THE CYTOLOGICAL BACKGROUND OF *CYCLAMEN* BREEDING

R. A. H. LEGRO

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PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD
VAN DOCTOR IN DE LANDBOUWKUNDE
OP GEZAG VAN DE RECTOR MAGNIFICUS IR. W. DE JONG,
HOOGLERAAR IN DE VEETEELTWETENSCHAP,
TE VERDEDIGEN TEGEN DE BEDENKINGEN
VAN EEN COMMISSIE UIT DE SENAAT
DER LANDBOUWHOGESCHOOL TE WAGENINGEN
OP DONDERDAG 4 JUNI 1959 TE 16 UUR

DOOR

R. A. H. LEGRO

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WOORD VOORAF

Zowel U, geachte lezer, als ik, zijn bekend met de formele stijl waarin men een voorwoord van een proefschrift pleegt te schrijven. Echter, indien U mij van nabij kent, zult U weten, dat zulks mij heel slecht ligt. Vergun mij derhalve, om juist nu, nu ik zovelen mijn grote erkentelijkheid en dank wil betuigen, toch maar mijzelve te zijn.

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THE CYTOLOGICAL BACKGROUND OF *CYCLAMEN* BREEDING

Met een samenvatting

DE CYTOLOGISCHE ACHTERGROND VAN DE *CYCLAMEN*-VEREDELING

by/door

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CONTENTS

CHAPTER I

GENERAL INTRODUCTION

1. DISTRIBUTION AND NOMENCLATURE OF THE GENUS *CYCLAMEN*

The plant explorer, who goes botanizing in the Mediterranean area, will undoubtedly come across a low growing plant with more or less heart-shaped leaves, and lovely nodding pink or white flowers with reflexed petals on elegant thin stalks. There is every chance that determination proves that he found a wild species of *Cyclamen*, for countries around the Mediterranean form the present distribution area of the genus *Cyclamen* which belongs to the family of the *Primulaceae*.

The *Cyclamen* was already known to the Greeks and Romans: they called it *Cyclaminus* after its disk-shaped corm. The Greek 'Kyklos' means disk. In 1700 TOURNEFORT introduced the name *Cyclamen* (45, p. 6) for the genus which has been kept ever since.

According to DOORENBOS (8) 14 species and a few varieties have to be distinguished. Some have a large distribution area, others have a small one. This indication in combination with others e.g. the high polyploidy and the various 'missing links' in the divergent chromosome numbers, makes one suppose that it is a very old genus, that many forms became extinct and that the nowadays species have to be explained as glacial relicts.

The present known species are:

C. balearicum WILLK.	*C. pseudibericum* HILDEBR.
C. repandum SIBTH. et SM.	*C. neapolitanum* TEN.
C. creticum HILDEBR.	*C. purpurascens* MILL.
C. cilicium BOISS. et HELDR.	*C. persicum* MILL.
C. coum MILL.	*C. africanum* BOISS. et REUT.
C. cyprium KY.	*C. graecum* LK.
C. libanoticum HILDEBR.	*C. rohlfsianum* ASCH.

Although DOORENBOS (8) mentions the names *C. orbiculatum* MILL. and *C. europaeum* L., SCHWARZ (46) has shown that these two names are incorrect; the correct names should be *C. coum* MILL. and *C. purpurascens* MILL., respectively. Therefore, these names will be used throughout this paper.

2. INTRODUCTION INTO WESTERN EUROPE AND ITS CONSEQUENCES

Without entering into details (9, 45) it may be said that the first species appeared in Western Europe in the sixteenth century. FUCHS (1551), DODOENS (1554), DE L'OBEL (1576) and others gave descriptions and pictures.

The most important species, which was introduced, was *C. persicum* MILL. Although it was a collector's plant for a long time after its first introduction in Europe (about A.D. 1600), as the wild form still is today, it was cultivated in increasing numbers. Its popularity grew more intense and by the undiminished diligence of amateurs, gardeners and breeders, there was a significant assortment soon after 1850. But the development of cultivars went on. It increased to the present assortment of over 200 cultivars with its large variation in colour and other characteristics. Germany alone is growing 119 cultivars[1])! (33).

Not only are *Cyclamen* very important pot plants in Germany, but they are also in great demand in other European countries. In The Netherlands it is the most widely grown pot plant.

In 1957 as a cut flower it had the 22nd place among 32 others, but the demand for *Cyclamen* as a cut flower is increasing.

Among the garden plants some hardy species as *C. coum*, *C. cilicium*, *C. purpurascens* and *C. neapolitanum* are of some interest as alpine garden plants, particularly in England (24). Generally speaking, they are more or less unknown, but perhaps this will change in the future, as some of the wild species, e.g. *C. coum*, *C. neapolitanum* and *C. graecum* (57) are no doubt of direct value as ornamental plants.

3. SCOPE OF INVESTIGATIONS

In 1946 WELLENSIEK started an extensive study of *Cyclamen* at the Wageningen Horticultural Laboratory, with the main purpose to investigate breeding in its broadest aspects. Apart from genetical problems, taxonomy (DOORENBOS) and cytology (DE HAAN) were studied. The present author took DE HAAN's place in 1951. The cytological problems, which were attacked by him, can be grouped in three parts:

1. the cytology of the cultivars;
2. the cytology of the crosses between diploid and tetraploid cultivars of *C. persicum* (and reciprocal);

[1]) According to a resolution of the 14th International Horticultural Congress in 1955, the name cultivar is used to indicate a cultivated variety.

3. the cytology of species and species crosses.

The results of these investigations will be described in the following chapters. In each part the proper cytological problems will be presented first, followed by a discussion of their consequences for breeding.

CHAPTER II

DESCRIPTION OF TECHNIQUE

1. Cytological methods

1.1. *Selecting the material*

For the study of the meiosis in young pollen mother cells, anthers were used, while egg mother cells were examined in connection with the results of crosses between diploid and tetraploid cultivars.

The anthers were taken from young flower buds ranging in size from $2\frac{1}{2} \times 4$ mm in *C. balearicum*, to $3\frac{1}{2} \times 6\frac{1}{2}$ mm in *C. persicum*. It is difficult to give the exact size of the bud which gives the greatest chance of finding dividing pollen mother cells. Another, more useful indication for the correct dividing stage is the length of the protuding petals outside the calyx. If the visible tips of the petals are about 1 mm, the pollen mother cells are usually in the process of dividing. The five anthers in this stage are slightly yellow coloured, and somewhat glassy. The anthers of any one bud vary only slightly or not at all in any one stage of development.

With pot culture of some species, as *C. repandum, C. balearicum* and *C. libanoticum*, the flower stalks start growing underground and they emerge just shortly before the opening of the flowers. So, one must carefully remove the soil near the surface in order to locate the correct buds.

Of the cultivar 'Wit met oog' flower buds of different sizes were selected for the examination of the reduction division in egg mother cells. Photo 1[1]) shows the correlation between bud sizes and various division stages.

For chromosome counts in somatic tissue the tips of the lateral roots ($\pm$ 1 mm thick), and the tips of very thin secondary lateral roots ($\pm$ 0.5 mm thick) are useful. The very thick adventitious roots usually have no divisions. Brown root tips have no divisions either. White root tips are an indication of good growth and corresponding cell divisions. In plants kept very wet, these white tips are mostly absent, but one can obtain them by knocking the plants out of the pots and replacing them. Thereafter the soil must be kept rather dry for one or two weeks. In this way the growth of new rootlets is stimulated.

The time of day of selecting anther material seems to be of minor importance. Although it is generally accepted that meiosis in pollen mother cells takes place mostly in the morning hours, very good division stages have frequently been found in anthers, selected in the afternoon. Selecting the root tips can take place at any time of the day.

1.2. *Pretreatment*

It happens very often that chromosomes lie very strongly in confusion, or that they are not orientated in one plane in metaphase. This is particularly the case when there is a great number of relatively long chromosomes. Counting is then very difficult, often even impossible. In any

[1]) All photographs are placed at the end.

case, the certainty about the exact number is disputable. To avoid these difficulties, one can give a pretreatment to the subject with a substance of which the action is twofold: first, destroying the spindle figure, by which procedure the chromosomes are mostly spread and can be brought in one plane by pressing, and secondly, contraction. Some of these substances which are known, are: colchicine (5), 8-hydroxyquinoline (42, 52), a-bromenaphthalene (32) and paradichlorobenzene (34).

The chromosomes of *Cyclamen* species with a low number are relatively long and mostly in strong confusion (see photo 2), whereas in species with a large number the chromosomes are relatively short, but lay very closely together (see photo 3). These facts make correct chromosome counting very difficult. Therefore, a pretreatment of the root tips was always applied. Previous experiments (30) showed that a treatment of 4–5 hours with 8-hydroxyquinoline was the best, and it was therefore used throughout this study. The solution was prepared according to TJIO and LEVAN: a 0.002 Mol solution (that is 0.29 g/l) in distilled water.

1.3. *Fixation*

Most of the time fresh material of root tips or anthers was used. Anthers were stained immediately. Root tips were hydrolised after a pretreatment. Particularly with the FEULGEN staining, better results were obtained if no fixation was applied. This is in accordance with the observations of HILLARY (23) and GERSTEL (13). If the material had to be fixed, it was done in CARNOY's fixation fluid (5).

1.4. *Staining*

Trials were carried out with orcein, aceto-carmine, crystal-violet, lacmoid, 1. basic fuchsin and haematoxyline as staining solution. It proved that staining with aceto-carmine or orcein gave the best results in the case of anthers. With root tips, staining with 1. basic fuchsin after FEULGEN, modified after DE TOMASI (7) gave very nice and well stained preparations. Thus, this method was preferred. The staining solutions were all prepared according to the formulae of DARLINGTON and LA COUR (5, 29); the dyes used were from G. T. GURR, London.

1.5. *Making the preparations*

Anthers. – As mentioned in 1.3, no fixation was applied. After removing the sepals and petals with a needle, the anthers were carried to a clean slide. After adding a drop of aceto-carmine, the material was squashed and, after removing thick tissue, supplied with a cover slip. The preparation was then gently heated, pressed and the superfluous solution was blotted away. Thereafter it was ready for examination.

Root tips. – After selection, the root tips were put into an 8-hydroxyquinoline solution during 4–5 hours. Hydrolysis followed in 1 N HCl at 60 °C for 10–12 minutes. Then the tips were dried with blotting-paper and brought into small porcelain cups with a fuchsin solution, where the root tips remained over night. The next morning they were rinsed in tap water. The extreme tips were cut off with a razor blade and transferred to a slide in a drop of 45 % acetic acid. After placing a cover slip, the tips were squashed by pressing and tapping softly with a wooden match stick. After blotting away the superfluous solution, the preparation was then ready for examination.

For a short preservation the cover slip was mounted at the edges with paraffin. In this way the preparation can be used for several weeks. If it was desired to make a permanent preparation, it was prepared by mounting in euparal according to DARLINGTON and LA COUR (5).

The usual period of hydrolysis of 4–6 minutes proved to be too short for a good FEULGEN staining of *Cyclamen* root tips. This period should be 10–12 minutes for an intensive staining of the chromosomes. Besides, the customary time of staining in the fuchsin solution of 1 to 2 hours proved to be too short as well. A better effect was obtained with 4–16 hours.

Originally root tip preparations were made by the paraffin section method and the crystal-violet staining. This method however was soon abandoned, since the author found that for counting chromosomes the squash method was more preferable than the paraffin method. Not only is the squash method much faster, but the results are even better most of the time. This is in full agreement with KAPPERT (26, p. 108).

2. MICROSCOPY AND MICROPHOTOGRAPHY

2.1. *Microscopy*

For studying and counting chromosome numbers microscopes of CARL ZEISS, Jena and of ZEISS OPTON, Oberkochen, were used. Both had phase contrast light. Both microscopes had 10 × oculars, but the former had objectives of 10 ×, 40 × and 90 ×, while the latter had objectives of 16 ×, 40 × and 100 ×.

If the chromosomes in the preparation were not laying in one plane or if they were covering each other, an attempt was made to spread them for the purpose of microphotography. This was performed by gently pressing the material with a sharp steel needle under the objectives 10 × or 16 ×. It demands a certain routine, but very excellent results can be obtained as shown in photos 4, 12, 14 and 27.

Although staining is not necessary for chromosome counting under phase contrast light, the author finds that staining benefits the observations. In some cases, for instance when the chromosomes overlap each other, it is easier to study under normal light conditions, because they are more easily distinguished from one another.

2.2. *Microphotography*

The microphotographs were made with a CARL ZEISS "Vertikal Kamera" in combination with a 100 Watt lamp and the microscope. "Replica" plates of the size 9 × 12 were used, and the negatives were developed with a normal hydrochinone developer.

The photographs were always made with phase contrast light and green filter, as these gave a sharp contrast. When an objective of 40 × was used, the exposure time was 45 seconds and in the case of a 90 × or 100 × objective, the exposure time was 90 seconds.

CULTIVARS

1. Introduction

It was Heitz (21) who published for the first time, in 1926, some chromosome numbers of *Cyclamen*. He recorded for the *C. persicum cult. hor.* the haploid number of 42–44 and the diploid number of 88. This cultivar belonged to the 'Splendens-Giganteum' type. In 1939 Glasau (14, p. 543) published the number 2n = 98 for a plant of the cultivar 'Salmoneum'. As the wild species had 2n = 48, he supposed that the number would probably be 2n = 96. This last-mentioned number was proved to be correct by Kappert (26) in 1941 for 'Käthchen Stoldt', 'Leuchtfeuer' (from Schneider), 'Pregetter lachsdunkel' and 'Rokoko rot', and in 1951 by de Haan (20). The latter counted the number 96 in cultivars with a red shade in their flowers, namely: 'Perle von Zehlendorf', 'Rood', 'Rood rococo', 'Rose van Aalsmeer' and 'Vuurbaak'. In the fringed and strongly fringed white ones, 'Wit fimbriata', 'Wit rococo' and 'Wit met oog fimbriata', the chromosome number was also 2n = 96 which must be considered as tetraploid.

The diploid number 2n = 48 was counted by Kappert (26) in 'Weisze Dame', 'Reinweisz' and 'Fenstercyclamen', by de Haan (20) in 'Sylphide', 'Sylphide cristata', 'Wit', 'Wit cristata' and 'Wit met oog' (purple and white, their crested forms and white with a crimson base). The cultivars investigated by de Haan were all raised at the Horticultural Laboratory at Wageningen and were grown from commercial seeds.

The author counted chromosomes of the same cultivars as de Haan (20) did and confirmed his results (photos 4 and 5).

2. Material

In the frame work of the International Registration of *Cyclamen* Cultivars by the Horticultural Laboratory at Wageningen, a large number of West-European cultivars was brought together, in all 242 groups. This extensive collection was studied cytologically by the present author. Not all groups are different cultivars, however: several are identical, but raised by different growers. But, on the other hand, similar cultivar names do not always imply identical material. For instance, there are six German 'Leuchtfeuers' that look alike from all outward appearance, but one of them has an aberrant number of chromosomes. Another example is 'Mauve Queen': Blackmore & Langdon's has 2n = 48, whereas Sutton's 'Mauve Queen' is tetraploid with 2n = 96. In this case the cultivars are morphologically different also: the first looks just the same as the Dutch 'Sylphide' (2n = 48), the second is more like 'Cattleya' with 2n = 96. And speaking about 'Cattleya', the Danish one differs morphologically from other 'Cattleyas'. As it looks identical with the above mentioned 'Sylphide' and also has 2n = 48, this material apparently bears the incorrect name.

3. Cytology

All of the above 242 groups were investigated in 1956–1958 in addition to another 33 cultivars of Dutch origin and 3 French ones, which were investigated in former years. Counts were all made of root tip material according to the technique described in Chapter II. In a majority of the cases from four to ten plants of each group were used for examination. In a few cases fewer plants were examined, but only when the group consisted of two or three plants. The chromosome numbers are based on various counts ranging between three and six complete metaphase plates for each plant. In the following sections these numbers will be described in detail. Table 1 gives a classification of these cultivars based on their somatic chromosome numbers. Below is a list of growers who contributed seeds or plants for the collection. The names of the growers are indicated in the tables 1 and 2 (see pages 10–14) with an abbreviated code for each according to country.

3.1. *The external morphology of the chromosomes*

The chromosomes in somatic metaphase appear as very small rods, about 1–2 μ long. In shape and in size they are slightly different from one another, while centromeres are difficult to distinguish. Most *Cyclamen* chromosomes have a median or submedian, or an almost terminal or subterminal centromere. In the diploids two of the chromosomes have a secondary constriction, in this way forming two satellites [not mentioned by GLASAU (14) and DE HAAN (20)], which are a little smaller than the chromosomes themselves and are egg-shaped. As this secondary constriction is a very deep one, the satellites mostly appear in squash preparations as if connected to the chromosome by a very thin chromatin thread, or as "fragments" (see photo 4). Very often, in the middle stage of the somatic metaphase, two of them are divided into two parts, although the other chromosomes and satellites are not yet in longitudinal division (see photo 6). Another remarkable fact is, that one can see very often that a satellite seems to be attached to the middle of the side of a chromosome,as shown in photo 7.

There is no difference between the chromosomes of the diploids and those of the tetraploids. However, the satellite chromosomes are four in number in the tetraploids (see photo 5). In the diakinesis, the stage in which the chromosomes are laying in pairs, called bivalents, there are 24 and 48 bivalents, respectively. Univalents or multivalents have never been observed, except in artificial triploids. An attempt was made to study the chromosome pairing, which however, was rather difficult as the chromosomes in meiosis are even smaller than those in mitosis and have the tendency to accumulate.

3.2. *Haploids and hemiploids*

It is a well known fact that on rare occasions some plants may originate with a haploid number of chromosomes in their somatic tissue. Often this accompanies polyembryony. KAPPERT (26) for instance found haploids in twin seedlings of flax and refers to other examples in rye, rice, grasses and potato. However, to the author's knowledge there is no report in the literature of the discovery of haploid *Cyclamen* with 24 chromosomes in the somatic tissue. KAPPERT (26) examined several twin seedlings of *Cyclamen* but did not find any haploid. After many years of investigation the author has never found a haploid *Cyclamen* either and therefore is of the opinion that haploids probably do not exist in diploid *Cyclamen*.

On the other hand, hemiploids, tetraploids with half the complement of chromosomes, have been reported. KAPPERT (26) mentions that the salmon pink *C. persicum parviflorum* (also called 'Neurosa', 'Neurosa von Reichart') has 48 chromosomes in its somatic tissue and was originally discovered in a group of 'Neurosas' with $2n = 96$! Another real hemiploid with 48 chromosomes in the somatic tissue was found among tetraploid plants named 'Kiel'. In both cases the plants were fertile.

In 1956 the author discovered within a group of 'Rosa von Marienthal' a plant with smaller flowers and thinner flower stalks than are normally found in this cultivar. Examination of the root tips revealed a chromosome number of 48, whereas the other plants had 96 chromosomes. Artificial pollination did not result in fruits and unfortunately the plant was lost.

In 1957 a Dutch grower presented to the Wageningen Laboratory of Horticulture a salmon scarlet plant which had a typical dwarf growth, very small leaves, flowers only slightly larger than the wild type and very short and thin flower stalks. The anthers contained hardly any pollen and all attempts of pollination failed. This plant had 24 chromosomes in pollen mother cells and 48 in root tip cells, whereas comparable scarlet flowering cultivars had 48 and 96 chromosomes, respectively.

Since several million *Cyclamen* are raised every year, undoubtedly there are many more hemiploids in existence, but in practice they are nearly always discarded, because they are plants with little or no commercial value to the grower. The only known positive case in which a breeder recognized something of value is the discovery by REICHART of the hemiploid 'Neurosa'.

