plateau** mantingue (région de Kita) grâce à l'existence de parefeux naturels: les seuils rocheux.

Cette façon d'interpréter les faits, loin d'être **une** vue **de l'esprit, fut** défendue par des auteurs aussi **avertis** que **Lane Poole, A. CHEVALIER, H. HUMBERT, A. AUBRÉVILLE,**

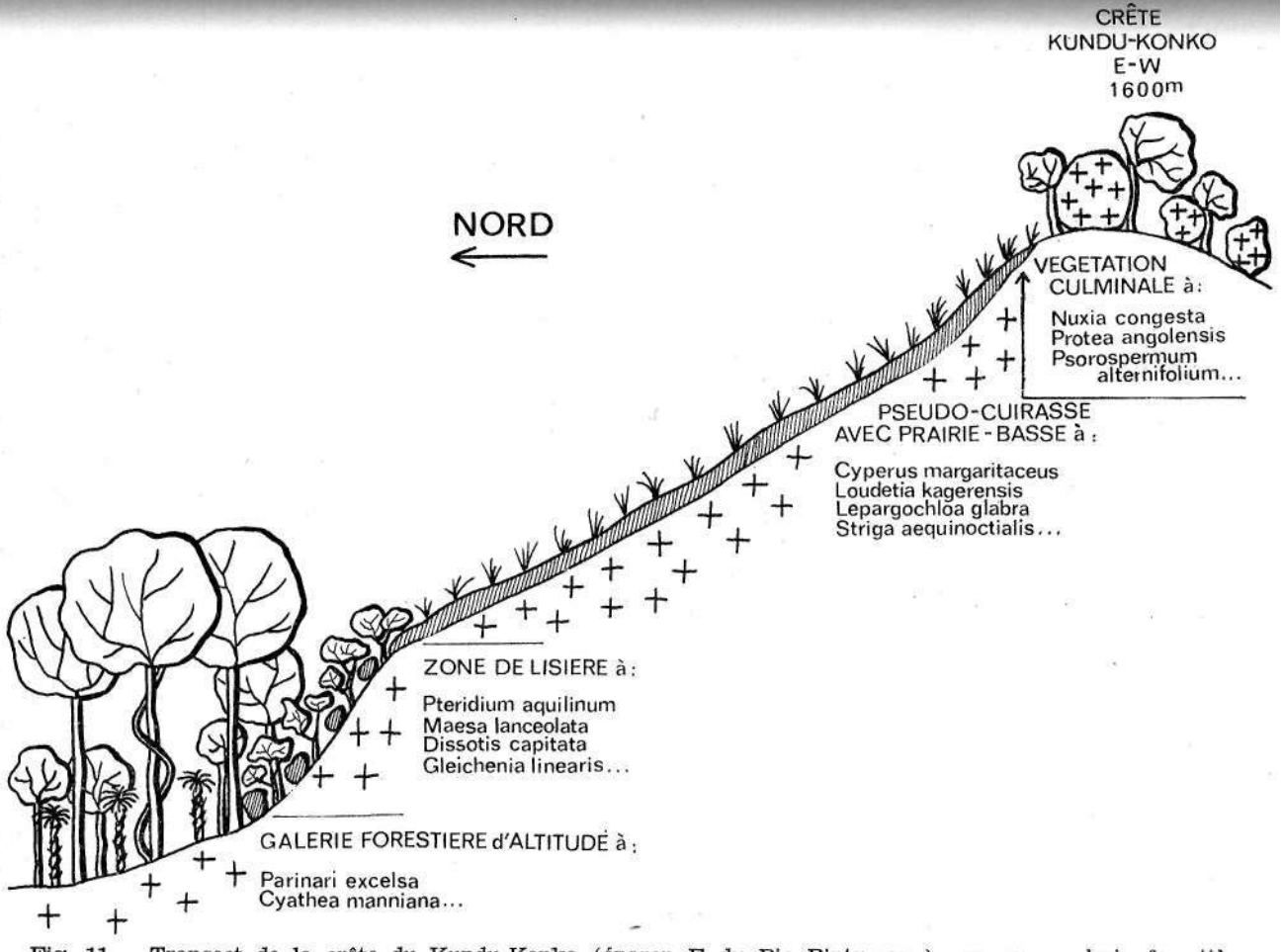
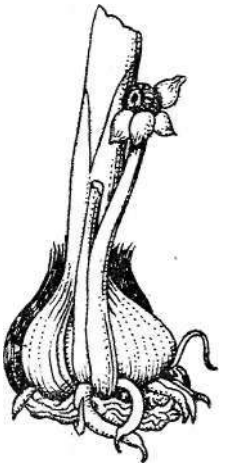


Fig. 11. — **Transect de la crête du Kundu-Konko (éperon E du Pic Bintumane) vers une galerie forestière d'altitude du versant N, affluent du Sonfon. Remarquer l'étendue de l'horizon «cuirassé» en pente.**

H. RICHARD-MOLARD,, R. SCHNELL, R. Letouzey... et de nombreux autres, fondant leur assertion sur l'observation et l'expérience.

H. HUMBERT, en 1931 (57), déclare: «Les vastes herbages, arborés ou non, où régnent, soit régulièrement, soit sporadiquement, les feux de brousse, sont toujours secon-



2 cm

Fig. 12. — *Gladiolus aequinoctialis* Herbert var. *aequinoctialis* (Irid.) Bulbe avec tige feuillée et florifère (coupée) et deux axes à bulbilles, l'un d'eux étant sectionné. Le bulbe est superposé à un élément ancien aplati et vidé. Présence, autour de l'appareil souterrain, d'une tunique fibreuse (partiellement enlevée) percée par les racines (E. HUBEE dess. d'après éch. herbier P. JAEGER).

daires, et doivent leur existence même à ce régime des feux... Il n'existe pas, en Afrique équatoriale, dans la végétation primaire, de steppes de hauts plateaux ou de montagne caractérisant des subdivisions territoriales naturelles» (cité d'après R. SCHNELL).

A. AUBRÉVILLE, en 1949, parle d'une savanisation anthropique de régions initialement boisées. — «La végétation climacique de l'Afrique intertropicale recevant plus de 600 à 700 mm de pluie par an est, d'après H. RICHARD-MOLARD, une

végétation forestière». Selon R. SCHNELL «dans les régions intertropicales le climax est, de façon générale, constitué par des forêts» (122).

Des expériences poursuivies, pendant plusieurs années, au Nigeria, en Côte d'Ivoire et dans l'Ouganda ont prouvé qu'il suffisait de protéger des feux des parcelles de savanes arbustives pour qu'une brousse forestière fermée se constitue (122).

Ainsi, par analogie, on peut être amené à penser que la prairie montagnarde, au même titre que la savane de piedmont, n'est pas une formation climacique, mais un *paraclimax* conditionné par les feux. Localisée dans l'étage submontagnard, elle s'est substituée à une forêt climacique éminemment fragile et elle se perpétue telle quelle grâce au déferlement périodique de la vague ignée. A l'appui de cette thèse A. AUBRÉVILLE fait ressortir ce fait, selon lui capital, que dans les régions tropicales humides... la végétation ligneuse dense et fermée prend toujours possession du sol, et ceci si abruptes que soient les pentes: et l'auteur de poursuivre: «c'est le feu qui a détruit les formations montagnardes primaires» (12). Pour R. SCHNELL (117) : «Une origine naturelle de la prairie des crêtes ne nous parait pas devoir être envisagée».

Le recul de la forêt montagnarde

Dans les ravins et vallées la forêt montagnarde à *Parinari excelsa* recule sous nos yeux; elle succombe au feu allumé par l'homme, et elle est remplacée, non par la prairie, mais par une formation secondaire herbeuse et buissonnante, dense, épineuse, difficilement penetrable, riche en espèces où, parfois, persiste encore un témoin de l'ancienne forêt définitivement détruite. Cette formation de «lisière», périodiquement ravagée par les feux est caractérisée par des herbacées érigées hauts de 1,5 à 2 m ou davantage comme : *«Pteridium aquilinum, Pavonia wrens, Laggera gracilis, Pycnostachys volkcensii, Triumfetta tomentosa...* par des lianes, les unes inermes comme: *Ipomoea involucrata, Adenia lobata, Stephania abyssinica, Mikania scandens...* les autres

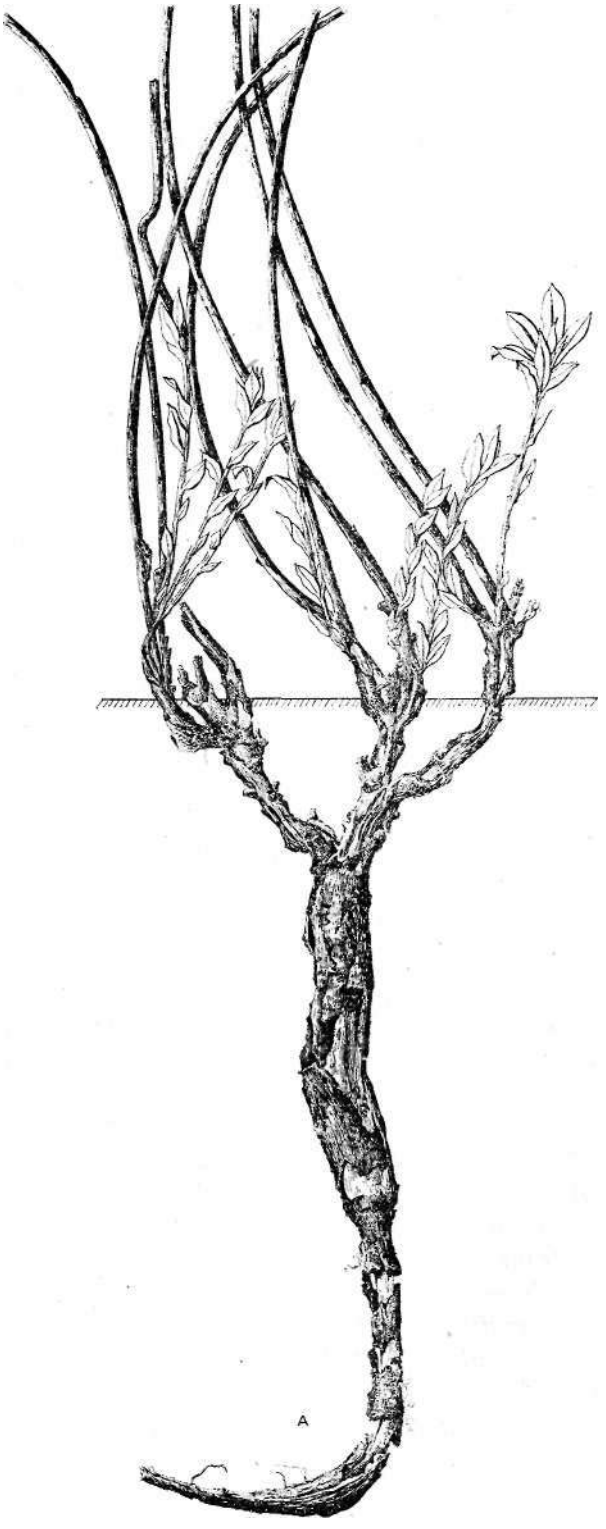


Fig. 13.—*Euphorbia depauperata*... A (voir legende dans la page suivante).

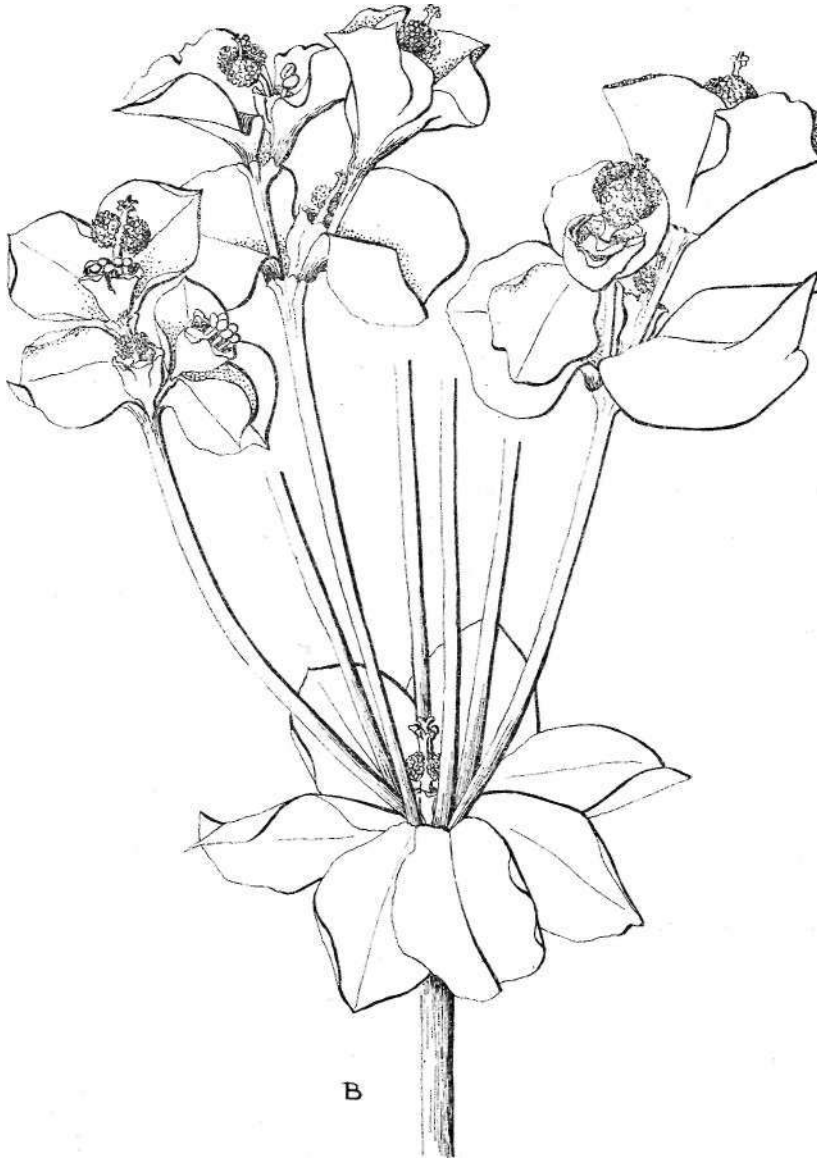
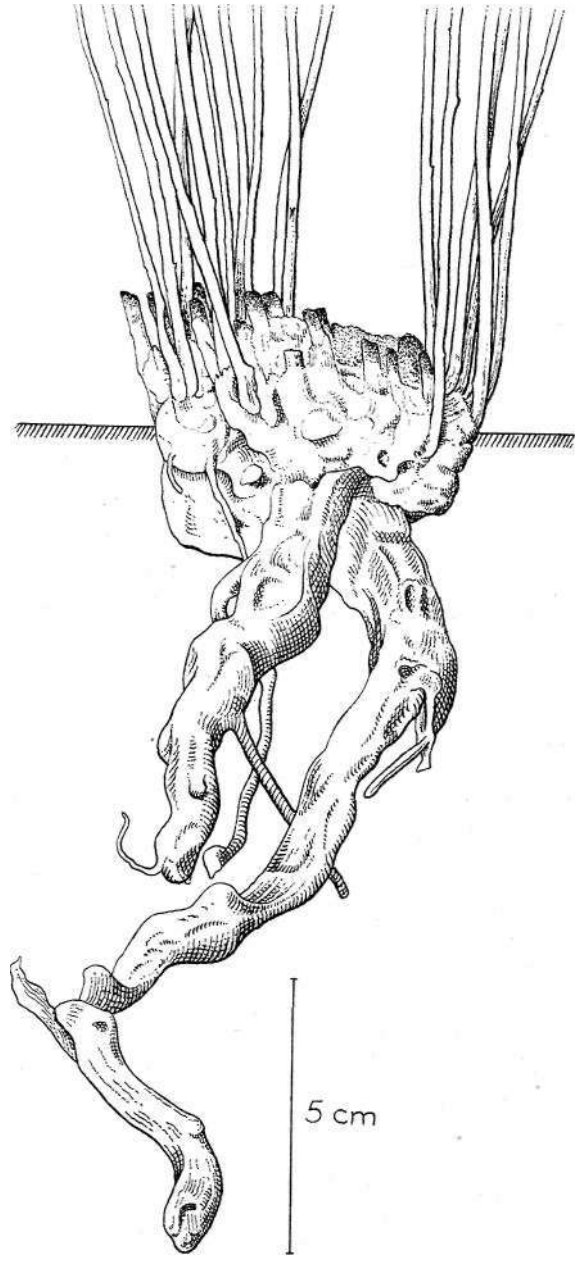


Fig. 13.—*Euphorbia depauperata* Höchst, ex A. Rich. (Euphorbiacées). Ä — Sous-arbrisseau caractérisé par son appareil souterrain napiforme. Aspect de l'appareil aérien après le passage du feu: moignons carbonisés dressés et jeunes pousses feuillées et florifères. Espèce prairiale à aire disjointe. B — Sommité fleurie (janvier) (K. WATEÉ dess.; Herbier P. JAEGER).

épineuses comme *Smilax kraussiana* et *Asparagus racemosus;* citons aussi le *Rubus féllatae,* buisson sarmenteux et épineux qui rend ces fourrés pratiquement impénétrables (69).

Il n'en va pas de même en dehors des ravins et des vallées où de vastes étendues sont couvertes uniformément par la prairie d'altitude dépourvue d'arbres et d'arbustes; et il est quelque peu malaisé de se faire une idée sur la nature des forêts qui, primitivement, ont dû recouvrir ces vastes étendues actuellement occupées par la prairie montagnarde. Toujours est-il que le caractère paraclimacique de la prairie d'altitude du Loma semble attesté par l'existence, au sein de la nappe herbacée, de nombreux phanérophytes mutés en chaméphytes ou en hémicryptophytes à port multicaule *(Protea occidentals, Psorospermum alternifolium...),* par l'existence aussi, en dehors des ravins isolés dans les étendues herbaeuses des hautes pentes (bassin du Denkali) de lambeaux forestiers à *Parinari excelsa;* mieux, sur les crêtes et les pentes supérieures du ¡Ziama et du Nimba SW, R. SCHNELL (117) signale une forêt relictuelle basse qui revêt parfois l'aspect d'un taillis; assez peu humide en saison sèche, elle livre passage au feu; on y remarque: *Eugenia leonensis, Ouratea reticulata, Trichilia heudelotii, Maesa nuda, Nuxia congesta, Hymenodictyon floribundum...*

Au Loma on ne peut pas ne pas être frappé par l'existence, sur les sommets granitiques, de ce maquis à *Dissotis leonensis* que l'on retrouve, comprimé à l'extrême, autour des blocs rocheux épars en prairie d'altitude. La Mélastomatacée, d'une grande sensibilité aux feux, n'a pu se maintenir que grâce à la protection que lui confèrent ces pare-feux naturels. Et on peut supposer, qu'en l'absence de feux, elle fera irruption hors de ces «réservoirs» pour peupler, avec son cortège de buissons et de petits arbres, une grande partie de ces étendues correspondant à l'actuelle prairie d'altitude. Et, tout comme sur les hauts sommets granitiques, ce fourré arbustif a dû être disloqué par des «clairières édaphiques» correspondant à l'affleurement de dalles rocheuses, de sols cuirassés... autant de stations éminement propices **aux** orophytes non forestiers.



Pig. 14.—*Eupatorium africanum* Oliv, et Hiern (Composées). Sous-arbrisseau à souche ligneuse, vivace, étalée au ras du sol en un «plateau» hérissé de moignons carbonisés qui, après le passage du feu, donne naissance à un faisceau de tiges dressées, feuillées et florifères (E. HUBER dess.; Herbier P. JAEGER).

Le maquis à *Dissotis leonensis* qui, selon nous, a dû recouvrir jadis de vastes surfaces de l'étage culminai du Loma, aurait été refoulé par la vague ignée vers ces stations de repli où nous l'observons encore aujourd'hui. Ainsi, grâce au feu, la prairie en s'étendant progressivement aurait atteint sa configuration actuelle, pendant que tout un cortège d'herbacées banales à prédominance graminéenne, originaire des savanes de piedmont, faisait irruption en altitude. Hautement compétitifs et envahissants ces éléments se seraient installés au détriment de la forêt primitive, processus de savanisation guère favorable à la survie des orophytes.

Comme le feu semble être le facteur primordial responsable de la genèse, de l'extension et du maintien de la prairie d'altitude, cherchons à connaître le cycle annuel de ces espèces soumises périodiquement au traumatisme igné et aussi, si possible, leur comportement en prairie soustraite à l'action de feux.

Le cycle annuel de la végétation en prairie montagnarde soumise au feux

Dès le début de la saison sèche (novembre-décembre) quand les Graminées et autres herbacées se dessèchent, la vague ignée déferle à travers prairies et savanes laissant derrière elle un paysage de désolation. Cependant, à cette époque de l'année qui coïncide avec l'entrée en état de repos de la végétation, le dommage causé par les feux est minime, les plantes ayant pour la plupart, bouclé leur cycle reproducteur, fruits et graines étant disséminés. Contre toute attente, 15 jours ou 3 semaines après l'incendie, des jeunes pousses vertes et tendres apparaissent et, sans tarder, plusieurs vagues de floraisons se succèdent au cours de la saison sèche et même durant la saison pluvieuse suivante dont la fin est invariablement marquée par la floraison et la fructification des Graminées; et le cycle recommence (67).

L'irruption du feu a pour effet de régénérer la prairie par l'apparition d'un gazon de jeunes pousses vertes, tendres et d'une haute valeur nutritive. Aussi ces pâturages, ainsi

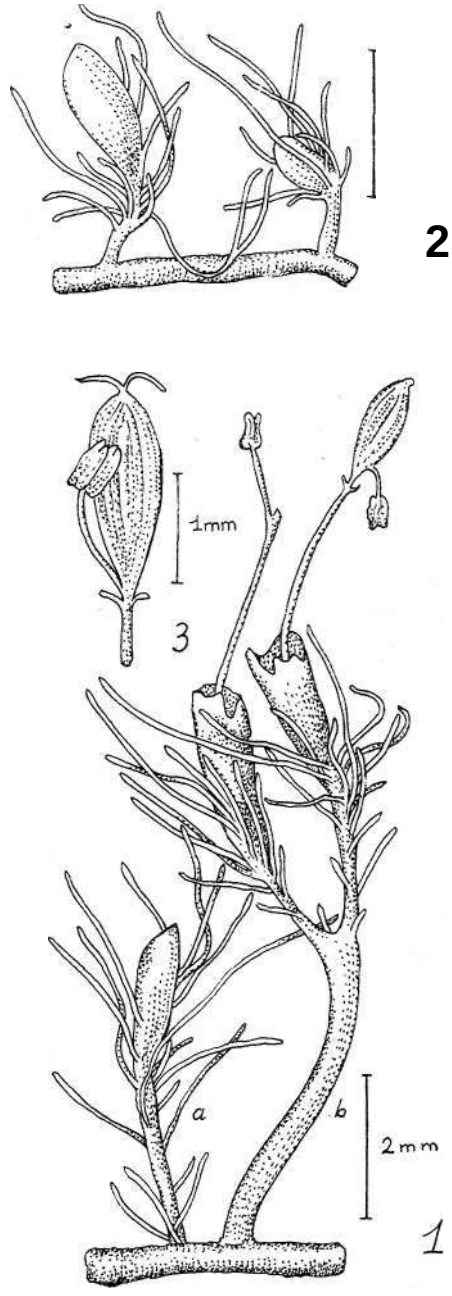


Fig. 15. — *Ledermanniella jaegeri* C. Cusset sp. nov. (Podostémonacées).

1 — à gauche: individu jeune; à droite: individu en fleurs; 2—individu avec boutons floraux; 3 — fleur. Lit torrentiel du Miramira vers 1400 m (janvier) (C. CUSSET dess.; Herbier P. JAEGER n.° 8789).

périodiquement renouvelés, sont-ils le lieu de rendez-vous de troupeaux de Buffles et d'Antilopes qui, en pleine saison sèche, à un moment de l'année où les réserves alimentaires s'épuisent, ont à leur portée des herbes d'une qualité supérieure; avec J. LEBRUN on peut suggérer qu'en l'absence de feux, les Ruminants seraient manifestement en régression sur ces hauteurs.

Le comportement de la prairie montagnarde non incendiée

La répercussion de la cessation des feux sur les comportement du tapis végétal en prairie montagnarde n'a pu être observée que dans quelques cas très peu nombreux: au Loma vers 1600 m, au Nimba guiñeen entre 1000 et 1700 m et dans la région de Macenta entre 900 et 1000 m.

Le cas du Loma — il s'agit d'une observation unique, isolée dans le temps et l'espace — se rapporte à une parcelle de quelques centaines de m^2 qui, lors de l'incendie périodique de novembre-décembre (1965) fut respectée des feux.

La strate herbacée parvenue au stade de repos, est sèche et prête à brûler. Mais, la vague ignée étant déviée, ce stade de repos, habituellement virtuel, se prolonge jusqu'aux premières pluies d'avril; à ce moment la prairie incendiée commence déjà à verdoyer et, chose inattendue, on constate également une reprise de la vie au niveau du lambeau non incendié: les Graminées rejettent de souche et les chaumes secs, apparenmment dévitalisés, engendrent des feuilles au niveau des noeuds supérieurs.

Des observations étalées sur plusieurs années et, partant, plus complètes, ont été effectuées par l'un de nous (J. G. ADAM) dans la région de Macenta et de N'Zérékoré (Guinée) ; elles ont montré qu'en cas de cessation des feux le cycle annuel était profondément perturbé.

Dans la région de Macenta, sur la ligne de partage des eaux Niger-Atlantique, entre 900 et 1000 m, de vastes savanes herbeuses ont remplacé depuis moins d'un siècle l'ancienne forêt guinéenne. Ces savanes qui, pendant trois années consécutives (1947-49) n'ont pas été incendiées sont constituées principalement par des Graminées vivaces appartenant aux

genres *Hyparrhenia, Andropogon, Schizachyrium, Elymandra, Pennisetum.* Dans la région de N'Zérékoré (Mt. Nimba), entre 1000 et 1700 m des prairies, vraisemblablement très anciennement anthropogènes, n'ont pas été incendiées pendant cinq années consécutives (1967-1971). Par leur composition floristique elles s'apparentent aux formations précédentes.

A l'approche de la saison sèche les Graminées et les autres herbacées se dessèchent, mais au lieu de devenir la proie des flammes, elles persistent telles quelles durant toute la saison sèche, couvrant les vastes étendues prairiales d'une nappa herbacée à allure de paille. L'aspect terne et monotone de ces prairies contraste singulièrement avec celles, vertes et richement fleuries qui, au préalable, ont été balayées par les feux. Ce n'est qu'avec les premières pluies que la feuillaison démarre; les chaumes apparemment dévitalisés, verdissent en émettant de jeunes feuilles à leur base et au niveau des noeuds; ce phénomène prend de plus en plus d'ampleur pour atteindre son apogée au plus fort de la saison pluvieuse; puis, avec le retour de la saison sèche, ces plantes montrent à nouveau les premiers signes de flétrissement. Mais ce cycle, fait inattendu, est marqué par l'absence de floraison et de fructification.

Exclusivement végétatifs, imposés par l'absence des feux, ces cycles se sont répétés durant cinq années consécutives. Les touffes herbacées primitivement espacées se sont développées pour occuper, en fin de compte, la presque totalité du sol. Les diaspores de ligneux amenés par le vent, les animaux... se développent en dépit de la concurrence des Graminées et, n'étant plus soumis à l'incendie périodique, rien ne semble s'opposer à leur développement: *Albizzia gummifera, A. zygia, Harungana madagascariensis, Trema guineensis...* En 1972, la prairie altimontane du Nimba fut à nouveau incendiée et l'année suivante elle était émaillée de fleurs.

