

THE CYTOLOGY OF THE GENUS *CYCLAMEN*

by

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## I. INTRODUCTION

This investigation of the cytology of the genus *Cyclamen* was started to find out the possibility of crossing different species, in order to use these for breeding purposes. This made it necessary to determine at first the chromosome numbers of the different species and varieties, see KAPPERT (9). It was self-evident that simultaneous with this investigation the systematic relationships of the different species were studied (DOORENBOS).

Taxonomists chiefly use easily distinguishable macroscopical characters for the classification of species and genera. After the discovery, however, that chromosomes are bearers of genetical characteristics, these also have been used in the study of relationships. Especially after it became clear that in many cases differences in species can be traced back to differences in chromosome arrangement, and none the less after new species were bred by intentional species crossings, more and more attention was paid to chromosome number and chromosome morphology as an aid to classification.

In the different *Cyclamen* species many different chromosome numbers are found. It is natural, therefore, that besides macroscopically distinguishable morphological characters attention should be paid to chromosome numbers also, as GLASAU (5) has done.

In the publication by WELLENSIEK, DOORENBOS and DE HAAN (16) it was already discussed, that the three monographs on *Cyclamen* were not in agreement about the number of species that could be distinguished, and there was no agreement on the nomenclature either. The taxonomy and nomenclature have since been revised by DOORENBOS (3). The results of the cytological investigations are published in the following.

## 2. MATERIAL

### 2.1. Nomenclature and taxonomy

In this paper we use the same nomenclature and taxonomic division as in our previous publications (3, 16). However, we call attention to the following.

As pointed out previously (3), the species usually indicated as *Cyclamen europaeum* L. in all probability should be called *C. purpurascens* MILL. We did not then know that shortly before SCHWARZ (14) had also discovered this mistake in the application of the rules of nomenclature. However, this author has, no more than we, been able to study the material preserved in the herbarium of LINNAEUS, which, in our opinion, will finally have to settle the question, to which species the name *C. europaeum* belongs. Therefore, we maintained this name in the present paper.

The taxonomic division too is almost the same as we gave in 1950. The only difference is formed by *C. creticum* HILDEBR., which we then, following SCHWARZ, looked upon as a subspecies of *C. repandum*. However, the results of a morphological and cytological study of living plants, imported from Crete, led us to the conclusion that this form indeed deserves specific rank. The order in which the species are mentioned is altered slightly. These changes mainly concern *C. cyprium*, which we now consider related to *C. cilicium*, not to *C. neapolitanum*.

Further taxonomic problems arose with *C. orbiculatum* and *C. pseudibericum*.

*C. orbiculatum* is a variable species with an extensive synonymy, which on account of some persistent *nomina nuda* has become very complicated. The paper of SCHWARZ (14) has been very illuminating, but matters have again been confused by a recent article of POBEDIMOVA (12), who instead of this one species mentions four for the northern part of its distribution area alone. These he indicates as *C. vernum* SWEET, *C. coum* MILL., *C. elegans* BOISS et BUISE and *C. circassium* POB. In our opinion these forms do not deserve the rank of species, but they may be grouped into subspecies as proposed by SCHWARZ. As the living material at our disposal for this study had not been imported directly from the natural habitat, we did not make such a division, but only mentioned a number of garden forms. Concerning *C. pseudibericum* SCHWARZ wrote to us that he had found in the natural surroundings (not known previously for this species) all transitions from this species into *C. libanoticum*, so that in his opinion there is only one species, which for reasons of priority has to be called *C. libanoticum*. We studied the material that may be obtained under these names in the trade, and in which both forms show such differences that a division into two species is indicated. Therefore, until we can form an opinion on the forms discovered by SCHWARZ, we maintain both species.

## 2.2. Origin of the material

*C. repandum*. Tubers obtained from the Italian trade. *C. repandum* var. *album*: from the bulb-growers-firm VAN TUBERGEN at Haarlem.

*C. balearicum*. Tubers and seed from the Botanical Institute at Barcelona which imported it from the Balearic Islands.

*C. creticum*. Twenty-two tubers from Crete.

*C. libanoticum*, *C. pseudibericum* and *C. cilicium*. Tubers from the firm of VAN TUBERGEN.

*C. orbiculatum*. Tubers from the Agricultural Institute at Ankara, from several collections in England and from VAN TUBERGEN. From the latter firm we obtained all garden varieties.

*C. cyprium*. Three tubers from the collection of Mr C.C. MOUNTFORT at Wimborne, Dorset, England.

*C. europaeum*. Material collected in the wild in Samoens (Haute Savoie) and Merligen (Switzerland); tubers from the Faculty of Agriculture and Forestry at Zagreb, and from an Italian trade firm.

*C. neapolitanum*. A tuber from the wild in Greece; further tubers from several collections and nurseries (among these the var. *album*).

*C. africanum*. Tubers from Algeria.

*C. persicum*. Many tubers from the natural habitat in Israël, Syria, Cyprus and Tunisia; furthermore two tubers, one under the name *C. aleppicum* var. *punicum* from the Botanical Institute at Kiel (GLASAU).

The commercial strains were all obtained from nurseries in the centre of floriculture Aalsmeer.

*C. Rohlfianum*. Two small tubers from the collection of Mr C. C. MOUNTFORT, who obtained the seed from Cyrenaica. Not studied yet.

*C. graecum*. A great number of tubers collected in the wild in Greece.

## 2.3 Forms not studied

As can be seen from the foregoing, we did not see all the forms described in the taxonomical literature. We point out that to complete our study we would have liked to have at our disposal living material of the following forms:

*C. mirabile* HILDEBR. This species has only been found once; it was not kept alive. Judging from morphological characteristics (especially the nervation of the sepals, see p. 160) we suppose it belongs to the group of species with 30 chromosomes.

*C. europaeum* var. *ponticum* ALB. This form, according to SCHWARZ a subspecies of *C. europaeum* and even raised to specific rank by POBEDIMOVA, is as far as we know not in cultivation, and therefore could not be investigated cytologically. The same can be said of the apparently closely related *C. parviflorum* POB.

*C. numidicum* GLASAU<sup>1)</sup> and *C. Gaydurowrysi* GLASAU apparently are no longer cultivated either. These forms will be discussed below (see pp. 161, 162).

Also, it will be clear that in relation to the taxonomical problems mentioned sub 2.1 we should have liked to study material of *C. orbiculatum* from different parts of the area of distribution and *C. pseudibericum* from the localities where it was found by SCHWARZ.

<sup>1)</sup> We maintain this name here, but point out it is not legitimate. It is a later homonym of *C. numidicum* GANDOGGER, Bull. Soc. Bot. France 36, 1889: 432.

