

1956

Cytogenetic Studies in the Genus *Cymbidium*

Donald E. Wimber

Claremont Graduate University

Recommended Citation

Wimber, Donald E.. (1956). *Cytogenetic Studies in the Genus Cymbidium*. CGU Theses & Dissertations, 3.
http://scholarship.claremont.edu/cgu_etd/3. doi: 10.5642/cguetd/3

This Open Access Dissertation is brought to you for free and open access by the CGU Student Scholarship at Scholarship @ Claremont. It has been accepted for inclusion in CGU Theses & Dissertations by an authorized administrator of Scholarship @ Claremont. For more information, please contact scholarship@cuc.claremont.edu.

CYTOGENETIC STUDIES IN THE GENUS CYMBIDIUM

By

Donald E. Wimber

A Dissertation presented to the General Faculty
of the Claremont Graduate School in partial
fulfillment of the requirements for the
degree of Doctor of Philosophy

1956

XC
10.22
W715

TABLE OF CONTENTS

	Page
LIST OF TABLES	iv
LIST OF ILLUSTRATIONS	v
INTRODUCTION	1
THE PROBLEM AND ITS LIMITS	4
SURVEY OF THE LITERATURE	6
Orchid Embryology	
Megasporogenesis and the Female Gametophyte	
Microsporogenesis and the Male Gametophyte	
Fertilization	
Embryogeny	
Orchid Cytology	
Meiosis in Species and Hybrids	
Haploidy and Polyploidy	
Karyotypes	
Cytological Investigations within the Genus	
Cymbidium	
MATERIALS AND METHODS	27
TAXONOMY	30
HYBRIDIZATION	33
CYTOLOGY	35
Karyology	
Meiosis in the Species	
Meiosis in the Primary Hybrids	
Cymbidium Lotta G.	
Cymbidium Gottianum G.	
Cymbidium Pauwelsii G.	
Cymbidium Doris G.	
Cymbidium Ceres G.	
Cymbidium Eberneo-lowianum G.	
Cymbidium Lowio-grandiflorum G.	
Cymbidium Coningsbyanum G.	
Cymbidium Hanburyanum G.	
Cymbidium Albanense G.	
Cymbidium Jean Brummitt G.	
Cymbidium Minuet G.	
Meiosis in the Complex Hybrids	

DISCUSSION	67
Resemblances of the Karyotypes	
Fertility in the Species	
Origin of Polyploidy	
Fertility of the Hybrids	
The F ₁ Hybrids	
The P ₁ Hybrids	
The Complex Hybrids	
The Tetrad and Evolution	
The Species Problem in Cymbidium	
SUMMARY	82
ACKNOWLEDGMENTS	84
LITERATURE CITED	85

LIST OF TABLES

Table	Page
1. Orchid Species Having Polyploid Varieties or Races . . .	16
2. Chromosome Numbers of <u>Cymbidium</u> Species	23
3. Chromosome Numbers of <u>Cymbidium</u> Primary Hybrids	25
4. Number of Recorded Crosses with each <u>Cymbidium</u> Species Through 1955	34
5a. Diploid Chromosome Numbers of <u>Cymbidium</u> Species Determined in this Study	37
5b. Diploid Chromosome Numbers of <u>Grammatophyllum</u> Species Determined in this Study	37
6. Chromosome Separation during Microsporogenesis in <u>Cymbidium</u> Species	45
7. Number of Sporads with Micronuclei in <u>Cymbidium</u> Species	46
8. Chromosome Separation during Microsporogenesis in <u>Cymbidium</u> Primary Hybrids	53
9. Number of Sporads with Micronuclei in <u>Cymbidium</u> Primary Hybrids	55
10. Number of Sporads with Micronuclei in <u>C. Pauwelsii</u> G. # 3	57
11. Chromosome Separation during Microsporogenesis in Triploid <u>Cymbidium</u> Hybrids	64
12. Number of Sporads with Micronuclei in <u>Cymbidium</u> Hybrids	65