Parmi les plantes qui s'adaptent au mieux à ce rythme exclusivement végétatif citons: *Hyparrhenia subplumosa, Rhytachne rottboellioides, Panicum eciklonii, Cyperus tenuiculmis* var. *guineensis...*

La suppression des feux, si elle entraîne une perturbation profonde du cycle annuel de la végétation en prairie montagnarde, est cependant incapable — sans doute en raison d'une durée insuffisante de cette période de cessation des feux — de nous faire assister à une reforestation effective de ces vastes étendues herbeuses.

Aussi y a-t-il lieu d'admettre que l'existence même et la pérennité de la prairie montagnarde, la restauration des pâturages en saison sèche et, partant, la présence des herbivores, sont conditionnées par les feux. La prairie altimontane du Loma se présente comme un groupement secondaire, une enclave dans l'étage forestier montagnard, dont la stabilité serait garantie par le déferlement périodique de la vague ignée.

VI — LA FLORE MONTAGNARDE; SES ORIGINES

Le tapis végétal de la prairie d'altitude du Loma où 248 espèces, sous-espèces ou variétés ont pu être dénombrées [1], s'avère d'une réelle complexité à la fois pour ce qui est de la composition floristique et de l'origine des taxons. Comme celui du Nimba (117) le peuplement végétal de l'étage culminai du Loma se distingue par une remarquable dualité: aux orophytes inféodés aux massifs montagneux et sans affinités avec les taxons de basse altitude, s'oppose un lot d'espèces planitaires banales originaire des pays de piedmont ou de régions plus lointaines.

A) Les orophytes

Les orophytes du Loma, comme ceux des autres massifs de la dorsale sont très variés quant à leurs exigences écologiques et quant à leur origine. Suivant les cas, il peut s'agir d'espèces prairiales ou forestières, de saxicoles ou de plantes hygro- ou hélophiles. Certaines, peu nombreuses, ne se trouvent que dans le seul massif du Loma; plus nombreuses sont

[1] Chiffre provisoire susceptible d'être modifié ultérieurement à la suite de nouvelles prospections.

celles qui sont propres à l'étage montagnard de l'ensemble des hauteurs ouest-africains allant du Fouta-Djalon à la chaîne camerounaise. Une attention spéciale méritent les

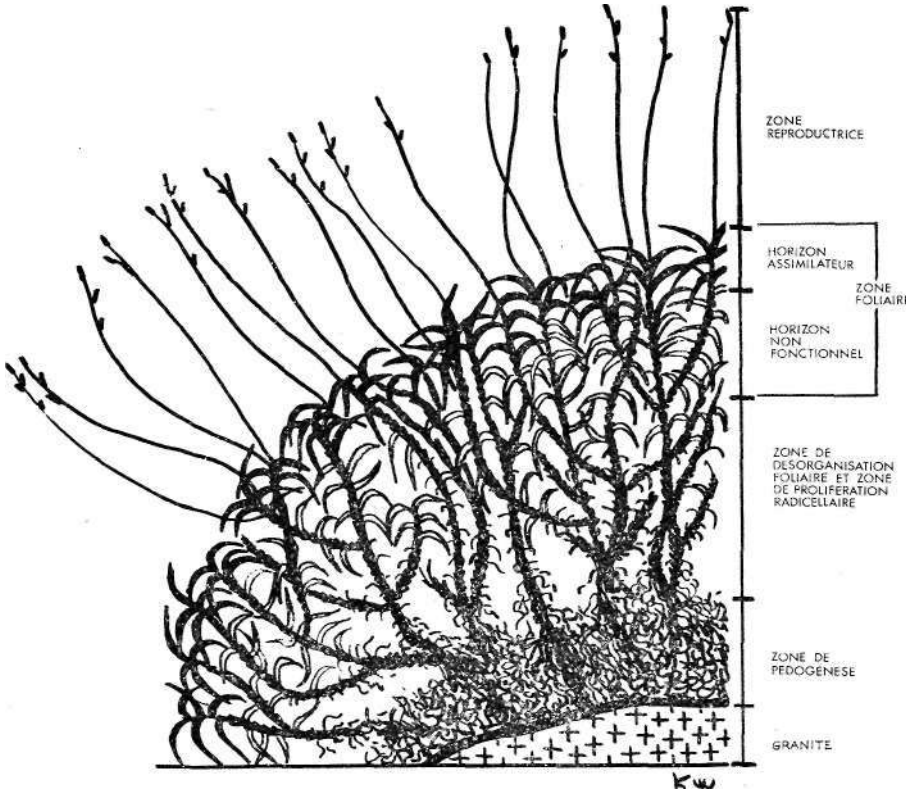


Fig. 16. — Dessin semi-schématique à travers une motte *d'Afrotrilepis jaegeri* J. Raynal (Cypéracées), orophyte saxicole, endémique des monte Loma-Tingi. Remarquer la spécialisation fonctionnelle des diverses zones disposées concentriquement (K. WATRÉ dess.; Herbier P. JAEGER).

espèces à aire disjointe que l'on trouve simultanément au Loma et, à plusieurs milliers de km de là, dans les massifs E ou SE africains.

Au Loma la forêt montagnarde à *Parinari excelsa* et, plus particulièrement les galeries forestières qui s'en détachent pour s'élancer en direction des sommets, ne recèlent

pas une seule espèce qui leur appartienne en propre; il n'en est pas de même de la prairie ni des milieux rocheux ou marécageux qui en dépendent où nous avons inventorié *neuf* espèces endémiques; ce sont:

Afrotrilepis jaegeri
Digitaria phaeotricha var. *patens*
Dissotis sessilis
Gladiolus leonensis
Ledermanniella jaegeri
Loudetia jaegeriana
Loxodera strigosa
Schizachyrium minutum (— S. brevifolium)
Scleria montícola

Ce chiffre, qui ne représente que 3,6% de l'ensemble des espèces inventoriées en prairie montagnarde (enclaves comprises) doit être considéré comme provisoire ; il est susceptible d'être modifié, dans un sens ou dans l'autre, au hasard des prospections; en effet, en raison de leur exiguïté certaines espèces risquent de passer inaperçues.

Le *Dissotis sessilis* mis à part, toutes les endémiques du Loma sont des herbacées; ce sont en majorité (7 sur 9) des Monocotylédones et près de la moitié sont des Graminées ; notons l'absence de Fougères de ce cortège qui, quoique peu important, permet cependant de souligner l'originalité floristique du Loma et de l'opposer à celle des autres massifs de la dorsale. Les endémiques du Loma se distinguent aussi entre elles par une hétérogénéité manifeste sur le plan écologique. Si le *Digitaria phaeotricha* var. *patens,* le *Loxodera strigosa* et le *Scleria monticola* sont des prairiales typiques, la vocation saxicole de l'*Afrotrilepis jaegeri* ne saurait être mise en doute. Le *Gladiolus leonensis* a été observé sur la tourbe édifiée par *Afrotrilepis pilosa* et les affinités du *Schizachyrium minutum* pour les sols marécageux et tourbeux semblent manifestes au même titre que celles du *Ledermanniella jaegeri* pour les eaux agitées et oxygénées des cascades et cascadelles. Le *Dissotis sessilis,* par contre, jalonne les ruisselets dévalant les pentes herbeuses du Pic Bintumane et le *Loudetia jaegeriana* s'installe dans les mottes moussues accrochées **aux parois** rocheuses **des dômes granitiques.**

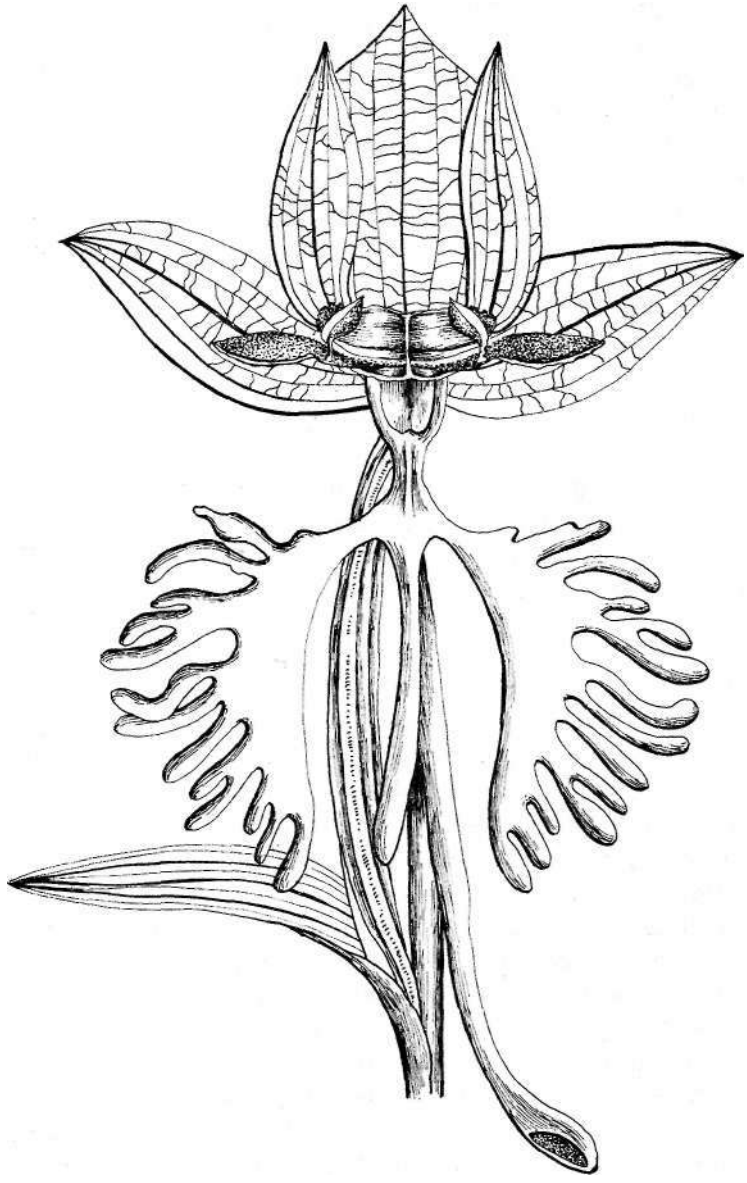


Fig. 17.—*Habenaria jaegeri* Summerh. (Orchidacées). Fleur vue de face; remarquer le labeile trilobé, les lobes latéraux sont profondément divisés; l'éperon (4-6 cm) est entamé à son extrémité inférieure. La plante, assez abondante en prairie d'altitude, fleurit en saison pluvieuse (K. WATRÉ dess.; Herbier P. JAEGER).

C. FAVARGER (39), à juste titre, met les chercheurs en garde de se prononcer sur l'âge et l'origine des endémiques sans les avoir, au préalable, soumises à l'examen caryologique. Dans notre cas l'endémisme, exclusivement de rang spécifique ou tout au plus infraspécifique, ainsi que la limitation de ces taxons au seul massif du Loma-Tingi, plaident en faveur d'une origine récente.

Peu nombreux sont les orophytes, endémiques ouest africains dont l'aire s'étend du Fouta-Djalon aux massifs camerounais et, parfois même, au-delà (Gabon) ; en raison du cachet étrange que ses touradons impriment au paysage, citons *Yl'Afrotrilepis pilosa,* espèce saxicole et héliophile (108).

Les affinités entre le Loma et le Fouta-Djalon sont soulignées par des orophytes pratiquement limités à ces deux massifs comme: *Dissotis leonensis,* élément caractéristique du bush montagnard relictuel des sommets granitiques; *Nerophila gentianoides,* Mélastomatacée à fleurs jaunes, couleur inconnue chez les autres représentants africains de cette famille; *Leocus pobeguinii,* sous-abrisseau remarquable par ses fleurs voyantes, entomophiles, d'un bleu «Aconit.», *Utricuïaria tetraloba* ancré au fond rocheux des cascades.

Des orophytes endémiques ouest-africains comme: *DoIichos nimbaensis, Kotschya lutea, Nemum bulbostylidoides, Veronia nimbaensis, Xyris festucifolia...* sont communs aux prairies d'altitude du Loma et du Nimba.

Pour d'autres comme *Andropogon mannii, Pennisetum monostigma, Coreopsis camporum, Lobelia kamerunensis, Mesanthemum jaegeri...* le Loma correspond à une station isolée à l'ouest des hauteurs du Nigeria et du Cameroun; il constitue, par le fait même, le point extrême W actuellement connu, de l'aire de répartition de ces espèces.

La prairie d'altitude du Loma, comme celles d'autres sommets ouest-africains (Nimba, FON…), héberge bon nombre d'espèces, généralement herbacées et vivaces, que l'on retrouve dans les massifs E et SE africains distants des premiers de plusieurs milliers de kilomètres; citons: *Cynanchurn praecox, Disa welwitschii, Drimia zombensis, Drosera pilosa, Euphorbia depauperata, Gynura miniata, Helichrysum nudifolium* var. *leiopodium, H. mechovianum,*

Hypoxis angustifolia, Leocus lyratus, Melanthera abyssinica, Pycreus atrorubidus, Sopubia mannii var. *tenuifolia, Trichopteryx elegantula...* Un cas comparable se retrouve dans le domaine de la faune orophile: le *Nectophrynoides occidentalis* Angel découvert par M. LAMOTTE sur les crêtes du Nimba, se rattache à des formes vivant sur les sommets est-africains (monts Usambara et Uluguru). Ce Batracien vivipare n'a pas été observé au Loma.

La présence des mêmes espèces — exceptionnellement il s'agit de taxons infra-speécifiques— en des points géographiquement aussi éloignés pose au biogéographe un problème ardu. L'hypothèse d'un transport à grande distance par l'intermédiaire du vent ou des oiseaux, n'a pas été retenue par la plupart des auteurs. Par contre, pour expliquer la disjonction spatiale de ces orophytes beaucoup de chercheurs dont A. AUBRÉVILLE, A. CHEVALIER, J. L. GUILLAUMET, J. LEBRUN, J. K. MORTON, R. SCHNELL... ont fait appel aux variations climatiques survenues au cours des époques révolues.

Les vicissitudes climatiques du Pleistocene, loin de rester limitées aux seules régions septentrionales, se ont répercutées sur la presque totalité du globe. Aux glaciations boréales auraient correspondu, sous les tropiques, des périodes pluviales marquées par une chute thermique et un accroissement des précipitations, phénomènes qui auraient entrainé un abaissement des étages de végétation et, partant, un rétrécissement des discontinuités spatiales, ce qui ne pouvait que faciliter les migrations. Ainsi, des reliefs modestes, véritables relais entre les massifs Est et Ouest africains, devenaient-ils susceptibles de donner asile aux orophytes. «De très hautes altitudes ne sont donc pas nécessaires dans les régions tropicales pour qu'il existe une flore montagnard» (26).

En considérant les orophytes prairiaux à aire disjointe on est surpris par le fait que les mêmes plantes se rencontrent identiques, à l'échelon spécifique, sur divers massifs de la dorsale Loma-Man et sur certains autres de l'Est, du Centre et du S.E. africain c'est-à-dire en des points séparés par un intervalle de plusieurs milliers de kilomètres. En

raison de leur isolement géographique sur les sommets occidentaux, on aurait pu s'attendre à une diversification au moins infraspécifique.

Aussi le cas de l'*Hàbenaria jaegeri* Summ, déjà signalé par R. SCHNELL en 1961, est-il particulièrement suggestif à cet égard. Il s'agit d'une Orchidée des prairies montagnardes du Loma et du Fon (121) ; elle est très proche de *VH. splendens* Rendle du Kilimandjaro, de *YH. praestans* Rendle du Ruwenzori et du Mozambique et de *YH. macrantha* Höchst. d'Abyssinie. Ces diverses espèces, très proches les unes des autres, sont caractérisées, par le même labelle lacinié-pectiné et ne diffèrent entre elles que par des variations mineures. Elles «appartiennent manifestement à un même phylum qui s'est répandu à une époque ancienne (peut-être au Tertiaire) sur les divers sommets africains» où l'isolement géographique a été particulièrement favorable aux mutations différenciatrices. La présence de la même espèce au Loma et au Fon parle en faveur d'un échange récent qui, selon cet auteur, a dû être consécutif aux variations climatiques du Quaternaire. L'absence de cette espèce au Nimba ne fait que confirmer l'individualité floristique et phytogéographique des divers massifs de la dorsale guinéenne.

Frappé par la complexité du peuplement végétal des montagnes de l'Afrique tropicale A. CHEVALIER (26) pense que «les orophiles de l'Afrique occidentale existent... aux points où nous les observons depuis un lointain passé». Et J. LEBRUN (80) d'affirmer que «le noyau de la flore orophile africaine porte... un cachet de grande ancienneté». D'après R. SCHNELL (124) la flore orophile prairiale «serait à considérer comme un témoin d'une époque ancienne plus sèche, conservé jusqu'à nos jours grâce à des conditions édaphiques favorables».

B) Les planitiaires

Diverses espèces, prairiales ou forestières, apparemment inféodées à l'étage culminai du Loma, se retrouvent à basse altitude en pays de piedmont, parfois même, en milieu côtier. Ainsi, au pied du Nimba libérien, dans une savane à *Rhy-*

tachne sp. vers 500 m, nous avons récolté *Pkyllanthus alpestris* et *Striga aequinoctialis.* Le *Pkyllanthus odontadenius* s. L, espèce à affinités montagnardes, se remarque à basse altitude à proximité des villages et le long des pistes. Des espèces qui forment de véritables peuplements au Plateau comme *Eupatorium africanus,* se retrouvent en savane de piedmont, d'autres comme *Gladiolus unguiculatus* sont largement répandues en Afrique tropicale.

De même, des plantes comme *Psorospermum alternifolium, Scutellaria paucifolia* et *Leocus lyratus* fleurissent et fructifient tout aussi bien en prairie montagnarde qu'en savane de piedmont.

Certains ligneux apparemment liés au milieu montagnard, prolifèrent tout aussi bien à basse altitude; le cas le plus spectaculaire est celui du *Parinari excelsa.* Cette Chrysobalanacée qui constitue des peuplements presque purs vers l'extrémité supérieure des galeries forestières où elle fleurit et fructifie abondamment, se retrouve en plaine, en milieu côtier (Casamance). Cette bipolarité a été particulièrement mise en relief par des auteurs comme A. AUBRÉVILLE (11), J. MIÈGE (95), et R. SCHNELL (124). Une étude cytotaxonomique des deux types, montagnard et côtier, de cette espèce, serait éminemment souhaitable.

Dans les vastes étendues herbeuses de la zone culminale du Loma on remarque aussi, quoique disséminées, des espèces endémiques de la savane ouest-africaine, comme: *Polygala cristata* et *P. baikiei.* Signalons aussi le cas du *P. lecardii,* plante commune dans tout l'ouest africain qui, en plaine et en montagne, a trouvé refuge dans les stations marécageuses.

Attirons enfin l'attention sur tout un cortège de plantes herbacées, essentiellement graminéennes, dont la pénétration en montagne est, incontestablement, l'oeuvre des feux:

Andropogon gay anus, A. schirensis, Hyparrhenia diplandra, H. rufa, Loudetia kagerensis, Panicum praealtum... et il semble qu'il en soit de même des Cypéracées de la prairie altimontane dont la moitié environ (17 sur 31) couvrent des aires très vastes dans les savanes de l'Afrique tropicale et australe:

Ascolepis protea, Bulbostylis oritrephes, Cyperus angolensis, Fuirena stricta, Fimbristylis schweinfurthiana...

CONCLUSION

La Prairie d'altitude du Loma, comme celle du Nimba, peut être considérée comme une enclave herbacée établie au sein de l'étage submontagnard forestier. Elle correspond à une formation secondaire, paraclimacique, qui doit son existence et sa pérennité, non pas au climat, mais au déferlement périodique de la vague ignée.

Grâce au feu, la prairie se serait substituée à une forêt basse, à un bush montagnard où le *Dissotis leonensis* dominant, s'associait à tout un cortège de buissons et de petits abres. Ce fourré, une originalité du Loma, était troué de «clairières édaphiques», espaces non boisés, où des orophytes non forestiers se seraient maintenus depuis une époque déjà fort ancienne.

Cette disposition, dans son ensemble, correspond à celle que R. SCHNELL (119) a observée au Nimba où, cependant, la Mélastomatacée n'a pas été observée.

D'une extrême sensibilité aux feux, ce maquis a succombé aux assauts répétés de la vague ignée ; éliminé ainsi de vastes surfaces désormais occupées par la prairie montagnarde, cette formation n'a subsisté, à l'état relictuel, que sur les hauts sommets granitiques où les seuils rocheux lui assurent un certain degré de protection.

Ces hauts sommets rocheux comparables à autant d'îles émergeant au-dessus l'océan forestier ont dû jouer, au même titre que les «clairières édaphiques», le rôle de bastions de refuge pour espèces relictuelles, mais aussi de creusets où, à la suite de mutations ou d'hybridations, de nouveaux taxons ont vu le jour. Ils ont contribué à faire du Loma un centre, il est vrai secondaire, de repli et de diversification, voire un réservoir d'où, à certaines périodes, se seraient écoulés, vers la prairie voisine, des taxons jusque là maintenus à l'abri des seuils rocheux.

A l'heure actuelle l'intensification des feux, encore attisés sur le versant Est par le souffle de l'harmattan, accélère la

savanisation de l'étage culminai en favorisant l'irruption en altitude de tout une cortège herbacé, surtout graminéen, originaire des savanes de piedmont. Ces plantes, envahissantes et fortement compétitives risquent de mettre en péril la flore orophile déjà ancienne et d'effacer ainsi les derniers vestiges d'une documentation susceptible de nous renseigner sur l'histoire du peuplement végétal de ce massif.

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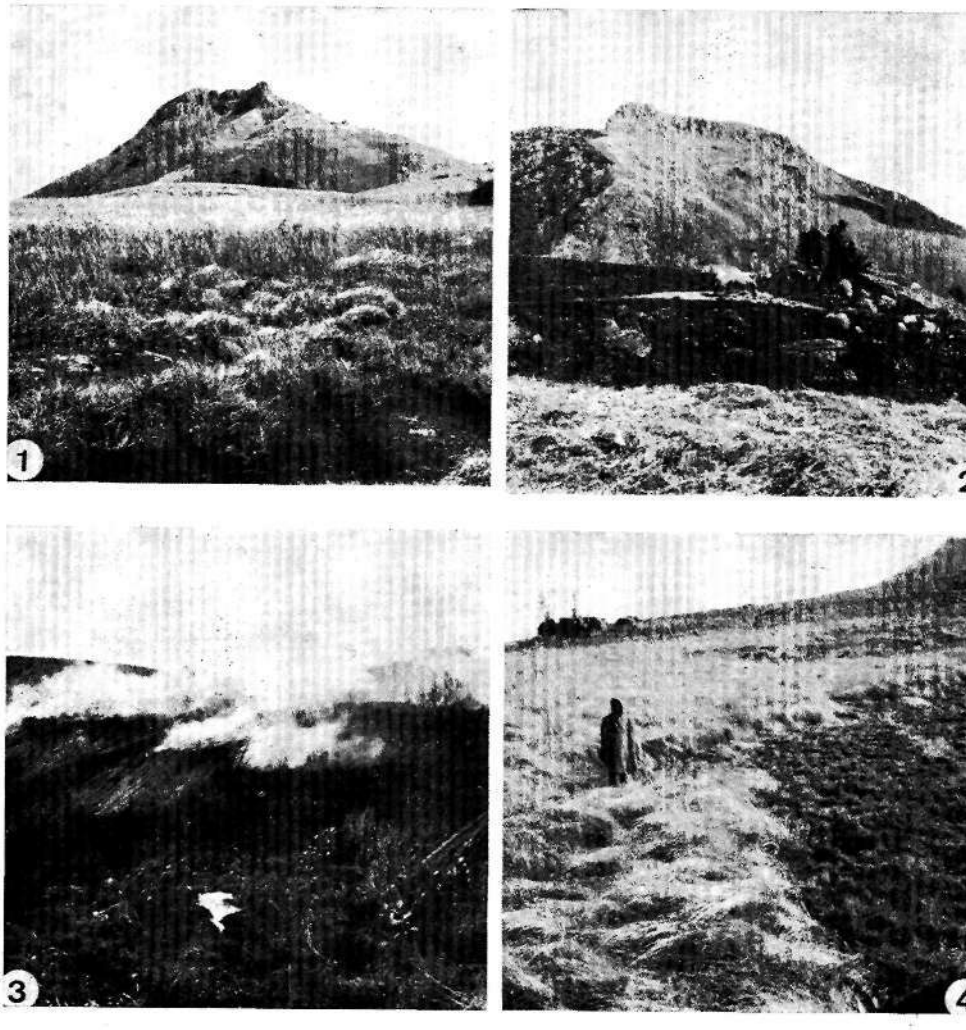
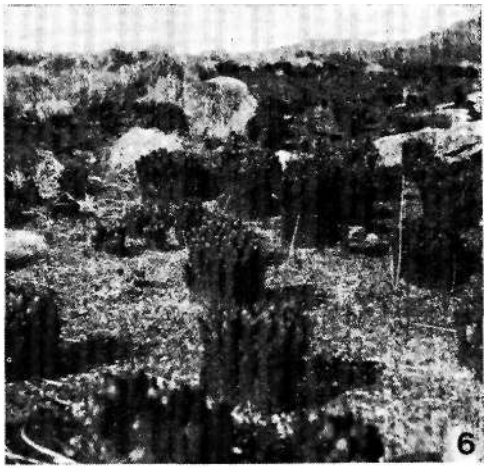
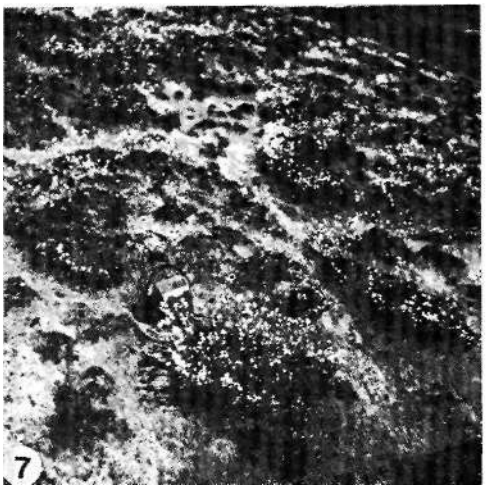
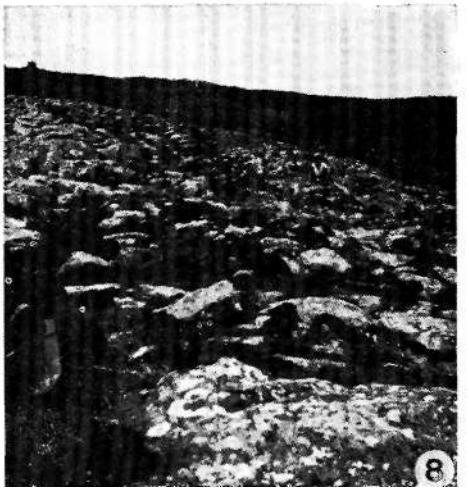


1. — Prairie d'altitude du rebord NW du Plateau, des pentes sud à ouest du Pic Bintumane, avant le passage du feu (4 déc. 65).
2. — Prairie d'altitude du rebord E du Plateau et de la pente Sud du Pic Bentumane, partiellement incendiées (6 déc. 65).
3. — Feu de brousse en prairie d'altitude (secteur Miramira) vers 1600 m (6 déc. 65).
4. — Prairie d'altitude près du rebord NW du Plateau; faciès à *Loudetia kagerensis* (K. Schum.) C. E. Hubb. ex Hutch, à g.: parcelle non incendiée; à dr.: parcelle incendiée et verdoyante (30 mars 65).