## 3. METHODS

Of most species the somatic divisions could be studied in the cells of roottips, the meiosis in pollen mothercells. Root tips of well growing plants were fixed in the Nawashin-Karpechenko mixture of the following constitution:

<i>Solution A:</i>	<i>Solution B:</i>
acetic acid, 50 ml	alcohol 96 %, 50 ml
chromic acid, 5 grams	formalin 30 %, 150 ml
dest. water, 425 ml.	dest. water, 275 ml.

Before use equal parts of solutions A and B were mixed. The preparations were brought in the usual way through different aethylalcohol concentrations and butyl alcohol in paraffine wax of a melting point of 58° C. The preparations were cut with a thickness of 12 – 15  $\mu$  on a microtome and stained in crystal-violet.

To study the meiosis young pollen mothercells were squashed in a drop of carmine-propionic acid on a slide with the end of a bone needle-holder. Carmine-propionic acid was made according to the same formula as carmine-acetic-acid, viz. DARLINGTON and LA COUR (1).

The cells remained on the slide under a cover slip, for 5 – 10 minutes in the staining solution, after that a piece of filterpaper was laid on the cover slip and gently pressed, and the superfluous solution blotted away.

The preparations were made permanent by dehydration in alcohol 80 %, 100 %, 100 % and mounted in euparal, viz. DARLINGTON and LA COUR (1).

Instead of carmine-acetic-acid, carmine propionic-acid was chosen, because the chromosomes swell less in this solution.

## 4. RESULTS

GLASAU (5) tried to classify the different species of the genus *Cyclamen* according to the size and number of their chromosomes as proposed by DELAUNAY (2), according to whom the more primitive species have a smaller number of large chromosomes compared with the more recent species. We deal with the different species in sequence of their chromosome-number beginning with the species with the lower numbers, to see if the chromosome numbers can be used as a characteristic in classification.

In the following review the names are used as given by DOORENBOS after his critical investigation. The species are arranged according to their probable relationship as judged by their morphological characteristics and their chromosome numbers.

*Cyclamen repandum*, 2 n = 20

In the cells of root tips 20 chromosomes were counted. The somatic chromosomes are very long,  $\pm 4 - 4.5 \mu$ , the ends are club-shaped as those of *C. balearicum*. The drawing of figure 1 shows the somatic chromosomes. Of the meiosis we got preparations of the diakinese stage, where 10 bivalents could be easily counted, see figure 2. In the stage metaphase II, 10 chromosomes (haploid) could be counted, see figure 3.

GLASAU uses the name *C. hederifolium* for *C. repandum*, see DOORENBOS (3). He states that the chromosome number amounts to 2 n = 24. We cannot make

out if this is a mistake in counting, or if his material was not identical with ours. The last assumption is less probable, because we never found the number 24 in any of the species.

*Cyclamen balearicum*,  $2n = 20$

The somatic chromosomes were studied in the cells of roottips. A drawing of one of the preparations is given in figure 4. The chromosomes of *C. balearicum* are very large, about  $4 - 4.5 \mu$  long; the chromosomes are rod-shaped. One of the ends is usually club-shaped. Twenty chromosomes could be counted easily in the rootcells.

During the meiosis of pollen mothercells, 10 bivalents could easily be counted. Figure 5 gives a drawing of the diakinese-stage. Also 10 bivalents can easily be seen in the photograph of figure 6.

Without doubt the chromosome number of *C. balearicum* is  $2n = 20$ . GLASAU gives the number as  $2n = 18$  which must be a mistake.

*Cyclamen creticum*,  $2n = 22$

The somatic chromosomes are long,  $4 - 5 \mu$ , some of them are „V”-shaped, others are bent, see figures 7 and 8.

Because they are seldom orientated in one plane, the counting of the somatic chromosomes is very difficult. We had not expected the number  $2n = 22$ , it seemed at first very improbable to us. For this reason we also fixed roottips in a weak solution of 8-hydroxyquinoline and hydrochloric acid, and stained with orceine, using the method of TJO JOE HIN and LEVAN (15). See also PRAK-KEN and SWAMINATHAN (12.1). In these preparations 22 chromosomes could be counted without doubt, see figure 9. The meiosis of pollen mothercells, in the diakinese stage showed clearly 11 bivalents, see figure 10.

The chromosome number of *C. creticum* amounts without doubt to  $2n = 22$ , which with the morphological characteristics is an argument for considering *C. creticum* as a separate species.

*Cyclamen libanoticum*,  $2n = 30$

The somatic chromosomes of *C. libanoticum* are rod-and „V”-shaped; they are somewhat shorter than those of the group of *C. balearicum*, *repandum* and *creticum*; the length is  $3 - 4 \mu$ .

The chromosome number of the somatic cells is  $2n = 30$ ; figure 11 shows the somatic chromosomes in the roottip cells.

We obtained good preparations of pollen mothercells at the stage of metaphase II, in which 15 chromosomes (haploid) could be counted easily, the photograph of figure 12 gives a good picture of these.

GLASAU mentions the numbers  $n = 18$  and  $2n = 36$ , our photograph of figure 12 shows however that the haploid number is 15.

*Cyclamen pseudibericum*,  $2n = 30$

The chromosomes are  $3 - 4.5 \mu$  long, the ends are „S”-curved, they are „V”-shaped and very often we saw the ends split. This makes counting very difficult, especially since we never found them lying in one plane. A good photograph could not be made. In order to contract the chromosomes to make counting somewhat easier, we fixed roottips in a cold „Craf”-solution at  $3^\circ \text{C}$ . Figure

13 gives a picture of such a preparation. In preparations of cold-fixed material we could easily count 30 chromosomes. GLASAU (5) gives the somatical chromosome number as 36.

Owing to shortage of flowers we did not succeed in observing the reduction division.

*Cyclamen cilicium*,  $2n = 30$

The chromosomes of *C. cilicium* are exactly of the same shape and size as those of *C. libanoticum* and *C. pseudibericum*. They are  $3 - 4 \mu$  long and mostly hooked or „V”-shaped, the ends clubbed and often split. We found no metaphase-plates, where they laid in one plane, so a photograph showing all the chromosomes could not be made. Figure 14 gives a drawing of a somatic metaphase plate. The meiosis was studied in pollen mothercells. In these preparations which were in the stage of metaphase I, 15 haploid chromosomes could be counted, see the photograph of figure 15.

GLASAU (5) gives the number as  $2n = 38$  for *C. cilicium*. This statement is not correct, the number of  $2n = 28 - 32$  given by HEITZ (6), is nearer to the exact number.