LIST OF ILLUSTRATIONS

Figure		Page
1.	Chromosomes from root tip of <u>Cymbidium grandiflorum</u> . . .	38
2.	Chromosomes from root tip of <u>Cymbidium lowianum</u>	38
3.	Idiograms of the chromosomes of <u>Cymbidium grandiflorum</u> and <u>C. lowianum</u> taken from figures 1 and 2	39
4.	Chromosomes from root tip of <u>Grammatophyllum scriptum</u> .	40
5.	Chromosomes from root tip of <u>Cymbidium devonianum</u>	40
6.	Chromosomes from root tip of <u>Cymbidium iridifolium</u>	40
7.	Chromosomes from root tip of <u>Cymbidiella rhodochila</u> . . .	40
8.	Chromosomes from root tip of <u>Cymbidiella rhodochila</u> . . .	42
9.	Microsporogenesis, metaphase I showing 3 univalents. <u>Cymbidium lowianum</u> var. <u>concolor</u>	42
10.	Microsporogenesis, telophase II showing univalents or fragments. <u>Cymbidium lowianum</u>	42
11.	After microsporogenesis, numerous micronuclei visible. <u>Cymbidium lowianum</u> var. <u>concolor</u>	42
12.	A diagramatic representation of the crosses examined in this study	48
13.	Microsporogenesis, interphase showing 2 bridges. <u>Cymbidium Pauwelsii</u> G.	58
14.	Microsporogenesis, interphase showing micronuclei. <u>Cymbidium Pauwelsii</u> G.	58
15.	Microsporogenesis, telophase II showing univalents. <u>Cymbidium Pauwelsii</u> G.	58
16.	Microsporogenesis, telophase I showing bridge, <u>Cymbidium</u> (<u>Pauwelsii</u> G.) ' <u>Comte d'Hemptinne</u> '	58
17.	Microsporogenesis, telophase II showing bridges. <u>Cymbidium</u> (<u>Pauwelsii</u> G.) ' <u>Comte d'Hemptinne</u> '	59

Figure	Page
18. Microsporogenesis, telophase II showing univalents. Cymbidium (Pauwelsii G.) 'Comte d'Hemptinne'	59
19. Microsporogenesis, telophase I and interphase showing univalents and micronuclei. Cymbidium Ceres G.	59
20. Microsporogenesis, interphase showing micronuclei. Cymbidium Ceres G.	59
21. Microsporogenesis, interphase showing bridge. Cymbidium Ceres G.	60
22. Microsporogenesis, telophase I showing bridge. Cymbidium Ceres G.	60
23. Microsporogenesis, telophase II showing univalents. Cymbidium Eburneo-lowianum G.	60
24. Microsporogenesis, telophase II, normal division. Cymbidium Eburneo-lowianum G.	60
25. Microsporogenesis, metaphase II and telophase II. Cymbidium Eburneo-lowianum G.	61
26. After microsporogenesis, 2 tetrads and 1 dyad visible. Cymbidium Hanburyanum G.	61
27. Microsporogenesis, metaphase I showing univalents. Cymbidium Minuet G.	61
28. Microsporogenesis, undeterminable stage. Cymbidium Minuet G.	61
29. Microsporogenesis, undeterminable stage. Cymbidium Minuet G.	62
30. After microsporogenesis, "tetrad" stage showing numerous nuclei. Cymbidium Minuet G.	62
31. After microsporogenesis, "tetrad" stage showing numerous nuclei. Cymbidium Minuet G.	62
32. Compound pollen grain at pollen division Cymbidium Minuet G.	62
33. Microsporogenesis, metaphase I showing univalents. Cymbidium (Olympus G.) 'Rex' (3n)	66
34. Microsporogenesis, metaphase I and interphase showing univalents. Cymbidium Fantasia G. (3n)	66

Figure		Page
35.	Microsporogenesis, telophase II showing univalents. Cymbidium York G.	66
36.	Microsporogenesis, telophase II showing univalents. Cymbidium York G.	66

INTRODUCTION

The orchids known today make up one of the largest Angiospermous families in the world. Recent estimates place the number of genera at about 450 which embraces between 10,000 and 15,000 species (some authorities go as high as 20,000). They are without doubt one of the most highly specialized groups of green plants. Botanically the flowers are of more than passing interest for they deviate so distinctly from the norm of the Monocots. They are the possessors of a number of unique structures that are found in no other family of flowering plants. The ovaries spew forth a prodigious number of seeds, several million in some cases, proembryos with little covering, that can be carried by air currents for hundreds of miles. The seed is little adapted for survival and indeed, cannot germinate in nature unless invaded by the hyphae of a mycorrhizal fungus. Yet of the copious numbers produced, enough survive the rigorous elimination procedure to carry on the species. The production of such immense numbers of seeds involves the usual sexual methods (with the exception of some few apomictic forms), but the powdery pollen of other seed plants would rarely be transferred to stigmas in quantities sufficient to engender millions of ovules. This problem has been solved by the transference of the entire pollen mass, the pollinia in toto, to the stigmatic surface by animals. It is this quota of pollen that initiates seed development, for it is not until pollination that the orchids form ovules, thus a drain on the vitality of the plant is prevented until seed production is more or less assured.