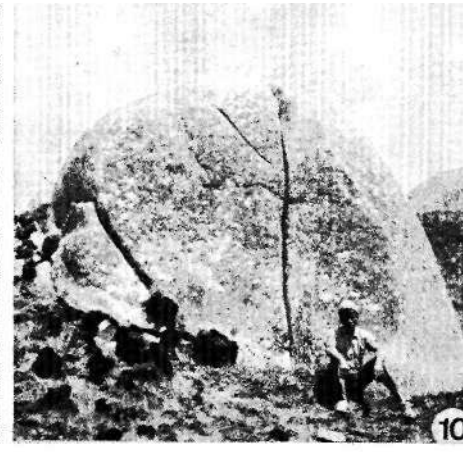


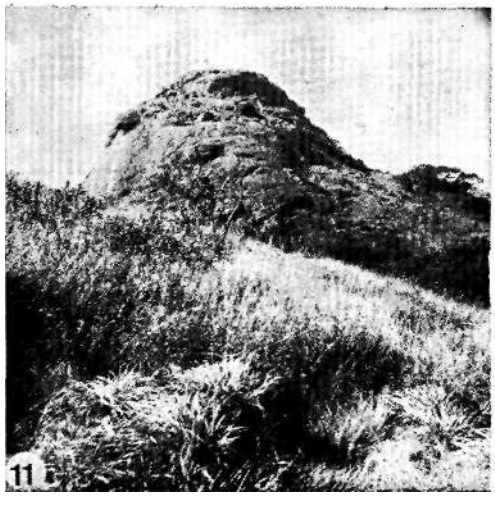
5. — *Afrotrilepis pilosa* J. Raynal (Cypéracées) en saison pluvieuse (24 sept. 64) avant le passage du feu: les touradons portent une épaisse touffe de longues feuilles retombantes; sur le sol tourbeux édifié par la Cypéracée on remarque quelques individus fleuris de *Mesanthemum prescottianum.* Corniche granitique versant W Loma vers 580 m.

6.—*Afrotrilepis pilosa* J. Raynal (Cypéracées) en saison sèche (23 déc. 65) après le passage du feu. Remarquer les touradons noirs, coralloïdas, débarrassés de la touffe foliaire. Corniche granitique du versant W du Loma vers 580 m (23 déc. 65).

7. — Touffes de *Cyperus nduru* Cherm. (Cypéracées) en fleurs après le passage du feu; versant sud du Pie Bintumane et Plateau attenant vers 1650 m, 3 février 52).

8.—Vue partielle du plateau sommital en forme d'auge du Pic Bintumane; affleurement de dalles doléritiques avec touradons noircis *d'Afrotrilepis pilosa* J. Raynal (Cypéracées); au fond à gauche point géodésique correspondant au sommet des monts Loma (26 février 66).



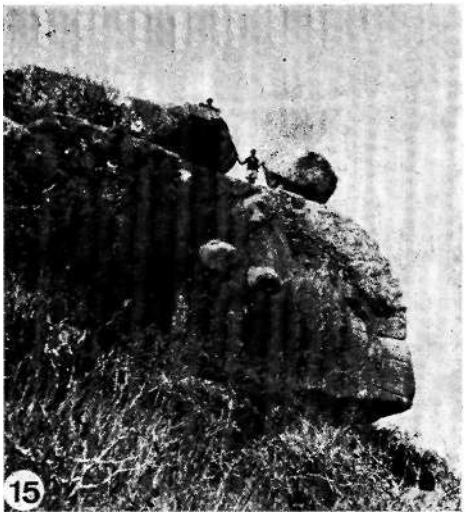


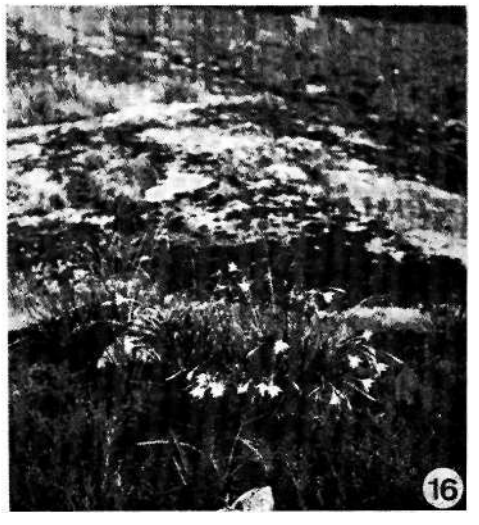
9- — Bastion rocheux à la limite des falaises à exp. Sud et Ouest du Pic Bintumane. Au 1er plan: fragment de prairie incendiée; le feu est bloqué dans sa progression par la parci rocheuse; cependant, par l'intermédiaire de couloirs herbeux il parvient au plateau sommital.

10. — Bloc granitique fracturé avec, au pied, des touradons calcinés *d'Afrotrilepis pilosa* J. Ra;ynal (Cypéracées). Prairie d'altitude du Kundu-Konko vers 1650 m (4 mars 66).

11. — Calotte granitique du Serelen-Konko (versant N); au premier plan: la prairie d'altitude non encore incendiée (20 déc. 65).

12. — *Dissotis leonensis* Hutch, et DaIz (Mélastomatacées) chargé d'Usnées; crête sommitale du Puen-Kcli vers 1400m (28 sept. 64).





13. — Individu isolé de *Eugenia pobeguinii* Aubrév. (Myrtacées) à la limite supérieure d'un lambeau de forêt montagnarde versant E du Pic Bintumane vers 1900 m. Remarquer l'état souffreteux de l'arbre, son port tortueux, la cime partiellement desséchée et chargée d'epiphytes (Usnées).

14. — Bush montagnard à *Eugenia pobeguini* Aubrév. (Myrtacées); buissons inclinés par l'action du vent. Sommet du Serelon-Konko vers 1480 m (20 déc. 65).

15. — Rocher granitique de la crête du Da-Oulen (vers 1470 m). Remarquer dans l'axe du personnage, accolées sur la paroi verticale à exposition sud, trois mottes *d'Afrotrilepis jaegeri* J. Raynal (12 déc. 65).

16. — Touffe de *Gladiolus leonensis* W. Marais (Iridacées) sur tourbe à *Afrotrilepis pilosa* en bordure d'une dalle granitique affleurante en prairie d'altitude du Plateau vers 1600 m (16 avril 66).

17.—Di*ssotis sessilis* Hutch, ex. Brenan et Keay (Mélastomataceae), endémique du Loma. Plateau sommital du Pic Bintumane, dans un amoncellement de blocs doléritiques près du déversoir du Sonfon (juin 64).

18. — Peuplement de *Mesanthemum jaegeri* Jac-Pélix (Eriocaulacées), en fleurs dans le maquis à *Dissotis leonensis* au sommet du dôme granitique du Serelen-Konko vers 1480 m; orophyte ouest-africain (27 sept. 64).

19.—*Habenaria jaegeri* Summersh. (Orchidacées), en fleurs; saison pluvieuse. Prairie d'altitude du Plateau vers 1660 m (31 juillet 64).

Bol. Soc. Brot., Sér. 2, 53 (2): 1397-1405 (1981).

NOVA *AGAVE* SUBESPONTÂNEA EM PORTUGAL

por

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SUMMARY

The A. found on waste calcareous places, mainly near the sea, in C. and S. Portugal, many specimens of *Agave atrovirens* Karwinski ex Salm-Dyck, which grow eubspontaneously. They are well distinguishable from the common *A. americana* L. by the broad dark green leaves, uncinate-conduplicate at apex and prolonged into a very stout spine 4-10 cm long, and by the short panicle three to five times shorter than the stout peduncle, flowers with syle twice as long as stamens and capsule oblong but contracted above into a short neck.

Em Fevereiro de 1975 notámos, em sebes nos arredores de Portimão (Algarve), que umas piteiras *(Agave* spp.) tinham as folhas verde-escuras, bastante grossas e largas, ainda que uncinado-conduplicado-sulcadas no ápice que terminava em longo espinho negro, pelo que se distinguiam sem qualquer dúvida da vulgar *Agave americana* L., também existente no sítio. Esses exemplares estavam todos reduzidos às rosetas foliares, excepto um à borda duma senda ou caminho vicinal com um escapo encimado por uma curta inflorescencia paniculada, tudo já marcescente e, consequentemente, sem elementos concretos de identificação. Porém, esta inflorescencia também chamou a nossa atenção pelo facto de o pedúnculo ser muito mais comprido (pelo menos, o

* Comunicação apresentada ao «Simposio Conmemorativo del II Centenario del Nacimiento de Lagasca», Sevilha, Out. 1976.

triplo) do que a parte paniculada, esta com um contorno piramidad pouco largo.

Ao regressar a Lisboa, procurámos identificar a espécie, de certo distinta da *A. americana* L., mas a parcimónia dos elementos colhidos não nos permitiu ir longe. Em todo o caso, pareceu-nos ser ou estar próxima da *A. cochlearis* Jacobi, que mais tarde viemos a verificar não passar dum sinónimo da *A. atrovirens* Karwinski ex Salm-Dyck.

Observando com mais pormenor as piteiras que se encontram nas sebes e sítios áridos, sobretudo junto do mar, quer em Lisboa quer em toda a região que desta vai até Cascais, fomos verificando que estas piteiras de folhas verde--escuras e uncinado-conduplicadas no ápice eram muito mais frequentes do que a princípio se poderia supor. Até na própria Tapada da Ajuda, em que está situado o Instituto Superior de Agronomia em Lisboa, vários indivíduos foram detectados na encosta calcária acima do lago pequeno a Nascente do Viveiro da Silvicultura. Entre estes, também havia alguns com inflorescencias marcescentes e acinzentadas, cujas panículas eram cerca de 1/4 do pedúnculo. Pelos elementos agora disponíveis, ainda que poucos, fomos avolumando a convicção de poder tratar-se de *A. atrovirens* Karwiski ex Salm-Dyck.

A frequência com que estas plantas foram sendo encontradas, coadjuvada pela cor verde-escura das folhas grandes, fez-nos levantar a estranheza de que nunca nenhum botânico se lhes tivesse referido anteriormente. Esta falta supomos, no entanto, poder relevar-se pela inadequada observação minuciosa das piteiras, tidas aprioristicamente como pertencendo a *A. americana* L. ou pela simples suposição que não passariam duma forma mais verde desta última. Por outro lado, como estas espécies monocárpicas só florescem com longos intervalos de anos, também aqui certamente o motivo da sua não identificação há mais tempo.

Em Janeiro de 1976, muitas piteiras verdes da região litoral entre Santo Amaro de Oeiras e Cascais entraram em profusa floração, durando esta até Maio conforme os sítios. Não há dúvida que este ano seco de 1976 foi um ano excepcional para a abundância de floração nas piteiras, o

que também sucedeu, ainda que mais tarde (Junho e Agosto), com a *A. americana* L.

Na presença de plantas completamente floridas e frutificadas, foi-nos possível recolher todos os elementos para descrição e identificação. Consultados A. BEUGER, *Die Agaven* (1915) e A. J. BREITUNG, *The Agaves* (1968), confirmámos a nossa suposição, a planta em estudo era de facto a *Agave atrovirens* Karwinski ex Salm-Dyck, e estávamos assim em presença de mais uma espécie subespontânea na região litoral calcária do Centro e Sul de Portugal. Percorridas outras regiões mais interiores do Centro de Portugal, verificámos que as piteiras nas sebes eram, de facto, só pertencentes a *A. americana* L., facilmente reconhecível pelas suas folhas glaucas rectas e panícula estreitamente oblongo-ovoide tão comprida ou um pouco mais do que o pedúnculo, além de ser planta de floração obviamente mais serôdia (Junho a Agosto, em vez de Janeiro a Maio).

A espécie *A. atrovirens* Karwinski ex Salm-Dyck é originária das regiões secas do Sul do México, onde é conhecida por «pulque». A sua boa adaptação em Portugal, com muitos indivíduos de certo já nada novos ou provenientes de propagação de outros anteriores, faz-nos supor que se trata de planta introduzida por volta dos fins do século XIX. Como certamente esta espécie não foi introduzida na Europa restritamente em Portugal em condições de ar livre, parece-nos muito provável a sua existência também em diversos pontos da região litoral do Mediterrâneo, pelo menos ocidental, isto é, da Espanha, França e Itália, incluindo provavelmente as ilhas das Baleares, Córsega, Sardenha e Sicília, onde deve proceder-se à sua busca.

Em qualquer das duas espécies que estudámos, bem como parece também suceder nas demais do género, as flores são distintamente protândricas, verificando-se um acentuado alongamento do estilete após a deiscência das anteras da própria flor.

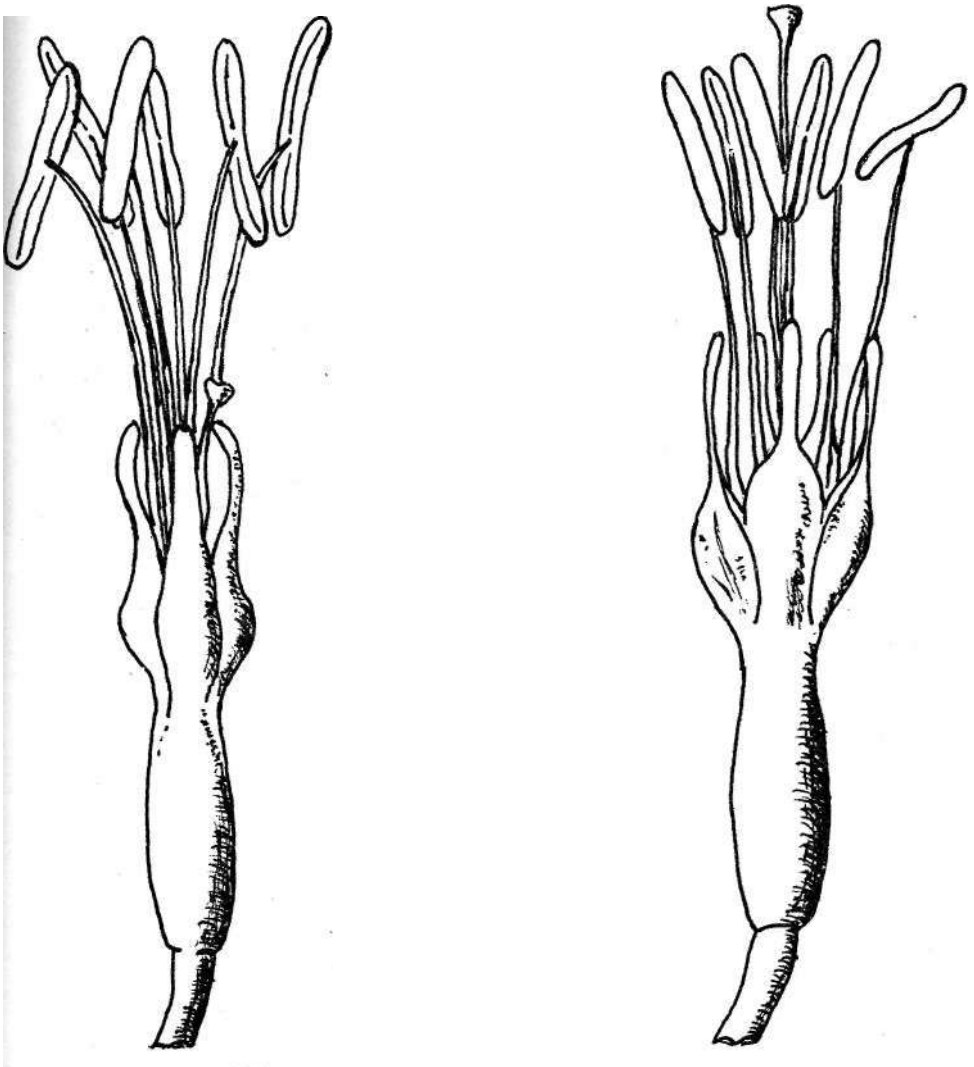
Para facilitar o reconhecimento e identificação das piteiras que podem encontrar-se subespontâneas em Portugal, apresentamos as seguintes chaves e descrições:

Folhas glaucas, lanceolado-oblongas, rectas e terminadas em espinho de 2-3 cm; pedúnculo igual ou menor do que a panícula oblongo-ovoide; estilete subigualando por fim os estâmes; cápsula oblongo--obovoide, obtusa . . . . . *1. A.* americana

Folhas verde-escuras, oblanceoladas ou lanceolado-acuminadas, uncinado-conduplicadas no ápice e terminadas em espinho de 4-10 cm; pedúnculo 3 a 5 X tão comprido como a curta panícula piramidal; estilete por fim exserto até ao dobro dos estâmes; cápsula oblonga, subcostada e contraída no cimo num colo . . . . . 2. A. atrovirens

1 A, americana L., *Sp. Pl.* 323 (1753).

Hemicriptófito arrosetado monocárpico muito robusto, rizomatoso-estolhoso propagando-se vegetativamente por numerosos rebentos hipogeos; caule grosso, curto, carnudo mas por fim lenhoso. Folhas com 100-200 X 15-25 cm, carnudas, glaucas, lanceolado-oblongas, rectas, subplanas apenas subconduplicadas apicalmente, um tanto contraídas junto à base dilatada, espessa e invaginante, remotamente espinhoso--dentadas com dentes de 5-10 mm; ápice terminado em espinho com 2-3 cm, anegrado, vulnerante, robusto, roliço-cónico ainda que sulcado na ½ basal na face superior. Escapo (incluindo a inflorescencia) com 4-7(-10) m, erecto; pedúnculo com 8-15(-20) cm de diâmetro na base, glauco em novo, tornando-se laranja-amarelado ou avermelhado na maturação, depois marcescente e acastanhado, por fim lenhoso, do mesmo comprimento ou menor do que a panícula, revestido de numerosas brácteas estéreis foliáceas, afastadas e não imbricadas, erecto-patentes ou por fim retroflectidas, triangular-assoveladas, da mesma cor do pedúnculo, inteiras e terminadas em mucrão grosso até 1.5 cm, as proximais até 50 cm. Panícula oblongo-ovoide, frouxa, com 20-25 ramos patentes, delgados, por vezes parcialmente bulbilíferos. Flores (incluindo o ovário) com c. 7 cm e assentes em pedicelos com 10-15mm, cilíndricos, glaucos e robustos; tubo do hipante com c. 35 mm, verde; perianto de segmentos com 30-35 mm, oblongo-lineares, obtusos, erectos, amarelo-esverdeados, um pouco convexos no $1/_3$ proximal; estâmes muito exsertos, com os filetes de 70-80 mm, inseridos no cimo do tubo do hipante, levemente divergentes distalmente, glabros, amarelo-esver-



Plores de: 1 — *Agave americana* L. (SINTRA: Rio de Mouro, numa sebe a Norte da E. N. 249, pouco além do ramal para as Mercês). 2— *A. atrovirens* Karwinski ex Salm-Dyck (CASCAIS: Estoril, plataforma calcária litoral entre a Pedra do Sal e o Forte de Santo António, em S. Pedro do Estoril) (X 1).

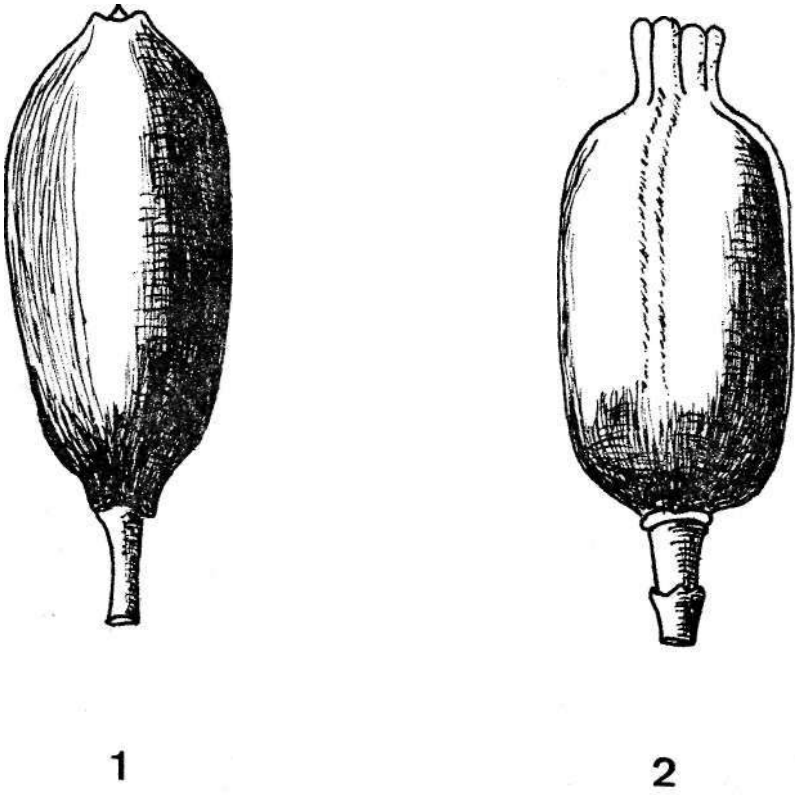
deados e brilhantes; anteras com 30-35 mm, dorsifixas, ± introrsas, deiscentes por 2 fendas longitudinais; estilete de princípio muito mais curto do que os estâmes, após a ântese igualando-os; estigma capitado, levemente emarginado. Cápsula loculicida com 45-65 X 20-30 mm, oblongo-obovoide, obtusamente trigonal, lisa, verde em imatura tornando-se castanho-anegrada na maturação, assente num pedúnculo curto subcilíndrico.

Subespontânea com muita frequência nas sebes, valados e sítios pedregosos áridos do Centro e Sul de Portugal; originária do Sul do México.

Com certa frequência encontram-se disseminados indivíduos com as folhas marginadas de amarelo (cv. *'Marginata'),* mesmo em sítios onde a espécie não está declaradamente cultivada.

2. A. atrovirens Karwinski ex Salm-Dyck, *Hort. Dyck.* 7 *(nom. nud.),* 302 *(cum descript.)* (1834).

Hemicriptófito arrosetado monocárpico muito robusto, rizomatoso-estolhoso propagando-se vegetativamente por alguns rebentos hipogeos; caule grosso, curto, carnudo mas por fim lenhoso. Folhas com 100-250 X 20-40 cm, carnudas, verde-escuras, lanceolado-acuminadas ou por vezes obovado--lanceoladas em indivíduos novos, uncinado-sulcado-conduplicadas na região apical, um tanto contraídas junto à base dilatada, espessa e invaginante, remotamente espinhoso--dentadas com dentes de 5-10 mm; ápice terminado em espinho de 4-10 cm, castanho-anegrado, rígido e muito vulnerante, robusto, subroliço-cónico ainda que lateralmente um tanto comprimido, na face superior estreitamente sulcado no 1/3 basal. Escapo (incluindo a panícula) com 7-10 m, erecto; pedúnculo com 15-22 cm de diâmetro na parte basal, verde tornando-se purpurascente e por fim acinzentado e lenhoso, três a cinco vezes o comprimento da panícula, revestido de numerosas brácteas estéreis foliáceas, imbricadas e ± aplicadas (por fim retroflectidas), triangular-agudas, da mesma cor do pedúnculo, inteiras e terminadas em mucrão negro com 10-25 mm, as proximais até 75 cm. Panícula piramidal,



Cápsulas de: 1 — *Agave americana* L. (CASCAIS: Estoril, São João do Estoril, lado ocidental da cerca do Forte de Santo António). 2 — *A. atrovirens* Karwinski ex Salm-Dyck (CASCAIS: Estoril, plataforma calcária litoral entre a Pedra do Sal e o Forte de Santo António, em S. Pedro do Estoril) (X I)-

curta, frouxa, com 15-20 ramos patentes, grossos, por vezes parcialmente bulbilíferos. Flores (incluindo o ovário) com 8-9 cm e assentes em pedicelos com 15-20 mm, aclavados, verdes e robustos; tubo do hipanto com 25-35 mm, verde; perianto de segmentos com 25-35 mm, oblanceolados mas contraídos acima num apêndice com c. 20 mm, linear-oblongo, erectos, verde-amarelados, convexos na metade dilatada; estames muito exsertos, com os filetes de 55-65 mm, inseridos no cimo do tubo do hipanto, levemente divergentes distai-

mente, glabros, amarelo-esverdeados e brilhantes; anteras com 20-25 mm, dorsif ixas, ± introrsas, deiscentes por 2 fendas longitudinais; estilete de principio mais curto do que os estames, após a ântese muito exserto e excedendo os estâmes em cerca do dobro. Cápsula loculicida com 60-65 X 25-30 mm, oblonga, obtusamente trigonal, subcostada nos ângulos, contraída no cimo num colo de 10-12 X 10-12 mm, verde em imatura e castanha na maturação, assente num pedúnculo curto cilindro-aclavado.

Subespontânea em sebes e sítios rochosos calcários secos e áridos não longe do litoral, no Centro e Sul de Portugal.

Exemplares estudados:

1) Estremadura — LISBOA: Tapada da Ajuda, fendas das rochas calcárias na vertente sobre o pequeno lago a Este do Viveiro da Silvicultura, OEIRAS: sebes a Norte da estrada entre Sassoeiros e S. Domingos de Rana, à Quinta da Costa, terreno calcário pedregoso; cabeço descampado seco junto ao Forte do Arieiro, a SW da Praia de Santo Amaro de Oeiras; talude calcário árido entre a estrada marginal Lisboa--Cascais e o Forte de São Julião da Barra, por cima da Praia de Carcavelos, CASCAIS: Parede, cimo das arribas calcárias litorais (entre a praia da Parede ou da Agua Doce e a ponta da Vigia); Estoril, plataforma calcária árida litoral (Baforeira; Pedra do Sal; Tranibeque; entre este e o Forte de Santo António; S. João do Estoril, em volta do Forte da Cadaveira, a Este da Praia da Poça, e a Oeste desta junto do Forte de S. Pedro); Cascais, plataforma calcária árida litoral (entre Oitavos e o Cabo Raso), MAFRA: Ericeira, cimo das arribas calcárias litorais, a Norte da Praia da Baleia.

2) Algarve — PORTIMÃO: sebe em terreno calcário junto ao ramal novo da estrada de acesso a Oeste da cidade e a Sul da ponte sobre a linha férrea; sebe em terreno calcário plano a Sul do caminho velho que segue para o Vau.

Ainda que muito menos frequente que o tipo, também aparecem uns exemplares com folhas marginadas de amarelo (cv. *Marginata'),* como os que observámos na plataforma calcária a Este da Enseada da Baforeira, em frente da Quinta da Condessa d'Edla, na Parede (Cascais).

Agave americana L.
Estoril: Praia da Poça, lado W.

Agave atrovirens Karwinski ex Salm-Dyck.
S. Pedro do Estoril.

Bol. Soc. Brot, Sér. 2, 53 (2): 1407-1436 (1981).

SOBRE AS RODOFÍCEAS DA RIA DE AVEIRO

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INTRODUÇÃO

O estudo das Rodofíceas da costa de Portugal mereceu a atenção dos naturalistas portugueses e estrangeiros desde os princípios do século XIX. Na sua Flora Lusitanica, BROTERO (1804) enumerou as seguintes espécies: *Conferva corallina* L. (= *Ceramium rubrum* Ag.), no Tejo, pág. 432; *Fucus longissimus* Gmel. [= *Gracilaria verrucosa* (Huds.) Papenf.], junto à foz do Tejo e no litoral, entre Setúbal e Sesimbra, pág. 436; *Fucus cartilagineus* L. (= *Gelidium cartüagineum* L.) ibid., pág. 437; *Fucus laceratus* Gmel. (= *Cryptopleura !acerata* !Kütz.), junto à foz do Tejo e do Douro, pág. 437; *Fucus filicinus* Wulf. (= *Grateloupia füicina* Ag.), junto à foz do Tejo, pág. 437. Não consta que BROTERO tivesse visitado a Ria de Aveiro.