*Cyclamen orbiculatum*,  $2n = 30$

GLASAU (5) gives the chromosome number of this species as  $2n = 30$ , this number tallies with the number we found. HEITZ (6) gives the haploid number as 14 which is not correct. The shape of the chromosomes is similar to those of the other species of this group with 30 chromosomes. The drawing of figure 16 shows the somatic chromosomes in a cell of the roottips. Counting the hooked chromosomes is very difficult. For this reason plants were kept for 12 hours at  $4^{\circ}\text{C}$ , before the roottips were fixed in a cold „Craf”-solution at  $4^{\circ}\text{C}$ . This treatment contracts the chromosomes as shown in the photograph of figure 17 and makes counting easier. At the meiosis of pollen mothercells 15 chromosomes (haploid) could be counted.

GLASAU (5) mentions that he often found one extra-ordinarily large chromosome in pollen mothercells, during the metaphase II, of the meiosis. I cannot confirm this, but I observed a noteworthy grouping of the meiotic chromosomes. In the photograph of figure 18 we see 5 groups of 3 chromosomes in one cell, and in the other 3 groups of 3, 2 groups of 2 and 2 chromosomes lying separate. If this „secondary association” is due to the homology of the chromosomes, it means that 5 is a basic number. How the other chromosome numbers are derived from this number is still a problem which is dealt with on page 159.

*Cyclamen cyprium*,  $2n = 30$

The chromosome number  $2n = 30$ , which we found in the somatic cells of roottips differs so much from the number mentioned by GLASAU (5),  $2n = 98$ , that this cannot be an error in counting. GLASAU must have had another species under the name *C. cyprium*.

As the morphological discription of GLASAU tallies with our material we cannot assume how this confusion has arisen. The somatic chromosomes of *C. cyprium* are  $2 - 3.5 \mu$  long, they are „S” or „V”-shaped, they thus have the same shape and size as those of the other species with the chromosome number of  $2n = 30$ . The photograph of figure 19 and the drawing of figure 20, made from the same preparation, give a picture of the chromosomes.

Unfortunately the number of available flowerbuds was too small to study the meiosis.

*Cyclamen europaeum*,  $2n = 34$

GLASAU (5) mentions that he could estimate with reasonable certainty the diploid chromosome number of *C. europaeum* as  $2n = 34$ . We also found this number in the somatic cells. HEITZ (6) gives the diploid chromosome number as  $2n = 28 - 32$ .

From the unexact number given by HEITZ and the uncertain counting of GLASAU (5) it follows, that the counting of the exact number is not easy. The chromosomes of this species are  $\pm 2 - 3 \mu$  long, they are rod and V-shaped.

When the metaphase plates are not well spread, counting is very difficult. This difficulty was surmounted by cooling the plants during one night at  $5^{\circ}\text{C}$  before fixing the roottips in a cooled fixation-solution.

Good preparations were obtained of pollen mothercells in the stage of metaphase II. The photograph of figure 21 shows clearly that the haploid number is 17.

It could thus be stated with certainty that the diploid chromosome number of *C. europaeum* is 34.

*Cyclamen neapolitanum*,  $2n = 34$

This species belongs to the same group as *C. europaeum* with the chromosome number of  $2n = 34$ . GLASAU (5) writes that all his material of different origin showed a diploid chromosome number of  $2n = 36$  and a haploid number of 18.

HEITZ (6) gives the approximate number of  $2n = 32 - 38$ . The statements of both authors are, accordingly to our countings, not correct. The number of 34 chromosomes was counted by us in metaphase plates of roottips which had been exposed to a temperature at  $4^{\circ}\text{C}$  and which were fixed in a cold fixation-solution. This contracts the chromosomes and lessens the chance of making errors in counting. The morphology of the somatic chromosomes of *C. neapolitanum*, closely resembles that of *C. europaeum* and *C. africanum*, they are rod-shaped or „V“- or „S“-curved; the length is  $\pm 2 - 3 \mu$ . Figure 22 shows a somatic metaphase plate. The meiosis was studied in pollen mothercells; preparations were obtained in the stage of metaphase II, where 17 haploid chromosomes could be counted very easily as shown in the photograph of figure 23.

*Cyclamen africanum*,  $2n = 68$

The chromosomes of *C. africanum* are very similar in size to those of *C. neapolitanum*. This fact alone makes the relationship of these two species highly probable. The chromosome number  $2n = 68$  is exactly twice that of *C. neapolitanum* ( $2n = 34$ ). It is probable that *C. africanum* in a tetraploid of a species nearly related to *C. neapolitanum*, see page 00.

GLASAU (5) gives for *C. africanum* the number of  $2n = 72$ . I have not been able to count more than 68 chromosomes in the roottip cells. How HEITZ (6) established a chromosome number of  $2n = 38$  for this species is not clear.

Unfortunately the number of flowerbuds available was too small to study the meiosis of *C. africanum*.

*Cyclamen persicum*,  $2n = 48$ , 96

The diploid chromosome number of the wild *C. persicum* was found to be 48,

this number tallies with the observations of HEITZ (6) and GLASAU (5). Cultivated strains have been obtained by selection from wild plants of *C. persicum*. Among the former are tetraploids with  $2n = 96$  chromosomes. According to GLASAU the cultivated strain „Kätchen Stoldt” has a chromosome number of  $2n = 130$ . Due to lack of material of this strain we could not verify this number.

The chromosomes of *C. persicum* are very small rods, only  $1 - 2 \mu$  long. The drawing of figure 24 illustrates a somatic metaphase plate of the strain „Sylphide” with  $2n = 48$ , and figure 25 shows a somatic metaphase plate of „Perle von Zehlendorf” with  $2n = 96$  chromosomes.

The history of the cultivated strains of *C. persicum* was studied by DOORENBOS (4). Of the cultivated strains the following have a chromosome number of  $2n = 48$ : „Wit”, „Wit cristata”, „Sylphide”, „Sylphide half cristata”, „Wit met oog”.

The chromosome number of  $2n = 96$  is found in strains with the colour red in their flowers, for instance: „Perle von Zehlendorf”, „Rose van Aalsmeer”, „Rood”, „Rood rococo”, „Vuurbaak”.

Exceptions are „Wit rococo”, „Wit fimbriata” and „Wit met oog fimbriata” which lacking red have also 96 chromosomes.

#### *Cyclamen aleppicum* ssp. *puniceum*, $2n = 48$

GLASAU maintains the species *C. aleppicum* FISCH, a form of *C. persicum* with pure white flowers. The character of the flower colour is systematically of no importance, but according to GLASAU this form should have 54 chromosomes. He found the same number in *C. puniceum* POMEL which form is identical with *C. persicum* in respect to the other characteristics also, that is why he called this species *C. aleppicum* ssp. *puniceum*. On our request we received material of *C. aleppicum* var. *puniceum* from Kiel, the same material as used by GLASAU. The diploid chromosome number of this plant is according to our determinations 48. This is the same as that of *C. persicum*. To our opinion, *C. aleppicum* ssp. *puniceum* is identical with *C. persicum*.