LIST OF GROWERS WHO SUPPLIED THE EXAMINED CULTIVARS (SEE TABLES 1 AND 2)

Austria:	HÜBNER (H), Ernstbrunn; SCHWARZROCK (S), Mödling.
Belgium:	DE TROYER (T), Ukkel.
Denmark:	DAEHNFELT (D), Hunderup; OHLSEN (O), Kopenhagen; RASMUSSEN (R), Horsens.
England:	BLACKMORE & LANGDON (B), Bath; MAARSEN (M), Osgadby; SUTTON & SONS (S), Reading.
France[1]):	HENNY et FILS (H), Vaucresson.
Germany:	BINNEWIES (B), Alfeld; BUBECK (Bu), Fellbach bei Stuttgart; FISCHER (F), Wiesbaden; FUSZ (Fu), Wernigerode im Harz; HANNSEN (H), Bremen; KLEE (K), Bielefeld; MAYER (M), Bamberg; MELZER (Me), Chemnitz; NESKE-SCHENK (N), Hohenhameln; NUPHAUS (Nu), Hamburg; STOLDT (S), Hamburg-Wandsbek; STRÜVE (St), Herford; SÜPTITZ (Su), Hamburg-Eidelstedt; TAGMANN (T), Hannover.
The Netherlands:	D. BAARDSE (B), Aalsmeer; Wed. P. EVELEENS (E), Aalsmeer; G. LODDER (L), Vleuten; Gebr. MAN (M), Aalsmeer; D. DE RIDDER (R), Aalsmeer; Laboratorium voor Tuinbouwplantenteelt (T), Wageningen.
Switzerland:	MOLL (M), Zollikon; SCHWARZ (S), Bern.

[1]) The French cultivars marked in table 2 with E were imported by the firm EVELEENS, Aalsmeer, but the origin is unknown.

TABLE 1. Chromosome numbers of West-European *Cyclamen* cultivars

	Austria	Belgium	Denmark	England
2n = 48	Reinweisz S	Decora T New Perle T Ondulatum gemengd T Oranje As T Pink Pearl T Superba T Sylphide T Triomph van Brussel T Wit oculatum T Zilverwit T	Cattleya D Reinweisz O Reinweisz frühe Stamme O Weisz no. 5 R White Hunderup strain D	Burgundy B Cyclamen persicum mixed S Firefly S Giant White B Mauve Queen B Sylphide M White S
2n = 90				
2n = 92			Dunkelrot O Fliederblau O Kirschrot O Leuchtfeuer O Medium salmon O Perle von Zehlendorf O Perle von Zehlendorf frühe Stamme O Rococo-mischung O Rosa von Zehlendorf O Rosa von Zehlendorf frühe Stamme O Weisz mit Auge O	
2n = 94				
2n = 95				

TABLE 1. Chromosome numbers of West-European *Cyclamen* cultivars

France	Germany	The Netherlands	Switzerland
Blanc H	Butterfly F Erika B Goldlachs N Kirschlachs B Lachs mit weiszem Rand B Pfirsichblüte T Reinweisz B, M Reinweisz lang Su Silberlachs N	Anneke T Barbarossa T Lodder strains 18 numbers L Ridson lila R Ridson rose R Ridson wit R Sonja T Sylphide T Willie T Wit T Wit cristata T Wit met oog T Wit met oog cristata T	Reinweisz S
	Lachsscharlach B		
Carmin E Rose foncé H Vermillon E, H	Brillantlachs B *Cattleya-Rosa* M Cherry Nu Dunkelrot M, N Flamingo B Flieder F Hellrosa mit Karminauge B Lachsdunkel B, N, St Lachshell M, N, St Lachsscharlach B Lavendel B Leuchtend Blutrot M Leuchtendrot M, N Leuchtendrot mit Silbersaum N Leuchtfeuer B, M, N, S, St Morgenröte B, N, St Neulachsrosa B, St Orange B Reinrosa N Rokoko N Rosa mit Auge N Rosa von Wandsbek M, S Ruhm von Wandsbek S Safraninrot B Safraninrot mit Silbersaum B Seidenrosa K Viktoria B		Helvetikum lachs M Helvetikum rosa M
	Andenken an Gottlieb Bubeck Bu Rot mit Lachsschein S Dunkelrot mit Silbersaum N		
	Lachshell B		Gefranste M, S

TABLE 1. (Continued).

	Austria		Denmark		England		France	
2n = 96	Lachsdunkel	H, S	Aase, rosa mit Øje	R	Afterglow	B	Graines Saumon	H
	Lachshell	H, S	Cherry Red	D	Bath Pink	B	Rose Cyclamen	H
	Lachsscharlach	H, S	Perle von Zehlendorf	D, R	Blackmore's Frilled Picottee Edged	B	Saumon Clair	E
	Leuchtend Dunkelrot	H	Rosa von Zehlendorf	D, R	Cattleya	M	Violet	H
	Leuchtendrot	S	Rose van Aalsmeer	D	Crimson	S		
	Leuchtfeuer	H, S	Salmon rex	D	Dark lilac	M		
	Reinweisz	H	Scarlet rex	D	Firebrand	S		
	Safraninrot	H	Torch light	D	Giant Crimson	B		
	Weisz mit rotem Auge	H	Vuurbaak laks	R	Giant Salmon Scarlet	B		
	Zartrose	S			Grandiflora	B		
					Hydrangea Pink	S		
					Light Rood	M		
					Lilac	B		
					Maarse's Purper	M		
					Mauve Queen	S		
					Perle von Zehlendorf	M		
					Pink Pearl	S		
					Reinrosa	M		
					Rosa von Marienthal	M		
					Rose Queen	S		
					Rose van Aalsmeer	M		
					Salmoneum oculatum	M		
					Salmon Picottee	S		
					Salmon Pink	S		
					Salmon Scarlet	S		
					Scarlet rood	M		
					Shell Pink	B, S		
					Sutton's Unique	S		
					Sweet scented	B		
					Vuurbaak	M		
					White	M		
					White with crimson base	S		

TABLE 1. (Continued).

Germany		The Netherlands		Switzerland	
Apfelblüte	B	Baardse's Wonder	B	Cattleya	S
Brockenfeuer	Fu	Baardse's Wonder fimbriata	B	Lachsdunkel	S
Cattleyen Mehrblüten-blättrig	Su	Carmin salmoneum	E	Lachs Silberblatt	S
Dunkelblutrot	B	Cattleya	T	Leuchtend Dunkelrot	S
Dunkellachs	Su	Cattleya fimbriata	E	Leuchtfeuer	S
Dunkelrot mit Silbersaum	N	Cyclamen purper	T	Leuchtfeuer Silberblatt	S
Fleischfarbe	Nu	Dirk Baardse	B	Neulachs	S
Gerhard Bubeck	Bu	Donkerrood	T	Reinlachs	S
Käthchen Stoldt	S, Su	Donkerrood fimbriata	E, T	Rosa mit Auge	S
Lachsdunkel	M	Donkerrood rococo	E	Weisz mit Auge	S
Lachs gefranst	M, Su	Harlekijn	M		
Lachshell	B	Harlekijn fimbriata	B		
Lachsrosa	N	Jubileum	B		
Lachsscharlach orange	H	Lavendel	E		
Leuchtendrot	B, S	Lichtrood	E, T		
Leuchtfeuer	Su	Lichtrood fimbriata	E		
Leuchtfeuer Mehrblüten-blättrig	Su	Lichtrood rococo	T		
Neue gefranste	B	Maarse's Purper	M		
Neulachsrosa	S	Maarse's Purper fimbriata	M		
Neurosa	B	Oculatum fimbriata	E		
Reinrosa	B	Oculatum rococo	E		
Rokoko erecta	B	Perle von Zehlendorf	E		
Rosa von Marienthal	S	Perle von Zehlendorf fimbriata	E, M		
Schöne Dresdenerin	Me	Perle von Zehlendorf zilverblad	T		
Striata	B	Reinrosa	T		
Viktoria	N	Reinrosa fimbriata	E		
Weisz mit Auge	B, M, N, S	Rood	E, T		
		Rood fimbriata	E, T		
		Rood rococo	E, T		
		Rood met rand	T		
		Rosa von Marienthal	E, T		
		Rosa von Marienthal fimbriata	M		
		Rosa von Marienthal rococo	T		
		Rosa von Zehlendorf	E		
		Rosa von Zehlendorf fimbriata	E		
		Rose van Aalsmeer	E, T		
		Rose van Aalsmeer fimbriata	E		
		Rose van Aalsmeer rococo	T		
		Salmoneum oculatum	E, T		
		Salmoneum oculatum fimbriata	E		
		Salmoneum oculatum rococo	E		
		Victoria	M, T		
		Vuurbaak	M, E, T		
		Welriekend licht	T		
		Welriekend rood	M		
		Wit	T		
		Wit fimbriata	E, T		
		Wit rococo	M, T		
		Wit met oog fimbriata	E, T		

TABLE 2. Diploid and tetraploid chromosome numbers in equivalent *Cyclamen* cultivars

	Austria	Belgium	Denmark	England	France	Germany	The Netherlands	Switzerland
White $\boxed{2n = 48}$	Reinweisz S	Zilverwit T	Reinweisz O Weisz no. 5 R	White S Giant White B	Blanc H	Reinweisz B, M Reinweisz lang Su	Wit T Wit cristata T	Reinweisz S
$\boxed{2n = 96}$	Reinweisz H			White M		Käthchen Stoldt reinweisz S, Su	Wit T	
White with crimson base $\boxed{2n = 48}$		Wit oculatum T					Wit met oog T	
$\boxed{2n = 96}$	Weisz mit rotem Auge H		Aase, rosa mit Øje R Weisz mit Auge (2n = 92) O	White with crimson base S		Weisz mit Auge B, M, N, S		Weisz mit Auge S

3.3. *Diploids*

As already mentioned in section 1 (p. 7), DE HAAN (20) investigated 13 Dutch cultivars and found 'Wit', 'Wit met oog', 'Sylphide', and their eventual crested forms to be diploid. The others were tetraploid. Today 62 cultivars, grown in The Netherlands have been studied and their results are the same with one exception, namely, a tetraploid white cultivar.

This latter exception also holds true for the Austrian 'Reinweisz' and for the German 'Käthchen Stoldt', as shown in table 2, while all white with crimson base cultivars ('Wit met oog'), except the Dutch and the Belgian one, are tetraploids.

For the moment, it is impossible to make certain which percentage of the total number of cultivars is diploid, as we do not know yet which cultivars are identical (see section 2, p. 7). However, about 20% of the cultivars grown in The Netherlands may be estimated to be diploid.

3.4. *Triploids*

Spontaneous triploidy in *Cyclamen* has not been described in literature, nor has any been observed in the investigated cultivars and in several large offsprings raised at the Horticultural Laboratory.

3.5. *Tetraploids and aneuploids*

As mentioned in section 1 all Dutch cultivars other than 'Wit', 'Wit met oog', 'Sylphide' were classified by DE HAAN as tetraploid. This classification is principally correct. As one can see from table 1, however, the author found some exceptions. DE RIDDER's 'Ridson Rose', a rosy-pink flowering cultivar, for instance, is diploid, while at LODDER's nursery scarlet flowering 'Rex' *Cyclamen* as well as salmon and pink-rococo types are present with $2n = 48$.

Exceptions on this classification were found also in the other, West-European cultivars. The remarkable English cultivars 'Firefly', intense salmon-scarlet, and 'Burgundy', violet, are diploid as well as the German salmon coloured 'Goldlachs', 'Silberlachs', 'Lachs mit weiszem Rand' and 'Kirschlachs'. The salmon coloured cultivars of the Belgian grower DE TROYER are also diploid.

In 1957 root tips of some recently imported French cultivars were gathered at the nursery of the firm EVELEENS. The first view in the microscope gave the impression that there were less chromosomes than normally present in tetraploids. Exact counts proved this opinion to be right; instead of 96 there were only 92 chromosomes in the somatic tissue of two of them (see photo 8). This number $2n = 92$ corresponds with $n = 46$, counted during meiosis in anthers. Later, this aneuploid chromosome number was also found in 1 other French, 2 Swiss, 11 Danish and 27 German cultivars.

It is not likely that all these different cultivars originated from only one and the same aneuploid (see also section 4.1.2, p. 19). It is more acceptable to postulate that this chromosome mutation occurred at different nurseries.

In some cases both numbers $2n = 96$ and $2n = 92$ were found in groups, bearing the same name, but grown at different nurseries. These cases are mentioned in table 3. A good example is 'Leuchtfeuer': five German growers are raising an aneuploid form, but only one has this cultivar with $2n = 96$. No doubt that the latter is not identical to the first ones.

TABLE 3. Sources of German cultivars each of which have the number $2n = 96$ and $2n = 92$

Cultivar	$2n = 96$	$2n = 92$
'Lachsdunkel' . .	MAYER	BINNEWIES, NESKE-SCHENK, STRÜVE
'Lachshell'	BINNEWIES	MAYER, NESKE-SCHENK, STRÜVE
'Leuchtendrot' . .	BINNEWIES, STOLDT	MAYER, NESKE-SCHENK
'Leuchtfeuer' . . .	SÜPTITZ	BINNEWIES, MAYER, NESKE-SCHENK, STOLDT, STRÜVE
'Neulachsrosa' . .	STOLDT	STRÜVE
'Reinrosa' . . .	BINNEWIES	NESKE-SCHENK
'Viktoria'	NESKE-SCHENK	BINNEWIES

The fact is remarkable that all the Danish cultivars from OHLSEN are aneuploids with $2n = 92$, while the other Danish cultivars are not. It can be hypothesized that the aneuploid ones have been raised from some plants imported from Germany.

Besides these aneuploids with a chromosome number of $2n = 92$, others have been found. One plant of 'Lachsscharlach' (normally $2n = 92$) showed the number $2n = 90$. The number $2n = 94$ was established in one plant of 'Dunkelrot mit Silbersaum' (normally $2n = 96$), while this number was common for 'Andenken an Gottlieb Bubeck' and 'Rot mit Lachsschein'. One plant of 'Lachshell' (normally $2n = 96$) showed $2n = 95$ (see photo 9), whereas the same number was found in 'Gefranste' from both MOLL (one plant) and SCHWARZ (several plants).

It is remarkable that no aneuploids were found in the large number of English, Dutch and Austrian cultivars nor have there been found any aneuploids between diploid cultivars.

3.6. *Hexaploids and higher numbers*

GLASAU (14) recorded that 'Käthchen Stoldt' had about 130 chromosomes in somatic tissue; he supposed it to be some aneuploid form of a hexaploid. KAPPERT (26, p. 111) could not confirm this, as he counted only 96 chromosomes in this cultivar. The author's own examination confirmed that 'Käthchen Stoldt' has exactly 96 chromosomes in somatic tissue. Higher numbers than 96 have never been found in the cultivars, nor mentioned in literature, except by GLASAU.

4. THE CYTOLOGICAL BACKGROUND OF THE ORIGIN OF SOME CULTIVARS

4.1. *Ancient cultivars*

In the beginning of the last century *C. persicum* was only a collector's plant. Improvements in its culture (shortening of the growing period), better economical circumstances after 1850 and the development of new forms by breeding led to the success of the present-day *Cyclamen* (9). These new forms were brought about by colour variations (especially in the beginning) and mutations (salmon colour!), tetraploidy and gene recombinations.

The main period of colour variations, which started about 1739 and lasted until 1870, was a time of slow advance. According to DOORENBOS (9), the catalogue of N. VAN KAMPEN, Haarlem, of 1739 mentioned three forms, which were white (with crimson base), rubicund and rosy, respectively. Other rosy shades followed, but they did not bring real changes in colour. Thereafter during the years 1867–'70 great progress was made. In England it was WIGGINS, Isleworth, who especially introduced red forms as 'Rubrum', 'Oriflamme' and 'Firefly', the lilac 'Mauve Queen', the pure white 'Purity' and the first marginated *Cyclamen, C. pers.* 'Marginatum' (9, 45). H. LITTLE, an amateur from Twickenham, exhibited in 1870 his 'Queen of the Crimsons' and 'Purpureum', while J. WELCH brought 'Kermesinum', carmine-rose.

At the same time in Germany almost the same variations originated at the nursery of HAAGE and SCHMIDT, Erfurt. They offered 16 different forms, among them: 'Flore Rubro', 'Lilacinum Grandiflorum', 'Marginatum' (!), 'Carmineum Superbum' and 'Kermesinum' (!).

It was about 30 years later that a real aberrant colour appeared: the salmon colour. It is remarkable that this new shade originated in 4 different places within 6 years: 'Salmon Queen' in 1894 with SUTTON & SONS, Reading, England; 'Ruhm von Wandsbek' in 1898 at STOLDT's, Wandsbek, Germany; 'Salmoneum' in 1900 at FRÖBEL's, Zürich, Switzerland and 'Giganteum Salmoneum' at VACHEROT-LECLOUFLE's, Boissy-Saint Léger, France (45).

In the period before 1870 little or nothing is known about flower enlargement. But from 1870 until 1900 it is known that in England and Germany, *Cyclamen* with large flowers were sold at the market. From the first decade 'Giganteum' from EDMONDS, 'Unicum' from HAAGE & SCHMIDT, 'Universum' from GRAFF, and MÜLLER's 'Splendens' are well known as giant forms (9, 45). From 1880 until 1900 a great number of cultivars with large flowers came into existence. Important cultivars of that period, which are still cultivated today, are STOLDT's 'Rosa von Marienthal', 'Leuchtend Dunkelrot', 'Käthchen Stoldt' and 'Ruhm von Wandsbek'.

4.1.1. The first tetraploids

Polyploidy has played an important role in the evolution of the genus *Cyclamen*. Thus one can expect that polyploidy has participated in the development of the cultivars too. It appears that even most of our present cultivars are tetraploid. It is difficult to say when or where the first tetraploid originated. As chromosome counts were not made in earlier centuries, of course, one never has full certainty about the chromosome numbers of these old cultivars. Yet it is possible to ascertain with fairly great accuracy in which period the first tetraploids came into existence. Going back into history, the following indications for the appearance of tetraploidy are at our disposal. The breeding data mentioned hereafter are according to SCHNEIDER and MAATSCH (45).

In 1907 KIAUSCH introduced the present-day cultivated 'Perle von Zehlendorf' and 'Rosa von Zehlendorf', which both are tetraploid. These were descendants from *C. pers.* f. *splendens* 'Salmoneum', raised by FRÖBEL, Zürich. From this cultivar, GLASAU (14) in 1939 counted the chromosomes of a 40-years-old plant and found the number of about 98 in somatic tissue. Where the wild *C. persicum*, the ancestor, has 48 chromosomes diploid (see Chapter V), this 'Salmoneum' of 1899 approached a tetraploid.

From 1881 until 1889 STOLDT, Wandsbek-Marienthal, raised the cultivars 'Rosa von Marienthal' (1881), 'Leuchtend Dunkelrot' (1882) and 'Käthchen Stoldt' (1889), which are still in existence today (although their habits have certainly been improved). In these cultivars the author established the number $2n = 96$ in root tips.

As these still raised old cultivars are tetraploids, derived from narrowly related crosses (except 'Käthchen Stoldt'), one may almost be sure that the parents were also tetraploid, or at least one of them. As parents for 'Rosa von Marienthal' and 'Leuchtend Dunkelrot', Stoldt used some old forms of *C. persicum* and 'Splendens', a white with crimson base, raised by Müller, Dresden-Striessen, and put on the market in 1873. From this, one can assume that 'Splendens' must have been tetraploid.

The author found another argument for this supposition in Müller's (36) report of his crossing work concerning his 'Splendens'. Speaking about one of the used cross parents of 'Splendens', namely 'Robustum' (synonym *C. aleppicum* and 'Unicum'), he reported as follows:

"Diese Sorte nahm die Befruchtungen anderer, schlankerer Varietäten des *C. persicum* niemals an, doch wirkte umgekehrt der Pollen derselben sehr kräftig auf andere *Cyclamen persicum* ein. Aus diesen Kreuzungen erzog ich meine ersten splendens, welche ich 1873 in den Handel brachte.

Dieses Befruchtungsverhältnis dem *C. persicum* gegenüber hat sich auch auf mein *C. persicum splendens* vererbt und scheint auch den verwandten *C. persicum giganteum* und Universum eigen zu sein, welche jedenfalls ähnlichen Ursprungs sind".

Thus it was not possible to fertilize 'Robustum' with pollen of other, slender (!) forms of *C. persicum*. But on the other hand, pollen of 'Robustum' "acted very strongly" upon those other *C. persicum* forms.

Müller now examined the same fertilisation relation with respect to *C. persicum* of his own 'Splendens' and suggests this to be characteristic too for 'Giganteum' and 'Universum'[1]).