Passados 44 anos (1848), WELWTTSCH, de origem austríaca, colheu na Ria de Aveiro *Gracüaria confervoiães* Ag. [= *Gracüaria verrucosa* (Huds.) Papenf.] e *Ceramium rubrum* (Huds.) Ag., as quais foram também colhidas por HENRIQUES, em 1874. Este continuou as suas pesquisas na Ria e em 1878 colheu *Gelidium corneum,* (Turn.) Thuret. var *sesquipedale.* Além das três espécies, os ditos naturalistas colheram ainda *Pterosiphonia complanata* (Ciem.) Falk., *PoIysiphonia elongata* (Huds.) Harv. e *Anfeltia plicata* (Huds.) Fries. Todas estas espécies foram publicadas por HENRIQUES, em 1881, nas Contr. Fl. Crypt. Lusit.

De 1883-1911, a Sociedade Broteriana publicou as espécies que foram aparecendo em várias localidades, mas nenhuma proveniente da Ria de Aveiro.

HAUCK, em 1889, publicou um trabalho sobre Rodofíceas do Norte de Portugal, limitando, porém, os estudos a materiais colhidos na Foz do Douro, Leça e Pampolide.

Em 1912, AUGUSTO NOBRE, JAIME AEREIXO e JOSÉ DE MACEDO publicaram o «Relatório oficial do regulamento da Ria de 28 de Dezembro de 1912», em que vêm assinaladas as seguintes Rodofíceas para a Ria.

Fam. BANGIACEAE

Wildemania umbilicalis Kütz.

Fam. GEODEACEAE

Gelidium corneum Lamour. (possivelmente vindo de outro lado).

Fam. GIGARTINACEAE

Ahnfeltia plicata Fries.

Fam. SPHOEBOCOCCACEAE

Gracilaria confervoides Grev.

Fam. BHODOMELACEAE

Pterosiphonia complanata Falk.
Potysiphonia havanensis Mont, (nas estacas da Ria).

Fam. CEBAMIACEAE

Geranium, rubrum Ag. (enrocamento da Barra).

O «Relatório» cita, por conseguinte, mais três espécies: *Wildemania umbilicalis* Kütz., *Gelidium corneum* Lamour. e *Potysiphonia havanensis* Mont., além das mencionadas por J. HENRIQUES nas Contr. Fl. Crypt. Lusit.

O facto de o «Relatório» referir que *Ceramium rubrum* Ag. se encontrava no «enrocamento» da Barra mostra que,

provavelmente, nessa data ainda não existiam no mesmo enrocamento *Chondrus crispios* (L.) Lyngb., nem *Gigartina stellata* (Stackh.) Batters, etc., que actualmente chamam a atenção pela sua abundância.

As condições da Ria antes de 1912 eram, portanto, muito diferentes das actuais, o que se deve à grande quantidade de blocos de pedra vermelha do Reciano, que para ali foram transportados, a fim de proteger o molhe central contra a violência das ondas nas marés cheias.

FR. ARDRÉ (in Portug. Acta Bk)L, Sér. B, 10, 1-4: 137-555, 1969) publicou um trabalho intitulado «Contribution à l'étude des algues marines du Portugal», que nos mereceu a melhor atenção não só pela parte histórica que o acompanha, mas também pelo número de locais indicados para as Rodofíceas, obtidos a partir do estudo das colecções conservadas nos herbarios de Lisboa, Porto e Coimbra, a literatura existente sobre cada espécie, etc. Verificamos, no entanto, que o número de referências à Ria de Aveiro era reduzido. Pelo contrário, o número das outras localidades e as algas existentes em cada uma delas era perfeito para a data e por isso foi para nós de muito valor a dita publicação. De acordo com ela, o ano de 1969 marca, por conseguinte, uma data em que o número de taxa de Rodofíceas inventariadas para a Ria de Aveiro era de 9 espécies.

Em 1973, o Director do Porto e Ria de Aveiro, Senhor Eng.^{0} JOÃO DE OLIVEIRA BARROSA e o Reitor do Liceu, Senhor Dr. ORLANDO DE OIIVEIRA, convidaram-nos a fazer pesquisas na Ria, como vínhamos fazendo noutras localidades das margens do Vouga. Passado algum tempo, um grupo de estudantes do Liceu de Aveiro, escolhidos pelo seu Reitor para efectuar colheitas, tinha o prazer de 1er, nas páginas do Bol. Soc. Brot. **51,** sér. 2: **91-106 (1977),** a descrição de três Rodofíceas novas para a Ciência e uma nova para Portugal. As pesquisas continuaram e, nesta data, estão assinaladas para a Ria trinta e uma espécies de Rodofíceas, cuja descrição apresentamos em vernáculo a fim de facilitar aos estudantes o seu conhecimento.

* * *

Ao Ex.mo Senhor Prof. aposentado, Doutor ABÍLIO FERNANDES, que sempre nos orientou no estudo das algas, corrigiu os nossos trabalhos e pôs ao nosso dispor livros e aparelhagem de investigação, protestamos a nossa sincera gratidão.

Ao Ex.mo Senhor Eng. JOÃO BE OLIVEIRA BARROSA que, por todos os meios ao seu alcance, possibilitou e facilitou as pesquisas na Ria, agradecemos a sua grande dedicação à causa do estudo da Ria, que resultou em auxílio para nós.

Ao antigo Senhor Reitor do Liceu, Dr. ORLANDO DE OLIVEIRA, que teve a amabilidade de escolher os melhores dos seus alunos para os delicados e árduos trabalhos da investigação, a nossa cordial estima e consideração.

ENUMERAÇÃO E DESCRIÇÃO DAS PLANTAS COLHIDAS

COMPSOPOGONACEAE Schmitz

Compsopogon lusitaniens P. Reis (vide Bol. Soc. Brot. 51, Sér. 2: 91, 1977). — Esteiro de Canelas, 27-VIII-1974, P. *Reis* 630 (coi).

CHANTRANSIACEAE Rabenh.

Rhodochorton purpureum (Lightf.) Rosenv. in Bot. Tidsskr. 23: 75 (1900). — De Toni, SyII. AIg.: 1510 (1903). — Gayral, AIg. Côt. Franc.: 361 (1966). — Pr. Ard. in Portug. Acta Biol., Sér. B, 10: 192 (1968).

Byssus purpurea Lightf., Fl. Scot., 2: 1000 (1792).

Conferva purpurea DiUw., Brit. Conf.: t. 43 (1809).

Callithamnion Rothii Lyngb., Hydr. Dan.: 129, t. 41 (1819).

Trentepohlia purpurea Ag., Syst. AIg: 36 (1824). — Hook, Brit. Fl. 2: 382 (1833).

Caïlithamnion purpureum Harv., Man.: 116 (1841).

Talo de 1-10 mm de altura, formando um estrato aveludado, subcrustáeeo, mediante filamentos curtíssimos, horizontais entrelaçados, emitindo filamentos erectos, simples ou pouco ramificados, de 8-12 u de diâmetro, constituídos por células cilindroides, dispostas topo a topo, de comprimento 2-4 vezes o diâmetro, com plastos parietais, desprovidos de pirenóides. Tetrasporângios 15-20 X 21-30 u, formados em tufos de ramúsculos.

Porto de Aveiro, 14-X-1978, *P. Reis* (COI).

Obs. Esta espécie, assinalada aqui pela primeira vez para a Beira Litoral, foi encontrada também no DOURO LITORAL: FOZ do Douro, VII-1879, *Newton* (coi).

GELIDIACEAE Harv.

Gelidium pusilum (Stackh.) Le Jolis var. pulvinatum (Ag.) Feldm. & Hamel in Rev. AIg. 9: 113, fig. 19-e (1936). — Gayral, AIg. Côt. Franc.: 375, t. 84 (1966).

Sphaerococcus pulvinatus C. Ag., Spec. AIg.: 1: 284 (1823).

Acrocarpus pulvinatus Kütz., Sp. AIg.: 762 (1849) ; Tab. Phyc. 18, t. 37, a-h (1868).

Gelidium, pulvinatum Thuret in Bornet, Mém. Soc. sci. Nat: 268, t. 28 (1892).

Talo reptante, roliço, com filamentos erectos de 5-15 mm de altura e de 50-150 u, de diâmetro, de cor vermelha intensa, irregularmente ramificado. Ramos subulados ou aclavados; ramúsculos geralmente subfoliáceos, obovados ou ligulados, por vezes com margem denticulada. Rizinas no centro dos ramúsculos. Tetrasporângios distribuídos irregularmente nos ramúsculos com 25-30 *p.* de diâmetro.

Próx. do farol da Barra, 17-Vni-1977, P. *Reis & M. Vieira* 707 (coi).

Esta espécie só tinha sido assinalada até hoje noutro local da costa portuguesa: ESTREMADURA, Rio Tejo, Porto Brandão, 11-1842, *Welwitsch* s. n. (LISU).

Gelidium pulchellum (Turn.) Kütz., Tab. Phyc. 18, t. 53, fig. 1-f (1868). — Feldm. & Ham. in Rev. AIg. 9: 119, fig. 23, t. 2, fig. 2 e 3 (1936). — Newton, Handb. Brit Seaw.: 263 (1931). —Gayr., AIg. Cot. Franc.: 377, t. 85 (1966). —Fr. Ard. in Portug. Acta Biol. 10: 202 (1969).

Gelidium corneum var. *pulchellum* Turn., Fuci: 146, fig. 256 (1819).

Talo de 3-10 cm, em tufos de cor vermelha intensa, fixado ao substrato mediante rizoides. Ramificação alterna ou oposta, uni-bipinada. Filamentos geralmente com uma só ordem de pínulas curtas, semelhantes entre si (2-5 mm), nascendo ao longo dos ramos primários ou secundários, sendo uns e outros roliços ou comprimidos. Estrutura interna constituída por três estratos: cortical, muito ténue, formado por células pequeníssimas, arredondadas, de 5-6 *p*, de diâmetro, dispostas em filas verticais; subcortical, formado por células maiores arredondadas entre as quais existem rizinas; e central, constituído por células alongadas atenuadas nas extremidades e por algumas rizinas. Tetrasporângios esféricos com 30 u, de diâmetro, produzindo tetrásporos tetraédricos ou irregulares. Cistocarposporângios em pínulas que se tornam fusiformes.

Molhe central da Barra, 17-VII-1977, *P. Reis & M. Vieira* 700 (coi).

Esta espécie é frequente na costa portuguesa, visto ter sido colhida mais nas seguintes localidades: DOURO LITORAL: Póvoa de Varzim, VII-1878, *Newton* s. n. (coi); ibid. IX-1880, *Padrão* s. n. (coi); Leça da Palmeira, X-1848, *Welwitsch* s. n. (LISU); ibid. VIII-1872, *Henriques* s. n. (COi); Foz do Douro, l-VIII-1878, *Lacerda* s. n. (coi), BEIRA LITORAL: Buarcos, IX-1877, *Möller* s. n. (coi); ibid., IX-1877, *Padrão* s. n. (coi); ibid., XI-1890, *G. de Carvalho* s. n. (coi; Lisu); ibid., X-1929, X-1930, *T. Morais* s. n. (coi); ibid., VIII-1948, *M. de Carvalho* s. n. (coi); ibid., XI-1949, *Ernesto & Mendes* s. n. (coi); ibid., 6-IX-1953, *Rodrigues & Santos* s. n. (coi). ESTREMADURA: Ericeira, LX-1890, *Barros e Cunha* s. n. (COi);

ibid., VIII-1844, *Welwitsch* s. n. (LISU) ; Caxias, XI-1852, *Welwitsch* s. n. (LISU).

GRACILARIACEAE Kylin

Gracilaria verrucosa (Huds.) Papenf. in Hydrob. 2: 195 (1950). — Gayral, AIg. Cot. Franc.: 425, t. 106 (1966).— Fr. Ardre in Portug. Acta Biol., Sér. B, 10: 247 (1919).

Fucus confervoides L., Sp. Pl. ed. 2, 2: 1629 (1763).

Fucus verrucosus Huds., Fl. Angl., ed. 2: 588 (1778).

Gracilaria confervoides (L.) Grev., AIg. Brit: 123 (1830). —Harv., Phyc. Brit. 2: t. 65 (1846-1851). —De-Toni, SyII. AIg. 4, 1: 431 (1897). —Newton, Brit. Seaw.: 429, fig. 258 (1931).— Hauck in Rabenh.: 182 (1885, reimp. 1971).

Sphaerococcus confervoides (Ag.) Kütz., Sp. AIg.: 772 (1849); Tab. Phyc. 18: t. 72 (1868).

Sphaerococcus divergens Kütz., Tab. Phyc. 18: t. 74 (1868).

Var. verrucosa

Talo de 7-50 cm, de cor purpurescente, por vezes com zonas esverdeadas, simples ou em grupos, erecto ou prostrado (neste caso produzindo filamentos erectos, muito ramificados na parte inferior). Ramos alternos, dirigidos em todos os sentidos ou, por vezes, unilaterais, nus, flageliformes ou com alguns ramúsculos atenuados nas duas extremidades, fixo ao substrato mediante um pequeno disco e numerosos filamentos rizoides. Células da zona medular (observadas em secção) de paredes cada vez mais finas e diâmetro cada vez maior à medida que se aproximam do centro. Zona cortical constituída por células com 8-10 u de diâmetro. Tetrasporângios disseminados na região cortical. Cistocarpos-porângios sésseis, esferoides ou ovóides.

Esteiro do Carregal, 23 e 27-VII-1973, *P. Reis* s. n. (coi).

Esta espécie já tinha sido colhida na Ria por WELWITSCH em 1848 e por HENRIQUES em 1874. Existe ainda nas seguintes localidades:

DOURO LITORAL: Póvoa de Varzim, 1878, *Padrão* s. n. (coi) ; ibid., 2-IV-1958, *M. Rodrigues & A. Santos* 597 (coi) ; Foz do Douro, 1-1879, *Lima* s. n. (coi). BEIRA LITORAL: Figueira da Foz, IX-1877, *Möller* s. n. (coi) ; ibid., IX-1953, IX-1954, *A. Santos* s. n. (coi) ; Buarcos, X-1889, *J. de Carvalho* s. n. (coi) ; ibid., X-1939, *T. Morais* s. n. (coi) ; ibid., XI-1949, *Rodrigues* s. n. (coi) ; Cabo Mondego, XII-1939, *Lacerda,* s. n. (coi). ESTREMADURA: Lagoa de Óbidos, *Welwitsch* s. n. (Lisu) ; Ericeira, VIII-IX-1846, *Welwitsch* s. n. (LISU) ; ibid., IV-1959, *Neves, M. Rodrigues, Reis & Santos* s. n. (coi) ; Belém, Cascais, Paço d'Arcos, Parede, Pedrouços, Tróia, Cruz Quebrada, Estoril, Oeiras, Cabo da Roca, III--VIII-IX-1841-1846, IX-XII-1849, I-IV-1850, LX-1851, 1-1853, *Welwitsch* s. n. (LISU) ; São Julião, s. d., *Welwitsch* s. n. (LISU) .

Var. ramulosa Kütz., Tab. Phyc. 18: t. 72 (1868).

Esta variedade afasta-se do tipo, especialmente pelo eixo principal que é distinto e pode ultrapassar os 80 cm, com numerosos ramúsculos e alguns, poucos, compridos, nus ou quase, distribuídos no meio daqueles.

Taxon vivendo juntamente com o tipo nos vários esteiros, 27-IX-1979, *P. Reis* s. n. (coi).

Não colhida até hoje em Portugal.

Var. procérrima (Turn.).— Newton, Handb. Brit. Seaw.: 431 (1931).

Afasta-se do tipo pelos ramos muito longos (ca. 2,40m), geralmente simples e quase nus.

Colhida pela primeira vez em Portugal, no esteiro do Carregal da Ria, 28-30-VIII-1980, *P. Reis* s. n. (coi).

Gracilaria vieirae P. Reis in Bol. Soc. Brot., 51, Sér. 2: 91 (1977).

No esteiro da Costa Nova do Prado, 23-VII-1973, *M. Vieira* 692 (coi).

RHABDONIACEAE Kylin

Catenella repens (Lightf.) Batters in Journ. of Bot. 40 Suppl.: 69 (1902). — Newton, Britt. Seaw.: 119, fig. 251 (1931). —Gayral, AIg. Cot. Franc.: 437, t. 112 (1966).— Fr. Ardre in Portug. Acta BioL, Sér. B, 10: 240 (1969).

Fucus repens Lightf., Fl. Scot. 2: 961 (1792).

Fucus opuntia Good. & Woodw. in Trans. Linn. Soc. 3: 219 (1797).

Catenella opuntia Grey., AIg. Brit.: 166, t. 17 (1830).— Harv., Phyc. Brit., t. 88 (1846-1851). — Kutz., Spec. AIg.: 724 (1849); Tab. Phyc. 16: t. 71 (1866).— J. Ag., Sp. AIg. 3: 588 (1876).—Hauck in Rabenh. 2: 186 (1885, reimp. 1971).

Talo de 1-3 cm de altura, pulvinado-cespitoso. Base do talo filiforme, reptante com rizoides, emitindo ramos em di-tricotomias, sendo aquelas articuladas, por vezes fortemente constrictas nas articulações, roliças ou complanadas, de espessura irregular, de 0,5-1 mm, por vezes de 60-200 u, frequentemente com pequenos ramúsculos. Ramos nascendo ordinariamente nas constrições. Articulações alongadas, ovóides ou claviformes, 2-10 vezes mais compridas que espessas. Tetrasporângios numerosos nos artículos dos ramúsculos curtos.

Espécie asinalada pela primeira vez para a Ria. Foi encontrada na meia laranja do molhe central da Barra, 25-XI-1978, *P. Reis á M. Vieira,* s. n. (coi). Além desta localidade, também tinha sido encontrada na Figueira da Foz, X-1929, por *T. Morais* s. n. (coi) e ainda na ESTREMADURA: Tejo salgado, Almada, Porto Brandão, X-1842, II & III--1843, *Welwitsch* (LISU).

PHYLLOPHORACEAE Nägeli

Gymnogongrus griffithsiae (Turn.) Mart., Fl. Bras. 1: 27 (1833). —Kütz., Tab. Phyc. 19: t. 65, fig. e-g. (1869).— Hauck in Rabenh., Crypt. Fl. 2: 139, fig. 56 (1885). —De--Toni, SyIl. AIg. 4, 1: 242 (1897). —Newton, Handb. Brit.

Seaw.: 412, fig. 245 (1931). —Chemin, in Bull. Soc. Bot. Franc.; 755, t. 6-7, fig. 1-3 (1933). — Gayral, AIg. Côt. Franc.: 457, t. 120, fig. 58c (1966).— Schott, in Bull. Inst. Ocean.: 62, fig. 35-36 (1968).

Fucus griffithsiae Turn., Hist. Fuc: t. 37 (1808).
Sphaerococcus griffithsiae Ag., Sp. AIg. 1: 316.

Talo cespitoso, pulviniforme, de 2-5 em de altura, de cor avermelhado-acastanhada, cartilaginoso, roliço ou subcomprimido, indiviso próximo da base e em seguida irregularmente ramificado, fixado ao substrato mediante um disco. Ramificação dicotômica, densa, por vezes fascieulado-policótoma. Segmentos filiformes, curtos ou longos, sendo os terminais, quer alongados, quer curtíssimos e acuminados ou subulados ou ainda obtusos e então, muitas vezes, comprimidos. Nematécias dispersas irregularmente pelos ramos, nascendo muitas vezes nas axilas, esferoidais, finalmente extensas e amplexicaules.

Espécie encontrada pela primeira vez na Ria, 17-VHI-1977, *P. Reis & M. Vieira* 696 (coi) e pela segunda na BEIRA LITORAL, onde já era conhecida: Buarcos, X-1929, *T. Morais* (COi). DOURO LITORAL: Póvoa de Varzim, IX-1881, *Padrão* (PÓ, coi, Lisu) ; Foz do Douro, 27-VII-1879, *Newton* (PO). ESTREMADURA: rio Tejo, pr. Caxias, III-1849, *Welwitsch* (LISU) ; Santa Catarina de Ribamar, 27 & 28-1-1853, *Welwitsch* (Lisu) ; Tejo pr. Cruz Quebrada, XII-1849, *Welwitsch* (LISU).

Gymnogongrus norvegicus (Gunn.) J. Ag., Sp. AIg., 2, 1: 320 (1851-1863). —Chemin, in Bull. Soc. Bot. Franc. 71: 305, fig. 1-2 (1929). — Seoane in Inv. Pesquis. 29: 126, fig. 33, 6-7 (1965). —Gayral, AIg. Côt. Franc.: 455, fig. 58 (1966).— Schotter in Bull. Inst. Océanogr.: 47, fig. 23-27 (1868).— De-Toni, SyIl. AIg.: 246 (1897).

Fucus norvegicus Gunn., Fl. Norv.: t. 3, fig. 4 (1766--1772). —Turn., Syn. Fuc. 2: 222 (1802); Esp. Icon. Fuc: t, 153, fig. 1-4 (1797-1808).

Gondrus norvégicus (Gunn) Lamour., Essai: 39 (1813).
Sphaerococcus norvégicus (Gunn) Ag., Sp. AIg.: **255** (1823).
Oncotylus norvégicus (Gunn) Kütz., Phyc. Gen.: 411 (1843); Sp. AIg.: 789 (1849).

Talo de 3-5 cm, de cor coccíneo-purpúrea, cespitoso, arredondado na base, comprimido na parte superior e em seguida plano, de ramificação dicotômica. Axilas muitas vezes um tanto agudas. Ramos lineares, de 2-5 mm de largura, com os últimos segmentos obtusos, arredondados ou emarginados no ápice. Pseudo-nematécias hemisféricas, salientes nas duas páginas, medindo cerca de 1-2 mm de diâmetro.

Molhe central, pr. farol, 17-VII-1977, *P. Reis & M. Vieira* 697 & 698 (coi).

Encontra-se também no COURO LITORAL: Leça da Palmeira, VIII-1872, *Henriques* (coi), BEIRA LITORAL: Buarcos, IX-1977, *Moller* s. n. (coi) ; Figueira da Foz, Buarcos 7-LX--1953, *A. Santos* (coi), ESTREMADURA: Praia das Maçãs, VIII-1840, *Welwitsch* (LISU) ; Portinho d'Arrábida, 14-XII--1851, *Welwitsch* (LISU) ; Oceano Atlântico nas rochas pr. Oeiras, l-XI-1851, *Welwitsch* (LISU); Caxias, XI-1849, *Welwitsch* (LKU).

GIGARTINACEAE Hauck

Gigartina acicularis (Wulf.) Lamour. in Ann. Mus. Hist. Nat. Paris, 20: 44 (1813). — J. Ag., Sp. AIg. 2: 263 (1851).— Kütz., Tab. Phyc. 18: t. 1c-e (1868). — Harv., Phyc. Brit., 3: t. 104 (1846-1851). —De-Toni, SyII. AIg. 4, 1: 198 (1897). — Hauck in Rabenh., Meersalg.: 136 (1885). — Newton, Br. Seaw.: 406 (1931). — Gayral, AIg. Cot. Franc.: 470 **(1966).—** Fr. Ardré in Portug. Acta Biol., Sér. B, **10:** 258 **(1969).**

Fucus acicularis Wulf., Crypt. Aquat. 3: n.^{0} 50 (1803).
Sphaerococus acicularis (Wulf.) Ag., Sp. AIg. 2: **322** (1885).

Talo de 4-10 cm, vermelho-acastanhado, purpúreo ou violáceo-escuro, quando seco, roliço, cartilagíneo, com cerca de 1 mm de espessura, irregularmente ramificado em todos os sentidos, disposto em tufos. Ramos divaricados, curvos, ponteagudos, com ramúsculos ± curtos, patentes ou curvados, subulados, subatenuados na base. Cistocarpos quase esféricos, 1-4 nos ramúsculos, muitas vezes unilaterais. Tetrasporângios em ramúsculos um tanto engrossados.

Espécie muito frequente na costa portuguesa, mas assinalada pela primeira vez para a Ria, no molhe central pr. farol, 14-X-1976) P. *Reis & M. Vieira* (coi). Foi também colhida em:

DOURO LITORAL: Porto, Douro, *Newton (coi);* Apúlia pr. Póvoa de Varzim, 22-rV-1958, *M. Rodrigues & A. Santos* 662 (coi) ; ibid., IX-1880, *Padrão* (coi) ; Figueira da Foz, entre Buarcos e o Cabo Mondego, 8-IX-1953, *A. Santos,* 19 (coi); Buarcos, IX-1877, *Moller* (coi) ; ibid., VIII-1948, *J. Montezuma de Carvalho* (coi). ESTREMADURA, Cascais, 6-LX-1843, *Welwitsch* (coi).

Gigartina teedii (Roth.) Lamour. var. lusitanica M. Rodrigues in Bol. Soc. Brot., sér. 2, 32: 91 (1958). — Fr. Ardre in Portug. Acta Biol., Sér. B, 10: 262 (1969).

Talo de 5-20 cm, vermelho-acastanhado, cartilaginoso, aplanado, de 3-10 mm de largura, densamente ramificado, com pínulas subhorizontais, patentes, simples, agudas, subespinicentes, inseridas nas margens dos eixos e dos ramos, muito raramente na superfície de uns e outros. Exemplares férteis produzindo cistocarpos muito numerosos.

Este taxon tem sido citado como *Gigartina teedii* (Roth.) Lamour., mas não há dúvida que ele constitui uma boa variedade, como M. RODRIGUES notou. Ê vulgaríssima na costa portuguesa, mas não tinha sido ainda assinalada para a Ria. Foi colhida no molhe central, 17-VIII-1977, P. *Reis & M. Vieira* 694 (coi). É conhecida ainda de:

DOURO LITORAL: Póvoa de Varzim, Aguçadora, 22-IV--1958, *M. Rodrigues «& A. Santos* 606 (coi) ; Póvoa de Varzim, IX-1877, *Padrão,* ESTREMADURA: Cascais, 6-LX-1843, *Welwitsch* (coi) ; Tejo salgado, pr. Caxias, X-1851, *Welwitsch* (LiSU); Foz do Tejo, pr. S. Julião, 7-1850, *Welwitsch;* entre Cascais e Cabo da Roca, 11-1840, *Welwitsch* 61 (LISU) ; S. Julião da Barra e Oeiras, VIII-1845, *Welwitsch* (LEU) ; Cabo da Roca, 11-1840, *Welwitsch* (LISU) ; entre Cruz Quebrada e Caxias, IV-1843, *Welwitsch* (Lisu) ; Oeiras, XI-1851, *Welwitsch* (Lisu) ; Oeiras, XI-1851, *Welwitsch* (USU).

Gigartina stellata (Stackh.) Batters in Journ. of Bot. 40, Suppl: 64 (1902). —Newton, Handb. Brit. Seaw. 408, fig. 242 (1931). — Rosenving, Mar. AIg. Denmark, Rhodoph. 4: 509, figs. 474-476 (1931). — Gayral, AIg. Cot. Franc.: 467, t. 125 (1966).

Fucus stellatus Stackh., Ner. Brit.: t. 12 (1795).

Fucus mamillosus Good. & Woodw. in Trans. Linn. Soc. 3: 174 (1797).

Sphaeroccocus mamillosus C. Ag., Sp. AIg. 1: 260 (1823).

Chondrus mamillosus Grev., AIg. Brit.: 127 (1830).

Mastocarpus mamillosus Kütz., Phyc. Gen.: 398, t. 76, fig. 3 (1843), Sp. AIg.: 733 (1840); Tab. Phyc. 17: t. 39 (1867).

Gigartina mamilosa Hauck in Rabenh., Krypt. Fl. 2: 137, fig. 55 (1885). —De-Toni, SyIl. AIg. 4, 1: 218 (1897).