Through the curious forces of evolution fantastic flowers have been developed that serve to attract animals, whether it be for food, sex, shelter, etc., they have proved effective in exciting the attention of birds, insects or other small animals. It is by the actions of these agents and through the workings of some often inimitable devices possessed by the flowers that pollination is brought about.

The late Oaks Ames (1946) put it well when he wrote:

It is a long step in evolution from simple beginnings, to the *Masdevallia* which, for purposes of pollination, holds an insect temporarily in a gentle trap. It is perhaps a much longer step to the irritable *Catasetum* which flings its pollinia violently at co-operating insects. From the incomparable *Coryanthes*, its bucket-like lip holding the measured bath through which an insect must swim when bringing about pollination, to the Australian *Cryptostylis* whose lip resembles the female of a wasp and seemingly lures the male to attempted copulation, is still another long step. Indeed, these steps in organic evolution challenge our powers of credulity and make us wonder why they were taken.

The cultivation of orchids for their beauty has long been practiced. Among the first to seriously culture orchids were probably the Chinese and Japanese, for among the writings of Confucius the virtues of orchids are praised. More than a thousand years ago a Chinese scholar wrote a book on orchids, specifically *Cymbidiums*, and described therein a number of varieties. In Japan the first orchid book was published in 1772 and contained descriptions of a number of orchid genera. In the Western world the first introduction of tropical orchids seems to have been in 1731 when a specimen of *Bletia verecunda* R. Br. was sent to Peter Collinson from the Bahama Islands. It was planted and flowered the following year. The second edition of Miller's Dictionary of Gardening in 1768 mentions Vanilla cultivated in British hot-houses. Apparently several species of Epidendrum were grown in England for they are also enumerated. In 1778 Dr. John Fothergill brought home from

China Phaius grandifolius Lour. and Cymbidium ensifolium Sw. The nineteenth century brought a deluge of tropical orchids into Europe. Although orchid hybrids are well known in nature it was not until 1856 that the first hybrid "raised by hand" was flowered. This was Calanthe dominii G., a cross of C. masuca Lindl. and C. furcata Batem. ex Lindl., raised at the Exeter nursery of James Veitch and Sons in England. The following years increased the flood of interest in hybridization. Paralleling this rising surge of interest, much work was done with orchid embryology, gamete formation, cytology and genetics. To the amateur the significance of many of these studies is rather obscure, yet a great deal of important work has been done and can be found scattered through the literature. In order to complement the present studies it was thought advisable to summarize some of this work here, for bibliographies bringing it together are nonexistent for the most part.

THE PROBLEM AND ITS LIMITS

The present problem is restricted mainly to cytological studies within the genus Cymbidium, a much hybridized member of the Orchidaceae. In recent years among amateur and commercial growers alike, this orchid has gained increased interest and has become one of the major orchidaceous cut flowers. As a result, intensive hybridizing programs have been advanced, yet beyond a few facts involving chromosome numbers, nothing has been known of the cytological behavior within the genus to supplement these programs. The present study was initiated in an attempt to fill this gap and to gain an insight into some of the more subtle problems within the genus. Specifically the problem consists of the following lines of investigation.

1. Analysis of the karyotypes of the species of Cymbidium that have contributed most to the present-day hybrids.
2. An investigation of meiosis in the pollen mother cells of the species and the primary hybrids.
3. An examination of the results of meiosis, the compound pollen grains, in the species and the primary hybrids.
4. An investigation of meiosis in some of the more complex diploid hybrids and some of the higher polyploids.
5. An examination of the chromosome numbers within the genus and related genera.

Within the entire orchid family investigations of this nature are very rare. In scanning the literature only isolated references are

found to researches into the meiotic behavior of the orchids; karyotype analyses are a little more common.



SURVEY OF THE LITERATURE

Orchid Embryology

Of the more than 15,000 species of orchids probably less than 200 have been investigated embryologically even imperfectly. Nineteenth-century authors who figure prominently in these studies are Müller (1847), Hildebrand (1863), Pfitzer (1880), Guignard (1882), Strasburger (1888), and Treub (1879). Later workers using improved techniques have found clear cases of misinterpretation in the reports of some of these older investigators. Much of the work done during the nineteenth and the early years of the twentieth century deserved reinvestigation.