In a crossing experiment made by the author with diploid and tetraploid *Cyclamen* cultivars (which will be discussed extensively in Chapter IV), the following was observed:

After pollinating diploid plants with pollen of a tetraploid, all plants, except a poorly growing one, produced fruits, whereas 55 % of the pollinations succeeded in fruits. Selfings gave 33 % fruit set. *Thus pollen of a tetraploid "acts very strongly" upon a diploid.*

After pollinating tetraploid plants with pollen of a diploid, only 14 of 24 plants produced fruits, whereas 5 % of the pollinations succeeded in fruits. Selfings gave 56 % fruit set. *Thus in certain cases pollen of a diploid never acts upon a tetraploid, in other cases the results are poor at best.*

Comparing Müller's experiences with the above mentioned findings of the author gives:

C. 'Robustum' C. 'Splendens'	×	*C. persicum*	→ no results (Müller)
tetraploid	×	diploid	→ no results, or very poor results (Legro)
C. persicum	×	C. 'Robustum' C. 'Splendens'	→ very strong action (Müller)
diploid	×	tetraploid	→ very strong action (Legro)

[1]) According to Schneider and Maatsch (45) the forms 'Giganteum', 'Universum' and 'Splendens' were qualified in 1896 as synonyms of each other and were described as very large flowering white with crimson base.

In view of:

1. this agreement;
2. the fact that 'Splendens' as a cross parent gave tetraploid descendants;
3. the gigas-character;
4. the fact that fertilization incompatibility in cultivated *C. persicum* has never been pointed out (38, p. 20), one must conclude that 'Splendens' (1873) and also that 'Robustum' (1863) were *tetraploid Cyclamen*. Very probably 'Giganteum' (1870) and 'Universum' (1871) were also tetraploids, but as MÜLLER is not quite sure about their behaviour (and no other useful arguments are available), one must leave this question as it is.

There is just one point worthwhile mentioning. It is striking that all fringed cultivars, the so-called fimbriata-types, are tetraploid without any exception (see table 1). In 1827 fringed *Cyclamen* have already been described and it is not impossible at all that these old cultivars might have been tetraploid also. It is too speculative, however, to go farther into this point.

Summarizing, one may say that in the period from about 1863 until 1900 the chief source of the tetraploid parents of our present-day tetraploid assortment has originated. One of these parents was 'Splendens' and since it was used in crosses by several growers, it played a very important role in the development of this tetraploid assortment.

4.1.2. The first aneuploids

STOLDT selected in 1898 his 'Ruhm von Wandsbek' as a salmon pink mutation from 'Rosa von Marienthal', light rose. It was established in the research herein that the colour mutation was coupled with a *chromosome mutation*.

'Rosa von Marienthal' → 'Ruhm von Wandsbek' (STOLDT, 1898)
$2n = 96$ → $2n = 92$

And later on:
'Ruhm von Wandsbek' → 'Rosa von Wandsbek' (STOLDT, 1910)
$2n = 92$ → $2n = 92$

This 'Ruhm von Wandsbek' of 1898 is very probably the first aneuploid in *Cyclamen*. The most evident possibility is that 4 homologous chromosomes were lost, thus 'Ruhm von Wandsbek' should be a nullisome. Another possibility is the loss of two pairs of homologous chromosomes, in which case it would be a double monosome. To suppose that 4 non-homologous chromosomes disappeared, is the least acceptable possibility.

Another old aneuploid cultivar is 'Leuchtfeuer'. It is not certain how 'Leuchtfeuer' came into existence. According to SCHNEIDER and MAATSCH (45, p. 19) it originated at DLABKA's, Berlin-Zehlendorf in 1915, probably from the cross 'Perle von Zehlendorf' ($2n = 96$) × 'Dunkelrot' ($2n = 92$). If this is correct, it is believable that the hybrid ($2n = 94$) has extruded the 2 chromosomes of 'Perle von Zehlendorf', which were without partner. Anyway, selection in 'Leuchtfeuer' led to another aneuploid cultivar:

'Leuchtfeuer' → 'Lachsscharlach' (BINNEWIES, 1922).
$2n = 92$ → $2n = 92$

4.2. *Recent cultivars*

From the beginning of this century until now a very large number of cultivars has been produced by several breeders. In 1950 DOORENBOS (9) gave a list, in which among others, 160 fancy names, given since 1900, are mentioned. But the number should be much larger, because for example not all the fringed Dutch cultivars are recorded and, of course, neither are those which originated after 1950. It is beyond the scope of the present paper to discuss the origin of all the cultivars. Only the origin of a few well-known cultivars will be retraced with respect to their chromosome numbers.

SCHNEIDER and MAATSCH (45) mention the way of origin of several cultivars. Some of them originated from crosses, others from selections. A cytological examination of the parents and their descendants now gives the possibility to control the exactness of the described way of origin. Some interesting examples of agreement of data found in the literature and chromosome counts made by the author are as follows:

'Rosa von Wandsbek' × 'Ruhm von Wandsbek' → 'Neulachsrosa' (STOLDT, 1933)
$2n = 92 \quad \times \quad 2n = 92 \quad \rightarrow \quad 2n = 92$

It is noticed that the material obtained from STOLDT under the name 'Neulachsrosa' in table 1 was not the original cultivar. Within parentheses he mentioned the name 'Hellachs'. The 'Neulachsrosa' obtained from STRÜVE is the correct one, ($2n = 92$).

'Lachsscharlach' × 'Leuchtfeuer' → 'Orange' (BINNEWIES, 1934)
$2n = 92 \quad \times \quad 2n = 92 \quad \rightarrow 2n = 92$

'Reinweisz' × 'Lachs mit weiszem Rand' → 'Kirschlachs' (BINNEWIES, ?)
$2n = 48 \quad \times \quad 2n = 48 \quad \rightarrow \quad 2n = 48$

'Lachshell' × 'Neurosa' → 'Apfelblüte' (BINNEWIES, 1948)
$2n = 96 \quad \times \quad 2n = 96 \quad \rightarrow \quad 2n = 96$

The origin of BRAUKMANN's 'Flamingo' as recorded by SCHNEIDER and MAATSCH poses a remarkable problem for a cytologist. According to them, its origin is as follows:

'Flamme' × 'Leuchtfeuer' → 'Flamingo' (BRAUKMANN, 1929)
$2n = ? \quad \times \quad 2n = 92 \quad \rightarrow \quad 2n = 92$

It was impossible to count the chromosomes of 'Flamme' because it is no longer available, but since all its descendants are diploid (see the last paragraph of this section), one must conclude that 'Flamme' was also diploid. This means that the number of chromosomes of 'Flamme', which had been brought into the zygote, must have been doubled, followed by a chromosome loss. It is quite obvious to suppose that the two lost chromosomes are those of the doubled set of 'Flamme'.

This then would be the first known case in *Cyclamen* of a diploid × "aneuploid tetraploid" cross, which resulted in an "aneuploid tetraploid" descendant.

About the origin of the diploid salmon shades, the following can be said: LODDER informed the author personally that his so-called LODDER-strains (18 more or less different types) originated from some plants imported from Germany as "Liebhaberssorte". As BRAUKMANN's 'Goldlachs' and 'Silberlachs' (originating from 'Flamme') have the same chromosome number ($2n = 48$) and are morphologically similar with LODDER's strains, there is no doubt about the origin. All these diploid cultivars, including 'Kirschlachs' (BRAUKMANN, 1936), 'Barbarossa' (BRAUKMANN, 1939) and the cultivars of DE TROYER, which are quite the same as the LODDER-strains (although DE TROYER claimed they were bred by himself in 1938), must have the same ancestor, i.e., BRAUKMANN's 'Flamme', raised in 1922. It is supposed that the diploid 'Lachs mit weiszem Rand' from BINNEWIES, which is almost identical with some of the above mentioned cultivars, also belongs to this group.

5. CONSEQUENCES FOR BREEDING

Table 1 shows that most of the *Cyclamen* cultivars are tetraploid, while WELLENSIEK (55) proved their autotetraploid behaviour by a genetical analysis. It is this that makes breeding so very troublesome. KAPPERT (26) has made interesting calculations which illustrate these difficulties. WELLENSIEK (54, p. 775) says: "There is one result of general interest in our Cyclamen work to which I like to draw special attention and this is the difficulty of breeding autotetraploids". And farther on: "It would undoubtedly be of enormous importance if our tetraploid varieties could be transferred into diploid condition and could be bred according to diploid methods, but for the moment" – 1952 – "it is uncertain whether this will be possible on a sufficiently large scale".

At this moment, seven years later, however, the opportunity has presented itself to obtain an assortment of diploids with the same colour variation as in our present tetraploid assortment. There are several possibilities which in combination will certainly lead to this purpose. These possibilities are:

1. Recognizing the diploid assortment by cytological examination of all known cultivars. So far, besides white, white with crimson base and purple there were found more than 10 other shades in salmon, rose, scarlet crimson, dark crimson, cherry-red and dark purple colours and moreover the nice diploid rose 'Rococo' (obtained from LODDER), the latter giving the possibility of breeding other diploid rococo-types.
2. Selecting hemiploids by means of cytological examination of tetraploid offsprings (26).
3. Reducing the chromosome number by crossing diploids with tetraploids and crossing the obtained triploids with diploids, according to KAPPERT's method (26). Since in this way he obtained new colour shades in diploid *Cyclamen*, we may be hopeful about our 33 triploids (see next chapter).
4. Profiting from the greater knowledge of the genes concerning the flower colour, as in the results of earlier paperchromatographical investigations of SEYFFERT (47, 48) and the recent studies of VAN BRAGT (1).
5. Crossing the available diploids and the newly obtained diploids with each other.

No practical obstructions arise when selecting large flowering types since large flowers are present in diploid as well as in tetraploid cultivars (10).

Summarizing it can be concluded that it is possible to carry out a well defined breeding project with the purpose to enlarge the diploid assortment, if the above mentioned possibilities are exploited in combination with each other.

CHAPTER IV

CROSSES BETWEEN DIPLOID AND TETRAPLOID CULTIVARS

1. INTRODUCTION

In 1948 WELLENSIEK (58) started a series of crosses between diploid (2n) and tetraploid (4n) cultivars to study genetics and cytology of potential hybrids. The following crosses succeeded:

2n × 4n
'Wit' × 'Wit fimbriata' → one F_1 of 6 seeds
'Sylphide' × 'Wit fimbriata' → four F_1's of 1, 2, 4 and 45 seeds, respectively
'Wit cristata' × 'Wit fimbriata' → one F_1 of 1 seed
4n × 2n
'Perle von Zehlendorf' × *C. persicum* → one F_1 of 1 seed.

In all these crosses cytological investigations were made by the present author. The data are recorded in table 4. Except in one case of probable hemiploidy all the F_2 and F_3 generations, raised from tetraploid F_1's, were tetraploid too. In one diploid F_2, one case of spontaneous tetraploidy occurred.

The data in this table refer to six crosses 2n × 4n giving 1 triploid F_1 (2 plants) + 3 tetraploid F_1's (5 plants) + 2 mixed diploid and tetraploid F_1's (14 plants); one cross 4n × 2n giving 1 tetraploid F_1 (1 plant).

Genetical analysis of the consecutive generations by WELLENSIEK (55, 56) led to the conclusion, that some of the examined gene segregations were a direct evidence for an autotetraploid character in F_1 and further generations; in the cross 2n × 4n the egg cell, and in the reciprocal cross the sperm cell must have been unreduced or doubled after reduction.

2. EXPERIMENTS

For a cytological explanation of the obtained results, the above discussed material was too small. Therefore new experiments were conducted by the author.

It appeared at first that the castration method was not entirely successful, since some diploid F_1-plants came into existence, which were identical to the female parent. Furthermore, this method – that is removing the anthers with a pair of forceps just before the flowers open – took much time and was tedious. An indication for a simpler method was the fact that the anthers are connected with the base of the corolla. Therefore, when one removes the corolla, all the anthers are removed in one operation without the chance of tearing the anther tissue, as often occurred by castration with a pair of forceps. A castration experiment was conducted in 1953/54 in this way: 2,300 flower buds of the cultivar 'Donkerrood' were castrated by removing the corollas shortly before the flowers opened. The plants were placed in a greenhouse, isolated from other *Cyclamen*. Neither fruits (except two parthenocarpic ones) nor seeds were obtained. This method, therefore, proved to be fully reliable.

The crosses between 2n and 4n *Cyclamen* cultivars were started in the flowering season 1954/55. For the experiments, 24 (+ one control) plants 'Wit met oog', 2n = 48, and 24 (+ one control) plants 'Donkerrood', 2n = 96, were used. The female plants were placed in a greenhouse isolated from other

TABLE 4. Somatic chromosome numbers in consecutive generations from the crosses between diploid and tetraploid *C. persicum* cultivars made by WELLENSIEK. All plants not investigated died before they could be examined

Cross	F_1-plants			F_2-plants			F_3-plants		
2n × 4n	number	investigated	2n	number	investigated	2n	number	investigated	2n
'Wit' × 'Wit fimbriata'	4	1	48	69	10	48			
		3	96	14	13	96			
				37	35	96			
				43	38	96			
'Sylphide' × 'Wit fimbriata'	2	2	72	1	1	±82			
				1	1	86			
	1	1	96	111	95	96			
	3	3	96	7	4	96			
				61	31	96			
				81	53	96			
	10	6	48	7	7	48			
				10	7	48			
				11	4	48			
				14	11	48			
				21	18	48			
					1	96			
				38	37	48			
		4	96	2	2	96	338[1])	279	96
				6	6	96			
				24	19	96			
				45	35	96			
'Wit cristata' × 'Wit fimbriata'	1	1	96	112	67	96			
4n × 2n									
'Perle von Zehlendorf' × *C. persicum*	1	1	96	90	1	48			
					88	96			

[1]) Totaled number.

Cyclamen, whereas some male plants remained in an adjoining greenhouse, separated from the former. The castrations were carried out as described above. Throughout the experiments no open flowers appeared on the female plants. Since the experiments took place in winter time, there were no pollinating insects present. The flowers on the female plants were therefore left uncovered. Artificial pollinations were made 1–2 days after castration and repeated every 4–6 days. Most of the flowers received in this way 3 pollinations. A total number of 1,600 crosses was made and the results are recorded in table 5.

The percentage of parthenocarpic fruits, obtained from the cross 2n × 4n was very high. It is striking, however, that the reciprocal cross did not produce any parthenocarpic fruits at all! Besides, it was interesting to note that fruits

TABLE 5. Results from the cultivar crosses 2n × 4n and reciprocal

Selfings and Crosses	Number of treatments	Parthenocarpic fruits		Fruits with seed		Seeds	
		number	%	number	%	total number	average number per fruit
'Wit met oog' (2n) selfed	55	0	0	18	32.7	982	54.5
'Donkerrood' (4n) selfed	25	0	0	14	56.0	137	9.8
'Wit met oog (2n)' × 'Donkerrood' (4n)	900	424	47	68	7.6	138	2.0
'Donkerrood' (4n) × 'Wit met oog' (2n)	700	0	0	34	4.9	121	3.5

of the 2n × 4n cross, containing only one seed, were of normal size, while fruits of the reciprocal cross, containing one seed, were much smaller (see photo 10). It is noticed that, if no fertilization took place, the ovules would remain alive 2–4 months or even longer without dying, and that they very often formed pseudo-seeds. It is very likely that this great longevity of the ovules increases the parthenocarpic fruit set, as GORTER and VISSER (16) suggest for parthenocarpy in pears and apples.

3. CYTOLOGY

3.1. *General*

In the cross 4n × 2n only triploids and tetraploids came into existence, however in the reciprocal cross triploids, tetraploids and *also* diploids arose, as shown in table 6. Since these diploids and also some of the tetraploids did not show a hybrid-character, they were not tallied in the total number of F_1-plants to obtain the number of true hybrids, as given in table 7. Although the percentage of successful crosses was higher for 2n × 4n than in the reciprocal cross, the number of these true hybrids was equal for both cases, i.e., 7 hybrids/100 crosses.

TABLE 6. Total numbers of diploids, triploids and tetraploids in the F_1

Cross	Number of seeds	Germinated seeds		Raised F_1-plants	Examined F_1-plants	Diploid		Triploid		Tetraploid	
		number	%			number of plants	%	number of plants	%	number of plants	%
2n × 4n	138	80	57.6	73	73	10	13.7	8	11	55	75.3
4n × 2n	121	77	63.6	70	69	0	0	26	37.7	43	62.3

TABLE 7. Number of true hybrids (differing in flower colour from the female plant)

Cross	Number of crosses	Number of F_1-plants	Triploid		Tetraploid		True hybrids per 100 crosses
			number	%	number	%	
2n × 4n	900	63	8	12.7	55	87.3	7
4n × 2n	700	48	26	54.2	22	45.8	7

The relation of the fruits to the diploid, triploid and tetraploid F_1-plants was as follows:

'Wit met oog' (2n × 48) × 'Donkerrood' (2n = 96):
1 fruit → 5 tetraploids
4 fruits → 4 tetraploids each
3 fruits → 3 tetraploids each
4 fruits → 2 tetraploids each
16 fruits → 1 tetraploid each
1 fruit → 1 tetraploid + 1 diploid
8 fruits → 1 triploid each
1 fruit → 3 diploids
2 fruits → 2 diploids each
2 fruits → 1 diploid

'Donkerrood' (2n = 96) × 'Wit met oog' (2n = 48):
1 fruit → 5 tetraploids
3 fruits → 4 tetraploids each
1 fruit → 3 tetraploids
2 fruits → 2 tetraploids each
2 fruits → 5 tetraploids + 1 triploid
1 fruit → 2 tetraploids + 3 triploids
6 fruits → 1 tetraploid + 1 triploid each
1 fruit → 1 tetraploid + 2 triploids
1 fruit → 3 triploids
3 fruits → 2 triploids each
4 fruits → 1 triploid each

The 10 diploids, mentioned in table 6, were obtained from 5 fruits. The plants were identical to the female parents, except two. The latter showed a colour segregation as commonly occurs in heterozygous 'Wit met oog' plants. From the 69 tetraploid descendants from the 4n × 2n crosses, 21 were identical to the female parents. They originated from 7 fruits. In both cases some of these mother-like F_1-plants originated together with true hybrids from one and the same fruit!

On the basis of these results one would assume that in some cases self-pollination must have occurred. Although the author is fully aware that errors are always possible, he will not accept this supposition. On the one hand, the castration method (removing the corolla) proved to be fully reliable, as has been pointed out above. On the other hand, there were never any open flowers with anthers on the female plants, whereas an accidental wrong pollination would have resulted in a number of fruits with a large amount of seeds. Therefore, the above mentioned descendants must have originated in another way than by a normal fertilization. This point will be discussed further in Chapter VI.

3.2. *Chromosomes*

The diploid and tetraploid F_1-plants did not show any cytological differences in comparison with the diploid and tetraploid cultivars. Aneuploids were not present. All the triploid F_1-plants had a chromosome number of 2n = 72. The meiosis in their pollen mother cells was very irregular. In early metaphase I some multivalents and univalents were often observed, sometimes associated with chromosome bridges, and in metaphase II chromosome numbers were found ranging from 30 to 42 (see photo 11).

Although the first two triploids, obtained by WELLENSIEK (54) in 1952, were completely sterile, in 1956/57 one partially fertile plant was found among 33 triploids. After artificial self-pollination this plant gave 6 seeds, from which

3 seedlings were raised. One showed the number 2n = 72, one had 2n = 84, without doubt; this is 6 times 12 and 7 times 12, respectively. The third plant showed the quite irregular number of 2n = 55 (see photo 12). In all these cases 3 satellites were present, however most of the time they were visible as "fragments".

Selfing the above mentioned 33 plants for the second time in 1957/58, four of them produced 3, 4, 13 and 20 seeds, respectively. From these seeds only 4 plants could be raised. Three of them had 72 chromosomes in somatic tissue, and one was too immature yet to be examined.