In *C. graecum* 84 or 85 somatic chromosomes are most probable, but the definite number could not be ascertained.

## 5. DISCUSSION

It is apparent from the results mentioned above, that the chromosome numbers found in the various species, are in ascending progression: 20, 22, 30, 34, 48, 68, 96.

It is not easy to discern in this series a certain basic number, from which the other numbers could be derived. However, 20 and 30 are multiples of 10, and 68 and 96 are multiples of 34 and 48 respectively.

If one assumes that the various species have developed from one original type, it is reasonable to suppose that the most primitive species, that is (from a cytological point of view) *C. repandum* with 20 chromosomes, is most closely related to this ancestor, and that the different chromosome numbers of other species have arisen from the number 20.

However, this set of 20 chromosomes may in turn be derived from another, yet more primitive one; i.e. it could be a doubling of the number 10. In that case species with 20 chromosomes would be tetraploids of an original type with 10 chromosomes, and in each cell there would be during meiosis two homologous

groups of 5 homologous chromosomes. This homology might be visible in meiosis as a „secondary association” of these chromosomes (LAWRENCE, (10).

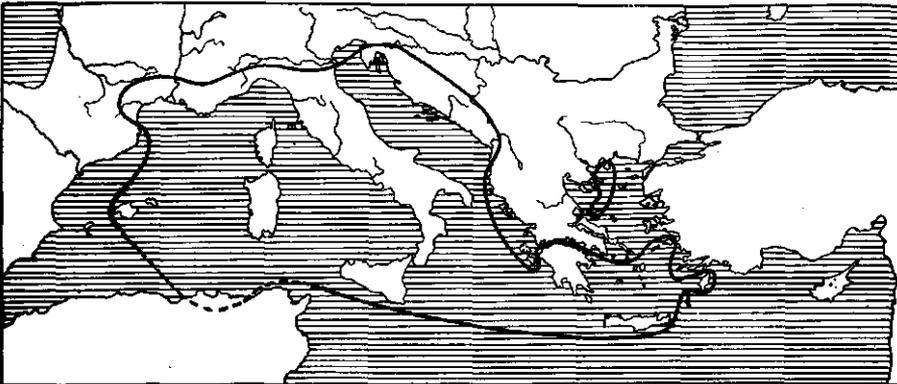
Indeed, in the stage metaphase II in meiosis of *C. repandum* ( $2n = 20$ ) and also of *C. orbiculatum* ( $2n = 30$ ) there are indications that this might be the case. In the photograph of fig. 3, showing the metaphase II in *C. repandum*, two groups of 5 chromosomes can be seen. Also the photograph of fig. 18, showing the metaphase II in *C. orbiculatum*, shows a typical grouping of chromosomes: in one cell five groups of 3 chromosomes occur, in the other three groups of 3, two groups of 2 and two single chromosomes.

According to the theory of secondary association this means that the species with 20 chromosomes are tetraploids of an original species which had 10 chromosomes, while the species with 30 chromosomes would be hexaploids of this primitive type. The good self-fertility of the species with 30 chromosomes is another indication that these are not triploids of a species with 20 chromosomes, but are more likely hexaploids. The relationship of the chromosome sets and with it that of the species may thus be explained.

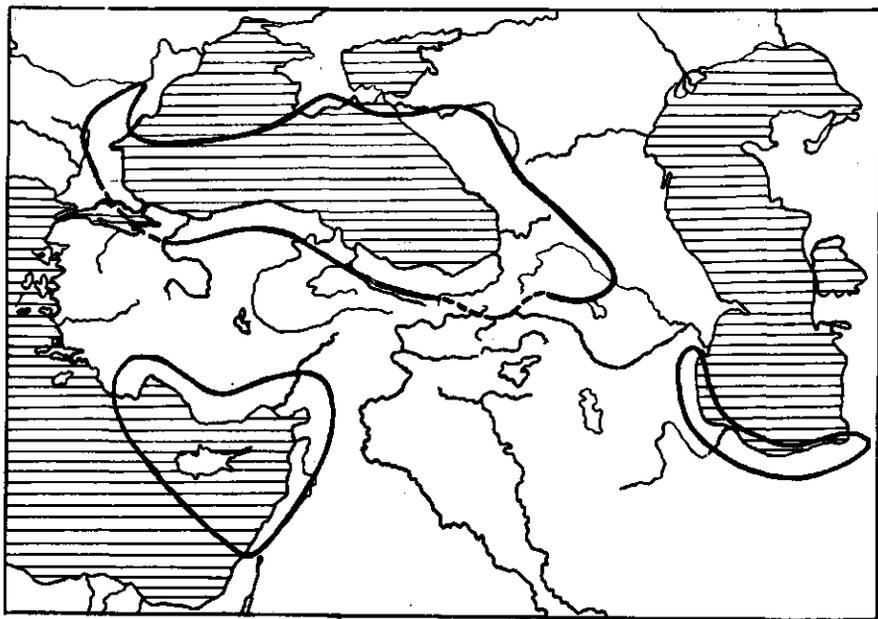
The species with 22 chromosomes, *C. creticum*, is closely related morphologically to *C. repandum*. It is plausible therefore, to suppose that it belongs to the same group as the latter species, having developed from this or from a common ancestor by the doubling of a pair of chromosomes.

The original *Cyclamen* must have carried all the characteristics of the present species, at least the possibilities for their development. The question arises, if the differentiation of these characteristics had taken place as early as that, or whether this occurred later. In other words: have there been several species with 10 chromosomes, or only one? In the first case several tetraploid types may have developed independently; in the latter case there can only have been one original tetraploid type, from which later several species have developed.

It is difficult to find good arguments for either case. Moreover, the problem arises with each group with a higher number of chromosomes. It is plausible to suppose, that at least the group of species with 20 and 22 chromosomes has developed from one original type. These species are closely related: they have many characteristics in common and one can well imagine, what the original type must have looked like. In this connection it is striking to notice that the species are mutually vicarious in their distribution. In the middle of the distri-



Textfigure 1. Distribution area of *C. repandum*, *C. balearicum* and *C. creticum*; the group with  $2n = 20$  and  $2n = 22$  chromosomes.



Textfigure 2. Distribution area of group with  $2n = 30$  chromosomes.

but in the distribution area one finds *C. repandum*; on the edge of this area in the East *C. creticum*, endemic on Crete, in the West *C. balearicum*, endemic or nearly so on the Balearic Islands. See textfigure 1. Perhaps one may assume that *C. repandum* is the closest relative of the original type.