Among recent workers the names of Afzelius (1916, 1922, 1928, 1932), Baranow (1915, 1918, 1925a and b), Pace (1907, 1909, 1914), Hagerup (1944, 1945, 1947), Hoffmann (1930) and Sharp (1912) are outstanding. Above these stands Swamy whose researches into orchid embryology are by far the most complete and comprehensive that we have (1941, 1942a and b, 1943a and b, 1944, 1945, 1946a and b and c, 1947a and b, 1948a and b, 1949a and b). Reviews of some of the more recent literature in orchid embryology have been given by Schnarf (1929, 1931), Swamy (1943a, 1949a and b) and Johansen (1950).

Megasporogenesis and the Female Gametophyte

Typically the orchid embryo sac contains eight nuclei of a monosporic origin. Usually a single hypodermal archesporial cell differentiates in the nucellus soon after pollination. A linear or T-shaped

tetrad of megaspores is formed, although frequently the micropylar cell of the dyad fails to undergo the homotypic division. The products of meiosis all degenerate save the lowermost cell; it undergoes further division to form the embryo sac. The nucleus of this cell generally divides three times to form the eight nucleate sac. The sac prior to fertilization contains three antipodal nuclei, two synergids, one egg and two polar nuclei, which may or may not fuse. The female gametophyte is formed in much the same manner in most of the orchids; in a few there is normally a bisporic origin of the embryo sac and sometimes a reduced number of nuclei, eg. Cypripedium and Paphiopedilum.

The ovules become anatropous at a very early stage of development. There are two integuments which are well developed by the time the embryo sac is mature, the inner coat consists of two or more layers of cells while the outer is single-layered. Soon after fertilization the inner integument completely disorganizes so that observation of the post-fertilization stages is greatly facilitated. The nuclei of the outer integument also degenerate so that at maturity the embryo is vested with only the papery remnants of the outer integument.

Microsporogenesis and the Male Gametophyte

A comprehensive summarization of the work done on the development of the male gametophyte in the Orchidaceae has yet to be made. Hoffmann (1930) observed the development of the pollen in a number of genera of the orchids, however his examples are rather isolated and his data are in most cases incomplete. Barber (1942) brings together some of the facts of the pollen-grain division with some of his own observations in a comparative account and finds some rather exceptional properties. The most complete, yet far from comprehensive, summary is given by

Swamy (1949a).

The microsporangium has in general, a wall of five layers, the epidermis, endothecium, middle layers and the tapetum. Inside this layer is found the mass of pollen mother cells. Reduction divisions are often accomplished in a simultaneous manner. The resulting tetrads are of an isobilateral, tetrahedral, linear or T-shaped arrangement. At maturity of the pollen grains four morphological forms can be recognized: (1) In the Cypripedieae and one or two exceptions in the Epipactieae the individual uninucleate pollen grains separate out of the tetrad soon after the reduction divisions and become invested with a thick wall. The grains are connected by only a few elastic threads, so the pollen mass is quite friable. (2) Four pollen grains derived from one pollen mother cell remain firmly attached together to form a compound grain. However, these individual tetrads are only loosely coherent. This is the general type that occurs in the Epipactieae. (3) In the tribe Orchieae and in a few members of the tribe Epipactieae, the compound grains are further aggregated into small bundles known as massulae. The walls separating the individual grains are thin and uncuticularized, yet around each packet there is developed a thicker wall. (4) In all the other tribes of the Orchidaceae the compound grains remain together in a mass called the pollinium.

Pollen grain division is by no means the same in all orchids. In the species with isolated pollen grains there is no synchronization of division between the individual grains. In those with tetrad pollen the grains of the tetrad may or may not divide at the same time. Those displaying massulae and pollinia show a synchronous type of division.

In the pollen grain division the generative cell is cut off towards the outer wall of the microspore. A cell-plate is laid down between the

vegetative and generative nuclei but is transitory in nature. The pollen matures and develops the characteristic thickenings. When pollination occurs the pollen tube emerges from any part of the germinal furrow. The vegetative nucleus usually enters the tube first and then the generative. While still in the gynostegium the generative nucleus divides to form the two male nuclei.