4. Possible explanations of the origin of the tetraploid F_1-plants

4.1. *Cytological*

Since in the above mentioned crosses tetraploid descendants arose, the diploid partner must have contributed twice the haploid number of chromosomes to the zygote. To determine if meiosis irregularities may have been the cause of the origin of the tetraploids, cytological investigations were made. However, during several years of these investigations in diploid *Cyclamen* anthers, only on one occasion a tetraploid pollen mother cell was observed. The chance of this occurrence can therefore be estimated to be extremely small, perhaps 1 to 100,000 or even smaller.

In megaspore mother cells the first or second reduction division could be observed (see photo 13). The view was always quite regular. The number of about 35 megaspore mother cells in which the divisions could be determined, is however far too little for any conclusion.

Cytological examinations did not lead to an explanation of the origin of the tetraploid descendants.

4.2. *Genetical*

Cytological processes as reduction division and chromosome doubling have always genetical consequences. As the cytological investigations did not lead to an explanation, a determination of the genetical results of the crosses may give a solution of the problem. Before going into the subject, something about the genes of the cross parents should be mentioned.

The two most important genes, which affect the flower colour in *Cyclamen*, according to Wellensiek (54, 55, 56) and Seyffert (47, 48), are:
W, responsible for general anthocyanin formation; *ww* means no colour at all.
S, in presence of *W*, responsible for the *limitation* of the crimson or purple colour to the *base* of the petals; *ss* gives *completely* coloured petals.

The female plants 'Wit met oog' were genetically different, i.e., some were *WWSS*, others *WWSs*. After self-pollination of the single male plant 'Wit met oog', used in the crosses 4n × 2n, the offspring segregated into 26 'Wit met oog' and 8 'Sylphide' (purple), thus closely approaching a 3:1 ratio. So the 'Wit met oog' male plant can be represented by *WWSs*.

The female and male plants 'Donkerrood' were homozygous *WWWWssss*. If the dominant gene *S* would have been present, this would have been observed directly as a different colour from the normal dark crimson.

For a genetical interpretation of the cross results, *W* has no value, because all hybrids will have *W* dominant. The observed segregations of the F_1's of the crosses in *reddish white with crimson base* and *purple* flowering plants, are the result of the presence or absence of *S*. Henceforth the term "reddish" will be used for reddish white with crimson base.

The author's above mentioned crosses are therefore symbolized as:
(1) 'Wit met oog' × 'Donkerrood' = 2n × 4n = *SS* or *Ss* × *ssss*;
(2) 'Donkerrood' × 'Wit met oog' = 4n × 2n = *ssss* × *Ss*.

The correctness of these symbolizations was established by the obtained triploids. In case (1) the triploid F_1-plants must be *Sss*, or *Sss* and *sss* (reddish and purple). Both types were observed, however not in any certain ratio since the mother plant material was heterogeneous. In case (2) the triploid F_1-plants must be *Sss* or *sss* and the segregation must approach 1:1 for the separate F_1's as well as for the totaled progenies. This has been observed, namely:

14 reddish : 12 purple
(13) : (13) c = 0.4

Since the *SS* female plants will contribute in any case *SS* into the zygote and the offspring will always be reddish, this type of plants will be left out of consideration in the following discussion.

4.3. *Cytogenetical*

Since crosses between diploid and tetraploid partners normally give only triploids, the occurrence of tetraploids must be the consequence of an abnormal course of events. The diploid partner undoubtedly contributes twice the haploid number of chromosomes in one way or another to the zygote. In general this is possible when either of the following occur:

A. Meiosis-abnormalities
B. Fertilization-abnormalities.

The different types of meiosis-abnormalities will be mentioned after PRAKKEN and SWAMINATAN (43); the fertilization-abnormalities as being dispermy, endogamy or endospermal embryo development, according to NEMEC (39), TISCHLER and PASCHER (51) and GUSTAFSSON (19), respectively.

A. Meiosis-abnormalities can be classified as:

1. Pre-meiotic disturbances by which the megaspore or microspore mother cells obtain the doubled chromosome number. A normal reduction division afterwards leads to diploid gametes.

The ♀ or ♂ plants *Ss* give *SSss* megaspores or microspores, which give *SS*, *Ss* and *ss* gametes in the ratio 1:4:1. Combination of these gametes with *ss* gametes of the tetraploid partner gives an F_1 of the type:

1 *SSss* : 4 *Ssss* : 1 *ssss* = *5 reddish* : *1 purple*.

2. Meiotic disturbances in the *first* division by which the uninucleate embryo sacs or pollen grains obtain the *unreduced* number and by which the homologous chromosomes remain together, e.g. after fusion of two M II plates. The ♀ and ♂ plants *Ss* give only *Ss* gametes, which give in combination with *ss* gametes of the tetraploid partner an F_1 of the type:

Ssss = *reddish*.

3. Meiotic disturbances in the *second* division by which the uninucleate embryo sacs or pollen grains obtain the doubled number. The homologous chromosomes are *separated* and each chromosome is present twice in germ cells.

The ♀ and ♂ plants *Ss* give then *SS* and *ss* gametes, which give in combination with *ss* gametes of the tetraploid partner an F_1 of the type:

1 *SSss* : 1 *ssss* = 1 *reddish* : 1 *purple*.

4. Post-meiotic disturbances by which the reduced nuclei in the male or female gametes re-develop the diploid number of chromosomes. In this case the doubling of the chromosome set of the haploid gamete happens before, at or after the moment of fertilization, while the presence of the double chromosome set of the tetraploid partner may or may not be a stimulus for this chromosome doubling.

The ♀ or ♂ plants *Ss* give then *SS* and *ss* gametes also, which give in combination with *ss* gametes of the tetraploid partner an F_1 of the type:

$$1\ SSss : 1\ ssss = 1\ \textit{reddish}: 1\ \textit{purple}.$$

B. Fertilization-abnormalities.

The different cases by which the diploid partner can contribute twice the haploid number of chromosomes into the zygote, are:

1. Endogamy. — In the 2n × 4n cross, endogamy – a *fusion* of 2 haploid embryo sac nuclei – before or at the fusion with the diploid gamete of the tetraploid partner, would give a 4n zygote.

In ♀ plants *Ss* the embryosac nuclei are either *S* or *s*. Fusion gives either *SS* or *ss* fusion products, which give in combination with *ss* gametes of the tetraploid partner an F_1 of the type:

$$1\ SSss : 1\ ssss = 1\ \textit{reddish}: 1\ \textit{purple}.$$

2. Dispermy. — In the cross 4n × 2n, dispermy – fertilization of the female gamete by two male gametes – would give a 4n zygote.

In ♂ plants *Ss*, gametes are normally *S* or *s*. If fertilization would take place by two male gametes, there is no reason why an *independant combination* would not take place. This gives in combination with *ss* gametes of the tetraploid partner an F_1 of the type:

$$1\ SSss + 2\ Ssss + 1\ ssss = 3\ \textit{reddish}: 1\ \textit{purple}.$$

3. Endospermal embryo development. — In the cross 2n × 4n an embryo development from an endosperm cell would give tetraploid descendants, since the endosperm is $(n + n) + 2n$ = tetraploid.

In ♀ plants *Ss* the embryo sac nuclei are either *S* or *s*. Fusion gives either *SS* or *ss* polar nuclei, which give after fertilization with *ss* second male nuclei endosperm and embryos of the type:

$$1\ SSss : 1\ ssss = 1\ \textit{reddish}: 1\ \textit{purple}.$$

5. Discussion

Tetraploid progenies from crosses between diploids and tetraploids have been observed in different plant genera as *Brassica* (41), *Campanula* (17, 18), *Petunia* (28), *Primula* (4), *Saccharum* (3), and *Solanum* (27, 43, 53). Of these cases, only the crosses between diploids and autotetraploids in *Brassica chinensis* and in *Campanula persicifolia* are equivalent with those in *Cyclamen persicum*. In *Petunia* and *Primula* only the 4n × 2n cross succeeded, whereas the other mentioned cases concern crosses between species. Some of the cases will be discussed together with the author's results in the following sections.

5.1. *2n × 4n*

The genetical results of the 2n × 4n crosses are given in table 8. The separate

F_1's cannot be summarized since the female plants were either heterozygous or homozygous for *S*.

TABLE 8. Diploid × tetraploid → tetraploid F_1. Segregation of the F_1's in reddish white with crimson base (reddish) and purple. The female plants (mp.) were *not* identical

F_1 \ mp.	677	682	683	684	686	689	707	709	723	724	726	732	753
Reddish	2	3	15	3	1	3	1	1	2	5	2	4	1
Purple	0	0	2	0	1	2	0	0	2	0	0	1	0

It is probable that the F_1 from mp. 683 – and perhaps the F_1's from mp. 724 and 732 also – originated as a consequence of tetraploid megaspore mother cells (possibility A.1). Furthermore, there were segregating and non-segregating F_1's. In the former one may conclude that the *first reduction division occurred*, thus possibility A.2 had not taken place. In the case of non-segregating F_1's very probably this happened also, but the small number of F_1-plants, or the homozygosity of the female parents may have prevented the outer perceptibility of the reduction.

Possibilities A.3, A.4, B.1 and B.3 may have occurred, since their effects are the same and since 1:1 segregations were observed. As it is impossible to decide the occurrence of these possibilities by genetical analysis, one cannot draw any conclusion, however.

Concerning the appearance of the first reduction division in general, the following can be said. BREMER (3) observed in *Saccharum* that the first reduction division always took place. The chromosome doubling occurred by endoduplication in either the chalazal tetrad nucleus (A.4) or in the chalazal dyad nucleus (A.3). BERGMAN (2) has examined in *Hieracium* also, that the first meiotic division took place in the usual way, while the second one failed to appear (A.3). NISHIYAMA *et al.* (41) also suggest that the first reduction division took place in *Brassica* and that doubling of the haploid set might occur in the early development of the 3n embryos. KOOPMANS *et al.* (27, p. 115) do not believe that in *Solanum unreduced egg cells* are responsible for the tetraploid offsprings. "Normally reduced gametes are doubled *afterwards*, by some cause which is not clear", and: "The only explanation possible seems to us, that doubling of a reduced gamete occurs after pollen has entered the eggcell". The author cannot fully support this theory. In regard to BREMER's observations and the fertilization-abnormalities mentioned above, this is surely not the "only explanation possible". However in the author's opinion, KOOPMANS *et al.* are correct when they doubt the explanation of PROPACH, IVANOV, IVANOVSKAJA and STELZNER. They are speaking of *unreduced egg cells*, while, as KOOPMANS *et al.* mention (27, p. 114), the meiosis of the megaspore mother cells has not, or in any case, has been insufficiently examined and the explanations are based on data of *pollen* meiosis alone! PRAKKEN *et al.* (43, p. 80 and p. 88) are also speaking about *unreduced egg cells* as origin of the tetraploid F_1's in *Solanum*, although further on (43, p. 89) they suggest that the increased gametic chromosome number mainly depends on post-meiotic disturbances.

It is clear from the above that in general crossing a diploid *Aa* with a tetraploid *aaaa*, followed by genetical analysis of the offspring can decide, if the first reduction division whether or not occurred.

Concerning *endogamy*, the two synergids have to be considered as possible fusion partners with the egg cell. They are lying close together with the latter and have also the capacity of being fertilized. The latter fact is shown in a long list of observed synergid-fertilizations by TISCHLER and PASCHER (51, p. 700–701). With regard to the fusion possibility, THOMAS (50) observed in embryo sacs of *Rubus nitidoides* nuclei-fusion in the egg cell region, whereby the "egg cell" got the double haploid number. Although further development of the "diploid" nuclei, thus originated, has not been traced yet, it is quite possible that they can be fertilized by pollen of a tetraploid partner. Such an origin of tetraploid descendants does not seem very likely, since such nuclei-fusions have been observed only once. One must not forget, however, that investigations about this subject are very scarce. Besides, the chance that tetraploid descendants are obtained from 2n × 4n crosses in *Cyclamen*, is extraordinarily small. Since an ovary of a diploid *Cyclamen* cultivar contains approximately 160 ovules and since from 900 crosses only 55 tetraploid hybrids were obtained, this chance is ± 0.04 %.

It is not unlikely that the supposed fusion between egg cell and one synergid (or perhaps among the two synergids) is influenced by the gamete of the tetraploid partner, or by the normal fertilization of the secondary embryo sac nucleus; or that when passing through one of the synergids, the male nucleus may draw the nucleus of the synergid with it into the egg. Anyhow, in connection with the later discussed dispermic origin of embryos in the 4n × 2n cross, as conformable "doubling mechanism", attention must be paid to the possible occurrence of endogamy.

Although *endospermal embryo development* occurs in nature, as GUSTAFSSON (19, p. 33) reported, its is doubtful to suppose an origin of the tetraploid F_1's of the 2n × 4n crosses in this way, because one can expect such a development in the reciprocal crosses as well. But then the endosperm will be pentaploid and the endospermal embryo also. However pentaploid F_1-plants have never been observed.

In summarizing:

(1) one or some F_1's may have originated as a consequence of tetraploid megaspore mother cells;

(2) in some cases, quite probably in all cases, the first reduction in the concerned megaspore mother cells took place, as was established by the segregating F_1's from *Ss* × *ssss* crosses;

(3) the process occurring after the first reduction division, by which the diploid ♀ parent brought twice the haploid number of chromosomes into the zygote, could not be determined by genetical analysis of the F_1's.

5.1. *4n × 2n*

The genetical results of the 4n × 2n crosses are given in table 9. The separate F_1's are too small as base for conclusions. It is allowed however to total them, since the female plants were identical (*ssss*) and were crossed with one male (*Ss*), although with the proviso that the F_1's as a whole do not form a heterogeneous progression. This can only be decided in connection with a comparison of the observed and the expected segregations. The ratios which approach the observed segregations may be 1:1, 3:1, or 5:1. In all these cases the several F_1's can be considered as forming a homogeneous progression at a 5 %-chance level, although in the question of a 1:1 and 5:1 ratio it becomes *doubtful*.

A segregation approaching a 5:1 ratio can be expected when pre-meiotic disturbances (A.1) took place and led to tetraploid pollen mother cells. There is however a serious objection to accept such a course of events. A prone pollen grain of 'Wit met oog' occupies approximately $250\,\mu^2$, an upright one $100\,\mu^2$. As the surface of the stigma is about $17{,}700\,\mu^2$, the approximate number of pollen grains lying in one layer on the stigma ranges between 75 and 175, with an average of 125. It is not likely that pollen grains from the 9th layer have a chance to participate in the fertilization. Then, in the case of the 4 F_1-plants of mp. 1261 and 1273 (only 3 are recored in table 9, since one did not flower), which originated from one fruit each, at least 4 diploid pollen grains in 1,000 haploid ones (8 times 125), or one in 250 must have been present. Moreover, these diploid pollen grains must then also have participated of the fertilization, which is certainly not usual. This consequence is however in contradiction with the cytological observations as mentioned in section 4.1, p. 26. An explanation of the origin of the tetraploid descendants by pre-meiotic disturbances (A.1) is therefore not acceptable.

The chance that the non-segregating F_1's would have segregated if they were in larger numbers, is very great. Consequently the chance that the first reduction division failed in these cases is very small. Considering this supposition and the segregating F_1's, the possibility A.2 may be excluded as a general explanation.

Totaling the F_1's on a 1:1 base gives a segregation which deviates significantly. Possibilities A.3, A.4, and B.3, causing 1:1 segregating offsprings must therefore be rejected. This is in agreement with the author's cytological examinations. In the preparations of *Cyclamen* disturbances in the second reduction division were never observed. Endospermal embryo development did not occur because this would have given pentaploid F_1-plants, which were not observed. It is noticeable that KOOPMANS *et al.* (27, p. 114) also observed the same with respect to their diploid cross partners: "Neither in *Solanum chacoense* nor in *S. phureja* we ever found a dyad." PRAKKEN *et al.* (43, p. 89) mention on this point: "The percentage of F_1 plants with an increased number of chromosomes usually seems to be much higher than the percentage of dyads or larger pollen grains." Moreover GUSTAFSSON (19, p. 18) declares that on account of meiotic disturbances this type of division leads probably to viable spores only on occasion.

TABLE 9. Tetraploid × diploid → tetraploid F_1. Segregation of the F_1's in reddish white with crimson base (reddish) and purple. The female plants (mp.) were identical. $c_{x:y}$ means mean deviation/observed deviation in connection with an x:y ratio. The recorded values have only a relative sense, except for the total. $P_{x:y}$ means the chance that the observed ratio corresponds with an expected x:y ratio

F_1 \ mp.	1255	1261	1272	1273	1275	1276	Total	Expected segregation
Reddish	1	4	1	3	4	3	16	15¾ = (3)
Purple	1	0	1	0	3	0	5	5¼ (1)
$c_{1:1}$	0	2	0	1.8	0.4	1.8	2.4	
$P_{1:1}$	50%	6%	50%	12%	27%	12%	1%	
$c_{3:1}$	0.8	1.2	0.8	1	1.1	1	0.1	
$P_{3:1}$	44%	20%	44%	30%	26%	30%	20%	
$c_{5:1}$	1.2	0.9	1.2	0.8	1.9	0.8	0.9	
$P_{5:1}$	28%	48%	28%	56%	8%	56%	14%	

The only possibility which then would remain to explain the tetraploid descendants is that of *dispermy* (B.2). The 3:1 segregation, which one can expect when dispermy occurs, is remarkably in agreement with the observed segregation, as table 9 shows.

Although dispermy is especially known in animals, dispermy in plants was already mentioned in 1910 by NEMEC (39, p. 437). Afterwards (40) he described this phenomenon for *Gagea lutea* in particular, whereby he often observed a triple fusion in the egg nucleus. ISHIKAWA (25, p. 295–297) also observed in the embryo sac of *Oenothera* a fusion of the egg nucleus with two male nuclei. He proposed "that the presence of excess nuclei is brought about by intrusion of two sets of sperm nuclei due to the attack of two pollen-tubes on a single embryo sac". He mentioned further that in angiosperms the entering of two or more pollen tubes into a single embryo sac happens sometimes and he enumerates 9 such cases. GERASSIMOVA (12, p. 127) reports that dispermy can also be expected in *Crepis capillaris*. According to MICHAELIS (35, p. 444) the best way to explain an obtained triploid hybrid of the cross *Epilobium hirsutum* × *E. luteum* is that another male nucleus of *E. luteum* has been supplied to the already fertilized *hirsutum* egg. SHARP (49, p. 347) mentions that "the fusion of two male gametes with the egg (dispermy) may possibly lead to triploidy in some cases". GLUSHCHENKO (15) proposes a polyfertilization to explain crosses made by him and other investigators in tomatoes, maize, wheat and *Mirabilis jalapa*. His observation of F_1's, which showed characteristics of two male parents, supports the conception of the occurrence of dispermy in plants.

The appearance of dispermy in some plant genera and the suggestion that it also occurs in the 4n × 2n crosses in *Cyclamen*, are not in contradiction with the fact that spontaneous triploidy (probably caused by dispermy) has never been observed so far. The reason may be that a fusion, giving a triploid zygote, seems to be extremely difficult since only 8 triploids originated from 900 crosses 2n × 4n. As an ovary contains approximately 160 ovules, the chances of such a fusion are 8 on ± 144,000, i.e., 0.0056 %. Besides, when a diploid plant is pollinated with haploid pollen, the concerned fusion depends on the occurrence of two pollen tubes in the embryo sac as well, which reduces these chances even more.

Moreover, it is quite probable that a certain influence of the plasma or egg nucleus is *preventing* in normal circumstances, i.e., after fusion of two equivalent haploid gametes, a fertilization by another gamete. In this connection, the formation of a cellulose membrane at the surface of the egg, the so-called fertilization membrane, is known. In order to bring about this membrane formation there must be a positively stimulating influence which is the reaction of a complete fertilization, i.e., a complete fusion of equivalent genomes. In the 4n × 2n crosses, this preventing influence may be fully or partly *disturbed by an incomplete* fertilization of a 2n-egg nucleus by an n-male nucleus alone, by which the chance to fertilize is created for another male nucleus.

From the above it is clear that the hypothesis of NEMEC – suggesting the origin of triploids from eggs, fertilized by two male nuclei – is certainly useful to explain the origin of the tetraploid descendants. The principle of fertilization by two male nuclei is the main point in this hypothesis. The author agrees with ISHIKAWA's view (25, p. 296): "In this respect, NEMEC's view seems to be very ingenious, who substituted two male nuclei for the diploid germ nucleus" and further on "NEMEC's view seems to be the most natural one among several hypotheses"!