Talo de 6-15 cm, de cor purpurascente ou vermelha intensa, cartilagíneo, plano ou canaliculado, roliço na base e fixado ao substrato mediante um disco, dicotomicamente ramificado, formando céspedes hemisféricas. Ramos patentes. Segmentos quer todos semelhantes, lineares, de 2-4 mm de largura, quer todos acunheados de 2-8 mm de largura ou os inferiores lineares e os superiores acunheados, os terminais emarginados ou bífidos, subacuminados ou muitas vezes um tanto obtusos. Papilas na parte superior da fronde e no disco, quer raras, quer numerosas curtas ou alongadas. Cistocarposporângios com pericarpo ovóide, de 2-5 mm de comprimento, curta ou longamente pedicelados.

Espécie muito abundante no molhe central da Ria, 17-VIII-1977, *P. Reis & M. Vieira* 695 (coi). Em 1912, porém, ainda ali não fora encontrada. Foi colhida também nas seguintes localidades:

Douro LITORAL: FOZ do Douro, s. d. *Newton* (PO). BEIRA LITORAL: Buarcos, XI-1889, *Goltz de Carvalho* (PO).

Chondrus crispus (L.) Stackh., Ner. Brit. XXIV (1997).—Lyngb., Hydr. Dam.: 15, t. 5A-B (1819). — Grev., AIg. Brit: 129, t. 15 (1830). —Harv., Phyc. Brit.: t. 63 (1846-1851).—Kütz.*, Sp. AIg.: 735 (1849); Tab. Phyc, 17: t. 49 (1867).—J. Ag., Sp. AIg. 2: 246 (1851). — De-Toni, SyII. AIg. 4, 1: 180 (1897). —Newton*, Handb. Seaw.: 404, fig. 241 (1931).—Gayral*, AIg. Côt. Franc.: 471, t. 127 (1966). —Fr. Ardre* in Portug. Acta Biol., Sér. B, 256 (1969).

Fucus crispus L., Syst. Nat. 2: 718 (1767) ; Mant. Plant.: 134 (1767).

Fucus filiformis Huds., Fl. Angl. : 585, ed. 2 (1778).

Sphaerococcus crispus C. Ag., Sp. AIg. 1, 2: 256 (1822) ; Syst. Alg.: 219 (1824).

Chondrus incurvatus Kütz., Phyc. Gen.: 399, t. 73, fig. 2 (1843); Sp. AIg.: 735 (1849); Tab. Phyc. 17: t. 50 (1867).

Talo erecto, de 8-15 cm, de cor violácea ou purpúreo-lívida, cartilagíneo, plano, filiforme na base, aderente ao substrato mediante um disco, de onde se elevam varias frondes. Ramificação dicótomo-flabeliforme. Dicotomias ± numerosas. Segmentos de largura muito variável (estreitos, lineares ou largos e acunheados), com ápices agudos ou obtusos, estreitos ou largamente arredondados; margens encrespadas, nuas ou com proliferações liguladas. Cistocarposporângios impressos no disco ou nas proliferações, quase ovais, de 2-2,2 mm de diâmetro. Tetrasporângios formando manchas semelhantes a carposporângios, na região cortical dos segmentos terminais.

Como *Gigartina stellata* (Stackh.) Batters, também *C. crispus* C. Ag. é muito vulgar no molhe central da Ria,

mas só em 17-VIII-1977 ali foi colhida pela primeira vez por *P. Reis & M. Vieira* 712 (coi). Foi herborizada ainda nas seguintes localidades:

DOURO LITORAL: Foz do Douro, 1-1878, *Lima* (coi) ; ibid. 1879, *Newton* (coi), BEIRA LITORAL: Cabo Mondego, XII-1939, *Lacerda* (coi) ; ibid., IX-1953, *A. Santos* (coi) ; Buarcos, IX-1877, *Moller (COi)* ; ibid., LX-1877, *Moller* (coi); ibid., X-1929, *T. Morais* (coi); ibid., VIII-1949, *M. de Carvalho* (coi); ibid., VIII-IX-1953, *A. Santos* (coi), ESTREMADURA: S. Martinho do Porto, V-1958, *M. Rodrigues & A. Santos* (COl) ; Ericeira, IV-1959, *Neves, M. Rodrigues, Reis & Santos* (coi) ; Paço d'Arcos, IX-1878, *Welwitsch* (coi) ; Cruz Quebrada: Cascais, II-III-IX-1872, s. n., *Welwitsch* (LISU).

Obs. Os autores assinalados com asterisco atribuem a combinação a LYNIGBYE, mas KYLIN atribui-a a STACKHOUSE.

LOMENTARIACEAE Nägeli

Lomentaria articulata (Huds.) Lyngb., var. linearis Zanardi, Syn AIg. Adriat.: 97 (1841); Saggio: 50 (1843).—De-Toni, SyIl. AIg. 4, 1: 554 (1897).

Lomentaria phalligera J. Ag., AIg. Med.: 110 (1842); Sp. AIg. 2: 727 (1852), non Kütz.

Lomentaria linearis Zanardi, Icon. Phyc. Adriat. 2: 161, t. 79 (1865).—Kütz., Sp. AIg.: 863 (1849); Tab. Phyc. 15, t 85 (1865).

Ghylocladia phalligera J. Ag., Sp. AIg. 3: 300 (1876).

Chylocladia articulata (Huds.) Grev. var. *linearis* (Zanardi) Hauck in Rabenh., Krypt. Fl. 2: 156 (1885, reimp. 1971).

Fronde erecta, de 3-12 cm de altura, róseo-purpúrea, empalidecendo no seco, irregularmente ramificada. Ramos primários um pouco contraídos na base, divididos frequentemente por dicotomias. Ramos secundários com ramúsculos opostos nas extremidades. Artículos roliços ou um pouco aplanados, tubulosos, de 4-6 vezes o diâmetro ou de 1-2 mm de largura, os terminais acuminados ou obtusos. Estrutura

interna constituída por duas zonas: a periférica formada por células muito pequenas e arredondadas, a subjacente por células grandes e irregulares. Tetrásporos tetraédricos, dispostos em torno de pequenas depressões corticais. Cistocarpos 1-3, ordenados em filas transversais.

Esta variedade, assinalada pela primeira vez para a Ria, no molhe central, 17-VIII-1977, *P. Reis d M. Vieira* (coi), é vulgar noutras localidades da costa portuguesa:

MINHO: Apúlia, Aguçadora, 22-IV-1958, *M. Rodrigues & Santos* (625 (coi). DOURO LITORAL: Foz do Douro, 27-VII-1879, *Newton* (PO, COi) ; Póvoa de Varzim, 27-IX-1881, *Padrão* (PO_7 coi, Lisu). BEIRA LITORAL: Buarcos, X-1929, *T. Morais* (COi) ; Cabo Mondego, I-XII-1939, *Lacerda* s. n. (coi), ESTREMADURA: São Martinho do Porto, V-1958, *M. Rodrigues d Santos* 718 (coi) ; Santa Catarina de Ribamar, 27 e 28-1-1853, *Welwitsch* (Lisu) ; Tejo, pr. Cascais, HI-1849, *Welwitsch* (Lisu).

CERAMIACEAE Reichenb.

Ceramium ciliatora (Ellis) Ducluz., Essai Conferv. Montpellier: 64, t. 53, fig. 1-4 (1905). — Lyngb., Hydroph. Dan.: 121, t. 37 (1819). —Harv., Phyc. Brit. 129 (1847). —J. Ag., Sp. AIg. 2: 133 (1851). — Ardiss., Phyc. Médit. 1: 117 (1883). — Hauck, Meersalg. 2: 110 (1885, reimpr. 1971). — De-Toni, SyII. AIg. 4, 2: 1473 (1903). — Gayral, AIg. Côt. Franc.: 529, t. 156 (1966). —Fr. Ardre in Portug. Acta Biol., Sér. B, 10: 280 (1969).

Conferva ciliata Ellis in Phil. Trans. 57: 425, t. 18, fig. h (1776) ; Lightf., Fl. Scot. 2: 998 (1792) ; DiIlW., Brit. Confer.: 77, t. 53 (1809).

Echinoceras ciliatum Kütz., Phyc. Gen.: 380 (1843).

Talo delicado, de 5-10 cm, de cor vermelha intensa, regularmente dicotômico, com segmentos patentes e as extremidades encurvadas em forma de torquez. Artículos dos ramos principais 2-4 vezes o diâmetro. Nós corticados, separados por espaços iguais ao diâmetro ou ultrapassando-o,

providos de uma coroa de espinhos, constituídos por 3 células, sendo os da parte inferior menores. Entrenós incolores. Tetrasporângios verticilados, ± proeminentes, muitas vezes alternando com os espinhos. Gonimoblastos laterais, frequentemente inseridos em proliferações e cercados de ramúsculos, 3-4 vezes mais longos, servindo-lhes de invólucro.

A Beira Litoral é assinalada pela primeira vez para esta espécie, 30-IX & 25-XI-1978, *P. Reis & M. Vieira* 720 (coi). Foi colhida ainda nas seguintes localidades:

ESTREMADURA: S. Martinho do Porto, l-V-1958, *A. Santos d M. Rodrigues* (coi) ; Tejo salgado, IV-1849, *Welwitsch* (LISU). ALGARVE: Cabo de S. Vicente, VI-1847, *Welwitsch* (LISU) ; Lagos, VI-1847, *Welwitsch* (LISU) .

Ceraminm flabelligerum J. Ag., Syst. AIg. Advers.: 27 (1844). — Harv., Phyc. Brit. 3: t. 144 (1847). — Kütz., Sp. AIg. 688 (1849); Tab. Phyc. 13: t. 14, fig. f-j (1863). — De--Toni, SyIl. AIg. 4, 2: 1482 (1903). — F . Miranda in Bol. Real Soc. Esp. Hist. Nat.: t. 29 (1929).— Newton, Handb. Brit. Seaw. 401 (1931). — Seoane-Camba in Inv. Pesquis.: 133, fig. 37 (1965). —Gayral, AIg. Côt. Franc.: 531 (1966). —Fr. Ardre in Portug. Acta Biol., Sér. B, 10: 282 (1969).

Ceramium spiniferum Kütz., Sp. AIg., 688 (1849).

Talo de 4-10 cm, de cor purpúrea. Eixos principais ramificados dicotomicamente. Ramos alternos, quase dísticos, ramificados em forma de leque ou flabelado-corimbosos na parte superior. Segmentos erecto-patentes, terminando em forma de torquez aberta, com uma espícula articulada e colorida, no lado externo de cada artículo. Artículos dos ramos principais de comprimento uma vez e meia maior que o diâmetro, sendo o dos ramos superiores igual a cerca de metade do diâmetro. Corticação geral. Tetrasporângios salientes da corticação, formando verticilos e tornando os artículos nodosos nas regiões terminais frutíferas. Gonimoblastos dispostos nos ramos superiores em grupos de dois a três, cercados de ramúsculos curtos que lhes servem de invólucro.

Molhe central da Barra, 17-VIII-1977, *P. Reis & M. Vieira* 713 (coi). Encontra-se, além disso, nas seguintes províncias: DOURO LITORAL: FOZ do Douro, VIII-1789, *J. Newton* (PO); Leça da Palmeira, IX-1880, *Newton* (coi, PO). ESTREMADURA: Rio Tejo, nas rochas basálticas, 9-III-1852, *WeZwitsch* (Lisu) ; Tejo salgado, pr. Caxias, 22-11-1952, *Welwitsch* (Lisu) ; Tejo, pr. Caxias, sobre as frondes de *Fucus vesiculosus*, 9-III-1952, *Welwitsch* (Lisu).

Ceramium arborescens J. Ag., Analecta Algol. 2: 33 (1894). —De-Toni, SyIl. AIg. 4, 2: 1472 (1903).— Newton, Handb. Seaw.: 399 (1931).

Talo arboriforme, de 8-9 cm, de cor intensamente vermelha. Eixos principais muito desenvolvidos. Ramificação dicotômica com os ramos progressivamente mais delicados, sendo os últimos quase capilares e corimbosos. Extremidades dos ramos principais geralmente alongadas e ligeiramente curvas. Proliferações numerosas. Corticação presente, formada a partir das duas margens (superior e inferior) de cada nó, sobre os respectivos entrenós e deixando uma zona nos artículos da parte superior do talo; na inferior corticação total. Artículos do ápice não corticados. Nós inferiores distantes, os superiores próximos. Artículos inferiores iguais a 2-3 vezes o diâmetro. Tetrasporângios verticilados, em série simples e imersos. Gonimoblastos raros.

BEIRA LITORAL: Aveiro, no molhe central da Barra, 17-VIII-1977, *P. Reis & M. Vieira* 711 (coi).

Obs. Esta espécie é assinalada pela primeira vez para Portugal.

Ceramium rubrum (Huds.) Ag., Syn.: 60 (1817); Sp. AIg. 2: 146 (1828). — Lyngb., Hydr. Dan.: 118, t. 62b, fig. 1 (1819). —Harv., Phyc. Brit: t. 181 (1848). —Mart, Fl. Bras.: 14 (1833). — Derb. & Sol., Mém. Physiol. AIg.: 71, t. 18, fig. 9-11 (1856).— Kütz., Tab. Phy. 13: t. 4 (1863).— J. Ag., Epier.: 100 (1876); Fl. Morphol.: t. 3, fig. 21-23 (1879); Anal. Algol. 2: 37 (1892). —-Ardiss., Phyc. Med.

1: 113 (1883). —De-Toni, Syü. AIg. 4, 2: 1476 (1903).— Gayral, AIg. Côt. Franc.: 535 (1986). —Fr. Ardr. in Portug. Acta Biol., Sér. B, 10: 289 (1969).

Conferva rubra Huds., Fl. AngL, ed. 2: 600 (1778).— DiUw., Brit. Conferva: t. 34 (1809).

Conferva tubulosa Huds., Fl. AngL, ed. 2: 660 (1778).

Boryna variabilis Bonnern, in Mém. Mus. 16: 53 (1828).

Talo de 20-25 cm, de cor intensamente vermelha, ramificado subdicotomicamente, totalmente corticado, inerme, subnoduloso. Extremidades dos ramos ligeiramente curvas ou rectas, afiladas. Artículos cilíndrico-elipsóidais, translúcidos na parte média da fronde. Nós obscuros e muitas vezes contraídos. Tetrasporângios mergulhados no estrato cortical em torno dos nós, dispostos em 1-2 séries transversais. Gonimoblastos 1-2, nascendo nos próprios segmentos ou muitas vezes em raminhos cercados de 3-5 ramúsculos encurvados, igualando ou ultrapassando os cistocarposporângios.

Trata-se de uma espécie vulgaríssima em toda a costa portuguesa, publicada por HENRIQUES in Contr. Fl. Crypt. Lusit: 25 (1881) por quem tinha sido herborizada em 1876, depois por MOLLER (1877) e por T. MORAIS (1929).

DOURO LITORAL: Póvoa de Varzim, VIII-1879, *Newton* (coi) ; ibid., IX-1880, *Padrão* (coi) ; Leça da Palmeira, VIII-1872, *Henriques (COI)* ; São João da Foz, VII-VIII-1858, *Henriques (COl)* ; Foz do Douro, VJ.H-1878, VII-VIII-1879, *Newton* (coi). BEIRA LITORAL: Buarcos, IX-1877, *Moller (coi)* ; ibid., VIII-1879, *Henriques (coi)* ; ibid., X-1929, *T. Morais* (coi). ESTREMADURA: S. Martinho do Porto, V-1958, *A. Santos (COi)* ; Ericeira, IV-1959, *Neves, M. Rodrigues, Reis & Santos* (coi) ; Pedrouços, Caxias, Cruz Quebrada, Tróia, VI-VIII--1849, *Welwitsch* (Lisu) ; Porto Brandão, 11-1843, *Welwitsch;* Arrábida, VI-1852, *Welwitsch* (LISU). ALGARVE: Faro, V-1847, *Welwitsch* (LISU).

SPYRIDIA Harv.

Spyridia filamentosa (Wulf.) Harv. in Hook., Brit. Fl. 2: 336 (1833); Phyc. Brit.; t. 46 (1846).

Vide P. REIS in Bol. Soc. Brot. 51, Sér. 2: 94 (1977).

Callithamnion tetragonum (Wither) C. Ag., Sp. AIg. 2: 176 (1823, reimp. 1969). — Harv., Phyc. Brit: 3: t. 136 (1847). —Kütz., Tab. Phyc. 12: t. 3, fig. c-d (1862).— Ardiss., Phyc. Med. 1: 74 (1883). — Hauck, Meeresalg.: 81 (1885, reimp. 1971). — Kylin, Stud. AlgenpL: 158 (1907).— Boerg., Mar. AIg. Canary IsI. 3: 46, fig. 17 (1930).—De-Toni, SyIl. AIg. 4, 2: 1320 (1903). — Feldm. Céram. Médit.: 473 (1940). —Fr. Ardre in Portug. Acta Biol., Sér. B, 10: 309 (1969).

Conferva tetrágono, Wither., Arrang. Brit. Pl. 5: 405 (1818).

Ceramium brachiatum Bonnern., Ess. Hydr. ed. 2: 87 (1828).

Phlebothamnion tetragonum Kütz., Sp. AIg.: 654 (1849).

Dorythamnion tetragonum Nägeli, Beitr. Morph. Syst. Ceram.: 344 (1861).

Talo erecto, de 6-8 cm de altura, de cor intensamente vermelha, cespitoso, subquadrangular na base, fixo ao substrato mediante rizoides. Eixos principais cobertos de ramos inseridos em espiral, alternos e pinados, os quais são igualmente cobertos de ramúsculos, que lhes dão um aspecto cilindroide. Pínulas encurvadas para a raquis e apiculadas. Base dos eixos principais inteiramente corticada com cerca de 360 u. de diâmetro; ramos com 110-190*p.;* os ramúsculos com 60-80 u, e as células terminais com 15-20u de diâmetro. Artículos de comprimento 2-3 vezes o diâmetro. Artículos dos ramúsculos cilindroides, igualando o diâmetro. Gonimoblastos inseridos nos ramos superiores. Tetrasporângio no lado anterior das últimas pínulas.

Apesar de pouco vulgar, herborizou-se no molhe central da Barra em 17-VIII-1977, P. *Reis & M. Vieira* 719 (COl).

Foi encontrado também nas seguintes localidades:

MINHO: Apúlia, Aguçadora, IV-1958, *M. Rodrigues <& Santos* s. n. (coi), DOURO LITORAL: Leça da Palmeira, VTII--1872, *Padrão* s. n. (coi) ; Foz do Douro, 1880, *Newton* s. n.

(coi), ESTREMADURA: Cabo da Roca, Parede, H-1842, IV-1850, *Welwitsch* s. n. (LISU).

DELESSERIACEAE Nägeli

Hypoglossum woodwardii Kütz., Phyc. Gen.: 65, fig. I (1843); Sp. AIg. 875 (1849); Tab. Phyc. 16: t. 11 fig. a-c (1866). — J. Ag., Sp. AIg. 3, 3: 189 (1898). — De-Toni, SyIl. AIg. 4, 1: 694 (1897). — Gayral, AIg. Côt. Franc.: 539, t. 161 (1966). — Fr. Ardre in Portug. Acta Biol., Sér. B, 10: 311 (1969).

Fucus hypoglossum Woodward, in Trans. Linn. Soc. 2: 30, t. 7 (1794). — Lamour., Ess. Thalassioph.: 39 (1813). — Grev., AIg. Brit.: 75, t. 12 (1830). — C. Ag., Sp. AIg.: 176 (1869). — J. Ag., Sp. Gen. Ord. AIg. 3, 1: 489 (1876). — Ardiss. in Phyc. Med. 1: 260 (1883).

Fucus hypoglossoides Stackh., Ner. Brit. ed. 2: tab. 13 (1816).

Delesseria hypoglossum (Woodward) C. Ag., Sp. AIg. 1: 176 (1823, reimp. 1969).

Delesseria lingulata Duby, Bot. Gall.: 946 (1830).

Talo de 6-8 cm, foliáceo, membranoso, de cor coccínea, vagamente ramificado, com proliferações nascendo das nervuras, fixado ao substrato por um disco comum a várias frondes. Ramos de 2-4 cm, lanceolado-lineares, acuminados numa e outra extremidade, de 1-3 mm de largura, inteiros. Nervuras cingidas muitas vezes por uma asa estreita, mesmo na parte inferior, raramente nuas. Nervuras secundárias ausentes. Lâmina formada por uma única camada de células. Cistocarporângios sésseis sobre a nervura, sub-esféricos, por fim apiculados. Tetrasporângios formando soros ao longo das nervuras.

Herborizou-se pela primeira vez no molhe central da Barra, 17-VIII-1977, *P. Reis & M. Vieira* 718 (coi). Aparece, no entanto, em toda a costa portuguesa.

MINHO: Montedor, 25-III-1963, *Fr. Araré;* 18-X-1963, *Fr. Ardré;* Viana, 24-III-1963, *Fr. Ardré.* DOURO LITORAL:

Póvoa, 1881, *Henriques* (coi) ; Foz do Douro, São João da Foz, VIII-1871, *Newton* s. n. (coi), BEIRA LITORAL: Buarcos, VIII-1879, *Henriques* s. n. (coi). ESTREMADURA: Nazaré (coi) ; Tejo salgado, Pedrouços, Caxias, Praia das Maçãs, Trafaria, 11-1841, 11-1942, 8-1849-VI-1853, *Welwitsch* s. n. (LISU), Arrábida, in-VII-1852, *Welwüsch* s. n. (Lisu) ; ibid., 1958, *PaIminha;* ibid,, IV-1959, *Neves, Reis & Santos,* s. n. (coi). ALGARVE: Sagres, 27-28-11-1960, 25-IV-1963; Lagos, 26-11--1960; Praia da Rocha, 25-11-1960, 27-IV-1963; Carvoeiro, 26-IV-1963; Albufeira, 15-VIII-1960, *FeUmomn.*

Cryptopleura ramosa (Huds.) Kylin ex Newton, Brit. Seaw.: 332, fig. 205 (1931). — Gayral, AIg. Côt. Franç.: 547, fig. 165 (1966).—Fr. Ardré in Portug. Acta Biol., Sér. B, 10: 319 (1969).

Uiva ramosa Huds., Fl. Angl.: 476 (1762).

Fucus laceratus Gmel., Hist. Fue: 179, t. 21, fig. 4 (1768).

Nitophyllum laceratum (Gmel.) Grev., AIg. Brit.: 83 (1830). — Harv., Phyc. Brit. 2, 1: t. 267 (1846-1851).— J. Ag, Sp. AIg. *S,* 3: 658 (1898). — De-Toni, SyIl. AIg. 4, 1: 663 (1897).

Cryptopleura lacerata (Gmel.) Kütz., Phyc. Gen.: 444 (1843); Sp. AIg.: 870 (1849); Tab. Phyc. 16: t. 25, fig. a-d (1866).—Kylin, Stud. Deless.: 86 (1924).

Talo de 6-12 cm, de cor vinoso-coccínea, plano, membranáceo, irregularmente ramificado e atenuado na base em estipe curto, de poucos milímetros de altura, fixo ao substrato mediante um disco. Ramificação pseudo-dicótoma ou, raro, penatiforme. Segmentos ondulados na margem ou subfimbriados. Lobos supremos arredondados ou emarginado--crenulados. Nervura do estipe grossa, formada por numerosas vénulas que, na parte inferior do talo, se separam irradiando e depois se aproximam, formando anastomoses ao longo da fronde. Vénulas simples, constituídas por uma única fiada de células cilindricas, dispostas topo a topo, só perceptível por transparência com o auxílio do microscopio.

Cistocarporângios hemisféricos, com orifício na parte superior, distribuídos ao longo das margens dos segmentos e dos lobos. Tetrasporângios agrupados em soros arredondados, independentes ou em linhas confluentes ao longo das margens dos segmentos e dos lobos.

B pouco frequente, mas existe em toda a costa, com excepção da zona do Cabo de S. Vicente a Vila Real de Santo António.

Encontrada no molhe central da Barra, 15-VIII-1977, *P. Reis & M. Vieira* 709 (coi).

Outras localidades:

MINHO: Ofir, Apúlia, IV-1958, *M. Rodrigues & Santos* s. n. (coi). DOURO LITORAL: Póvoa de Varzim, 14-VII-1878, *Newton* (PO) ; ibid., IX-1879, *Padrão,* s. n. (coi) ; Leça da Palmeira, VIII-1872, *Henriques* s. n. (coi) ; ibid., VIH-1879, *Newton* (COi) ; Foz do Douro, VIII-1879, *Newton* (COi) ; Foz do Douro, VIII-1879, *Newton* (coi), BEIRA LITORAL: Buarcos, LX-1877, *Möller,* s. n. (COI) ; ibid., VIII-1879, *Henriques,* ESTREMADURA: Peniche, X-1884, *Mendonça* s. n. (coi) ; Ericeira, V-1842, III-1844, *Welwitseh* s. n. (LISU) ; Tejo salgado, Paço d'Arcos, Cruz Quebrada, Belém, Cascais, 1841, 1842, 1847, 1850, 1852, *Welwitseh* (LISU) ; Arrábida, 1-1958, *Vieira s.* n. (COl) ; ibid., IV-1959, *Neves, M. Rodrigues, Reis & Santos* (coi).

RHODOMELACEAE Harv.

Polysiphonia elongata (Huds.) Harv. in Hook., Br. FL, 2: 333 (1833). — J. Ag., Sp. AIg. 2, 3: 1004 (1863). — Kütz., Phyc. Gen.: 428, t. 50, fig. 5 (1843); Sp. AIg.: 828 (1849); Tab. Phyc. 14, t. 4 (1864). — Ardiss., Phyc. Med. 1: 416 (1883). —Hauck in Rabenh., Meeresalg.: 227 (1885).— Falkenb., Rhodom.: 126, t. 21, fig. 6-9 (1901). — De-Toni, SyIl. AIg. 4, 2: 903 (1903). — Gayral, AIg. Cot. Franc.: 589 (1966).

Conferva elongata Huds., Fl. Angl.: 599 (1762).

Corradoria elongata (Huds.) Mart., Fl. Bras. I: 16 (1883). .

Hutchinsia elongata (Huds.) Ag., Sp. AIg. 2: 82 (1823).

Gramitia elongata (Huds.) Bonnern., Hydry. Loc. 16: 22 (1828).

Polysiphonia stenocarpa Kütz., Sp. AIg.: 830 (1849); Tab. Phyc. 14: t. 11, fig. d-f (1864).

Polysiphonia chalarophlaea Kütz., Sp. AIg.: 831 (1849); Tab. Phyc. 14, t. 12, fig. d-f (1849).

Polysiphonia clavigera Kütz., Sp. AIg.: 831 (1849) ; Tab. Phyc. 14, fig. a-d (1849).

Hutchinsia strictoides Lyngb., Hydr. Dan.: 114, t. 35 (1819).

Talo de 10-20 cm de altura, de cor vermelho-purpúrea nos ramúsculos jovens e acastanhada nos antigos. Eixos principais perfeitamente distintos, ramificados alternadamente em todos os sentidos, corticados até à ponta dos ramos, com 1-2 mm de diâmetro. Ramúsculos de última ordem nus, com 40-80 u, de diâmetro e quatro células pericentrais, cercadas de outras quatro mais pequenas (nos ramos mais antigos oito células terciárias envolvem as oito precedentes e são cercadas pelas células corticais).

Apesar de muito rara foi, no entanto colhida por MOLLER e HENRIQUES, na Ria, em 1876 e ultimamente em 17-VIII-1977, no molhe central da Barra, por P. *Reis & Vieira* s. n. (coi).