The map in textfigure 1, just as those in the following textfigures, was prepared from data, collected from the following sources:

- 1) Places of origin of our own importations.
- 2) Finding spots of dried material in the Rijksherbarium at Leiden, and the Herbarium of the Laboratory of Plant Taxonomy and Ecology at Wageningen.
- 3) Finding spots mentioned in literature. A great number of Flora's was examined; we will not mention them here, but point out that a complete list of all the Flora's of the Mediterranean area is given in RIKLI, *Das Pflanzenkleid der Mittelmeerländer*, Bd III, 1948, 1137-1150.

With the group of 30 chromosomes, however, evolution from one original hexaploid type is much less probable. The species have only the following characteristics in common:

Tubers rooted from the under side. Leaves crimson beneath. Sepals with 1-5 parallel veins with few branches, entire; corolla-lobes at the base with a dark spot, which does not reach to the tube (except in *C. cilicium*); anthers yellowish; style shorter than the corolla-tube (sometimes slightly longer in *C. libanoticum*).

The characteristic nervation of the sepals only occurs in this group. All other Cyclamens have sepals with one thick much branched vein (see the beautiful drawings of HILDEBRAND (7, 8)). For the rest one finds in this group species with hairy and with corky tubers, with smooth and „eared” corolla-lobes, some that flower in autumn and others that flower in winter, and it is not possible to indicate, which would have been the characteristics of an original type, should this have existed. The area of distribution of this group could be used as an argument for such an original type, as it is closed and a great part of it is not shared

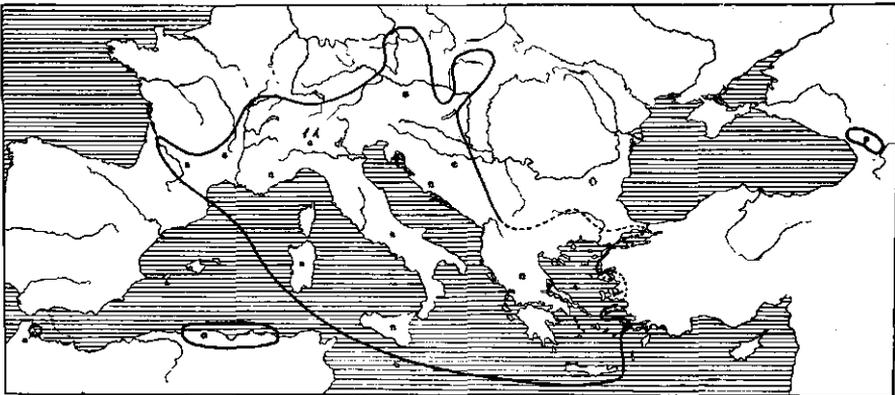
by other species. Little can be said of the distribution of the species, as all of them, except *C. orbiculatum*, are rare; in any case they are not strikingly vicarious. See textfigure 2.

To explain the higher chromosome numbers, one must recourse to even more speculations. In the first place one can assume that the medium-sized and small chromosomes have originated from the large ones by the process known as fragmentation. However, it is certain that doubling of chromosome numbers has also taken place.

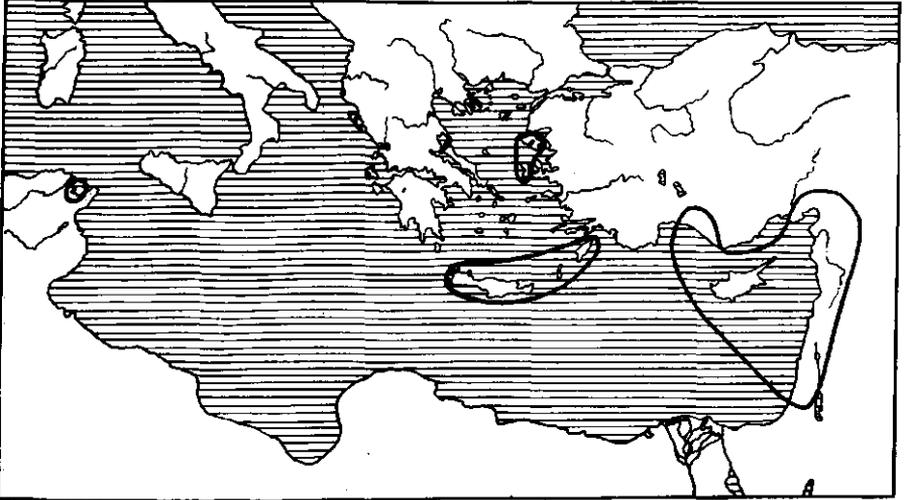
Medium-sized chromosomes are found in species with 34 or 68 chromosomes. There are two species with 34 chromosomes, *C. europaeum* and *C. neapolitanum*. It is curious that these have almost as many morphological differences as is possible inside the genus; moreover, the few characteristics they have in common occur in many other species. This does not indicate an evolution from one original type with 34 chromosomes; should this have been the case nevertheless, then many links must have disappeared.

On the other hand the relationship is very marked between *C. neapolitanum* and the only species with 68 chromosomes, *C. africanum*. These species have many morphological characteristics in common, i.e. almost identical flowers and a very characteristic rooting system; moreover, they have the same periodicity. Thus this argues well for the assumption that *C. africanum* originated from *C. neapolitanum*, after doubling of the number of chromosomes. We must point out however, that this might not be wholly true. GLASAU found among plants, originally imported from Algeria, one specimen with  $2n = 36$  chromosomes; he described it as *C. numidicum*. As to morphological characteristics this plant is closely related to, if not identical with *C. africanum*. Assuming that GLASAU made the same error as he did with *C. neapolitanum*, which he accredited with 36 chromosomes too, one is led to the supposition that *C. africanum* has to be looked upon as a tetraploid of *C. numidicum* GLAS. In that case the most plausible hypothesis is that *C. numidicum* is the ancestor of *C. africanum*, the former having been developed from *C. neapolitanum* as a southern vicarious species.

It may be pointed out, that *C. europaeum* is strictly vicarious with respect to *C. neapolitanum*; the areas of these species only overlap near Roche, where the spontaneity of *C. neapolitanum* is doubtful, see LUDI (11), and perhaps in northern Dalmatia. See textfigure 3.



Textfigures 3. Distribution area of *C. europaeum* and *C. neapolitanum* (the group with 34 chromosomes), of *C. africanum* with 68 chromosomes and *C. europaeum* var. *ponticum*.



Textfigure 4. Distribution area of the group with 48 chromosomes.

About the origin of *C. persicum* with 48 small chromosomes we are completely in the dark. The species is not only apparently unrelated to any species with a lower chromosome number, but it shows some characteristics (i.e.: fruitstems not coiled; anthers violet), which do not occur in these species. Its area of distribution consists of isolated parts, which points to high antiquity. See textfigure 4. Forms with 96 – or according to GLASAU even more – chromosomes occur in cultivation; among wild specimen these have not been found so far.