In brief, the author supposes that the most probable course of events in the crosses 4n × 2n was that:

(1) a preventing influence of the plasma or egg nucleus did not function by the incomplete fertilization of a 2n-egg nucleus with an n-male nucleus;
(2) if occasionally another pollen tube entered the embryo sac, its first generative nucleus fused also with the egg nucleus as consequence of the above mentioned circumstance (dispermy);
(3) the combination possibilities of the different gamete types (*S* and *s*) were based on independent distribution, which leads to a 3:1 segregation of the zygote types.

It must therefore be considered as being the most probable that in the 4n × 2n *Cyclamen* crosses, *dispermy* is the mechanism causing tetraploid descendants.

6. CONSEQUENCES FOR BREEDING

The crosses between diploid and tetraploid cultivars gave for the greatest part tetraploid descendants and these have no value with regard to the desired enlargement of the diploid assortment. The obtained triploids may have value in this connection. However, this point has been discussed already in Chapter III, p. 21.

CHAPTER V

SPECIES

1. INTRODUCTION

Since 1926 the *Cyclamen* species have been the subject of cytological investigations. It was HEITZ (21, 1926), who made the first chromosome counts, while later on GLASAU (14, 1939) and DE HAAN (20, 1951) examined the chromosomes

TABLE 10. Survey of chromosome counts in *Cyclamen* species in comparison with counts by DE HAAN (20)

Species	DE HAAN		LEGRO	
	n	2n	n	2n
C. balearicum	10	20	10	20
C. repandum	10	20	10	20
C. creticum	11	22	11	22
C. cilicium	15	30	15	30
C. coum	15	30	15	30
C. cyprium	–	30	–	30
C. libanoticum	15	30	15	30
C. pseudibericum . . .	–	30	15	30
C. neapolitanum . . .	17	34	17	34
C. purpurascens . . .	17	34	17	34
C. persicum	24	48	24	48
C. africanum	–	68	34	68
C. graecum	–	84–85	42–43	84, 85, 86
C. rohlfsianum	–	–	–	96
C. graecum	–	–	68	136

The most occurring synonyms are: for *C. repandum*, *C. vernale*; for *C. coum*, *C. orbiculatum* and *C. vernum*; for *C. neapolitanum*, *C. hederaefolium*; for *C. purpurascens*, *C. europaeum*.

of most of the species. KAPPERT (26, 1941) investigated only *C. persicum*. During 1951–1959 the author verified and continued the counts of DE HAAN. The results of these investigations are given in table 10, together with the numbers found by DE HAAN. The counts of HEITZ and GLASAU will be left out of discussion, since DE HAAN proved most of their counts to be incorrect.

DE HAAN (20) published extensively about his cytological investigations, so only a summary will be given in this chapter, completed as far as possible with some new details.

2. MATERIAL

The origin of the investigated material, consisting of 14 species and some varieties, present at the Horticultural Laboratory at Wageningen and collected by WELLENSIEK with the aid of the Ministry of Agriculture, Foreign Service, is given in table 11.

TABLE 11. Origin of the investigated *Cyclamen* species

Species	Source	Donated by
C. africanum . . .	Algeria	Dr. A. Dubois, Algiers (Algeria)
C. balearicum . . .	Balearics	Bot. Inst. at Barcelona (Spain)
C. cilicium	Turkey	Firm Van Tubergen, Haarlem (The Netherlands)
	Turkey	Mrs. D. E. Saunders, Farnsborough, Kent (Eng.)
C. coum	Turkey	Agr. Inst. at Ankara (Turkey)
C. coum varieties . .	cultivated	Firm van Tubergen, Haarlem (The Netherlands)
C. creticum	Crete	Dr. Th. Raptopoulos, Tessaloniki (Greece)
C. cyprium	Cyprus	Mr. C. C. Mountfort, Wimborne (England)
C. graecum	Greece	Dr. Th. Raptopoulos, Tessaloniki (Greece)
C. libanoticum . . .	?	Firm Van Tubergen, Haarlem (The Netherlands)
C. neapolitanum . .	Greece	Dr. Th. Raptopoulos, Tessaloniki (Greece)
C. neapolitanum var. *album*. . . .	cultivated	Several collections and nurseries
C. persicum	Israel	Mr. E. Vega, Tel Ganim (Israel)
	Tunisia	Serv. de l'Horticulture, Rabat (Tunisia)
	Cyprus	Dutch Ambassy, Cyprus
C. pseudibericum . .	?	Firm Van Tubergen, Haarlem (The Netherlands)
	Turkey	Dr. H. Demiriz, Istanbul (Turkey)
C. purpurascens	Switzerland	Own collection
	Yugo-Slavia	Fac. of Agr. & Forestry, Zagreb, (Yugo-Slavia)
C. repandum . . .	Italy	Italian Trade (Italy)
	Rhodos	Mr. V. Cohen, London (England)
C. repandum . . . var. *album*	cultivated	Firm Van Tubergen, Haarlem (The Netherlands)
C. rohlfsianum . . .	Cyrenaica	Mr. C. C. Mountfort, Wimborne (England)
		Dr. Fletcher, Woking (England)

3. CYTOLOGY

2n = 20 (photo 14), n = 10.

C. balearicum; *C. repandum*, *C. repandum* var. *album*.

These species have $4\frac{1}{2}$–6 μ long chromosomes, which can be grouped into 4 chromosomes with median centromeres, 4 with submedian, 8 with subterminal and 4 of which the position of the centromeres – subterminal or terminal – is not clear. Satellite chromosomes, as found in some other species, have never been observed.

A drawing of the metaphase chromosomes of *C. repandum* is given in fig. 1. The chromosomes were examined in a very clear mitotic division, which showed 5 groups of 4 closely similar chromosomes.

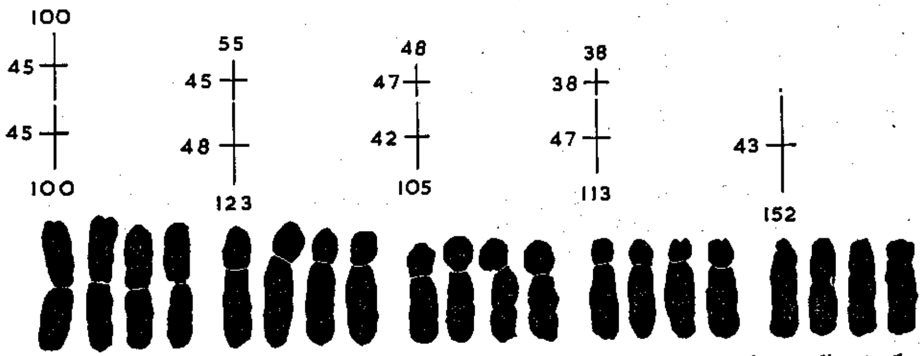

FIG. 1. Mitotic metaphase chromosomes in *C. repandum* (2n = 20) grouped according to the position of the centromeres, with the average measurements of the chromosome arms in each group. The average length of the chromosome arms of the first group has been fixed on the base of 100 units

2n = 22 (photo 15), n = 11.

C. creticum.

The chromosomes are like those of the above mentioned species. Instead of 8 chromosomes with a subterminal centromere there are 10 in *C. creticum.*

2n = 30 (photo 16), n = 15.

C. cilicium; *C. coum*, *C. coum* var. *coum*, *C. coum* var. *orbiculatum* (and their white and pink flowering forms); *C. cyprium*; *C. libanoticum*; *C. pseudibericum.*

The author was able to examine both meiosis and mitosis of all these species, except the meiosis of *C. cyprium*, since the flower bud material was very scarce. Also a white flowering form of *C. cilicium* obtained from Mrs. D. E. SAUNDERS and found by Mr. E. K. BALLS in Turkey, has been investigated.

The species have $3\frac{1}{2}$–5 μ long chromosomes, and possess the same type of centromeres as *C. repandum.* It is remarkable that from this group only *C. cyprium* has 2 satellite chromosomes. The fairly large satellites themselves are most of the time present as "fragments", as one can see on photo 17.

2n = 34 (photo 18), n = 17.

C. neapolitanum, *C. neapolitanum* var. *album*; *C. purpurascens.*

Especially long chromosomes are not present, their size ranges between 2 and 3 μ. However, 4 chromosomes are a little longer than the other ones. They show the same types of centromeres as mentioned above for *C. repandum.* In root tips of *C. neapolitanum*, once a cell with 35 chromosomes was found. Although many root tips were examined in this group with 34 chromosomes, satellite chromosomes were not found in *C. neapolitanum*; however, they were always present in *C. purpurascens* (see photo 18).

During metaphase II of the meiosis in both species, a noteworthy grouping of the chromosomes was often observed, in which the chromosomes sometimes formed chainlike figures (see photos 19 and 20). The connections between the chromosomes appeared more often as a pair of threads, but occasionally as a single thread. This "secondary association" in metaphase II has been observed by DE HAAN (20) in *C. repandum* and *C. coum* (syn. *C. orbiculatum*), as well as by the author in other species, described below.

2n = 48 (photo 4), n = 24.

C. persicum.

There is no difference between the chromosomes of the wild *C. persicum* and those of the cultivated diploid *C. persicum*. As the chromosomes of the latter have been discussed in Chapter III, section 3.1., no further description will be given here.

2n = 68 (photo 21), n = 34.

C. africanum.

The chromosomes of *C. africanum* are very similar in shape to those of *C. neapolitanum*, however *C. africanum* has 4 satellite chromosomes and *C. neapolitanum* has none. The meiosis view is quite the same as that of *C. neapolitanum*, as one can see from photos 22 and 23.

2n = 84–86 (photo 24), n = 42, 43.

C. graecum.

DE HAAN (20) could not ascertain the definite chromosome number in *C. graecum*, but supposed the somatic number to be 84 or 85 as the most probable. Exact counts of the present author proved that instead of one chromosome number, 84 *or* 85, there are three numbers, namely, 84, 85 or 86. Since also the number 2n = 136 occurs (see last paragraph of this section), which is 8×17, it is supposed that the proper number is 2n = 85, that is 5×17. Plants with 2n = 84 and 86 may have originated from plants with 2n = 85, which formed germ cells having either n = 42 or 43. During meiosis these haploid numbers were found in metaphase II of plants with 2n = 85, whereas one or more univalents could be observed in metaphase I.

The chromosomes of this species only measured 1–$1\frac{1}{2}\,\mu$ and it was extremely difficult to determine the exact number of satellite chromosomes. Occasionally 5 satellites were found, but on the other hand less than 5 were also observed.

GLASAU (14) described a species *C. gaidurowryssii* (?) – one plant – which was very similar to *C. graecum*. He counted the number 2n = $\pm$ 162 in the root tips. From the botanical garden at Kiel, Germany, seeds of this *Cyclamen* were obtained, from which three seedlings were raised. The author established the number 2n = 84 in root tips of all three plants. Since anaphase chromosomes in *Cyclamen* sometimes have the tendency to remain together for a short period before they separate to the poles, as photo 25 shows, very probably GLASAU counted such a group of anaphase chromosomes and in this way obtained almost twice the true diploid number.

$2n = 96$ (photo 26).

C. rohlfsianum.

De Haan (20) was not able to study *C. rohlfsianum.* Glasau (14) mentioned $2n = 72$ for this species. This number is not in agreement with the author's counts. The author consistently counted $2n = 96$ from many root tips of corms from two different sources.

The chromosomes are 1—2 μ long and are rather similar to those of *C. persicum.* Unfortunately the plants did not flower, so that the meiosis could not be studied.

$2n = 136$ (photo 27), $n = 68$.

C. graecum.

In 1952, when meiosis studies were carried out in a group of *C. graecum*, the haploid chromosome number of 68 was determined in anther material of a certain imported collection, consisting of 9 corms. This surprising chromosome number tallied with the diploid number of $2n = 136$ in root tips. The chromosomes in the metaphase stage of both meiosis and mitosis are very similar to those of *C. graecum* with $2n = 84–86$.

4. Origin of the chromosome numbers

During evolution many basic chromosome numbers in plant genera have been altered. This also may have been the case in *Cyclamen* as one can see from the 9 different chromosome numbers in this genus. Such modifications can be brought about by chromosome fragmentation, addition or loss, and also by polyploidy and hybridization. Usually polyploidy can be recognized the most easily as cause of the modifications. This is also the case in the genus *Cyclamen.* As polyploidy is also the most important cause of the varying chromosome numbers in this genus, it will be discussed first.

4.1. *Polyploidy*

Considering the species from a cytological point of view, it is not difficult to establish polyploidy. The 9 different chromosome numbers can be grouped into 3 polyploid series:

1. the numbers 20 (inclusive of 22) and 30 with the basic number of 10;
2. the numbers 34, 68, 85 (including 84 and 86) and 136 with the basic number of 17;
3. the numbers 48 and 96 with the basic number of 24.

The species which belong to the first and third series show no outer conformity as is commonly the case with diploids and their polyploid forms. In the second series one can observe not only a cytological relationship, but also a more or less close morphological relationship.

The first polyploid series. – At first sight one would conclude that the two species with $2n = 20$ are diploids and the 5 species with $2n = 30$ are triploids. This is however not very likely. De Haan (20, p. 159) supposed that the species with 20 chromosomes were ancient tetraploids and those with $2n = 30$ were ancient hexaploids, developed from an original type with 10 chromosomes. Thus it was postulated that the original basic number in *Cyclamen* was 5. He based this hypothesis on the observation of "secondary association" in *C. repandum* and *C. coum* (syn. *C. orbiculatum*). Indeed, the present author also observed on occasion a certain affinity between chromosomes in metaphase II. DE HAAN (20) also suggested that because of good self-fertility in the species with 2n = 30, they were hexaploids instead of triploids. This observation is in full agreement with the author's.

A third indication – and to the author's opinion the most important one – that the basic number may be 5, is the appearance of 5 groups of 4 almost similar chromosomes in the species with 2n = 20, as shown in fig. 1. In the same way, although with some difficulty, the author was able to examine in *C. pseudibericum* groups of 6 rather similar chromosomes. The similarity of the chromosomes within each group of the latter species, however, is not as eloquent as in the species with 2n = 20. The differences within the groups themselves are also much more variable than in *C. balearicum* and *C. repandum*.

The second polyploid series. – Considering 17 as basic number for this series, the species with 2n = 34, 68, 85 and 136 are diploid, tetraploid, pentaploid and octoploid, respectively. The meiosis views of these species are uniform, although when the chromosome numbers increase, the size of the chromosomes decreases. The chromosomes, both in metaphase I and II, are most of the time situated radially in concentric circles. Except for this cytological relationship, and excluding *C. purpurascens*, there is also a morphological one sometimes, which is not true for the other two series. *C. africanum* (2n = 68) especially looks like a real tetraploid in comparison to *C. neapolitanum* (2n = 34). The former has very leather-like leaves and its flowers, flower stems, fruits and seeds are much larger than those of *C. neapolitanum*. *C. graecum* (2n = 85) is only slightly larger in size than *C. neapolitanum*; *C. graecum* with 2n = 136 however has much broader flowers than the latter species.

At first glance one might suppose that all the polyploids with basic number of 17 arose in the past from *C. neapolitanum*, however there are arguments to the contrary. First, the species in the case in question are completely intersterile. Second, all attemps failed to reconstruct the pentaploid *C. graecum* by crossing the diploid *C. neapolitanum* and the octoploid *C. graecum*. Third, *C. neapolitanum* has no satellites, whereas all the other species in this series have.

From the above evidence it is more acceptable to postulate that the present-day polyploid forms originated from one or more species, which are probably now extinct.

The third polyploid series. – With respect to its chromosome number, 2n = 96, one might consider *C. rohlfsianum* as a tetraploid from *C. persicum* with 2n = 48. The relationship of the former with *C. persicum* is, however, doubtful and consists only of a cytological one. With respect to its morphology it is much more related to *C. africanum* and *C. neapolitanum*. Therefore SCHWARZ (46, p. 278) presumed that *C. rohlfsianum* would have a chromosome number of 2n = 68! Indeed, after the first count the author thought that the chromosome number was 102, which might have been a hexaploid form of 2n = 34, and thereby would classify it in the second polyploid series too. Further counts however proved that the number was consistently 96.

Thus, on the one hand there is a cytological relationship with *C. persicum*, on the other hand a morphological relationship with *C. africanum*. Besides, it is the only species of *Cyclamen* with long, projecting anthers, the origin of which cannot be traced and further is only present in the closely related North-

American genus *Dodecatheon* ($2n = 44$)! *C. rohlfsianum* therefore occupies a particular place among the *Cyclamen* species. So far its origin cannot be determined. The probable conclusion, one can draw, is that it came into existence as a tetraploid from a species with 48 chromosomes, or that it is an aneuploid form from an extinct species with $2n = 102$.

Speaking of *C. persicum*, DE HAAN (20, p. 162) said: "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.: fruit stems not coiled; anthers violet), which do not occur in these species. Its area of distribution consists of isolated parts, which points to high antiquity".

Undoubtedly *C. persicum* is descended from a species or form, whether extinct or not, with a lower chromosome number. It is obvious to assume that it originated as a tetraploid from a species or form with $2n = 24$, since the occurrence of polyploidy in the genus is quite normal. An origin of the number 48 by way of chromosome fragmentation, addition or loss from the other present-day species is certainly not acceptable, as also hybridization cannot give a clue to the origin of this number.

In considering the number 48 as a tetraploid from 24, it is, of course, possible that it is an octoploid from $2n = 12$. Since there is, however, a group of 4 instead of 8 chromosomes in *C. persicum*, which are clearly longer than the other ones, it is more acceptable to consider *C. persicum* as an auto-or allo-tetraploid. The fact that *C. persicum* normally functions as a diploid makes the latter suggestion the most likely.

4.2. *Other ways of origin*

In the previous section the hypothesis concerning the origin of the *Cyclamen* species from a primitive type with $2n = 10$ was discussed and the origin of several chromosome numbers was explained by polyploidy.

Polyploidy however is not the entire explanation for the origin of the numbers 22, 34 and the hypothetic number 24, from which the number 48 must have been derived. DE HAAN (20, p. 161) assumed that *fragmentation* (6) of the large chromosomes led to other chromosome numbers. Indeed, in this way the numbers 22, 24 and 34 could have been originated from the numbers 20 and 30, respectively. But the same result could have been obtained by *chromosome addition* as a subsequence of non-disjunction, thus by failure of separation of either paired chromosomes at meiosis or sister chromatids at mitosis, and their passage to the same pole. Such a case was reported once in *C. neapolitanum* where 35 chromosomes appeared instead of 34 in a mitotic metaphase. Non-disjunction was also observed several times during meiosis in *C. purpurascens*.

Moreover *chromosome loss* can give the numbers 22 from 24 and 34 from 36. During ancient times the chromosome numbers of 24 and 36 may have been the diploid and triploid predecessors of today's *C. persicum* ($2n = 48$). The evidence that chromosome loss occurs in *Cyclamen* without any disadvantageous consequences, is the appearance of aneuploids ($2n = 90, 92, 94, 95$) in tetraploid *Cyclamen* cultivars. It is however impossible to decide on the base of the present-day species, what process has taken place. First, because many forms are now extinct, which could only bridge the gaps among the species, even with the same chromosome number (20). Second, because the chromosome shape of the species may have been changed so many times during evolution, which is more clearly understood after comparing chromosome photographs of *C. repandum* and *C. persicum*, for instance. Third, because the chromosomes of the species with $2n = 34$ and higher are so small that it is very difficult and often impossible to recognize details, which could give some indication. One can therefore only vaguely suppose that the above mentioned numbers have originated by either fragmentation, addition, loss, or combinations.

Another process which can modify the chromosome number is *hybridization*. Crossing species with different chromosome number can give forms with another, new basic chromosome number. The more closely the parents are related to each other, as for instance in polyploids, the greater the chance is that the hybrids will be viable. Conversely the behaviour of hybrids, with respect to chromosome pairing for instance, can give an insight into the relationship of the parents. Therefore, if it were possible to hybridize the available present-day *Cyclamen* species, it might give some useful clues about the origin of species in former times. This possibility has been, among others, the reason for making species crosses which will be discussed in the next section.