Na zona entre S. Vicente e Vila Real de Santo António não foi ainda encontrada, mas existe em outras localidades. MINHO: Montedor, 5-IH-1963, *Fr. Ardré,* DOURO LITORAL: Póvoa de Varzim, VII-1878, *Newton* s. n. (coi) ; Leça da Palmeira, VIII-1872, *Henriques* s. n. (coi) ; Foz do Douro, VII-1879, *Newton* s. n. (coi)'; ibid., 1-1879, *Lima* s. n. (coi). BEIRA LITORAL: Buarcos, VII-1953, IX-1954, *Santos* (coi) ; ibid., IX-1877, *Moller* (coi) ; Figueira da Foz, 1-1870, *Simões* (coi). ESTREMADURA: Cascais, Paço d'Arcos, Caxias, II-III--1843, X-1849, III-1852, *Welwitsch* s. n. (LISU) ; Tróia, HI-IV--1850, *Welwitsch;* Arrábida, V-1958, *Neves, M. Rodrigues, Reis & Santos* (coi).

Polysiphonia havanensis Mont, in Ramon de la Sagra, Hist. Nat. Cuba 9: 34, t. 5, fig. 3 (1834).

Talo de 5 cm, cespitoso, intensamente ruivo-acastanhado. Filamentos primarios prostrados, reptantes, constituidos por artículos de comprimento igual a 1/2 do diâmetro, inflados nos genículos, produzindo filamentos secundarios, inseridos em ângulo mais ou menos recto, decompostos em subdicotomias (raras na parte inferior), longamente atenuados, pouco ou muito ténues nos ápices, descorticados, mais ou menos providos de ramos compostos e de ramúsculos simples, uns e outros constituídos por artículos 4-sifónados, de comprimento irregular, desde 1,5 até quase o triplo do diâmetro; genículos dilatados especialmente na parte inferior do talo. Ramos distantes na parte inferior, semelhantes aos filamentos secundarios, e aproximados na parte superior, misturados com ramúsculos um tanto mais simples longos ou curtos atenuados na base, por vezes muito curtos. Tetrasporângios ovoides, muito raros, um ou poucos (2-3) em cada ramúsculo.

Ria de Aveiro, nas estacas do porto da Vista Alegre, 3-IX-1975, *P. Reis* s. n. (coi).

Obs. Transcrição da Revista I. **D.** E. **S.** O. **4: 41 (1975).**

Polysiphonia pulvinata J. AIg., AIg. Med.: 124 (1842); Sp. AIg. 2, 3: 957 (1865).

Talo de 5 cm, em céspedes densíssimas, fusco-purpúreas. Filamentos primários prostrados, densamente entrelaçados, radicantes, constituídos por artículos cujo comprimento é igual ao diâmetro ou 1/2 deste, produzindo filamentos secundários mais ou menos erectos, com dicotomias raras, muitas vezes cobertos de ramúsculos de segunda ordem, sendo uns e outros articulados desde a base, 4-sifonados. Ápices fasciculado-tricoblastíferos. Ramúsculos inferiores alongados, mais ou menos patentes, sendo os superiores muitas vezes mais densos, patentes ou ascendentes. Artículos descorticados, sendo o comprimento igual ao diâmetro na parte inferior e até ao triplo na superior dos espécimes mais desenvolvidos; genículos dilatados especialmente na metade inferior dos eixos principais. Tetrasporângios dispostos em

série até 4-5 nos filamentos torulosos, abaixo dos ápices. Cistocarpos urceolados ou subpiriformes, raros.

Habita na Ria de Aveiro, nas águas tranquilas da praia do Carregal a cerca de 20 cm de altura de água, 25-VIII--1975, *P. Reis* 718 (coi).

Assinalada também para o DOURO LITORAI.: FOZ do Douro, VHI-1879, *Newton* s. n. (coi). ESTREMADURA : Cascais, Estoril e Parede, 1-1850, *Welwitsch* s. n. (LISU).

Obs. Transcrição da Revista I. D. E. S. O., 4: 42 (1975).

Polysiphonia fernandesiana P. Reis in Bol. Soc. Brot. 51, Sér. 2: 99 (1977).

Vide descript. loc. cit.

Ria de Aveiro, ll-VI-1972, P. *Reis* 652A (coi).

Bostrychia scorpioides (Gmel.) Mont., Hist. Bot. Cuba: 39 (1838). — Harv., Phyc. Brit, 2: Tab. 48 (1846). —Batters in Journ. Bot. London (Supl.: 77, 1902). — De-Toni, SyIl. AIg. 4, 2: 1164 (1903). —Newton, Brit. Seaw.: 332 (1931). — Fr. Ardre, in Portug. Acta Biol., Sér. B, 10: 344 (1969).

Fucus scorpioides Gmel., Hist. Fue: 135 (1768).

Fucus amphibius Huds., Fl. Angl. ed. 2: 590 (1778).

Rhodomela scorpioides (Gmel.) Ag., Sp. AIg. 1: 380 (1822). —Grev., AIg. Brit: 105 (1830). —Hook., Brit. Fl. 2: 294 (1833).

Alsidium scorpioides (Gmel.) J. Ag. in Linnea 15: 28 (1841).

Helicothamnion scorpioides (Gmel.) Kütz., Phyc. Gen.: 433, t. 43V (1843).

Talo de 5-10 cm, formando tufos de cor acastanhada, por vezes negra, aderindo às raízes de halófitos mediante rizoides. Eixo primordial ramificado irregularmente. Eixos secundários unilaterais, alternos ou dicótomos. Ápices em forma de cauda de escorpião. Sifão central estreito, cercado

de várias camadas de células de parede espessa, sendo estas envolvidas por 1-2 camadas de células semelhantes, mais pequenas e assimiladoras. Tetrasporângios tetraédricos, dispostos em verticilos sobre estiquídias fusiformes. Cistocarposporângios ovóides sobre os últimos ramúsculos.

Assinalada pela primeira vez para a BEIRA LITORAL: esteiro de Mira, a sul da Costa Nova do Prado, 2-IX-1979, *P. Reis* 725 (COi).

Existe também no DOURO LITORAL: FOZ do Douro, 1889, *Hauck s.* n., na ESTREMADURA: Seixal, Portimão, pr. Setúbal, 7-VIII-1847, VI-1852, *Welwitsch* s. n. (LISU) e em Faro, 1963, *Ginsburg-Ardré* s. n. in Contr. Etud. AIg. Mar. Port.

Laurencia pinnatifida (Gmel.) Lamour., Essai: 42 (1813). — Grev., AIg. Brit.: t. 14, fig. 1-5 (1830). — Ardiss., Phyc. Med. 1: 332 (1833). — Harv., Phyc. Brit. 2: t. 55 (1846).— Kütz., Sp. AIg.: 856 (1849); Tab. Phyc. 15: t. 66, fig. a-e (1865). — J. Ag., Sp. Gen. Ord. AIg. 2: 764 (1852): 3: 656 (1876). —Hauck in Rabenh. Krypt. Fl. 2: 208 (1885).— De-Toni, SyIl. AIg. 4, 2: 798 (1903). — Gayral AIg. Côt. Franc.: 563 (1966). —Fr. Ardré, in Portug. Acta Biol., Ser. B, 10: 357 (1069).

Fucus pinnatifidus Gmel., Syst. Nat. 2: 1385 (1768); Hist. Fue: 156, t. 16, fig. 3 (1768).

Condria pinnatifida (Gmel.) Ag., Sp. AIg. 1: 337 (1822).

Talo de 5-10 cm de altura, de cor vermelho-escura ou acastanhado-esverdeada, conforme a intensidade luminosa do ambiente, cespitoso, fixado ao substrato mediante um disco acompanhado de filamentos rizóidais. Ramificação 2-4-fida, alterna, muito raramente oposta ou unilateral. Ramos primários distantes ou aproximados, ± comprimidos, frequentemente de base adelgaçada e os ápices arredondados ou lobados. Ramúsculos numerosos, distintos, pinados. Pínulas ínfimas mais longas, as superiores mais curtas e as supremas confluentes com o ápice crenado. Ráquis principal quase roliça na base com 1-4 mm de largura, de ápice obtuso,

arredondado ou lobado; últimas ramificações com pínulas de 0,5-1 mm de largura. Tetrasporângios mergulhados na região cortical dos ramúsculos. Cistocarposporângios em forma de urna com poro apical, fixados lateralmente nos últimos ramúsculos. Carpósporos piriformes.

Frequente no molhe central da Barra, onde foi colhido pela primeira vez, em 17-VIII-1977, por P. REIS & V. VIEIRA 707 *(coi).*

Outras localidades:

MINHO: Apúlia, Aguçadora, IV-1958, *M. Rodrigues* •& *Santos* (coi), DOURO LITORAL: Póvoa, IX-1877, *Padrão* (ooi) ; Leça da Palmeira, XIII-1872, *Henriques* s. n. (coi). S. João da Foz, 1-1879, *Newton* (coi), BEIRA LITORAL: Cabo Mondego, XII-1939, *Lacerda* (COi) ; Buarcos, 1822, *Moller* s. n. (COi) ; ibid., IV-1930, *T. Morais* (coi) ; ibid., VÏÏI-1948, *M. de Carvalho* s. n. (coi) ; Figueira da Foz, XI-1949, *M. Rodrigues* (coi); ibid., IX-1953, IX-1954, *Santos* s. n. (coi), ESTREMADURA: Nazaré, XI-1883, *Padrão* s. n. (coi) ; S. Martinho do Porto, Ericeira, JV-1950, *Neves, M. Rodrigues, Reis & Santos* s. n. (coi) ; Tejo salgado, Cabo da Roca, Cascais, Caxias, XI-1849, II-xII-1851, 1852, *Welwitsch* (LKU). ALENTEJO: Arrábida, 1958, *Pálminha;* Vila Nova de Milfontes 1954, *Dizerbo.*

DISTRLBUIÇAO DAS ESPÉCIES DE RODOFÍCEAS DA RIA, NAS LOCALIDADES DA COSTA DE PORTUGAL

Na primeira parte deste trabalho, a introdução, expôs-se a história das pesquisas sobre as Rodofíceas da Ria de Aveiro. Na segunda, apresentou-se a descrição em português das espécies, a fim de facilitar aos estudiosos do grupo o seu conhecimento. Nesta terceira parte, pretende-se mostrar quais são as espécies da Ria representadas em cada uma das 56 localidades, onde, até à data, se têm efectuado colheitas de Rodofíceas; a média de espécies existentes nas principais zonas da costa; a maior frequência de algumas espécies; as que existem só na Ria; as que se encontram

em toda a extensão da costa portuguesa; as que não existem na zona norte, mas ocorrem nas outras e as que se estendem desde o norte só até várias localidades.

Por conseguinte, não se pretende saber qual é a localidade da costa de Portugal que tem maior ou menor número de espécies de Rodofíceas, nem mesmo se o número delas, em geral, aumenta ou diminui do norte para o sul. Só interessa a representação das Rodofíceas da Ria nas várias localidades e média de cada região; a sua presença em toda a costa portuguesa ou só dentro de certos limites.

De harmonia com o referido plano, a análise do quadro anexo mostra o seguinte:

1.° — Que o número de espécies assinalado para cada localidade é muito variável: vai de 1 espécie em Lagoa de Albufeira e no Cabo de S. Vicente até 31 na Ria de Aveiro.

2.° — A média das espécies pelas principais zonas da costa é de 10,2 do Rio Minho ao Douro; de 16,5 do Douro ao Mondego; de 7,9 do Mondego ao Tejo; de 6,6 do Tejo ao Cabo de S. Vicente e deste até Vila Real de Santo António é de 5,7.

Por conseguinte a média dos números médios de espécies das algas Rodofíceas da Ria de Aveiro, representados nas cinco zonas vai diminuindo para o sul e no rio Mondego encontra-se a máxima (16,5) ao norte e a maior (7,9) ao sul.

3.° — Em relação à frequência nota-se que as espécies correspondentes aos números 11-16 inclusive apresentam uma frequência de 3,9 para a 1.ª zona; 5,2 para a 2.ª; 2,9 para a 3.ª; 2,5 para a 4.ª e 1,1 para a 5.ª. As frequências restantes afastam-se pouco destas.

Também neste aspecto a Ria representa frequência mais alta que as outras quatro localidades.

A presença varia de 20 para *Chondrus crispus* (L.) Lyngb., a 45 para *Gigartina acicularis* (Wulf. Lamour. dentro

das 56 localidades, para as espécies de maior frequência ou seja 11-16. A presença das restantes está compreendida entre 19 e 43 ou é mais baixa que 19.

4.º — Existem espécies (n.os 1, 9, 18, 21, 26 e 29) que só são assinaladas para a Ria de Aveiro. Trata-se de espécies descritas recentemente que poderão existir noutras localidades, onde não poderão ter sido ainda colhidas ou ter sido consideradas como outras espécies.

5.° — A maior parte das espécies (n.os 2, 4, 6, 7, 11, 13, 16, 17, 19, 20, 22, 23 e 25) encontram-se em toda a extensão da costa portuguesa (costa ocidental e sul).

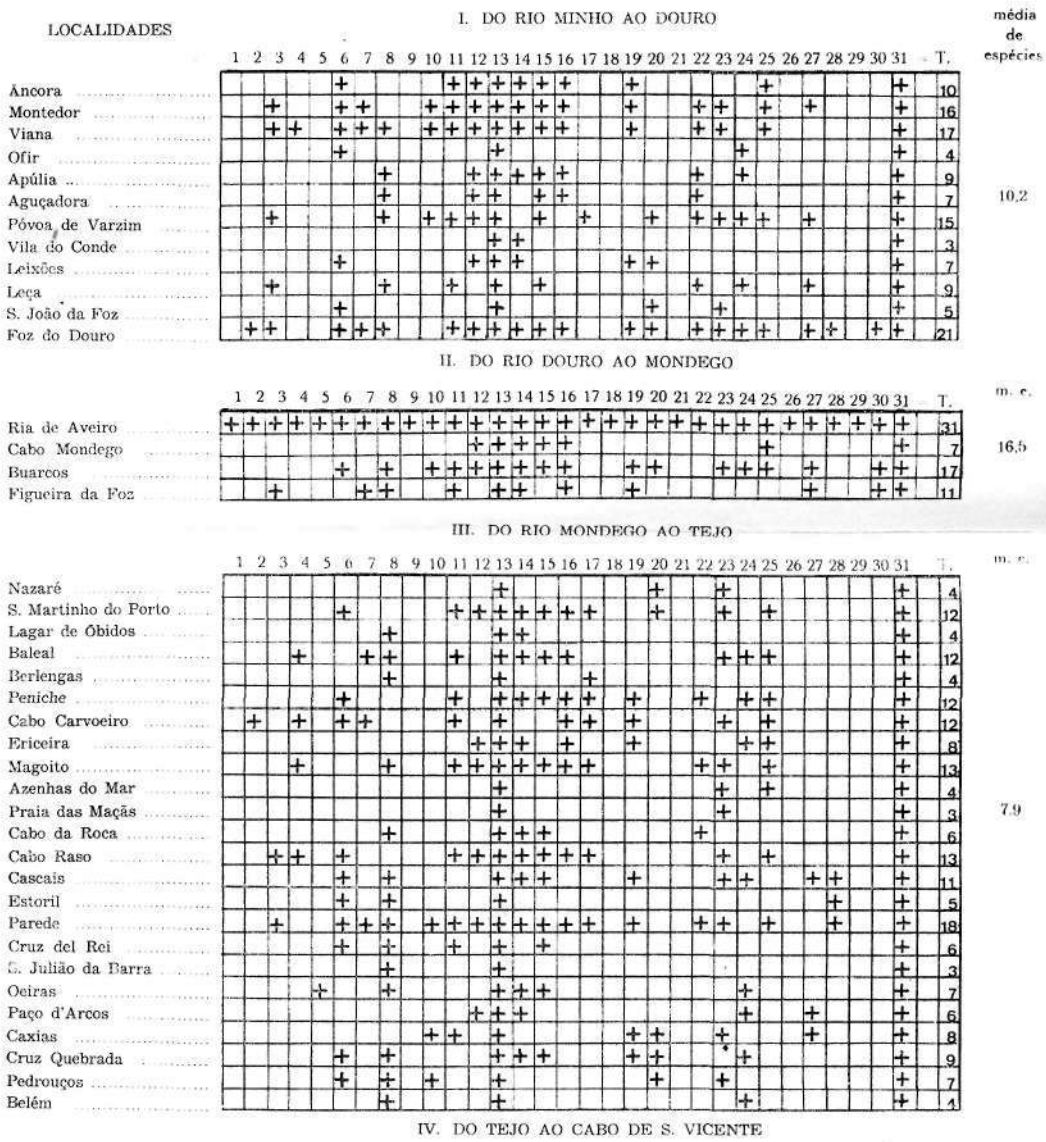
6.° — Há espécies (n.os 8, 12, 14, 15, 24, 27 e 31) que se estendem desde o norte até à zona do Tejo ao Cabo de S. Vicente inclusive. Outras só atingem a zona do Mondego ao Tejo inclusive (n.os 3, 10 e 28). A única espécie que não existe na zona do norte, mas atinge a zona do Mondego ao Tejo é a n.° 5.

7.° — A n.° 30 só foi encontrada na 1.ª, 2.ª e 5.ª zonas.

MAPA DA DISTRIBUIÇÃO DE RODOFICEAS EXISTENTES NA RIA DE AVEIRO

Géneros dispostos segundo a ordem de Kylin

31 Laurencia pinnatifida (Huds.) Mont.
30 Bostrychia scorpioides (Huds.) Mont.
29 Polysiphonia havanensis Mont.
28 P. pulvinata J. Ag.
27 P. elongata (Huds.) Harvey
26 P. fernandesiana P. Reis
25 Pterosiphonia complanata (Clemen.) Falken.
24 Cryptopleura ramosa (Huds.) Kylin ex Newton
23 Hypoglossum woodwardii Kiitz.
22 Callitamnion tetragonum (Withering.) C. Ag.
21 Spyridia filamentosa (Wulfen.) C. Ag.
20 Ceramium rubrum (Huds.) C. Ag.
19 C. flabelligerum J. Ag.
18 C. arborescens J. Ag.
17 C. ciliatum (Ellis) Duclus.
16 Lomentaria articulata (Huds.) Lyngb.
15 Gigartina teedii (Roth) Lamour. var. lusitanica M. Rodrigues
14 G. stellata (Stack.) Batters
13 G. acicularis (Wulfen.) Lamour.
12 Chondrus crispus (L.) Lyngb.
11 Gymnogongrus norvegicus (Grun.) J. Ag.
10 G. griffithsiae (Turn.) Martins
9 Gracilaria vieirae P. Reis
8 G. verrucosa Papenfus.
7 Catenella repens (Lightf.) Batters
6 Gelidium sesquipedale (Turner) Thuret
5 G. corneum Lamour. var. corneum
4 G. pusillum (Stackh.) Le Jolis
3 G. pulchellum (Turn.) Kütz.
2 Rhodochorton purpureum (Lightf.) Rosen.
1 Compsopogon lusitanicus P. Reis



Bol. Soc. Brot, Sér. 2, 53(2): 1437-1458 (1981).

INTROGRESSION IN WEST AFRICAN ORCHIDS OF THE GENUS *EULOPHIA*

by

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SUMMARY

Eulophia cristata Stead, and *E. millsoni* Summerhayes are two commonly occurring- ground orchids in west Africa. They differ in their ecological preferences, but on the coastal plains of Ghana, which have been considerably distributed through shifting peasant agriculture, both species intermingle and hybrid swarms occur. These species differ markedly in such characters as flower colour **and** morphology, number of flowers in the spike, and height of the inflorescence. Using these characters, hybrid indices and pictorial scatter diagrams have been produced for several populations to demonstrate the extent of introgression. It is a noteworthy feature of these populations that introgression is unilateral, for though backerosses with the *E. millsoni* parent abound, none involving the *E. cristata* parent hove been encountered.

INTRODUCTION

THE genus *Eulophia* consists of large terrestrial orchids. * In West Africa it contains 34 species (SUMMERHAYES in HEPPER, 1968). Most of these have large, colourful and often very beautiful flowers. They differ considerably from each other in details of flower structure and colour and do not, in West Africa, form a taxonomically critical genus of closely allied species, though in southern Africa several species complexes occur (HALL, 1965). Hence the discovery in 1956 of hybridization and apparent introgression between two of the commonly occurring species on the coastal plains

of Ghana was unexpected. The two species concerned (see Plate 1) are the pink and purple-flowered *E. cristata* (Sw.) Steud. **and** *E. millsoni* (Rolfe) Summerhayes which has cream coloured flowers with an orange tip to the spur. A subsequent examination of the literature and of herbarium specimens revealed that similar hybrids, between these species, had been collected by earlier workers and described as separate species. Following the discovery of the hybrids in 1958, a survey of the populations of these orchids on the coastal plains of Ghana revealed the occurrence of introgression in most of the populations. A detailed study of these populations was undertaken and demonstrated an unusual situation in which introgression is occurring in one direction only, from *E. cristata* into *E. millsoni.* Introgression is affecting this latter species in a variety of ways, including ecological adaptation, time of flowering and general morphology. The significance of these effects is considered in relation to the present day distribution of this species and its future evolution.

DISTRIBUTION

Both *E. cristata* and *E. millsoni* have a wide distribution in the savanna regions of tropical Africa. The range of *E. cristata* extends across West Africa from Senegal to the Cameroons, south into Zaire and east into Uganda, the Sudan and Ethiopia. *E. millsoni* occurs from the Ivory Coast to the Cameroons, east into Uganda and the Sudan, and from there south to Zimbabwi and Mozambique.

Location of hybrid populations

All the hybrid populations which I have encountered occur on the coastal plains of Ghana (the Accra Plains). Three such population were studied in detail. Also, populations located where only one of the species is present were examined for purposes of comparison with the hybrid populations. The three populations in which introgression was studied are situated in the coastal grasslands of Ghana:

1) *The Nsawam road colony* is situated about 750 m. west of the main road from Accra to Nsawam, about 16 km. from Accra.

2) *The Adantan colony* is situated on the Accra to Aburi road on the east side about 400 m. south of the village of Adantan, in a shallow valley on the Accra side of the village.

3) *The Aboadi colony* is situated around the lagoon at the back of the sandbar on the Sekondi side of Aboadi.

The first two colonies are only a few kilometres apart in the continuous grassland area of the Accra Plains, whilst the Aboadi colony occupies a small enclave of grassland about 190 km. to the west.

Ecology

Both species grow in the savanna regions of Africa but they occur in quite distinct habitats. *E. cristata* grows in well drained soils. In almost all the localities where I have seen it in West Africa it has been growing on latérite. This is the case on the coastal grasslands of Ghana where it is absent from the extensive areas of clay, but abundant over most of the latérite. *E. milsoni*, on the other hand, favours seasonally waterlogged grassland. It grows in valleys and depressions in the latérite areas where alluvial deposits of sand and silt have accumulated, overlying an impervious base. Also, it occurs scattered across the broad expanse of clay soils on the coastal plains of Ghana on either side of the Volta River (the Akuse clays). During the rainy season these habitats become waterlogged, but for the remainder of the year they are very dry, resulting in unfavourable conditions for most species of plants. These areas support a short and impoverished grassland which is dominated by the grass *Brachiaria falcifera* (Trin.) Stapf. Also characteristic of these situations are many geophytes including *Anthericum warneckei* Engl., *Murdannia simplex* (Vahl) Brenan, *Crinum ornatum* (Ait.) Bury, *Urginea ensifolia* (Thonn.) Hepper and *Curculigo pilosa* (Schum. & Thonn.) Engl.

Hybrid plants occupy a wide range of habitats intermediate between and including those of the parents. Usually they grow in the *E. millsoni* populations but in the western part of the Accra Plains, and around Winneba and Elmina, the hybrids occur scattered over the rolling grassland. In the Aboadi and Adantan localities the two types of habitat, laterite and alluvial depressions, adjoin and there is little intermediate habitat. In these situations the hybrids occur alongside *E. millsoni* but do not extend onto the laterite where only *E. cristata* grows. During this study F_1 hybrids were only encountered on four occasions, in each case growing in an intermediate type of habitat where a thin overlay of silt covered the laterite. In the Nsawam road colony there is a broad area of transitional habitat on the lower slopes of the hillside where the laterite is covered by silt. *E. millsoni* abounds in the lowlying seasonally waterlogged valley and in the transitional zone on the lower slopes. *E. cristata* is abundant on the laterite and also in this transitional area. Hence the conditions are ideal for the development of a hybrid population. The F_1 plants and considerable numbers of introgressed forms of *E. millsoni* occur in this transitional area.

Reproductive biology

Flowering in these orchids, as with many plants in the savanna regions of Africa, is stimulated by the effects of the annual fires which sweep through the grasslands during the dry season, and by the early rains which fall towards the end of that season. Flowering takes place between late February and May, after the first rains have occurred. The abundance of flowering spikes varies considerably in different years. For instance, 1956 and 1959 were years when flowering occurred in great profusion, whereas the intervening years were very poor with few visible spikes. In the two favourable years *E. cristata* was so abundant that it coloured the landscape in many of the grassland areas of the Accra Plains, and children from nearby villages gathered them by the armful for sale along the roadsides. The reasons for these

major fluctuations in flowering are not known. However, local changes in abundance due to the effects of burning were observed. Areas of grassland which did not burn in a particular year produced very few flowering spikes, whereas adjacent burnt areas produced a much larger number of spikes. Heavy rain is necessary to initiate the development of the dormant underground flowering shoots. On the Accra Plains the time, distribution and amount of the early rains, which occur towards the end of the dry season, is very variable and uncertain. On occasions an early local storm may initiate flowering, sometimes in areas which were not burnt that year. If the storm is followed by another dry spell, late burning will destroy the flowering shoots of these orchids in that year. I frequently observed this to happen on very localized areas of the Accra Plains. However, on these occasions a second flowering sometimes occurred.

These orchids, particularly *E. cristata* and the F_1 hybrids, reproduce rapidly by vegetative means. The rootstock consists of a branched chain of angular tubers about 4 X 3 cm. and 2.5 cm. thick. A new tuber is produced every year at the end of each branch. Hence a clone of plants rapidly develops, occupying a square metre or more of ground. Some idea of the rate of development of these clones can be gauged from observations on an albino plant which was discovered in 1956 in the Nsawan road colony. In this plant the pink and purple pigment was completely lacking from the flowers and inflorescence, so that the flowers were pure white with a green lip. In 1956 there were only three flowering spikes occupying an area about 1/2 m. in diameter. By 1959 there were eight flowering spikes occupying an area about **1.75** m. in diameter.

These orchids are adapted to pollination by large insects. The cap on the column is about 3 mm. broad and requires appreciable pressure to push it off. Each pollen mass on the pollinium is slightly over 1 mm. in diameter, hence, only large insects can bring about pollination. Each flower on the spike remains open for several days [DOCK & PROFITA (1975) give a mean duration of 10-14 days in 25. *cristata]* and each spike lasts for up to three weeks. The flowers of

both species are only slighty scented. During the day the scent is, at most, barely noticable, but after dark *E. cristata* produces a weak, pleasant sweet aroma, whilst that of *E. millsoni,* though still weak, is more pungent and not particularly pleasant. Visits were paid to several of the flowering colonies on the Accra Plains at various times of the day and night in order to collect and observe pollinating insects but remarkably few insect visitors were found. The only species which I observed freely visiting the flowers of both orchids was a large beetle belonging to the *Meloidae — Coryna hermanniae* L. This was often found at rest on the flowers and was frequently eating them. It flies freely in the sun, particularly when the winds are calm. It favours the flowers of *E. millsoni* but was also found on those of *E. cristata.* On two occasions these beetles were observed with pollinia sticking to the back of the thorax. Most of the flowers of *E. millsoni* in which the ovary had been fertilized and was swelling had the column partially eaten by these beetles. There seems little doubt that they are the main pollinating agent of *E. millsoni,* at least on the Accra Plains of Ghana where this orchid sets seed freely, with most of the flowers producing a capsule.