*Cyclamen Rohlfsianum*, with according to GLASAU (5) 72 small chromosomes, also has striking morphological characteristics, particularly the long projecting anthers, the origin of which cannot be traced, and occurs in a very limited area. Relationship with any of the present species with small chromosomes is not apparent, nor can next of kin among the other species be pointed out.

On the other hand it is not improbable that a relationship exists between *C. graecum*, with 83–88 small chromosomes according to DE HAAN (16), and *C. persicum*: there is some conformity in shape and rooting of the tubers and in shape of the leaf, while both species have violet anthers. Like the other species with small chromosomes, *C. graecum* has an area of distribution that is split up into small parts; in this respect the recently noted occurrence on Cyprus is interesting, if it is not an ancient import. See textfigure 5. *C. Gaydurowrysi*, described by GLASAU, can be looked upon as a tetraploid *C. graecum*, although it is not certain that this polyploidy extended to the whole plant, as GLASAU examined root tips only; he found  $\pm 162$  chromosomes.

Surveying the facts and hypotheses put forward in the foregoing, it is evident that little is known about the origin of the different chromosome numbers; in fact, so scanty is our knowledge, that we have no starting points for plausible theories. Especially in respect to the numbers 34, 48, 72, we are completely in the dark.

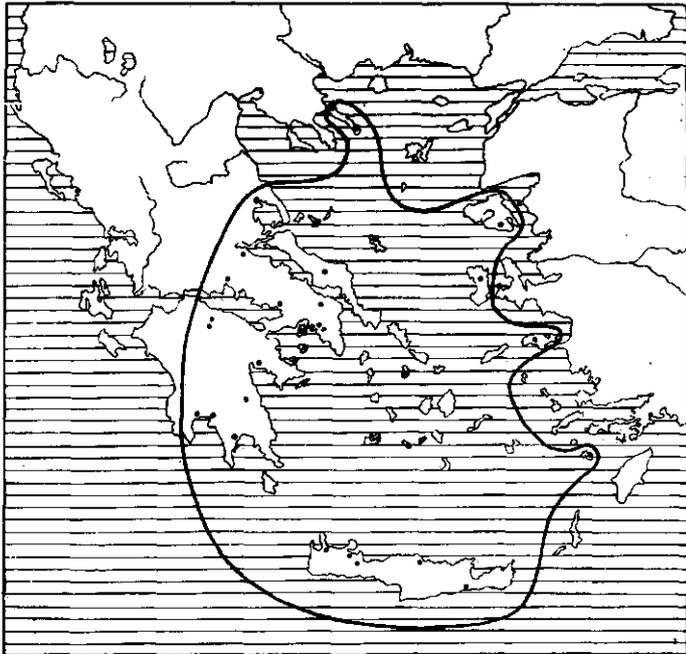
The fact that a regular series of chromosome numbers does not occur, may prove that a number of forms has become extinct. Among species of the same number of chromosomes also gaps occur which only can be bridged by forms

now extinct. That in the course of time the changing circumstances have played havoc with the genus *Cyclamen* is also proved by the splintered distribution areas of some of the species, and the extreme rarity of others.

It is very curious that the extensive development of the chromosome arrangement has not been accompanied by a development or at least a differentiation of the morphological characteristics. It is true that a difference in the chromosome set (except a simple doubling) is always combined with such morphological differences as justify specific rank, but in many cases these differences only consist in the combination of characteristics; very few characteristics occur in one species, or combined with one number of chromosomes only. Moreover, the differences existing between species with the same number of chromosomes are as large as occur in the genus.

The consequence of this is that the morphological relationship has no connection with the cytological relationship. The grouping of species given by SCHWARZ on morphological grounds, hangs in mid-air when compared with the cytological evidence. I.e. of the two species of the series *Fascipedia*, one has 30 big chromosomes, the other 83-88 small ones. The other species with 30 chromosomes are to be found in the other section, namely in both series of the subsection *Helicocaulon*. The first of these series also comprises one of the species with 34 chromosomes (the other one is in the other section), the second series also the species with 20 and 22 chromosomes.

From this example it is apparent that the morphological characteristics on which this classification is founded (and which up till now have been considered the most important differentiating characteristics in the genus *Cyclamen*) have no relation to the cytological characteristics. We have studied thoroughly the



Textfigure 5. Distribution of *C. graecum*. Dots indicate findspots.

question, whether there are other morphological characteristics which do show such a relation, but as can be seen from the foregoing, with very meagre results.

No important differentiation of physiological characteristics has gone with the development of the chromosome set, as may be readily inferred from the distribution areas of groups of species with the same number of chromosomes. These areas overlap in many cases, especially in the Egeian territory. Neither does it follow from the available data about the natural habitats of the separate species that a certain chromosome number goes with a particular ecological preference, at least not if one compares groups which comprise more than one species.

Thus very little is apparent of a directing of the evolution by external circumstances. At most such an influence can be detected within one group of species with the same set of chromosomes: we have already pointed out that within the groups with 20–22 and 34–68 chromosomes the species are clearly vicarious.

Of course one has to be mindful of the possibility that in ancient times, i.e. at the beginning of the Pleistocene or before, the situation may have been much clearer. Perhaps in the glacial age the genus *Cyclamen* did not only lose many species, but the morphological characteristics and the ecological preferences were levelled as well.

Judging from the evidence now at our disposal, however it seems that the evolution within the genus *Cyclamen* mainly concerns the chromosome set. It led to species greatly different in this respect, and as a result intersterile, notwithstanding the often close morphological conformity. Hybrids of species of *Cyclamen* are not known, not even among species of the same number of chromosomes, although the latter are not out of the question. Further research is wanted and has already been planned.

## 6. SUMMARY

1. All species and a number of garden varieties of the genus *Cyclamen* were examined cytologically, to establish the possibilities for hybridising.
2. In the discussion of the material some new data for the limitation and nomenclature of the species are brought forward.
3. The result of the cytological investigations may be summarized as follows:

<i>C. repandum</i>	20 big chromosomes
<i>C. balearicum</i>	20 big chromosomes
<i>C. creticum</i>	22 big chromosomes
<i>C. libanoticum</i>	30 big chromosomes
<i>C. pseudibericum</i>	30 big chromosomes
<i>C. orbiculatum</i>	30 big chromosomes
garden varieties	30 big chromosomes
<i>C. cilicium</i>	30 big chromosomes
<i>C. cyprium</i>	30 big chromosomes
<i>C. europaeum</i>	34 medium-sized chromosomes
<i>C. neapolitanum</i>	34 medium-sized chromosomes
<i>C. africanum</i>	68 medium-sized chromosomes
<i>C. persicum</i>	48 small chromosomes
garden varieties	48 or 96 small chromosomes
<i>C. graecum</i>	84 or 85 small chromosomes

4. The hypothesis is brought forward that species with 20 chromosomes are tetraploids, while those with 30 chromosomes are hexaploids of a primitive type with 10 chromosomes. The species with 22 chromosomes could have originated from one with 20 by the doubling of two chromosomes.
5. It is suggested that medium-sized and especially small chromosomes may have originated from large ones by the process known as fragmentation, while *C. africanum* ( $2n = 68$ ) developed from a form related to *C. neapolitanum* (perhaps *C. numidicum* GLASAU) by a doubling of the set of chromosomes.
6. An attempt is made to trace other steps in the evolution of the genus by comparing cytological evidence with morphological and geographical data. It seems, however, that very little relation exists between chromosome set and morphological characteristics or ecological preferences.
7. From this it is suggested that either the evolution of the chromosome set was highly independent of these other characteristics, or else in the course of time, especially in the glacial periods, many forms became extinct and the ecological preferences were levelled.
8. As a result of this process of evolution and elimination many of the present species, although in some cases closely related from the morphological point of view, are intersterile. Hybridising possibilities are therefore limited.