5. Species crosses

5.1. *Introduction*

For over a century many workers have occupied themselves with trials to hybridize the *Cyclamen* species. In the literature, data about species crosses, however, are very scarce. One of the first recordings of a species hybrid refers to the so-called *C. atkinsii* MOORE in the Gard. Comp. I of 1852, as cited by DOORENBOS (8). This article reported that the parents of this hybrid were *C. coum* ($2n = 30$) and *C. persicum* ($2n = 48$). Plants under the name *C. atkinsii* have been investigated by DE HAAN (20) and the present author, and the chromosome number was established to be $2n = 30$. With regard to this number, being the same as that of *C. coum*, as well as to its morphological conformity with the latter, the author fully agrees with DOORENBOS (8, p. 25), who saw also the original herbarium material at Kew and reports: "Considering that *C. coum* and *C. Atkinsii* both have 30 chromosomes, *C. persicum* on the other hand 48, we may assume that the cross never took place and the original plant was a mutation of *C. orbiculatum* var. *coum*". The form is now called *C. coum* var. *orbiculatum* f. *album* DOORENB.

MÜLLER (36), who started *Cyclamen* breeding in 1867, also tried several species crosses and reported in 1885 the following:

"Mit Kreuzungen zwischen verschiedene Arten, wie *Cyclamen persicum*, *C. coum*, *C. europaeum*" – synonym for *C. purpurascens* – "*C. hederaefolium*" – synonym for *C. neapolitanum*? – "und *C. africanum* habe ich mir früher, aber ohne allen Erfolg, viel Mühe gegeben, ich habe nicht eine hybride Pflanze erhalten. Vor Jahrzehnten wurden bereits Hybriden angeboten, so u.a. *Cyclamen Roebellianum* als Bastard von *C. persicum* und *C. africanum* (*macrophyllum*), was ich aber von verschiedenen Seiten unter diesem Namen erhielt, waren stets nur minderwertige Varietäten von *Cyclamen persicum* und ich glaube nicht, dasz überhaupt Bastarde existieren".

Thus MÜLLER was not able to obtain hybrids after many trials from the first mentioned species, whereas so-called hybrids which were offered to him earlier, always turned out to be inferior varieties of *C. persicum*.

HILDEBRAND (22, 1898) described two hybrids, obtained from the cross *C. neapolitanum* × *C. africanum*, later on called *C. hildebrandii* SCHWZ. by SCHWARZ (46). These hybrids differed only slightly from one another and looked very like the female parent, *C. neapolitanum*. The described characteristics in which these hybrids differed from *C. neapolitanum*, can be found, however, in the very heterozygous plant material of the latter as well! This fact, combined with the negative results of the author's own cross experiments, makes this case of hybridization very doubtful. It is also curious that HILDEBRAND never reported subsequently about any F_2-generation, although the crosses were made already in 1890 and the plants were both fertile. Further crosses made by HILDEBRAND, namely *C. coum* × *C. persicum*, *C. neapolitanum* × *C. graecum* and *C. africanum* × *C. graecum* all failed.

Under the guidance of WELLENSIEK (58) from 1947 until 1950, crosses were made between *C. persicum* and *C. neapolitanum* with the purpose to introduce the characteristic of the "eared" corolla-lobes of the latter into *C. persicum* and its cultivars. In total 88 crosses were made, resulting in 8 fruits with totally 47 seeds. Most of the seeds did not germinate and only 3 groups of F_1's were raised, consisting of 1, 3 and 15 plants, respectively. In all three cases, however, the plants were identical with the female parent, *C. persicum* and also had the chromosome number $2n = 48$.

In the reciprocal cross, *C. neapolitanum* × *C. persicum*, about 200 attemps were made to hybridize, but without any success.

SCHWARZ (46, 1955, p. 279) recorded that he obtained seeds and seedlings of the cross *C. coum* × *C. persicum*, but said that he first had to wait and see the flowering results to be sure that no errors with the pollination were made. Results from the crosses *C. coum* × *C. repandum* (syn. *C. vernale*), *C. persicum* × *C. repandum* and *C. neapolitanum* × *C. purpurascens*, which apparently succeeded also, will be recorded later, when first an F_2-generation is available.

From 1951 through 1957 the author started a series of crosses in *Cyclamen* species for the purpose of enlarging the knowledge about the pedigree of the species. Special attention was paid to:

1. the possible reconstruction of the existing chromosome numbers 34 and 85;
2. the realization of the missing links $2n = 51$ and $2n = 102$ (being the triploid and hexaploid, respectively, in the polyploid series with $n = 17$ as basic number), and $2n = 24$;
3. the degree of cytological relationship between the species with $2n = 20$, and $2n = 34$;
4. the possible resynthesis of *C. pseudibericum* by crossing *C. coum* and *C. libanoticum* to establish the supposition that the former species originated as a natural hybrid from the latter two.

5.2. *Experiments*

Preliminary results have been published before (31). Details will follow below. For the cross experiments, carried out in *Cyclamen* species, plants were used of the extensive collection at the Wageningen Horticultural Laboratory. The applied cross technique was similar to that described for cultivar crosses in Chapter IV, p. 22–23.

For the reconstruction of the chromosome number 34, crosses were made between *C. balearicum* ($2n = 20$), *C. repandum* ($2n = 20$) and *C. creticum* ($2n = 22$) on the one hand and *C. persicum* ($2n = 48$) on the other hand.

To reconstruct the number 85 and to realize the chromosome numbers 51 and 102, crosses were made between *C. neapolitanum* (2n = 34) and *C. graecum* (2n = 136), *C. neapolitanum* (2n = 34) and *C. africanum* (2n = 68), *C. africanum* (2n = 68) and *C. graecum* (2n = 136), respectively.

The realization of the number 24 was tried by crossing *C. repandum* (2n = 20) and *C. libanoticum* (2n = 30).

To determine the degree of cytological relationship between *C. balearicum* and *C. repandum*, both with 2n = 20, as well as between *C. neapolitanum* and *C. purpurascens*, both with 2n = 34, they were crossed together.

C. coum (2n = 30) and *C. libanoticum* (2n = 30) were crossed together to try to resynthesize *C. pseudibericum* (2n = 30) in the same way as this has been done for *Nicotiana*, *Brassica* and other species (37).

As a curiosity, the crosses *C. graecum* (2n = 85) × *C. neapolitanum* (2n = 34) and *C. graecum* (2n = 136) × *C. graecum* (2n = 85) were also made.

The above mentioned crosses and their results are recorded in table 12.

TABLE 12. Summarized results from crosses between *Cyclamen* species for the years 1951 through 1957

Cross parents	Number of				2n-numbers	
	crosses	fruits	seeds	F_1-plants	cross parents	F_1
C. balearicum × *C. repandum*	8	2	13	3	20 × 20	–
C. balearicum × *C. persicum*	130	10	73	30	20 × 48	20
reciprocally	43	0	0	0	48 × 20	–
C. repandum × *C. persicum*	59	5	38	0	20 × 48	–
reciprocally	244	2	2	1	48 × 20	48
C. creticum × *C. persicum*	20	0	0	0	22 × 48	–
reciprocally	30	0	0	0	48 × 22	–
C. repandum × *C. libanoticum*	37	0	0	0	20 × 30	–
reciprocally	28	3	4	2	30 × 20	30
C. coum × *C. libanoticum*	233	37	208	23	30 × 30	30
reciprocally	50	2	13	0	30 × 30	–
C. neapolitanum × *C. purpurascens*	900	0	0	0	34 × 34	–
reciprocally	107	0	0	0	34 × 34	–
C. neapolitanum × *C. africanum*	420	1	1	0	34 × 68	–
reciprocally	90	0	0	0	68 × 34	–
C. neapolitanum × *C. graecum*	688	0	0	0	34 × 136	–
reciprocally	90	0	0	0	136 × 34	–
C. purpurascens × *C. graecum*	4	0	0	0	34 × 136	–
C. africanum × *C. graecum*	74	0	0	0	68 × 136	–
reciprocally	72	0	0	0	136 × 68	–
C. graecum × *C. neapolitanum*	132	0	0	0	85 × 34	–
C. graecum × *C. graecum*	86	0	0	0	136 × 85	–

5.3. *Discussion and conclusions*

From the total of 3,545 crosses, recorded in table 12, only 62 fruits were obtained. These fruits were quite normal or sometimes only slightly smaller than fruits from self-pollinations. They always had a normally enlarged receptacle even when there was only one seed per fruit. Only in the cross *C. coum* × *C. libanoticum*, and especially in the reciprocal cross, the fruit stems did not coil like a corkscrew as normally occurs after selfing. This is a curious fact and at the same time an indication that no accidental self-pollination took place! In all crosses parthenocarpic fruits were observed, especially in crosses with *C. neapolitanum* as female parent. Sometimes over 90% of the crosses resulted in such seedless fruits which remained very often more than 3 months on the plants.

From the total of 352 seeds, which were well developed, only 59 germinated. The causes of this poor germination are unknown. Perhaps the time of sowing had influenced this result. Together with the other *Cyclamen* seeds, they were normally sown 2–3 months after ripening. SCHWARZ (46, p. 246) however points to the fact that seeds of some species, e.g. *C. repandum*, must be sown immediately after ripening: even some days of desiccation results in poor germination. A small germination experiment, carried out later on, confirmed this; the results appear in table 13.

TABLE 13. Percentages of seed germination of two *Cyclamen* species with reference to time of sowing after harvesting the seed

Species	Time between harvesting and sowing	Number of seeds	Germinated seeds	%
C. cyprium	2 months	25	4	16
	2 days	22	17	77,3
C. repandum	2 months	144	3	2,1
	2 days	34	27	79,4
	0 days	70	69	98,6

The F_1-plants, which could be raised, had all the same chromosome number as their female parent and were, except two, all morphologically identical to the mother plants. The two exceptions obtained from the cross *C. balearicum* × *C. persicum*, made in 1951, could be determined as *true hybrids*, although the author is almost certain that *C. persicum* was not the male parent. Both meiosis and mitosis in these hybrids were similar to those in *C. balearicum* or *C. repandum*. The purplish-pink flowers appeared in shape and in colour very much like *C. repandum* flowers (*C. balearicum* flowers are white). The leaves exhibited a close similarity to both *C. balearicum* and *C. repandum*. The four raised F_2-generations all segregated into types like both of these species, with a 3:1 segregation into purplish-pink and white flowers. Since the cross *C. balearicum* × *C. persicum* was made at the same time as crosses with *C. repandum* were made, it is very likely that a wrong pollination was applied. Considering these facts, the author is almost sure that the hybrids were derived from *C. balearicum* and *C. repandum*. To determine if this was well grounded, the author purposely made the cross *C. balearicum* × *C. repandum* to obtain seedlings for direct comparison with the above mentioned true hybrids. Unfortunately the 3 obtained F_1-plants from the cross *C. balearicum* × *C. repandum*, which could have been evidence for such a comparison, died in the seedling stage.

On the other hand the cross *C. balearicum* × *C. persicum* was remade in 1956 from which 67 seeds were obtained and 26 seedlings were grown. All of the plants obtained looked identical to *C. balearicum* in shape, flowering habit and chromosome number.

The F_1-plants from *C. persicum* × *C. repandum*, *C. libanoticum* × *C. repandum* and *C. coum* × *C. libanoticum* were, as has been said before, all identical to the female plants. Several F_2-generations, raised from the latter cross, did not alter things. On the basis of the large number of seeds and F_1-plants, one would assume there was self-pollination. Although the author is fully aware that errors are always possible, he will not accept this supposition in this case. There were never open flowers with anthers on the female plants. The castration method was controlled on *C. coum* and proved to be fully reliable: 100 buds were castrated, which did not give any fruit when left unpollinated. The fact that the fruit stems in *C. coum* × *C. libanoticum* (and reciprocally) did not coil, as mentioned before, proved also that a normal fruit set, as takes place after selfing, did not occur. An answer to the question how these descendants may then have originated will be given in the next chapter.

The conclusions one can make from the cross results are the following:

The reconstruction or realization of *Cyclamen* with numbers of 34, 85 and 24, 51, 102 chromosomes, respectively, is not possible with the present-day species available, which are the most suitable for this purpose.

From the negative results from the crosses with the octoploid *C. graecum* ($2n = 136$), one must conclude that there is not any cytological relationship other than the basic number 17 and the similar meiosis view, with regard to the species with $2n = 34$, 68 and 85, and the octoploid *C. graecum*.

From the negative results of the crosses between *C. neapolitanum* and *C. purpurascens*, one must conclude that since these species are incompatible, they probably have not evolved from one original type.

It is almost sure that between the species with $2n = 20$, besides having a close morphological relationship (20), there is also a close cytological one with respect to their mutual compatibility. The two obtained true hybrids point to this direction.

No cytological evidence could be obtained for the supposition that *C. pseudibericum* may be a natural hybrid of *C. coum* and *C. libanoticum*, since all attempts to resynthesize failed.

On the basis of his investigations, the author seriously doubts that the offsprings obtained by HILDEBRAND (22) and SCHWARZ (46) possessed a hybrid-character, as well as that hybrid-populations between *C. repandum* and *C. libanoticum* are present in nature, as SCHWARZ (46, p. 257) suggests. His next suggestion that *C. libanoticum* ($2n = 30$) would be a natural hybrid of *C. repandum* ($2n = 20$) and *C. coum* ($2n = 30$) is not only unacceptable, but is also incomprehensible since SCHWARZ knew their different chromosome numbers.

Generally speaking, hybridization possibilities in *Cyclamen* are very limited through interspecific incompatibility. The species crosses did not give any clue as to the possible origin of the different chromosome numbers by hybridization.

6. CONSEQUENCES FOR BREEDING

Crosses between wild species and cultivated ones can introduce sometimes one or another desired characteristic into the latter, e.g., resistance against diseases or a new flower colour. In *Cyclamen* species desirable characteristics are present, as for instance the fine odour in *C. purpurascens*, the attractive leaf colour and pattern of *C. graecum*, and the "eared" corolla-lobes of several species. Introduction of these characteristics into *C. persicum* cultivars has not been accomplished to date. Although not all cross combinations have been tried, one can almost be sure that hybridizing of *C. persicum* with the other species in a normal, direct way is not possible.

CHAPTER VI

FALSE HYBRIDS

Sometimes in crossing-experiments offsprings arise which show only the characteristics of the female parent, although they could not have been originated from self-pollinations. SHARP (49, p. 405) called these metromorphic (like the mother) descendants "false hybrids".

Also in *Cyclamen* crosses between diploid and tetraploid cultivars (see Chapter IV) and in species crosses (see Chapter V), besides true hybrids such metromorphic F_1-plants were produced. Since an origin by self-pollinating can be excluded, as has been pointed out in the previous chapters, the author will use the term "false hybrids" for these metromorphic descendants, after SHARP.

WELLENSIEK *et al.* (59) reported failure of seed formation after pollinating *Cyclamen* with various substances other than *Cyclamen* pollen. The author fully agrees that living *Cyclamen* pollen is necessary for seed formation. Furthermore, in the previous mentioned castration experiments for both cultivars and species, the female plants never produced seeds without pollination. Thus one may conclude that for the production of false hybrids living pollen is required.

Just as in other species as *Hypericum, Poa, Potentilla,* as PRAKKEN (44, p. 586) mentions, the pollination stimulus might consist of a fertilization of the fused polar nuclei by the second male nucleus.

"The development of metromorphic offspring induced by pollination, but without complete syngamy", is called, according to SHARP (49, p. 405), "pseudogamy". Therefore on the basis of the evidence above and the definition given by SHARP, it is appropriate to use the term "pseudogamic" for the origin of the false hybrids in *Cyclamen.*

SHARP (49) gives several possibilities for the development of pseudogamic seeds. The latter may arise for instance by reduced or unreduced parthenogenesis, whether or not followed by chromosome doubling, or by reduced or unreduced apogamy. It is unknown however what occurred in the embryo sacs of the *Cyclamen.* The only positive fact is that in two F_1-generations of the above mentioned cultivar crosses and also in two F_1's of *C. coum* × *C. libanoticum*, in which cases the female parent was heterozygous, segregation for a certain characteristic appeared. This indicates that the false hybrids arose from cells with the *reduced* chromosome number, although the plants had the somatic chromosome number of the female parent. Then only two possibilities remain, by which the false hybrids may have arisen:

by reduced (or haploid) parthenogenesis followed by chromosome doubling, or by endogamy, thus by fusion of two (haploid) embryo sac nuclei (as explained in Chapter IV).

In the non-segregating offsprings of false hybrids it is difficult to draw a conclusion whether reduced or unreduced parthenogenesis occurred. But it is the author's opinion that the same process took place in these cases as in the segregating offsprings.

SUMMARY

I. GENERAL INTRODUCTION

1. *C. persicum* has been cultivated in Western Europe since its introduction about 350 years ago.
2. Although it was initially only a collector's plant, soon after 1850 it became a very important pot plant. Today over 200 cultivars are known and the *Cyclamen* is the most widely grown pot plant in The Netherlands.

II. CYTOLOGICAL TECHNIQUE

Chromosome counts were made in root tips and anthers of both *Cyclamen* cultivars and species. The applied staining technique consisted of the FEULGEN staining and the aceto-carmine method. Root tips were always pretreated with an 8-hydroxyquinoline solution.

III. CULTIVARS

1. In *Cyclamen* cultivars the diploid and tetraploid chromosome numbers $2n = 48$ and $2n = 96$ are present.
2. By far the majority of the large number of investigated West-European cultivars, including the Dutch ones, was established to be tetraploid.
3. In 2 Swiss, 3 French, 11 Danish and 27 German cultivars the aneuploid chromosome number $2n = 92$ was determined. Also the aneuploid numbers $2n = 90$, $2n = 94$ and $2n = 95$ were found in Swiss and German *Cyclamen*. However, no aneuploids were found in Dutch, English or Belgian cultivars.
4. The first tetraploid cultivars appeared in the second half of the nineteenth century. It was concluded that 'Robustum' (1863) and 'Splendens' (1873) were already tetraploid.
5. Probably the first aneuploid with $2n = 92$ was 'Ruhm von Wandsbek' (1898).
6. Cytological data were compared with breeding data and found to be in accordance.

IV. CROSSES BETWEEN DIPLOID AND TETRAPLOID CULTIVARS

1. In $2n \times 4n$ and reciprocal crosses between *Cyclamen persicum* cultivars, true hybrids as well as false hybrids were obtained. The true hybrids consisted of triploids and tetraploids: 12.7 % and 87.3 %, respectively, in the cross $2n \times 4n$; 54.2 % and 45.8 % in the cross $4n \times 2n$.
2. Cytological evidence for the origin of the tetraploid descendants could not be obtained. Several hypotheses concerning the way of origin of these tetraploid descendants were discussed.
3. In the cross $4n \times 2n$ genetical evidence was obtained for the supposition that dispermy is the cause of the origin of the tetraploid descendants. Per analogy, endogamy in the cross $2n \times 4n$ is the most acceptable cause of origin of the tetraploid descendants, thus forming together with dispermy one kind of "doubling mechanism".

V. SPECIES

1. The chromosome range of 14 *Cyclamen* species was determined as follows: 20, 22, 30, 34, 48, 68, 84–86, 96 and 136. These numbers are in agreement with and extend the counts of DE HAAN.

2. The different chromosome numbers may be grouped into 3 polyploid series with the basic numbers n = 10, 17 and 24.
3. A new argument was brought forth concerning the hypothesis about the pedigree of *Cyclamen* species from a primitive type with 2n = 10.
4. Reconstruction of some chromosome numbers, as well as realization of missing links by crossing of the present-day species, was not possible. Therefore, evidence for a way of origin other than polyploidy of the today's different chromosome numbers in *Cyclamen* could not be proposed.
5. Two true hybrids were obtained from *C. balearicum* as the female parent and very probably *C. repandum* as the male parent, as well as a number of false hybrids. Any attempt to produce interspecific hybrids of other species failed, because of intersterility.