The situation in *E. cristata* is less clear. On the Accra Plains few flowers develop capsules. Many spikes are completely sterile, whilst those that are fertile rarely have more than 1 or 2 capsules, even though the spikes bear between 20 and 30 flowers. Insects were only rarely observed to visit the flowers of this species on the coastal plains and the pollinia and cap in fading flowers were usually intact, indicating that they had not been pollinated. In 1959 this orchid flowered about three weeks earlier than usual (it was in full flower on March 1st) and in great profusion. However, flowering occurred before the *Coryna* beetles emerged and even fewer capsules than usual were formed. Similar observations on this species, made in Guinea savanna localities in Ghana, showed that pollination occurred more frequently and that each spike usually had 5 or more developing capsules.

Since these observations were made, DOCK & PROFITA (1975) have reported on a study of pollination in *E. cristata* on the Accra Plains. They concluded that carpenter bees of the genus *Xylocopa* (especially *X. olivácea)* pollinate this orchid. They were able to observe pollination on four occasions and found that in 31 % of the 203 flowers that they examined pollinia have been removed, presumably by visiting insects. They concluded that the bees were attracted by the colour of the flowers, as no nectar appeared to be produced.

Pollination experiments (see Table 1) carried out on wild plants showed that both self and cross pollinations are sucessful. Also, crosses between the two species could readily be made and produced normal capsules.

TABLE 1

Pollination experiments on *Eulophia* orchids

	No pollination — spikes bagged	Selfed	Cross pollinated	Pollinated with *E. millsoni*	Pollinated with *E. cristata*
E. cristata	6 (0)	10 (10)	16 (16)	4 (4)	
E. millsoni	6 (00	7 (7)	6 (6)		4 (2)

Figures in parenthesis indicate number of resulting capsules.

Germination tests on the seed resulting from these crosses were not carried out. LOCK & PROFITA (1975) obtained similar results. They found that both cross and self pollinatio resulted in a somewhat reduced seed fertility (67 % and 65% in selfed flowers and 96% in cross pollinated flowers).

Chromosome **number**

The karyotype of these two orchids was examined in root tips using low temperature treatment (CHINNAPPA & MORTON, 1978) and in pollinia, using the method described by HALL (1965) — see Table 2. The most suitable stage in which to examine the chromosomes proved to be the metaphase of the pollen grain nucleus leading to the formation

of the generative and tube nuciei. This, of course, gives the gametic chromosome number and the chromosomes are relatively distinct, well spread and of reasonable size. *E. mülsoni* has a gametic number of 21 chromosomes, all very similar in size and shape. *E. cristata,* on the other hand, appears to have a gametic number of 27 chromosomes. Of these, 21 are large and similar to those of *E. millsoni,*

TABLE 2

Chromosome numbers in *Eulophia* orchids

	2n	n	Voucher	Locality
E. cristata	56		s.n. .(JKM)	Legon, Ghana
» »		C.27	s.n. (GC, JKM)	Achimoia to Pokoasi Rd., Ghana
» »		27	SL1234 (SL, K, GC, JKM)	Lunsar junct. to Port Loko, Sierra Leone
E. mülsoni		21	s.n. (GC, JKM)	Accra to Elmina Rd., Ghana
» »		21	GC 38109 (GC)	Ayikuma near Dodawa, Ghana

whereas 6 are smaller in size. Many nuclei of *E. cristata* were not easy to interpret and in several there was a possibility of there being more than 6 small chromosomes, giving a gametic number in excess of 27. Whether these small chromosomes are supernumerary of *ß* chromosomes is not clear at this stage. Root tips proved difficult to work with, but an excellent mitotic plate of *E. cristata* showed 56 chromosomes. However, ar-RUSHDI (1971) reports a sporyphytic chromosome number of 46 in this species in Nigeria. This is the only published chromosome count for either of these species. HALL (1965) gives chromosome numbers for 21 species of *Eúlophia* from southern Africa. In these the gametic numbers range from 20 to 60 with 21 and 27 the most commonly occurring numbers. Clearly, further work is required on the cytology of *E. cristata.* No information on the cytology of the hybrids is available.

Populations analysis

a) *Sampling.* The main purpose of this investigation was to demonstrate that introgression is occurring, and the direction in which gene flow is taking place. A random sampling of the orchid population, across a large tract of these coastal grasslands, would have been of little value because of the great difference in abundance of the two species and their hybrids. *E. cristata* vastly out numbers both *E. millsoni* and its hybrids, which are usually confined to restricted areas of suitable habitat. Also, only 4 small groups of F_1 hybrids were encountered. Hence, sampling was limited to areas occupied by hybrid populations and all individuals flowering that particular year (1956) were scored in the three populations selected for study.

b) *Scoring.* As these two orchids differ considerably in the colour of their flowers (see Plate 1), this provides a very suitable and convenient method of assessing hybridity. *E. millsoni* has entirely cream flowers, apart from the tip of the spur which is bright orange. No trace of purple or green colouration has been observed in the flowers of plants growing in pure colonies of this species. In hybrid plants the flowers frequently have purple veins on the lip, are pink or purple flushed, or have a pronounced greenish colouration. The two species differ in many other ways besides flower colour and several morphological characters were also scored. A condition normal to *E. millsoni* was scored as 0, and to *E. cristata* as 2; any intermediate stage as 1. The normal condition in each species was determined after examination of pure colonies from several localities remote from areas of hybridization. The following 10 characters were scored, and used both in the construction of the hybrid index and pictorial and scatter diagrams.

1) Height of inflorescence from ground to tip. 0=below 68 cm. 1 = 66 to 90 cm. 2 = over 90 cm.
2) Number of flowers in the inflorescence. 0 = below 9. 1 = 9 to 16. 2 = over 16.
3) Angle of lateral sepals to the ovary. 0 = over 120°. 1 = 46° to 120°. 2 = under 46°.

4) Angle of lateral lobes of the lip to the horizontal. 0 = below 91°. 1 = 91° to 110°. 2 = over 110°.
5) Colour of perianth wings. 0 = cream. 1 = cream tinged with pink to salmon pink. 2 = pure pink.
6) Colour of lateral lobes of the lip. 0 = cream. 1 — a pronounced greenish tinge or traces of pink or mauve. 2 = olive.
7) Colour of spur. 0 = bright orange. 2 = pink. 1 — a pronounced greenish colouration with the reduction of absence of the orange tip, or a pink colouration superimposed on the normal *E. millsoni* condition.
8) Ground colour of lip. 0 = cream. 2 = pale purple. 1 = cream with a pink or mauve tinge, to bright salmon coloured.
9) Colour of veins of lip. 0 = cream. 2 — deep purple. 1 = pale purple to mauve tinged ,or a pronounced greenish colouration.
10) Height of veins (calli) at base of the lip. 0 = under 0.7 mm. 1 = 0.7 to 1.4 mm. 2 = 1.5 mm. and over.

Analysis of results

The two methods which have been used to analyze the data are hybrid indices and pictorial scatter diagrams. These are simple to produce and provide an effective means of portraying the results.

The Hybrid Index of a plant consists of the sum of the values for the 10 characteristics which were scored. Thus *E. millsoni* normally has a value of 0 and *E. cristata* of 20. The hybrid indices have been plotted as histograms (Figs. 2-4) which show their frequency in each of the three populations. Colonies of each of the species were examined in areas where only one occurred and where no signs of hybridization were apparent. In these colonies *E. cristata* had an index within the range 19-20 with 87 % of the plants scoring 20. The colonies were situated near Nsawam on the coastal plains, near Ajena and Gambaga in the Guinea savanna regions of Ghana, and near Port Loko in Sierra Leone. Colonies of *E. millsoni* were examined near Dawa

and north of Adidome on the coastal plans of Ghana. The plants all had an index of 0 or 1 with 78 % of them scoring 0.

In the Pictorial Scatter Diagrams (Figs. 5-7) the characters used for the two axes are number of flowers in the inflorescence for the horizontal axis, and the heigh of the

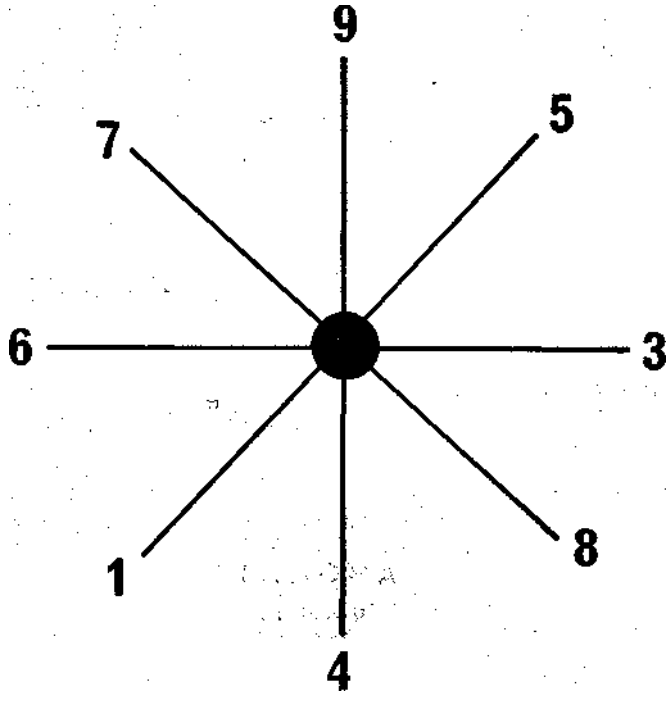

Fig. 1. — Symbol used in Pictorial Scatter Diagrams. For explanation see text.

veins at the base of the lip for the vertical axis. Each plant is represented by a dot, to which lines are attached to indicate the presence of the remaining characters. Absence of a line indicates an *E. milssoni* character, a short line an intermediate character, and a full length line an *E. cristata* craracter. The significance of each of the lines used in the symbols is shown in Figure 1 in which the numbers refer to the list of characters scored (see above).

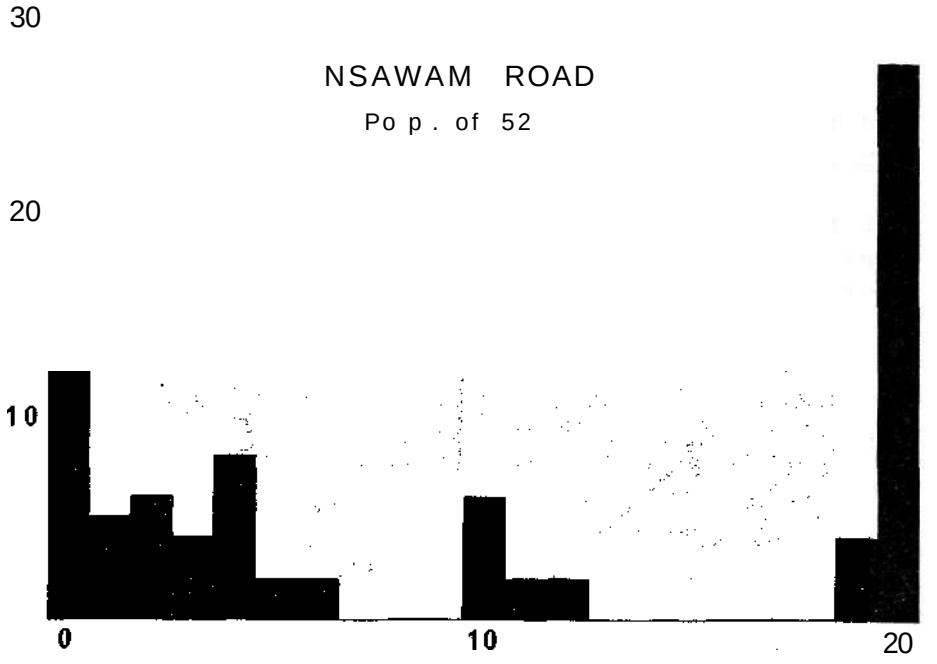

Fig-. 2. — Histogram of Hybrid Indices. The Nsawam Road population. Hybrid indices on horizontal axis, percentage occurrence on vertical axis.

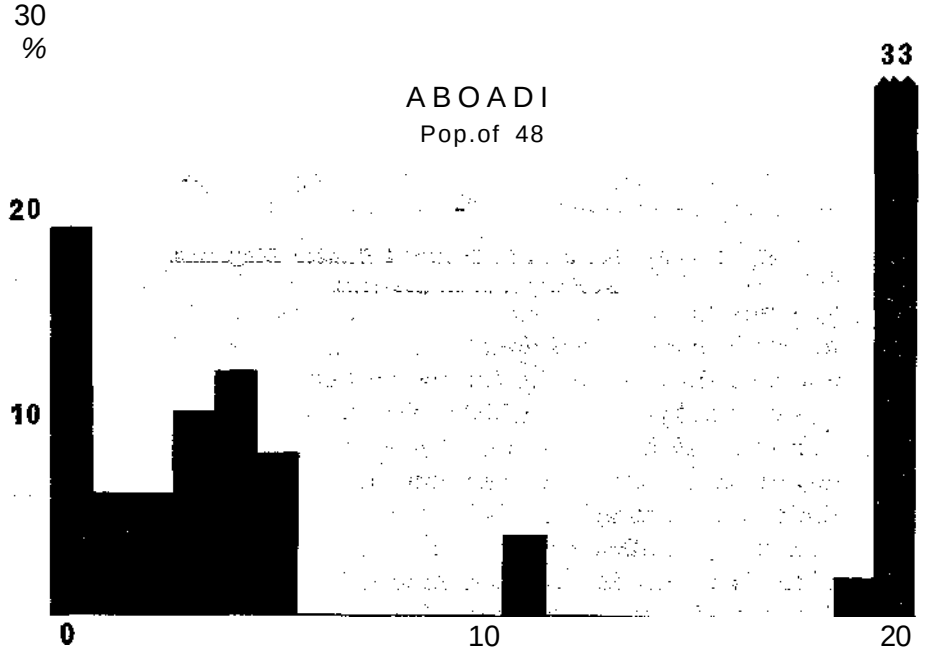

Fig-. 3. — Histogram of Hybrid Indices. The Aboadi population. Hybrid indices on horizontal axis, percentage occurrence on vertical axis.

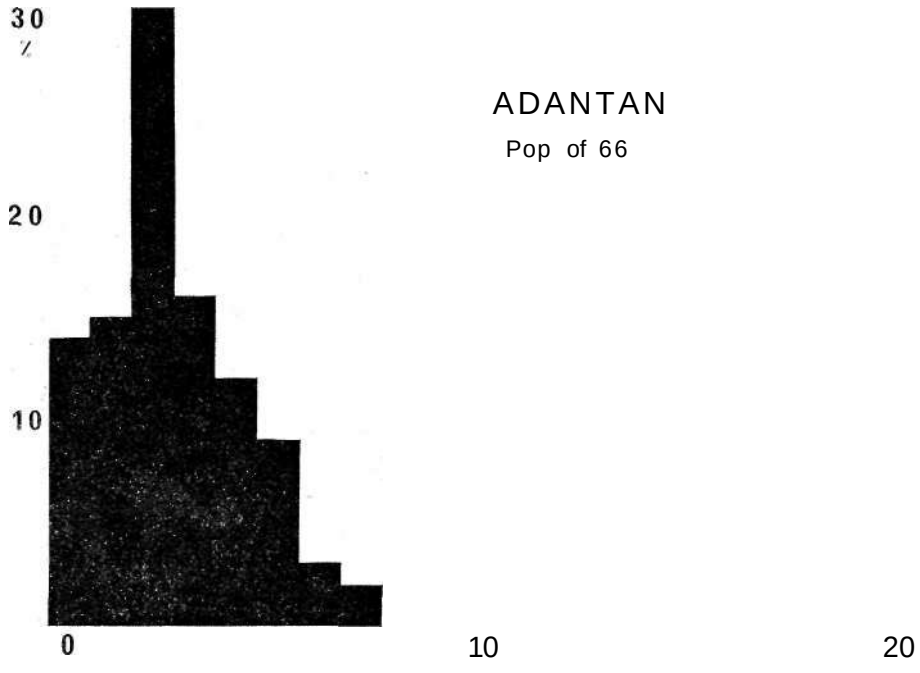

Fig. 4. — Histogram of Hybrid Indices. The Adantan population. Hybrid indices on horizontal axis, percentage occurrence on vertical axis.

DISCUSSION

These *Eulophia* hybrids provide an interesting example of unilateral introgression in which gene flow is in one direction only, leaving the donar species *(E. cristata)* unaffected (see Figs. 2-7). In the Aboadi and Nsawam road (Figs. 3,6 and 2,5 respectively) populations both species are present together with F_1 hybrids. The flow of *E. cristata* genes into *E. millsoni* is apparent. The Adantan population (Figs. 4 & 7) consists of introgressed forms of *E. millsoni* without either F_1 hybrids or the *E. cristata* parent. However, this species is present in the colony but was not flowering during the year when the population was sampled. Several flowering spikes were noted in a subsequent year.

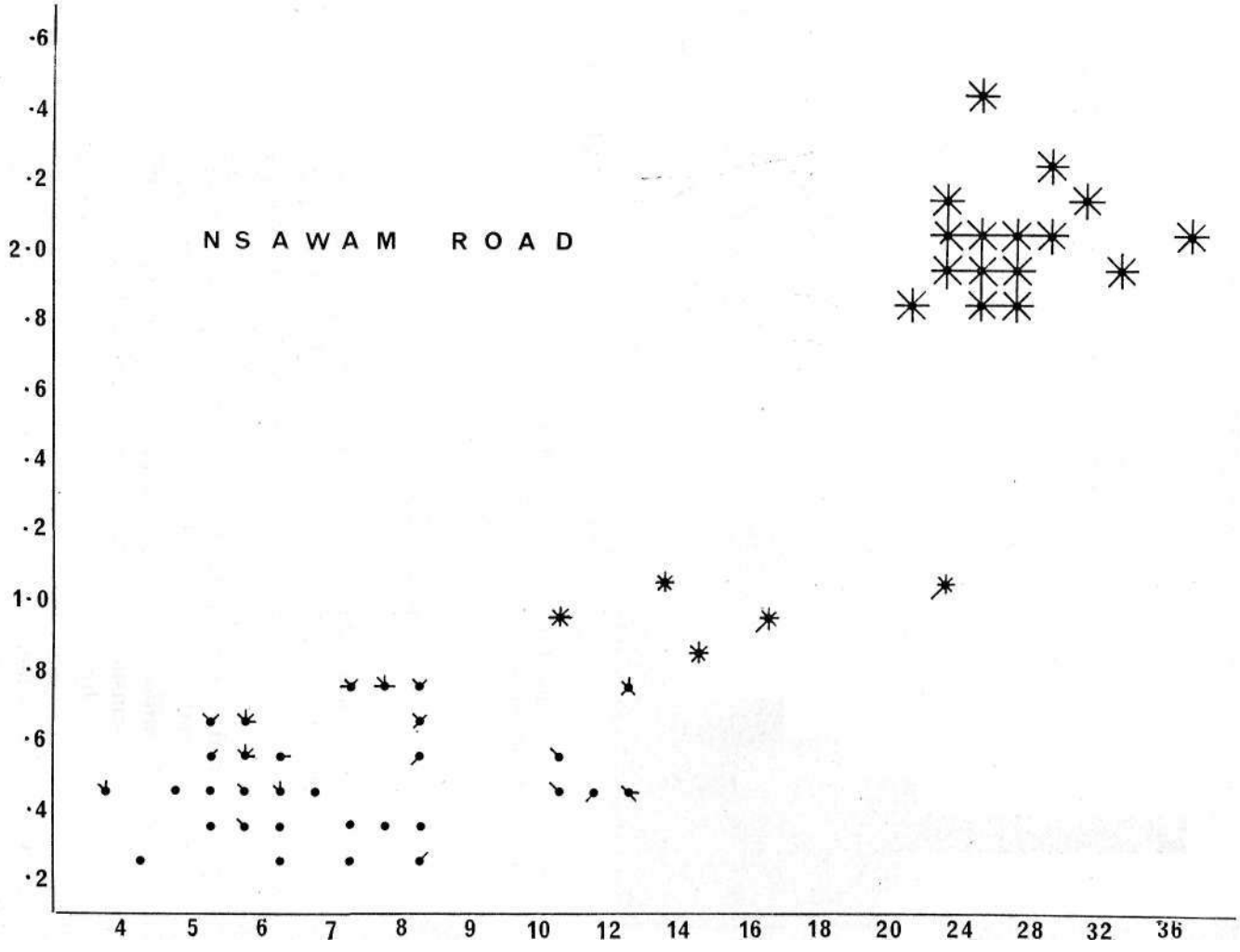

Fig. 5. — Pictorial Scatter Diagram. The Nsawam Road population.
Horizontal axis represents number of flowers in the spike. Vertical axis represents the height of the veins (calli) at the base of the lip petal.

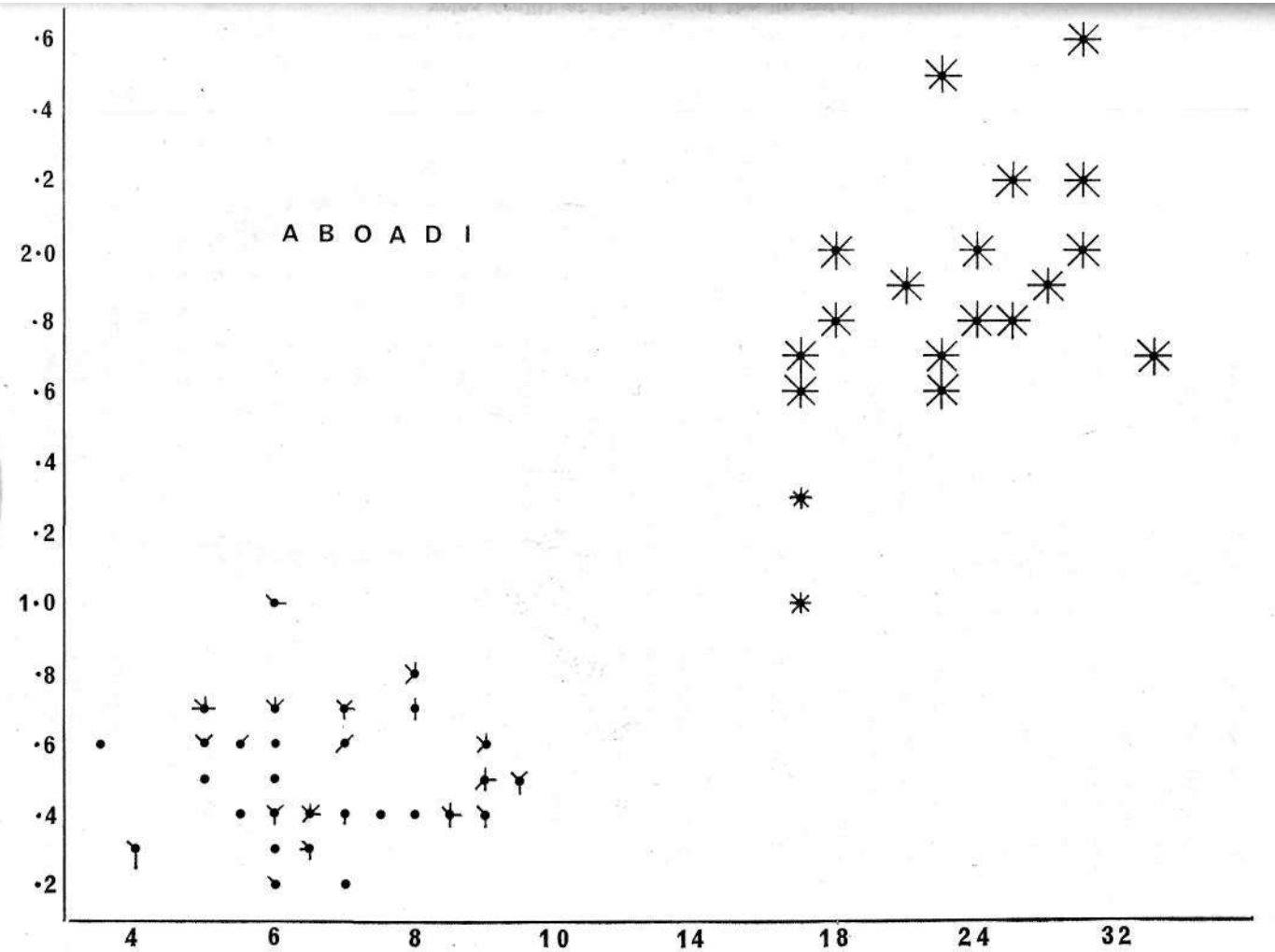

Fig. 6. — Pictorial Scatter Diagram. The Aboadi population.
Horizontal axis represents number of flowers in the spike. Vertical axis represents the height of the veins (calli) at the base of the lip petal.

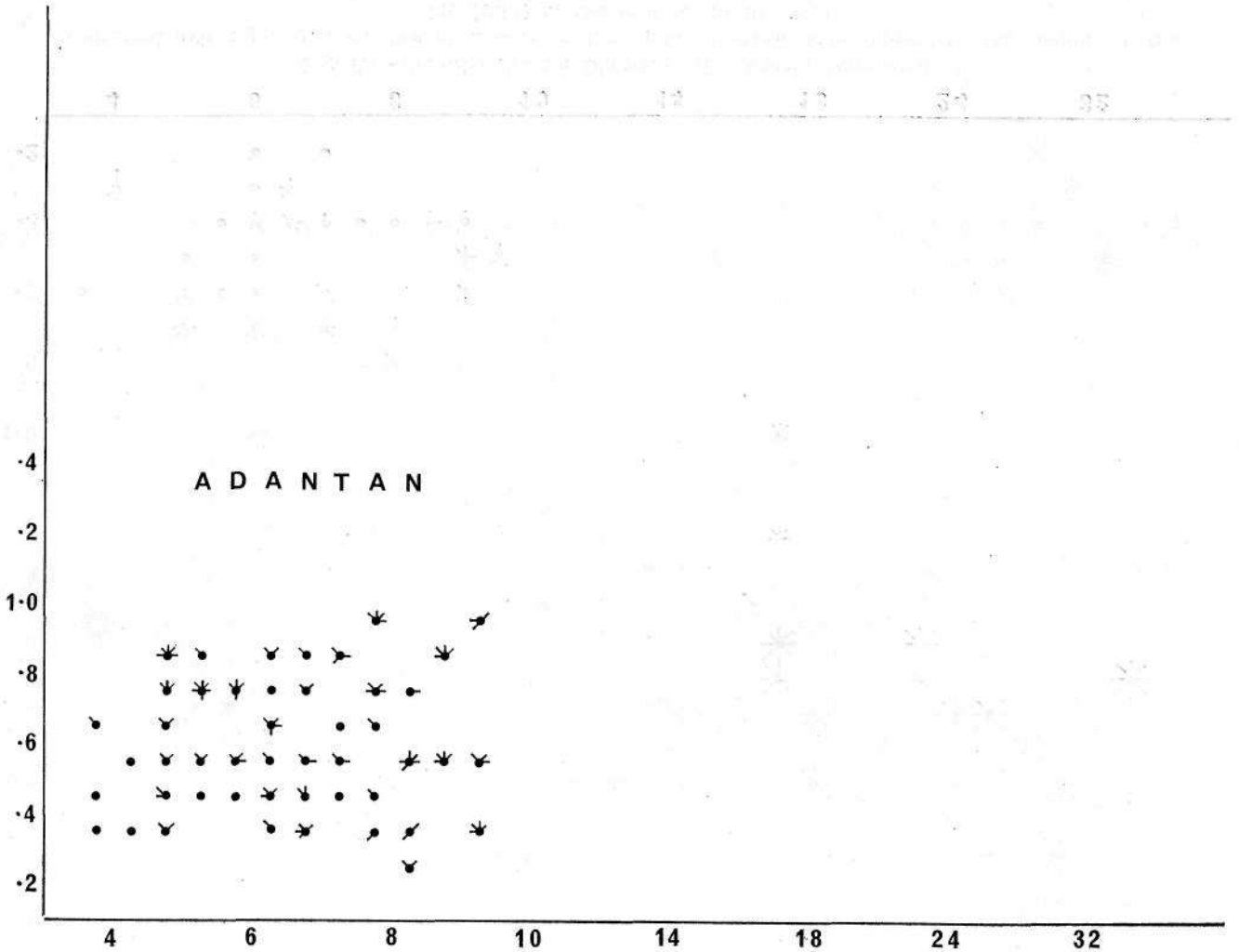

Fig-. 7. — Pictorial Scatter Diagram. The Adantan population.
Horizontal axis represents number of flowers in the spike. Vertical axis represents the height of the veins (calli) at the base of the lip petal.