#### Acknowledgement

Thanks are due to the Netherlands Organisation for Pure Science (Z.W.O.) for enabling the senior author to study cytological technique in Sweden (Prof. A. Müntzing) and England (Dr C. D. Darlington).

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## EXPLANATION OF FIGURES

## PLATE 1

- Fig. 1. *Cyclamen balearicum*. Somatic metaphase plate ( $2n = 20$ ).  
 „ 2. *Cyclamen balearicum*. Meiosis of pollen mothercell, diakinese, 10 bivalents.  
 „ 3. *Cyclamen balearicum*. Meiosis of 3 pollen mothercells, diakinese, 10 bivalents in each cell.  
 „ 4. *Cyclamen repandum*. Somatic metaphase plate ( $2n = 20$ ).  
 „ 5. *Cyclamen repandum*. Meiosis of pollen mothercell, diakinese, 10 bivalents.  
 „ 6. *Cyclamen repandum*. Meiosis of pollen mothercell, metaphase II,  $n = 10$ .

## PLATE 2

- Fig. 7. *Cyclamen creticum*. Somatic metaphase plate ( $2n = 22$ ).  
 „ 8. *Cyclamen creticum*. Somatic metaphase plate, drawing of the same cell as the photograph of figure 7.  
 „ 9. *Cyclamen creticum*. Roottip chromosomes treated with 8-hydroxyquinoline, fixed in hydrochloric acid and stained with orcein.  
 „ 10. *Cyclamen creticum*. Meiosis of pollen mothercell, diakinese, 10 bivalents.  
 „ 11. *Cyclamen libanoticum*. Somatic metaphase plate ( $2n = 30$ ).  
 „ 12. *Cyclamen libanoticum*. Meiosis of pollen mothercell, metaphase II,  $n = 15$ .

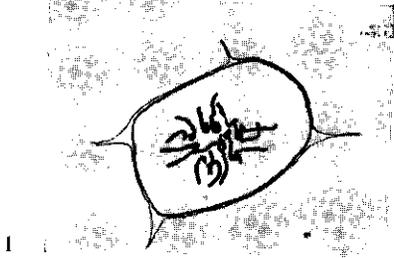
## PLATE 3

- Fig. 13. *Cyclamen pseudibericum*. Somatic metaphase plate,  $2n = 30$ .  
 „ 14. *Cyclamen cilicium*. Somatic metaphase plate,  $2n = 30$ .  
 „ 15. *Cyclamen cilicium*. Meiosis of pollen mothercell, metaphase I,  $n = 15$ .  
 „ 16. *Cyclamen orbiculatum*. Somatic metaphase plate,  $2n = 30$ .  
 „ 17. *Cyclamen orbiculatum*. Roottip chromosomes, kept 12 hours at  $4^{\circ}\text{C}$  before fixation,  $2n = 30$ .  
 „ 18. *Cyclamen orbiculatum*. Meiosis of pollen mothercell, metaphase II,  $n = 15$ .  
 „ 19. *Cyclamen cypricum*. Somatic metaphase plate,  $2n = 30$ .

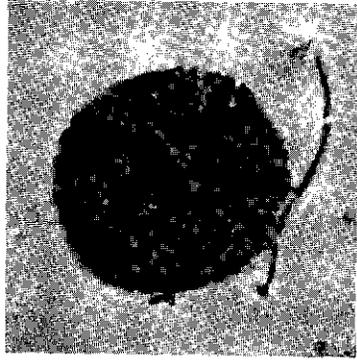
## PLATE 4

- Fig. 20. *Cyclamen cypricum*. Somatic metaphase plate,  $2n = 30$ ; drawing of the same cell as the photograph of figure 19.  
 „ 21. *Cyclamen europaeum*. Meiosis of pollen mothercell, metaphase II,  $n = 17$ .  
 „ 22. *Cyclamen neapolitanum*. Somatic metaphase plate,  $2n = 34$ .  
 „ 23. *Cyclamen neapolitanum*. Meiosis of pollen mothercell, metaphase II,  $n = 17$ .  
 „ 24. *Cyclamen persicum* „Sylphide“. Chromosomes of roottip,  $2n = 48$ .  
 „ 25. *Cyclamen persicum* „Perle von Zehlendorf“. Chromosomes of roottip,  $2n = 96$ .

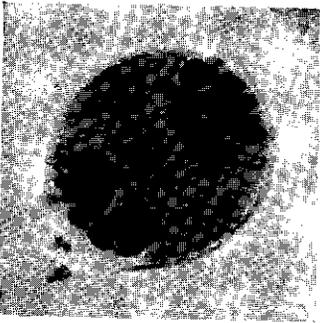
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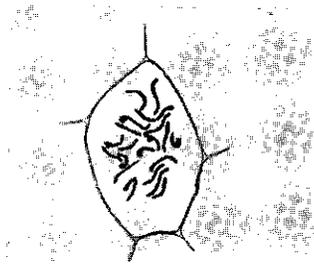
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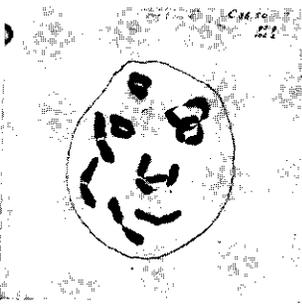
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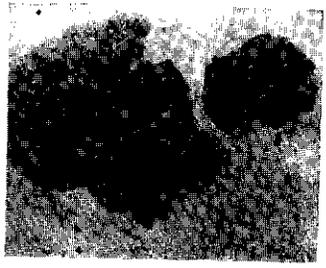
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PLATE 2