VI. FALSE HYBRIDS

1. In crosses between diploid and tetraploid cultivars and in species crosses, offsprings came into existence consistently, which were cytologically and morphologically similar to the female parents.
2. Since self-pollination must be excluded, the only way of origin is an apomictic one. The appearance of segregation of certain genes in some of the offsprings suggests a reduced parthenogenesis followed by chromosome doubling, or endogamy.

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SAMENVATTING

DE CYTOLOGISCHE ACHTERGROND VAN DE *CYCLAMEN*-VEREDELING

I. ALGEMENE INLEIDING

1. Ongeveer 350 jaar geleden werd *C. persicum* in Westelijk Europa ingevoerd en is sedertdien in cultuur.
2. Ofschoon aanvankelijk een plant voor verzamelaars, werd zij spoedig na 1850 een zeer belangrijke potplant. Op de dag van vandaag zijn meer dan 200 cultivars bekend en is de *Cyclamen* in Nederland de meest geteelde potplant.

II. CYTOLOGISCHE TECHNIEK

In worteltopjes en antheren van zowel *Cyclamen*-cultivars als soorten werden chromosoomtellingen uitgevoerd. Als kleurmethoden werden de FEULGEN-kleuring en de aceto-karmijn methode toegepast. Worteltopjes werden steeds voorbehandeld met een oplossing van 8-hydroxyquinoline.

III. CULTIVARS

1. In *Cyclamen*-cultivars zijn het diploïde chromosomenaantal 2n = 48 en het tetraploïde aantal 2n = 96 aanwezig.
2. Van het grote aantal onderzochte West-Europese cultivars, met inbegrip van de Nederlandse, is verreweg het grootste gedeelte tetraploïd gebleken.
3. In 2 Zwitserse, 3 Franse, 11 Deense en 27 Duitse cultivars werd het aneuploïde chromosomenaantal 2n = 92 vastgesteld. Eveneens werden de aneuploïde aantallen 2n = 90, 2n = 94 en 2n = 95 in Zwitserse en Duitse *Cyclamen* aangetroffen. Er bleken geen aneuploïde chromosomenaantallen onder de Nederlandse, Engelse en Belgische cultivars voor te komen.
4. De eerste tetraploïde cultivars verschenen in de tweede helft van de negentiende eeuw. Geconcludeerd werd dat 'Robustum' (1863) en 'Splendens' (1873) reeds tetraploïd waren.
5. Vermoedelijk was 'Ruhm von Wandsbek' (1898) de eerste aneuploïde met 2n = 92.
6. Cytologische gegevens werden vergeleken met gegevens omtrent de veredeling uit de literatuur en in overeenstemming met elkaar bevonden.

IV. KRUISINGEN TUSSEN DIPLOIDE EN TETRAPLOIDE CULTIVARS

1. In 2n × 4n en reciproke kruisingen tussen *Cyclamen persicum*-cultivars werden nakomelingen mèt en zònder een bastaardkarakter verkregen. De echte bastaarden bestonden uit triploïden en tetraploïden: resp. 12,7 % en 87,3 % in de kruising 2n × 4n en 54,2 % en 45,8 % in de kruising 4n × 2n.
2. Cytologisch bewijsmateriaal voor het ontstaan van de tetraploïde nakomelingen kon niet worden verkregen. Betreffende de ontstaanswijze van deze tetraploïde nakomelingen werden verscheidene hypothesen naar voren gebracht.
3. In de kruising 4n × 2n werd genetisch bewijsmateriaal verkregen voor de veronderstelling, dat dispermie de oorzaak is van het ontstaan van de tetraploïde nakomelingen. Per analogie is endogamie de meest aannemelijke oorzaak van het ontstaan der tetraploïden in de kruising 2n × 4n, op deze wijze met dispermie één type van „verdubbelingsmechanisme" vormend.

V. SOORTEN

1. De chromosomenaantallen van 14 *Cyclamen*-soorten werden als volgt vastgesteld: 20, 22, 30, 34, 48, 68, 84–86, 96 en 136. Deze aantallen stemmen overeen met de tellingen van DE HAAN.
2. De verschillende chromosomenaantallen kunnen worden gegroepeerd in 3 polyploïde reeksen met de grondtallen n = 10, 17 en 24.
3. Een nieuw argument ter staving van de hypothese omtrent de afstamming van *Cyclamen*-soorten van een oertype met 2n = 10, werd naar voren gebracht.
4. Reconstructie van enige chromosomenaantallen alsmede de verwezenlijking van "missing links" door kruising van de tegenwoordige soorten, bleek niet mogelijk. Bewijs voor een andere ontstaanswijze van de huidige chromosomenaantallen in *Cyclamen* dan door polyploïdie, kon niet worden overgelegd.
5. Twee echte bastaarden werden verkregen van *C. balearicum* als moederplant en zeer waarschijnlijk *C. repandum* als vaderplant, alsmede een aantal nakomelingen zonder bastaardkarakter. Elke poging om interspecifieke bas-

taarden van andere soorten te verkrijgen, mislukte tengevolge van intersteriliteit.

VI. De z.g. onechte hybriden

1. In kruisingen tussen diploïde en tetraploïde cultivars en in soortskruisingen ontstonden overeenstemmende nakomelingschappen, die cytologisch en morfologisch gelijk waren aan de moederplanten.
2. Aangezien zelfbestuiving moet worden uitgesloten, is een apomictische ontstaanswijze de enig mogelijke. Het optreden van splitsingen voor bepaalde genen in sommige van de nakomelingschappen suggereert het optreden van haploïde parthenogenesis gevolgd door chromosoomverdubbeling, of endogamie.

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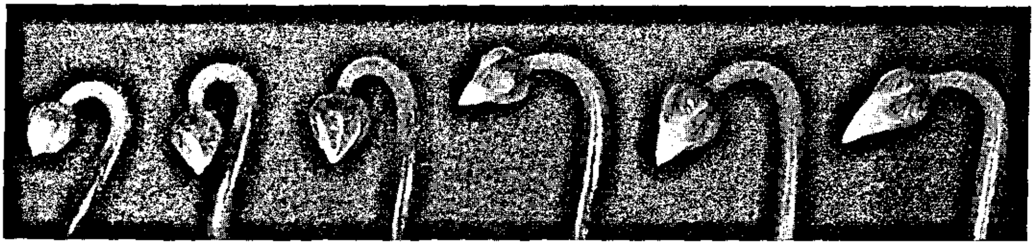

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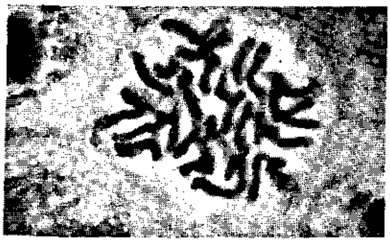

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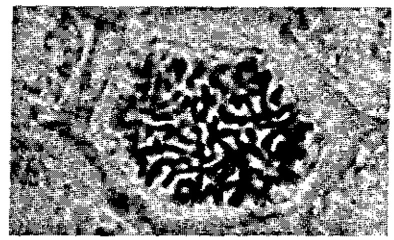
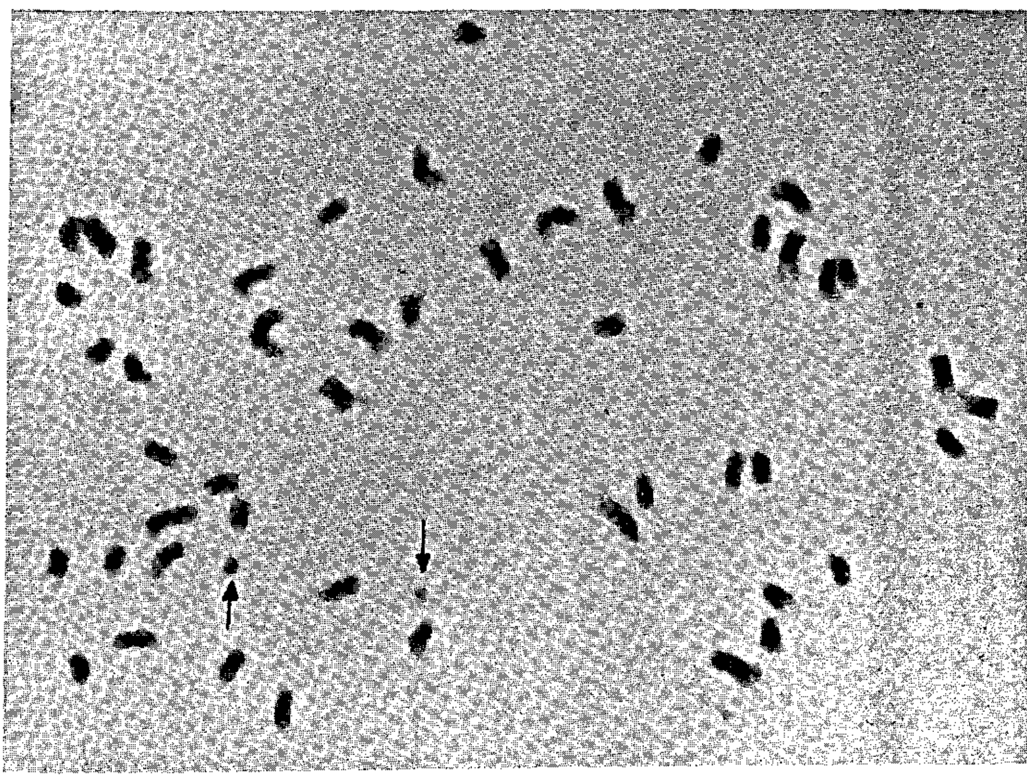




4

PHOTO 1. (p. 4). Outer size of flower buds of the cv. 'Wit met oog' in relation to stages of reduction division: a. P.M.C. resting; b. P.M.C. in reduction division; c. transition stage from tetrads into pollen grains, E.M.C. still resting; d. E.M.C. in reduction division; e. formation of embryo sac nuclei; f. whole process finished.

PHOTO 2 and 3. (p. 5). Chromosomes of *Cyclamen* species with a low number are relatively long and mostly strong confused (photo 2, *C. cyprium*, 2n = 30), whereas in species with a large number, chromosomes are relatively short, but lay very closely together (photo 3, *C. persicum* cv., 2n = 96). From crystal violet stained paraffin sections, after DE HAAN (20).

PHOTO 4. (p. 7). *C. persicum* 'Wit met oog', 2n = 48. Mitotic metaphase. Two satellites present (arrows). From pretreated FEULGEN stained squash. × 3500.

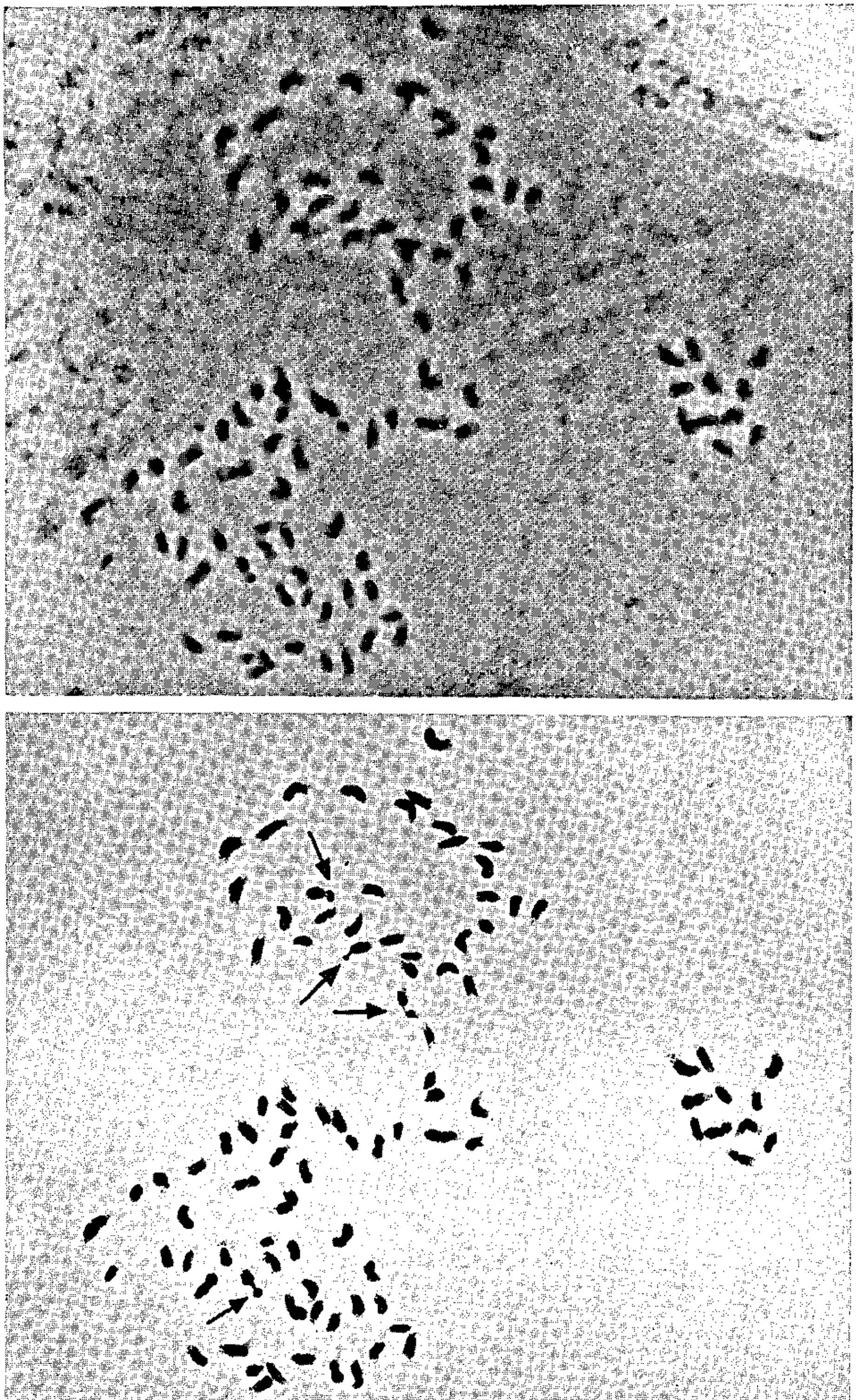
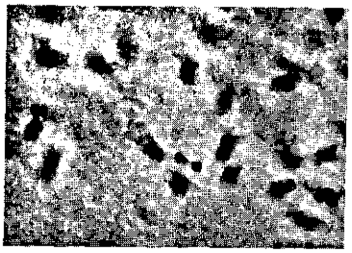


PHOTO 5. (p. 7). *C. persicum* 'Rosa von Marienthal', 2n = 96. Mitotic metaphase. Four satellites present (arrows). From pretreated FEULGEN stained squash. × 2400.



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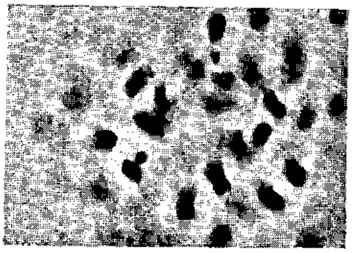

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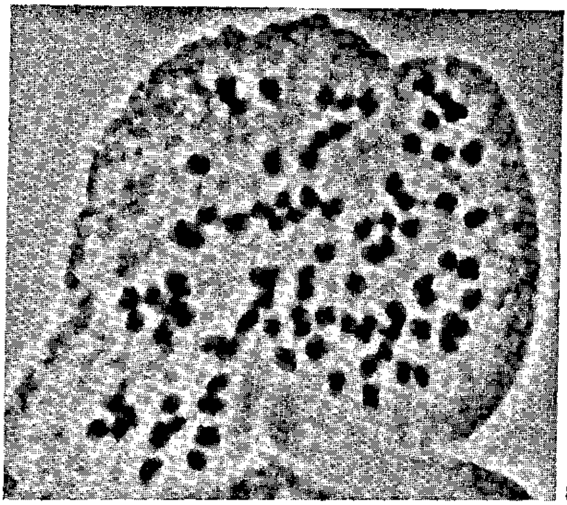

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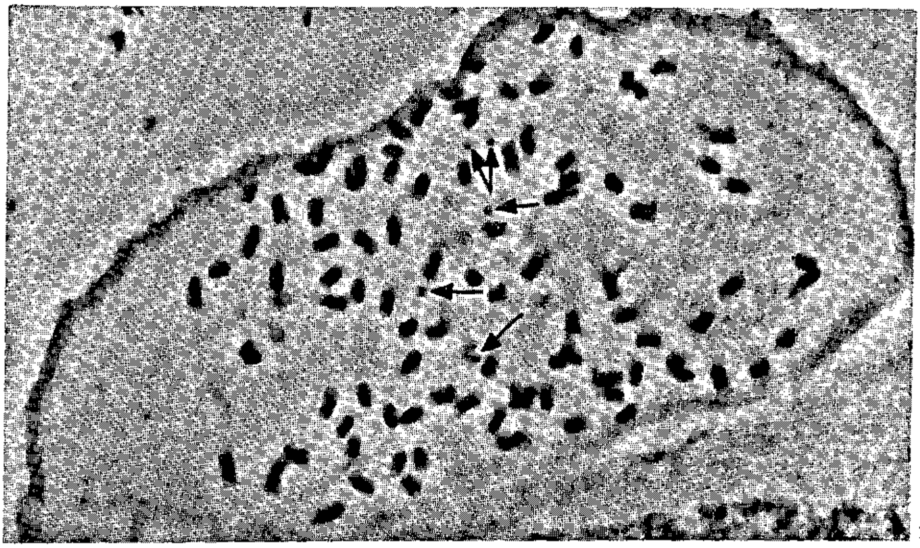

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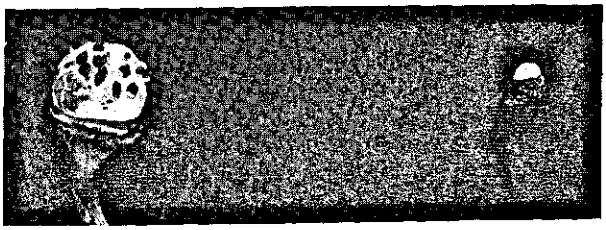

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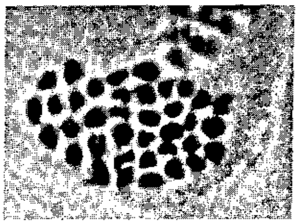


11

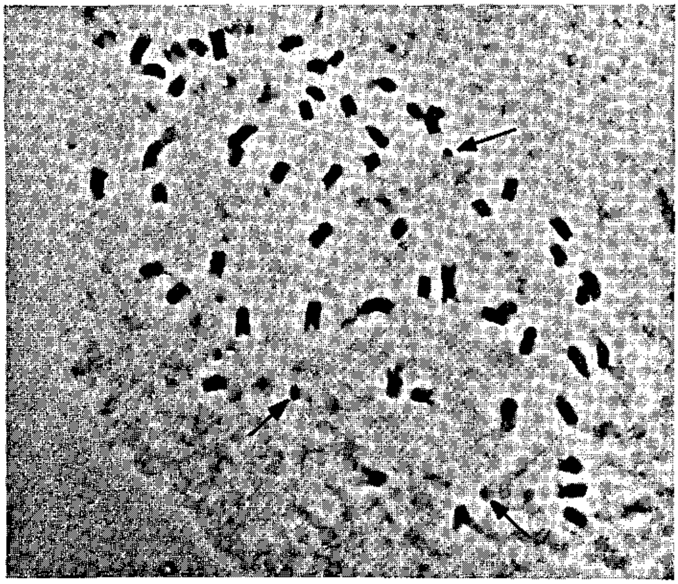
PHOTO 6 and 7. (p. 8). *C. persicum* 'Wit met oog'. Part of mitotic metaphase showing a commonly already divided satellite, whereas the chromosomes are not yet divided (photo 6), and a satellite which seems to be connected at the side of the chromosome (photo 7). × 2400.

PHOTO 8. (p. 15). *C. persicum* 'Vermillon', 2n = 92. Mitotic metaphase. × 2400.

PHOTO 9. (p. 16). *C. persicum* 'Lachshell', 2n = 96. Mitotic metaphase from plant with only 2n = 95. Four satellites (one divided) present (arrows). × 2400.