The reason for gene flow occurring in one direction only in these orchids is not known but may be due to several factors, including the low indicence of pollination in *E. cristata* and failure of the F_2 generation or of backcrosses with *E. cristata.* F_1 hybrids are of very rare occurrence. During 15 years of field work in West Africa I only encountered them in four localities — all on the coastal grasslands of Ghana. In each case only a single plant was involved, which had developed colonially into a group of tubers. As these orchids are perennial and reproduce vegetatively it is apparent that the initial crosses between the species very rarely succeed. The incidence of pollination in *E. cristata* on these coastal grasslands has been observed to be low, hence the chances of backcrossing with that parent are reduced. However, the rate of pollination in *E. millsoni* flowers is high, hence backcrossing in that direction will be favoured. Also, in the F_1 hybrid, the flowers are intermediate in colour and structure and may not be as attractive to the pollen vector of either species. In that event selfing would rarely take place and the level of backcrossing would be further reduced. A similar situation, in which introgression is in the direction of only one of the parent species, has been reported by Lee (1975) in hybrids between *Typha angustifolm* and *T. latifolia* in the Great Lakes region of North America.

The cause **of introgression**

The occurence of introgression is frequently associated with disturbances in the habitat of the species concerned, which cause changes in their distribution. Such is the situation in these *Eulophia* orchids. The coastal plains of Ghana have been subject, during the past century, to increasing pressures from human activity which have resulted in the destruction of extensive areas of thicket and the expansion and infiltration of the grasslands into the areas of thicket and forest. These grasslands can be divided into three types: a) those on clay soils — Akuse clay, b) those on the latérite, and c) those occurring on and immediately

behind the coastal strand and around lagoons. The first and the last of these types represent natural climaxes due to adaphic and climatic factors. Most of the grassland on the laterite, however, appears to be derived from the breakdown of more or less continuous vegetation of thicket and dry forest. The soil in this area is suitable for village farming in which the shifting pattern of agriculture, involving «slash and burn» methods of cultivation, has destroyed large areas of natural vegetation and favoured the development of grassland. When these farms are abandoned, due to declining soil fertility, they are rapidly colonized by grass. This burns annually as a result of both natural and man made fires. Regeneration of the thicket and forest is slowed down by the improverished soil, and in many areas it is completely prevented by the annual fires which sweep across the plains and frequently encroach on the remaining areas of thicket and forest. In years of severe burning the rate of encroachment can be considerable. 1959 was one such year. In the Nsawam road population the edge of the thicket was marked by metal stakes at the beginning of this study in 1956. No change had occurred between then and 1959, but in that year the thicket was driven back 84 m. in the area facing the severe wind-driven fires. Several thicket clumps were completely destroyed and others were considerably reduced in size.

Neither of these orchids can grow in areas of continuous thicket and forest. They require grassland or grassy enclaves. *E. cristata* is abundant in the grasslands on the laterite, especially in areas recently derived from thicket, but it is rare or absent from large areas on the clay soils, except at their northern tip where this species is again abundant. In this area the grasslands of the Accra Plains are now in contact with those of the Guinea savanna through the «Volta Gap» where the Volta River breaches the forested range of hills separating the Guinea savanna of the interior from the coastal grassland. This area of forest was only about 20 km. wide, but extensive felling associated with farming and construction of the Akosombo Dam have destroyed much of it and permitted the migration of Guinea savanna species onto the coastal plains. *E. cristata* occurs in the

Guinea savanna. The origin of the extensive colonies of this orchid on the coastal plains appears to be by migration from the Guinea savanna through the Volta Gap. This occurred when suitable habitats were created by the destruction of thicket and forest and the development of farms. *E. cristata* is also abundant in small areas of grassland at the back of the coastal strand and around lagoons. Several of these colonies, e. g. at Senya Beraku, Winneba, Elmina and Aboadi, are in isolated grassy enclaves separated by many kilometres of thicket and forest from the grassland of the Accra Plains. Hence it is probable that they are of different and older origin. However ,the low incidence of pollination in this species throughout the coastal plains, suggests that it is a recent arrival in an area where its pollinator does not occur.

Pure colonies of *E. millsoni* are confined to the grassland on the clay soils. This species also occurs along the coast in seasonally flooded areas behind the strand. However, *E. cristata* also grows in these locations and all the colonies show signs of introgression. *E. millsoni* appears to have followed the recession of the thicket and forest on the laterite soil. Again, most of these colonies show evidence of hybridisation. Hybrid colonies occur on the coastal grasslands wherever the two species come into close contact. This occurs throughout the grassland areas on the latérite, in the enclaves of grassland behind the coast, and around the edge of the grassland on the clay.

The significance of introgression

Introgression may have a significant influence on future evolution in these orchids. As long as there is a barrier to the formation of backcrosses with the *E. cristata* parent, this species will not be affected, but certain effects on the *E. millsoni* populations are already apparent. The introgression of *E. cristata* characters into *E. millsoni* has produced changes in morphology, flower colour, ecological adaptation and time of flowering. It is difficult to asses the selective value of changes in morphology and flower

colour, but the effects of other changes are more apparent. Introgressed forms of *E. millsoni* are frequently encountered away from the lowlying seasonally marshy land to which this species is normally confined. On the undulating grasslands which lie inland from Winneba, this species and its hybrids occur scattered over the whole area. Similarly, they are to be found on the higher ground of the Accra Plains around Achimota and Legon. Hence it is apparent that introgression has enabled *E. millsoni* to extend its area either by an increase in ecological tolerance or by a change in competitive efficiency.

Though both these species of *Eulophia* flower at approximately the same time of year, many of the hybrids come into flower several weeks later and are frequently only just opening when the remainder of the colony is past its peak flowering period. The selective importance of this may be considerable in an area subject to annual burning. These orchids flower soon after the annual fires which sweep the plains, and on a number of occasions I have observed flowering colonies badly damaged by late burning. Hence a delay in the time of flowering prevents damage from fires and increases reproductive success.

Nomenclature

The name *E. flavopurpurea* (Rchb. f.) Rolfe has replaced *E. millsoni* as an earlier homonym in recent literature (SUMMERHAYES in HEPPER, 1968). However, it is apparent both from the name and the type description of *E. flavopurpurea* that it is a hybrid between *E. millsoni* and *E. cristata*, hence the name *E. millsoni* should be retained for this species. The following is a partial synomy of *E. millsoni* and its hybrids with *E. cristata.*

E. **millsoni** (Rolfe) Summerhayes in Flora of West Tropical Africa ed. I. 2: 446 (1936). *Lissochilus millsoni* Rolfe in Flora of Tropical Africa 7: 79 (1897) —Type Millson 86 (K) from Ilorin in Nigeria (Rowland s. n. from llorín, in Herb. Kew and cited by Rolfe under this species is

a hybrid with *E. cristata). L. lacteus* Kraenzl. in Engler Bot. Jahrb. 43: (1908)—type Beauman 24 (K) from Misahoe in Togo.

E. cristata X E. **millsoni.** *E. flavopurpurea* (Rchb. f.) Rolfe in Flora of Tropical Africa 7: 65 (1897). *Gyrtopera flavopurpurea* Reichenbach in Beit. Syst. Pflanz. 5: 68 (1871) — type Schweinfurthe 3546 (sketch in K) from the Sudan — *«.Labellum dilute purpureostriatum, calcor apice flavimn, petala etc. flavidovirescentia». Lissochilus johnsoni* Rolfe in Kew Bull. 1910: 160 — type Johnson 854 (K) from the Accra Plains of Ghana appears to be the *F1* hybrid. *L. andersoni* Rolfe 1. c. 159 (1910) •—)type Anderson from Aburi in Ghana — this plant is illustrated in Bot. Mag. t. 8470. The illustration shows mauve stripes on the lip and greenish sepals and petals — clearly one of the backcrosses or segregates verging towards the *E. mittsoni* parent.

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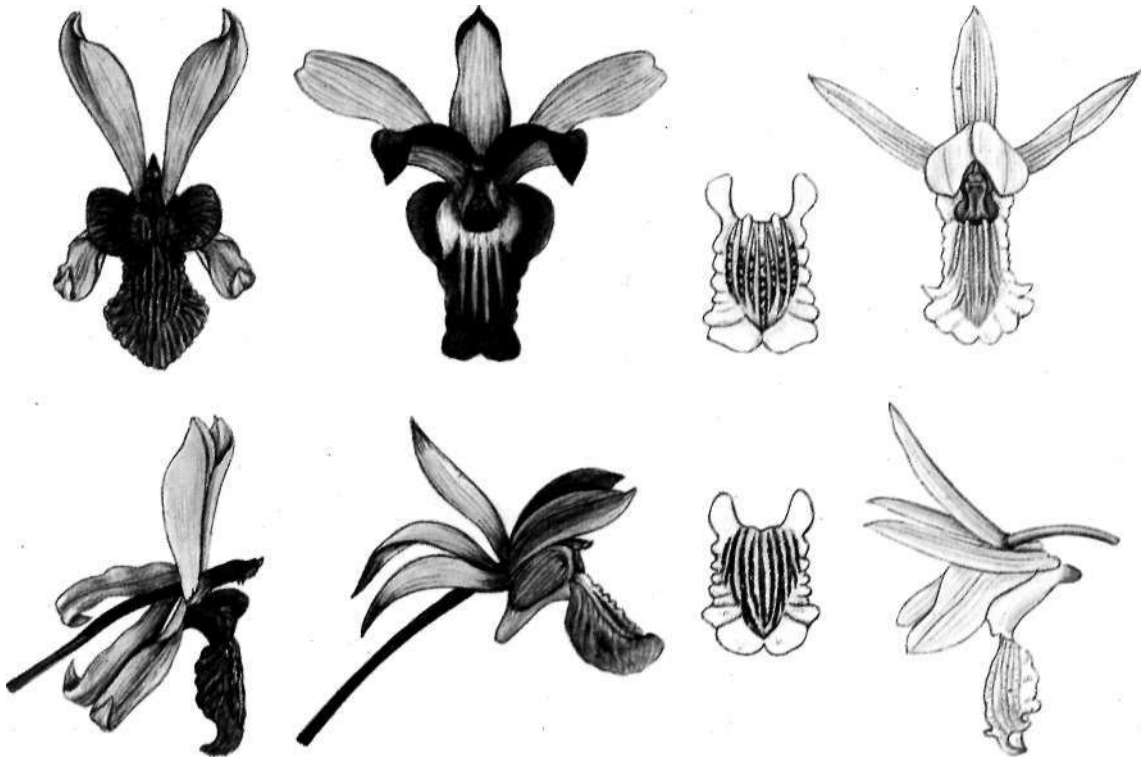

Flowers *of Eulophia.*

Left to right: *E. cristata, E. cristata* X *E. millsoni F1* hybrid, lip petals of segregates, *E. millsoni.* All X 2.

Bol. Soc. Brot., Sér. 2, 53 (2): 1459-1462 (1981).

POLYGALA FERNANDESIANA (POLYGALACEAE), A NEW SPECIES FROM TROPICAL AFRICA

by

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WHILE studying *Polygala capillaris* complex, 'which is widespread throughout tropical Africa and South Africa and also known from Madagascar and Comores Islands, I was able to isolate a new species which I name in honour of Prof. ABÍLIO FERNANDES. It can easily be recognised and appears to occur mainly in Central Africa.

The new species has been included so far in *P. micrantha* Perr. & Guill., which is a Central-Western African species. It is clearly distinguished from this species by having stipitate-glandular hairs. Moreover it has densely leaved stems, and 2-2.5 X 0.9-1.2 mm, ovate-elliptic wings which hide the stipitate capsule, whereas *P. micrantha* has sessile-glandular hairs, a sparsely leaved stem and 1.25-2 X 0.75-1 mm, narrowly obovate to spathulate wings, which are shorter or almost hiding the sessile-capsule.

P. *fernandesiana* is the only species of the complex with stipitate-glandular hairs, and it does not occur near the coast, whereas P. *micrantha* and the other six (excl. P. *africana* Chod. which has seeds with glochidiate hairs) species of the complex are frequently found in coastal regions. P. *sansi^ barensis* Gurke, for example, occupies a restricted area (East coast of Kenya and Tanzania and the North of Mozambique) along the coast.

Polygala fernandeslana J. Paiva, sp. nov.

Herba annua, caule erecto, ramoso, gracili, generaliter-4-angulato, 10-25 cm alto, pilis stipitato-glandulosis instructo. Folia alterna, breviter petiolata, petiolo c. 0.5 mm longo, glabro vel sparse stipitato-glanduloso piloso; lamina linearis vel lineari-lanceolata, 5-15 X 0.5-1.25 mm, ápice acuta, interdum reeurvata, basi cuneata, glabra, costa supra impressa, subtus conspicua. Flores lilacinei vel rosei, pedicellati, pedicellis c. 0.8 mm longis, glabris, in racemos terminales, raro laterales, rhachidi striata, glabra, 2.5-10 cm longa (pedúnculo 5-16 mm longo, semper $^{1/5-1/6}$*RACEMI* tanquam longo, stipitato-glanduloso piloso) dispositi; bractea lanceolato-linearis, c. 1 mm longa, glabra, caduca; bracteolae lineares, c. 0.5 mm longae, glabrae, caducae. Sepalum posterius lineare, 1.25-1.5 mm longum, glabrum; alae ovato-ellipticae, 2-2.5 X 0.9-1,2 mm, glabrae, capsulam complete opertentes (generaliter quam capsula duplo longiores); sépala anteriora libera, linearia, 0.75-1 mm longa, glabra. Pétala superiora obovata, 1.5-1.75 X 0.5 mm, carinam aequantia aut longiora; carina 1.5 X 0.5 mm, cristata, crista c. 0.5 mm longa. Stamina 8, parte libera filamentorum 0.2 mm longa, tubo 0.4-0.5 mm longo, glabro; antherae 0.1-0.2 mm longae. Ovarium aplanato-ellipsoideum, 0.3 X 0.25 mm; stylus 0.1-0.2 mm longus, ramo anteriore stigmatifero. Capsula 1.25-1.5 X 0.75 mm, subgloboso-ellipsoidea, stipitata, glabra. Semina ovoidea vel ellipsoidea, 0.75-0.8 X 0.4 mm, breviter pubescentia, ecarunculata.

Habitat in Africa Centrali (in regionibus vulgo dictis Chad, Nigeria, Cameroon, Central African Republic).

Typus: Cameroon, prope Nagaou Ndéré Plateau, 20.IX. 1967, *Jacques-Félix* 82220 (P, holotypus; YA, isotypus).

Affinis *P. micranthae* a qua caulibus pilis stipitato-glandulosis instructis et floribus majoribus (alae 2-2.5 X 0.9-1.2 mm nee 1.25-2 X 0.75-1 mm) praecipue differt.

Species in honorem praeclarissimi Prof. Dr. Abilii Fernandis, Botanicae eximii cultoris, dicata.

Polygala fernandesiana J. Paiva

Annual densely leafy herb, 10-25 cm tall, often much branched from the base; stems slender, 4-angled and covered with stipitate-glandular hairs. Leaves petiolate (petiole c. 0.5 mm long, glabrous or with slightly stipitate-glandular hairs); lamina 5.15 X 0.5-1.25 mm, linear-lanceolate, acute at the apex, cunéate at the base, sometimes arcuate, glabrous. Flowers pink or lilac, rarely whitish, arranged in terminal (occasionally some lateral) racemes, 2.5-10 cm long, with a glabrous, striate rachis (peduncle 5-16 mm long, always $^{1}/_{5}$-$^{1}/_{6}$ as long as the racemes, stipitate-glandular hairy), early caducous bracts (1 mm long, lanceolate-linear, glabrous) and bracteoles (0.5 mm long, linear, glabrous); pedicels (c. 0.8 mm long, glabrous. Posterior sepal 1.25-1.5 mm long, linear, glabrous; wing-sepals 2-2.5 X 0.9-1.2 mm, ovate-elliptic, glabrous and completely hiding the capsule (usually twice longer than the capsule); anterior sepals 0.75-1 mm long, linear, glabrous, free. Upper petals 1.5-1.75 X 0.5 mm, obovate, longer or as long as the carina; carina 1.5 X 0.5 mm, crest c. 0.5 mm long. Stamens 8, free part of filaments 0.2 mm long, staminal tube 0.4-0.5 mm long, glabrous; anthers 0.1-0.2 mm long. Ovary flattened-ellipsoid, 0.3 X 0.5 mm; style 0.1-0.2 mm long, anterior branch stigmatic. Capsule 1.25-1.5X0.75 mm, subglobose-ellipsoid, stipitate, glabrous, neither winged nor emarginate. Seeds 0.75-0.8 X 0.4 mm, ovoid-ellipsoid, rugose, with white or brownish short hairs, not carunculate.

CHAD: Folle, Mussopys, 13.111.1964, *Andru* 1199 (K); Lake Chad, IX.1902, *Chevalier* 5391 (P); Lake Chad, Mboukou, 24.IX.1902, *Chevalier* 5527 (K; p); Lake Chad, between Kenio and Tomi, 22.IX.1902, *Chevalier* 5693 (P); Lake Chad, Ungourras Plateau, 13.IV.1902, *Chevalier* 6116 (K; p); Lake Chad, Ungourras Plateau, 14.XI.1902, *Chevalier* 6117 (p); Lake Chad, IX.1957, *Koechlin* 4709 (P).

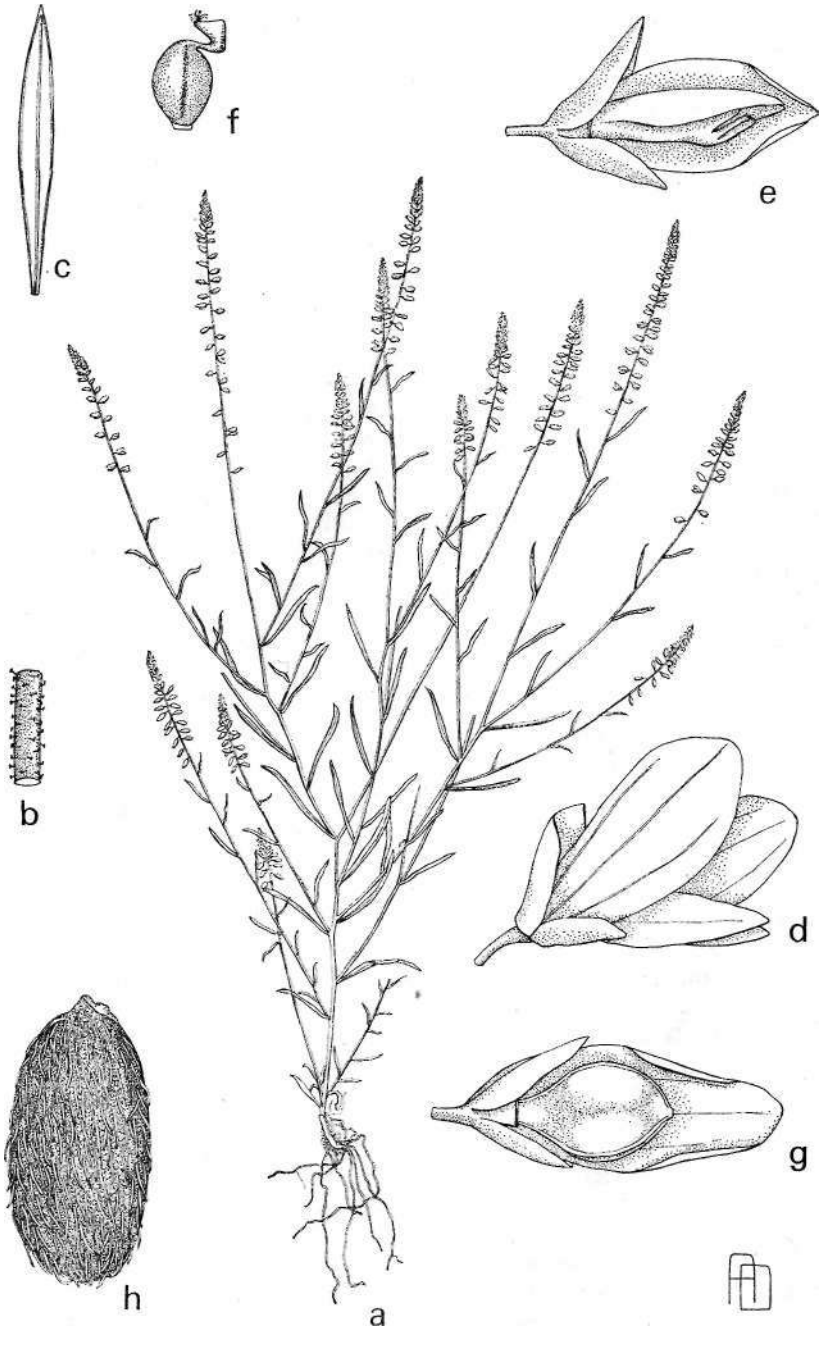
NIGERIA: Northern Region, Sardauna Prov., Mambilla Plateau, 4.VHI.1973, *Chapman* 52 (K); Adamawa Prov.,

Mambilla Plateau, Dorofi, VI.1958, *Chapman* 65 (K; P); Southern Region, Bamenda, N. of Bum, *Maitland* 1397 (K).

CAMEROON : Mango Distr., 7 km S. of Poli, 29.VII.1974, *Fotius* 2209 (P; YA); M. of Nagaou Ndéré Plateau, 20.IX.1987, *Jacques-Félix* 8220 (P; YA); Mbepit Distr., near Foimbot, *Letoiizey* 1811 (p); Maka, 40 km N. of Tibati, 21.LX.1963, *Letouzey* 5874 (K; P; YA); Grand YoIi,: near,, Mayo Darlé, 19.VI.1967, *Letcmzey* 8665 (P; YA); Massif du Mbepit, 9.VIII. 1932, *Letouzey* 11611 (P; YA);. W. loe. *Miläbraed* 10161 (P); 15 km N. of Bouat Laterita, VIII.1966, *Lebrun* 14162 (P); Mt. Ngamba, E. of Ngaou Ndéré, *Piot* 38 (P); Njen Tibati, 6.IX.1914, *Tessmann* 2723 (K).

CENTRAL AFRICAN REPUBLIC: Yalinga, 4.VIII.1921, *Le Testu* 2998 (BM; p); Zako, 18.XI.1922, *Le Testu* 4343 (BM; P); Waka, Kâga Mbigo, 60 km N. of Bambari, 13.LX.1922, *Tisseront* 56 (KM; P).

I am very grateful to Prof. A. FERNANDES, Mme. R. FERNANDES and Dr. E. LAUNERT, who have corrected the Latin and English languages. .



Polygala fernandesiana J. Paiva

a) habit (X^1A); b) part of branch (X 7.5); c) leaf (X 2); d) flower (X 10); e) flower with upper petal removed (X 10); f) ovary (X 10); g) developing fruit (X 12); h) seed (X 30). All from *Fotius* 2209 (P).

ÍNDICE

INSTRUÇÕES AOS COLABORADORES

1. O *Boletim da Sociedade Broteriana* é uma revista destinada à publicação de artigos originais em todos os domínios da Botânica. No entanto, artigos muito extensos sobre florística, fitogeografia e fitossociologia são publicados geralmente nas *Memórias*, enquanto que os trabalhos de divulgação científica e os referentes à história da Botânica são reservados para o *Anuário* — as duas outras revistas da Sociedade.

2. Destinado principalmente à publicação dos artigos elaborados pelo pessoal científico do Instituto Botânico de Coimbra, nele se inserem todavia trabalhos da autoria de membros da Sociedade, bem como os de outros investigadores, quer portugueses, quer de outras nacionalidades. A publicação de qualquer artigo, porém, está na dependência da aprovação da Comissão Redactorial.

3. Os manuscritos entregues para publicação devem ser dactilografados a dois espaços e possuir uma margem da largura habitual. Poderão ser escritos em português, inglês, francês, alemão, italiano ou espanhol. O nome do autor (ou autores) deverá figurar na primeira página, bem como o endereço da Instituição em que trabalha. Um resumo não excedendo aproximadamente 300 palavras, preferivelmente em inglês, deverá iniciar o artigo.

4. Os nomes latinos dos géneros, espécies e categorias infraspecíficas que figurarem no texto devem ser sublinhados uma só vez, enquanto que os nomes dos autores, quando não escritos em maiúsculas, devem ser sublinhados com um traço ondulado. As palavras em negro devem ser sublinhadas duas vezes. Os nomes dos autores citados no texto devem ser seguidos pela data da publicação entre parênteses.

5. No que respeita à ordenação e disposição da bibliografia, seguir as normas utilizadas em um dos volumes recentes desta publicação.

6. As figuras a intercalar no texto, geralmente reproduzidas em zincogravura, não deverão exceder a mancha tipográfica. As estampas *hors-texte* (em regra fotogravuras) serão impressas em papel *couché* e não deverão ultrapassar 13 X 18 cm. Sempre que as figuras sejam de pequenas dimensões, aconselha-se a sua reunião em estampas com as dimensões acima indicadas.

7. Cada autor (ou grupo de autores) receberá 50 separatas grátis, sendo as excedentes que pretender fornecidas ao preço do custo e pagas directamente à Tipografia.

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1. Le *Boletim da Sociedade Broteriana* est un périodique destiné à la publication d'articles originaux concernan: tous les domaines de la Botanique. Cependant, des articles très longs sur floristique, phytogéographie et phytosociologie sont en général publiés dans les *Memórias*, tandis que les travaux de divulgation scientifique et ceux concernant l'histoire de la Botanique sont réservés au *Anuário* — les deux autres revues de la Société.

2. Ayant particulièrement pour but la publication des articles élaborés par le personnel scientifique de l'Institut Botanique de Coimbra, ce périodique publie aussi les travaux des membres de la Société, ainsi que ceux d'autres botanistes, soit portugais, soit de quelque autre nationalité. Toutefois, la publication des articles est sous la dépendance de l'avis de la Commission de Rédaction.

3. Les manuscrits doivent être dactylographiés à deux espaces et avoir une marge. Ils peuvent être rédigés en portugais, anglais, français, allemand, italien ou espagnol. Le nom de l'auteur (ou des auteurs) devra figurer à la première page après le titre du travail, ainsi que l'adresse de l'Institution où il travaille. Un résumé ne dépassant pas 300 mots, de préférence en anglais, devra ouvrir l'article.

4. Les noms latins des genres, des espèces et des catégories infraspécifiques devront être soulignés une fois, tandis que les noms des auteurs, quand non dactylographiés en lettres majuscules, doivent être soulignés par une ligne ondulée. Les noms des auteurs cités dans le texte doivent être suivis de la date de la publication mise entre parenthèses.

5. En ce qui concerne la bibliographie, voir quelque volume récent du *Boletim*.

6. Les figures du texte, en général des dessins à l'encre de Chine, ne doivent pas, avec les légendes, dépasser 10,5 X 18 cm. Les planches hors-texte ne devront pas dépasser 13 X 18 cm. Les figures à petites dimensions doivent être réunies dans des planches aux dimensions ci-dessus mentionnées.

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