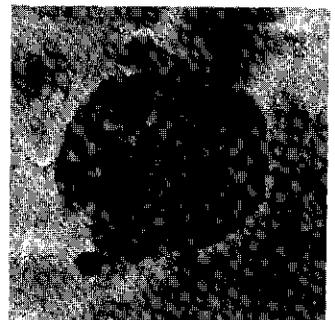
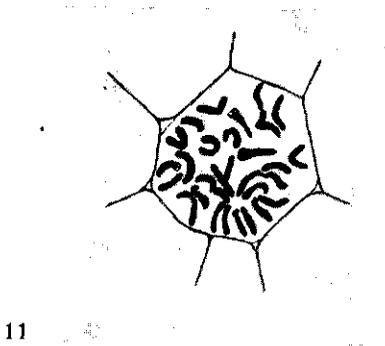
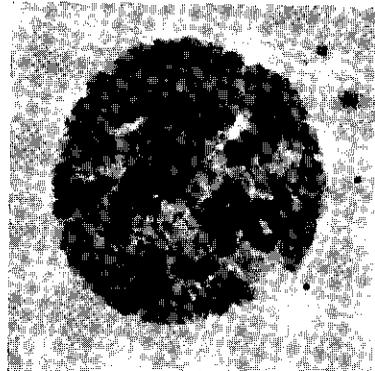
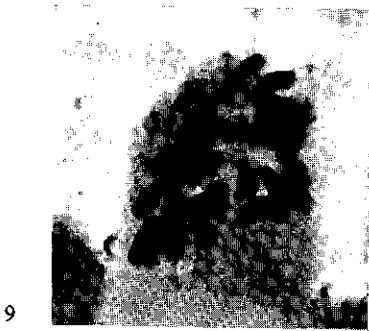
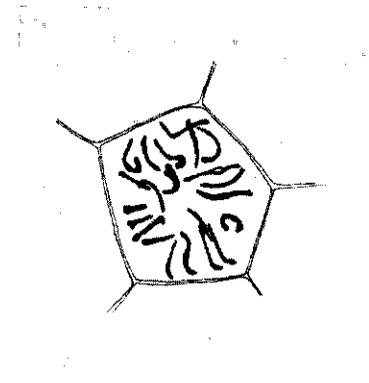
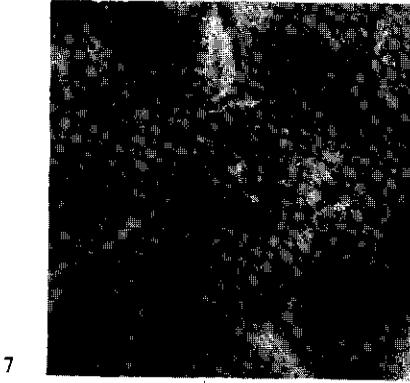
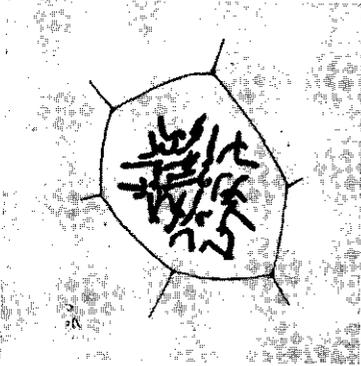
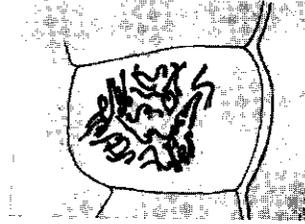


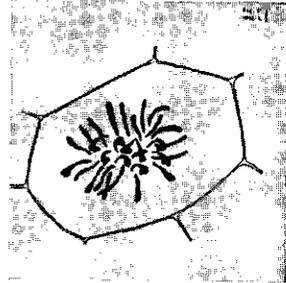
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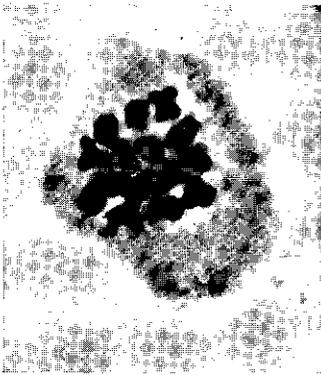
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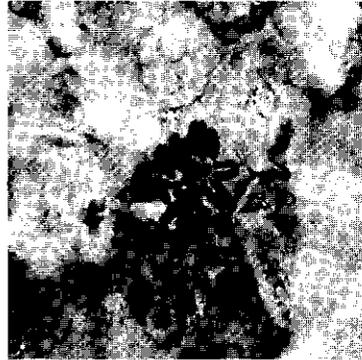
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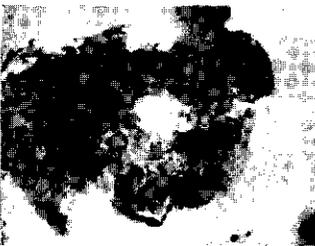
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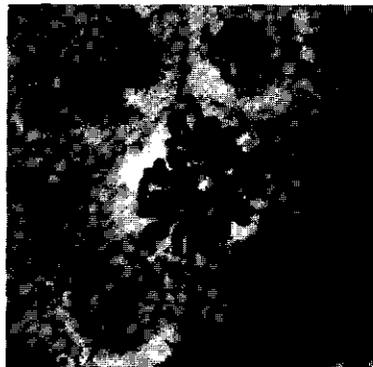
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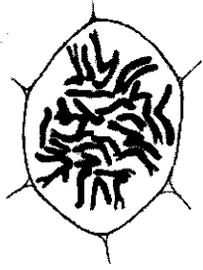


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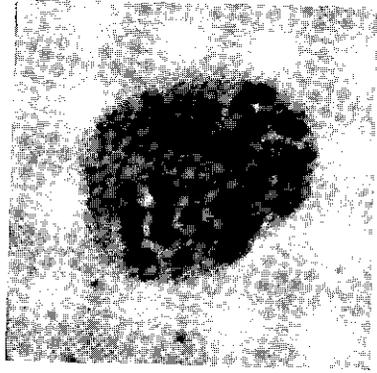


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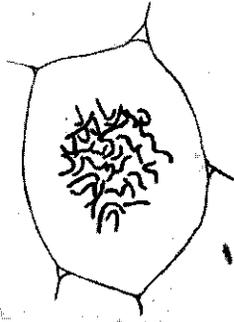
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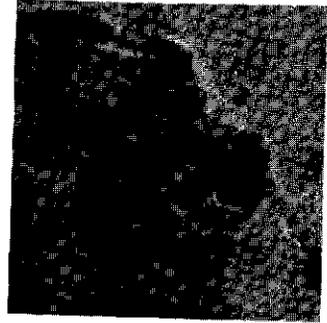
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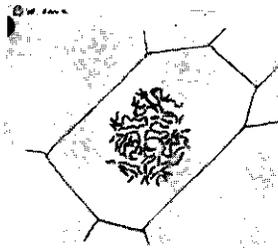
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