PHOTO 10. (p. 24). Opened *Cyclamen* fruits showing left the *enlarged* receptacle with one seed (lower left) from 'Wit met oog' × 'Donkerrood' (2n × 4n), and right the *contracted* receptacle with one seed from the reciprocal cross.

PHOTO 11. (p. 25). Metaphase II plate with n = 37 in P.M.C. of a triploid *Cyclamen*. × 2400. Photos 6, 7, 8 and 9 from pretreated FEULGEN stained squashes; photo 11 from aceto-carmine squash.



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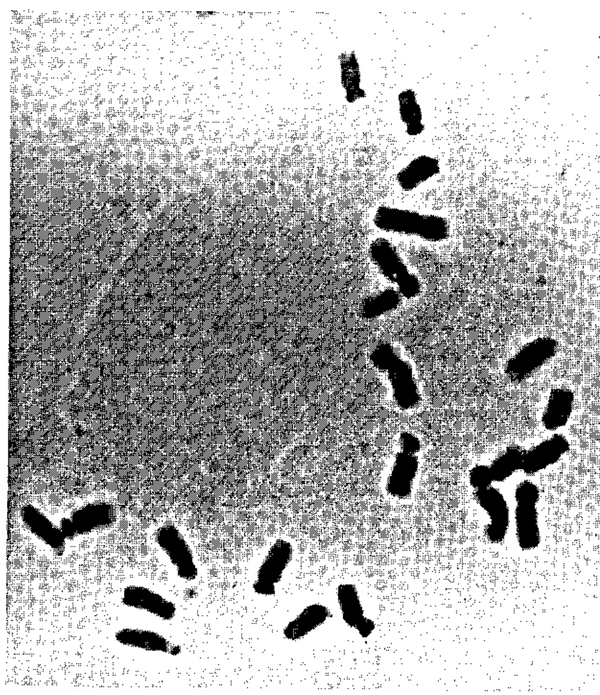

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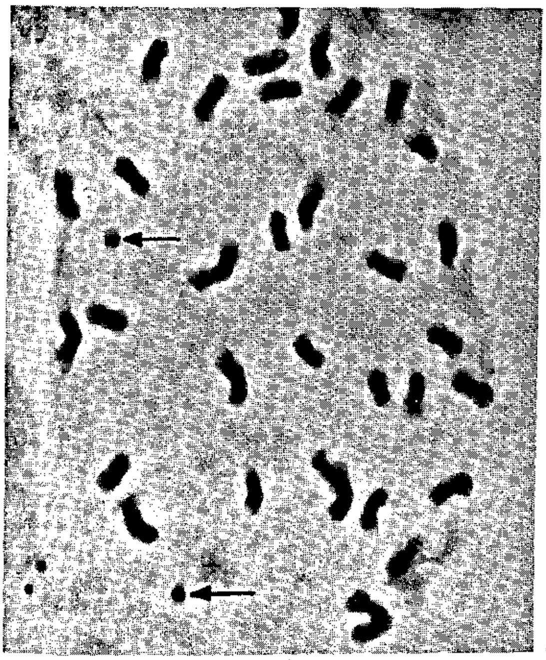
PHOTO 12. (p. 26). *C. persicum* F_2-plant from 'Donkerrood' × 'Wit met oog', 2n = 55. Mitotic metaphase. Three satellites present (arrows). The selfed F_1 had 2n = 72. × 2400.
PHOTO 13. (p. 26). *C. persicum* 'Wit met oog', 2n = 48. Anaphase I in E.M.C. × 960.
PHOTO 14. (p. 34). *C. repandum*, 2n = 20. Mitotic metaphase. × 2000.
PHOTO 15. (p. 35). *C. creticum*, 2n = 22. Mitotic metaphase. × 2000.
All photos from pretreated FEULGEN stained squashes.

16

17



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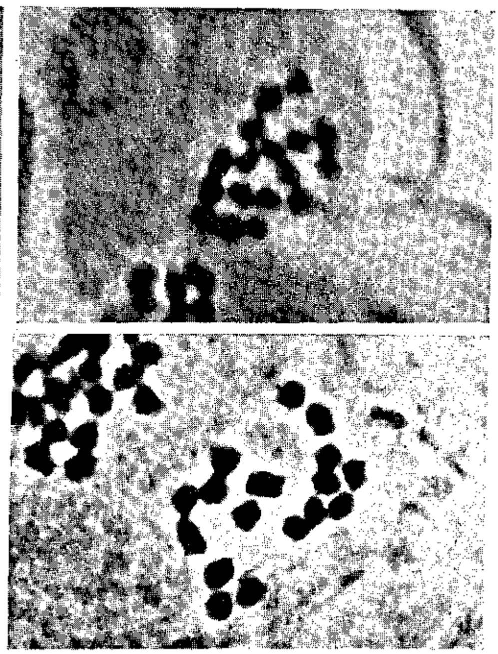
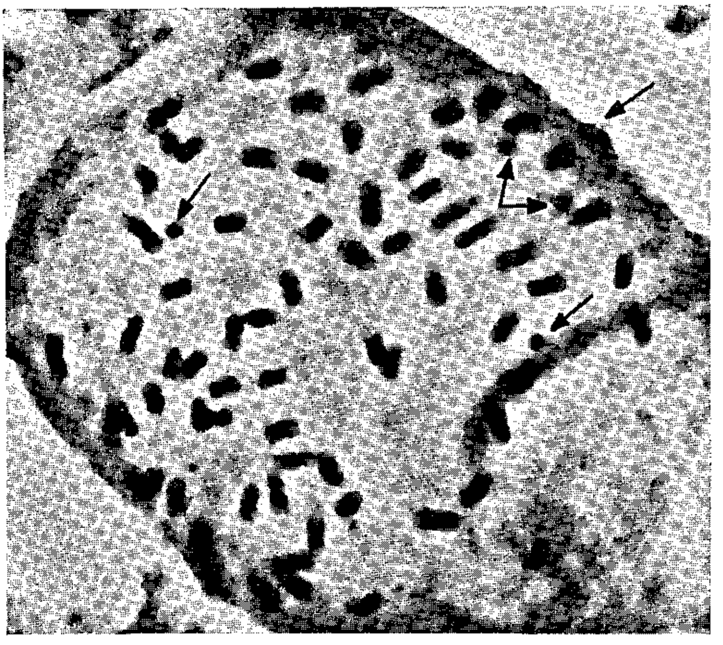


19 and 20

PHOTO 16. (p. 35). *C. pseudibericum*, 2n = 30. Mitotic metaphase. × 2000.
PHOTO 17. (p. 35). *C. cyprium*, 2n = 30. Mitotic metaphase. Two satellites present (arrows). × 2000.
PHOTO 18. (p. 35). *C. purpurascens*, 2n = 34. Mitotic metaphase. Two satellites present (arrows). × 2400.
PHOTO 19 and 20. (p. 36). Metaphase II plates, showing typical chain figures in P.M.C.'s from *C. purpurascens*, n = 17 (photo 19) and *C. neapolitanum*, n = 17 (photo 20). × 2400.
Photos 16, 17 and 18 from pretreated FEULGEN stained squashes; photos 19 and 20 from aceto-carmine squashes.



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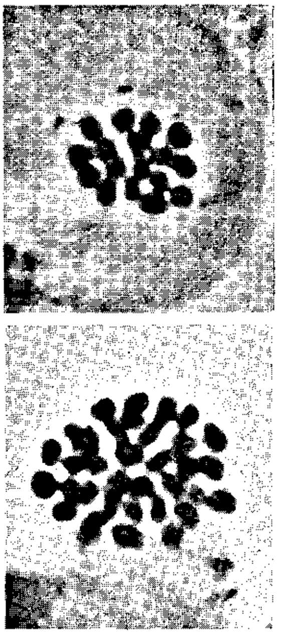

22 and 23

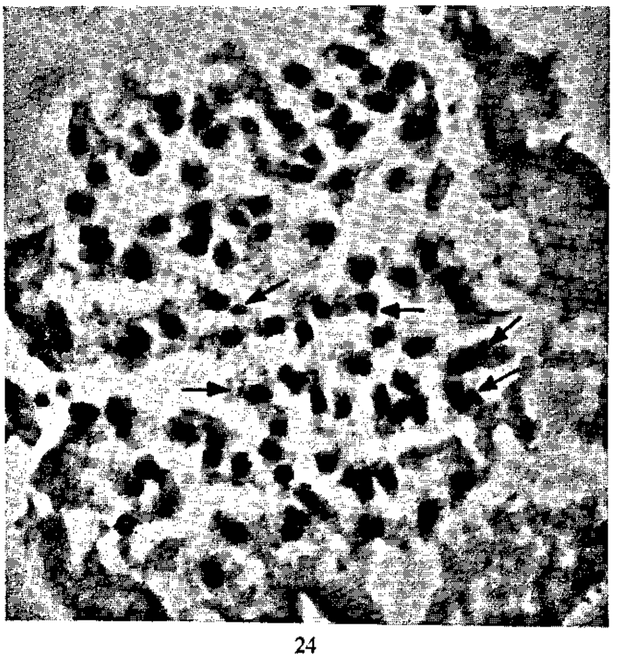

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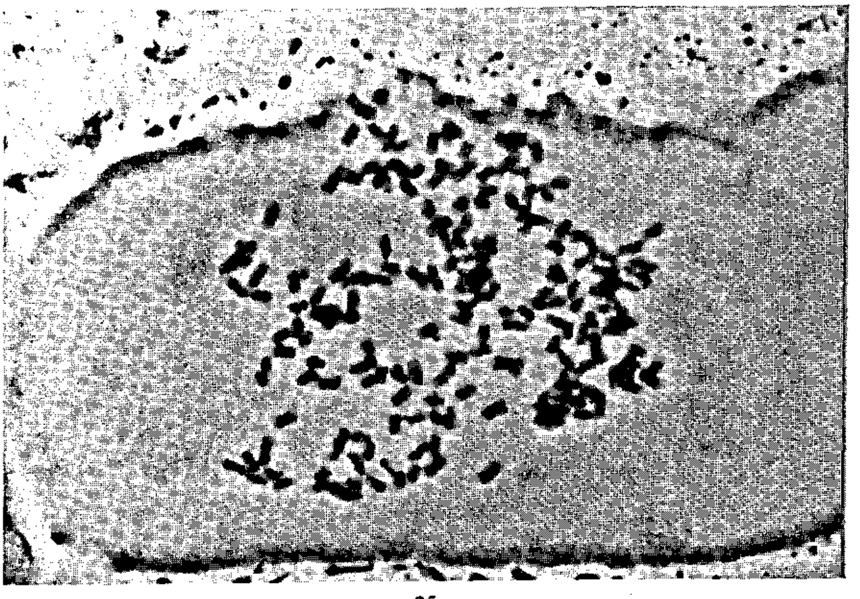
PHOTO 21. (p. 36). *C. africanum*, 2n = 68. Mitotic metaphase. Four satellites (one divided) present (arrows). × 2400.

PHOTO 22 and 23. (p. 36). Similarity of metaphase II plates in P.M.C.'s from *C. neapolitanum*, n = 17 (photo 22) and *C. africanum*, n = 34 (photo 23). × 2400.

PHOTO 24. (p. 36). *C. graecum*, 2n = 84–86. Mitotic metaphase. Five satellites present (arrows). × 2400.

Photos 21 and 24 from pretreated FEULGEN stained squashes; photos 22 and 23 from aceto-carmine squashes.



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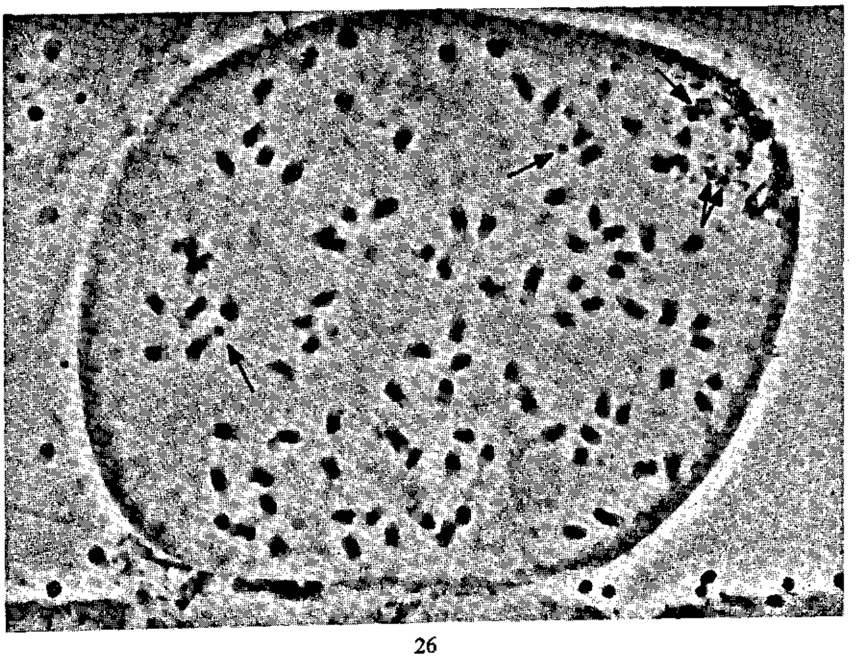

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PHOTO 25. (p. 36). Mitotic anaphase chromosomes of *C. rohlfsianum*, 2n = 96, remaining together for a short period before they separate to the poles. × 2400.

PHOTO 26. (p. 37). *C. rohlfsianum*, 2n = 96. Mitotic metaphase. Four satellites (one divided) present (arrows). × 2400.

Photos from pretreated FEULGEN stained squashes.

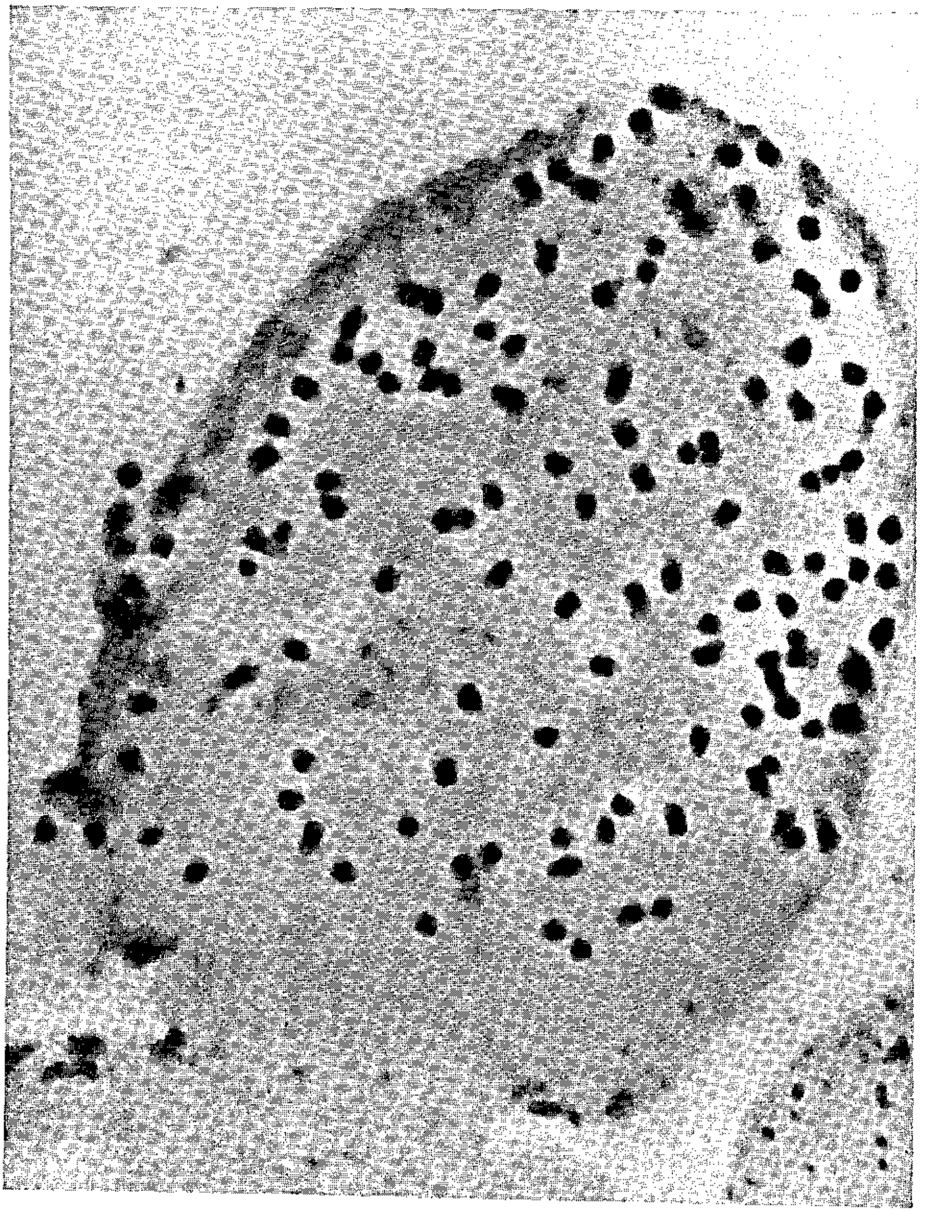

PHOTO 27. (p. 27). *C. graecum*, 2n = 136. Mitotic metaphase. × 3375. From pretreated FEULGEN stained squash.

STELLINGEN

I

De veronderstelling van DOORENBOS, dat het succes van kruisingen tussen kleinbloemige en de eerste grootbloemige *Cyclamen* zou aantonen, dat de laatste niet polyploïd waren, is onvoldoende gefundeerd.

DOORENBOS, J.: Meded. Landbouwhogeschool Wag. **50**, 1950:1-59.

II

Bij een eventuele integratie van Europa heeft de Nederlandse potplantencultuur in tegenstelling met de snijbloemencultuur geen ernstige concurrentie te verwachten.

III

Voor het genus *Cryptocoryne* kan, min of meer analoog met *Cyclamen*, een polyploïde reeks met het grondtal 7 worden vastgesteld.

IV

Het is niet verantwoord de term „ongereduceerd" te gebruiken in direct verband met eicellen en pollenkorrels, indien het al of niet plaats gehad hebben der eigenlijke reductiedeling niet door een genetische analyse bij de nakomelingschap is of kan worden uitgemaakt.

V

Het zou wenselijk zijn het ontstaan van haploïden in soortskruisingen grondig te bestuderen, in het bijzonder ten aanzien van de chemische achtergrond, zulks in verband met de mogelijkheid doelbewust het chromosomenaantal te kunnen reduceren.

VI

De suggestie van SCHWARZ, dat *Cyclamen libanoticum* HILDEBRAND het kruisingsprodukt zou zijn tussen *Cyclamen coum* MILLER en *Cyclamen repandum* SIBTHORP & SMITH, is onhoudbaar.

SCHWARZ, O.: Feddes Repertorium **58**, 1955:233-283

VII

Cyclamen graecum LINK, zoals deze thans algemeen wordt opgevat, bestaat tenminste uit twee taxa, die de rang van soort toekomen. Deze onderscheiden zich door pentaploïdie en octoploïdie, waarbij dit verschil in chromosomengarnituur vergezeld gaat van onderlinge sexuele incompatibiliteit, van verschillen in uiterlijk en vorm van de bloem, en van een verschillende habitus.

Diss. R. A. H. Legro
Wageningen, 1959

VIII

Kritische systematische studiën dienen enerzijds op zo uitvoerig mogelijk herbarium- en literatuuronderzoek te berusten, anderzijds steeds, indien dit uitvoerbaar is, te steunen op de cultuur en het onderzoek der levende planten onder verschillende levensomstandigheden. Soms is dit laatste onontbeerlijk om tot gefundeerde, systematische resultaten te komen.

IX

De wettelijke bescherming van de kwekerseigendom zal alleen goed tot zijn recht kunnen komen, indien een, bij voorkeur internationale, standaardcollectie van levend en gefixeerd materiaal aanwezig is.

X

Wanneer men in de orgelbouw meer stelselmatig gebruik zou maken van diverse houtsoorten voor lepels van tongwerken, als bijvoorbeeld teakhout, makoré, notehout en perehout, dan zou men een aantal muzikaal-bruikbare (of -waardevolle) klankkleuren verkrijgen.