Fertilization

The interval between pollination and fertilization in orchids varies considerably, from eight to ten days in some species to as long as three months in others. Hildebrand (1863) lists twenty species in which he observed the time from pollination to the onset of embryo formation. Schnarf (1929) has tabulated the findings of previous authors, and Swamy (1949b) has added ten observations of his own. The pollen tubes after passing down the gynostegium enter the ovary and generally grow down the wall of the ovary in three strings; these strings subdivide and ramify throughout the placental tissue. At the time of pollination the ovules are still undeveloped, being at the megaspore mother cell stage. Pollination stimulates the development of both the ovules and the ovary, so that by the time the pollen tubes reach the micropyle the embryo sac is usually mature. When the tip of the pollen tube enters the sac in most of the species observed, it apparently kills one of the synergids. One of the male nuclei fuses with the egg while the other may or may not fuse with the polar nucleus. Often the polar nuclei are already atrophied at the time of fertilization. If fusion does occur, endosperm development usually ceases at that point. However, in a few members of the family divisions may occur. A four-celled endosperm has been observed in some species of the Cypripedieae (Pace, 1907 and

Procina, 1930) and Swamy (1947a) has found as many as ten nuclei in Vanilla planifolia Andr.

Embryogeny

Although the types of embryo sacs in the orchids may be categorized rather easily, the simplicity ends with the numerous developmental patterns of the embryos. Swamy, who has probably worked in this field more than any other man, presents an excellent review of the subject (1949b). He divided the embryos into the suspensored and the suspensorless forms and then further subdivided those with suspensors into several classes depending on the mode and degree of development of the suspensor.

Some phylogenetic tendencies seem to be indicated in the developmental patterns of the embryos and the seeds:

1. In the so-called primitive tribes, the Cypripedieae and Epipactieae, are found the suspensorless embryos. These embryos precede those with unicellular suspensors and in the more specialized tribes, the unicellular suspensors become variously modified by different forms of multicellular organizations.
2. The developmental plan (the onset of divisions in a definite sequence) is more pronounced in the more primitive tribes, whereas in the more specialized the tendency to proceed in exact steps is less pronounced.
3. Endosperm development is reported only from the two primitive tribes, the Cypripedieae and Epipactieae.
4. A multicellular opaque and sclerotic seed coat is reported only in the primitive tribes.

These embryological trends exhibit a remarkable correlation between the morphological characters and the specialization tendencies that have been guiding factors for the systematic classification of the orchids.

Orchid Cytology

Meiosis in Species and Hybrids

Among the orchids, meiosis in the pollen mother cells has often been seen; studying the reduction divisions is one of the common methods for determining the chromosome number of the plant in question. At times in these investigations, discontinuities have been noted. Fuchs and Ziegenspeck (1924) were among the first to call attention to irregularities in meiosis from material of Orchis traunsteineri Saut. collected in the field. They were without doubt dealing with a group that was quite variable and probably of hybrid origin. In many plants they found variations in the reduction division resulting in microspores that deviated from the usual haploid number of ten. Many authors since then have tried to corroborate their cytological findings and have failed in almost every instance. Belling in the same year (1924) reported micro-pollen from plants of Cypripedium acaule Ait. that had been grown in a greenhouse. In this species with usually ten haploid chromosomes, microcytes were found at times with one chromosome and others with only nine. In Listera ovata (L.) R. Br. Tuschajakova (1929) recorded many abnormalities in the formation of the pollen resulting in dyads, triads and oftentimes micronuclei.

Francini (1931) examined the reduction divisions in Paphiopedilum leeanum G., a primary hybrid between P. insigne (Wall.) Pfitz. which had a diploid number of thirty-two and P. spicerianum (Rchb. f.) Pfitz. with a somatic chromosome number of thirty. The hybrid had a diploid number of thirty-one and at metaphase I showed fifteen bivalents and one univalent. An F_2 P. leeanum G. was later examined (1932) and was found to have a chromosome number of twenty-four. It was theorized that

irregularities in the reduction divisions in the F_1 hybrid yielded this plant of an aneuploid nature. Later Francini (1934) studied the cytology of P. villosum (Lindl.) Pfitz., P. barbatum (Lindl.) Pfitz. and their primary hybrid P. harrisianum G. P. villosum showed a diploid number of twenty-six. In an analysis of fifty microspores, chromosome numbers were found to range from twelve to fifteen with most at thirteen. P. barbatum had a somatic number of thirty-six. The primary hybrid P. harrisianum G. exhibited a diploid chromosome number of thirty-two. Here irregularities were prevalent at meiosis resulting in a greatly lowered fertility level. A compilation of the associations at diakinesis showed most commonly: four trivalents, seven bivalents and six univalents. McQuade (1949) studied the results of meiosis in P. maudiae G. and its parents, P. callosum (Rchb. f.) Pfitz and P. lawrenceanum (Rchb. f.) Pfitz. P. callosum had a diploid number of thirty-two while P. lawrenceanum had thirty-six. The hybrid P. maudiae G. displayed a somatic number of thirty-four. While both the parents underwent normal meioses, the primary hybrid showed disorders at anaphase I occasionally. These irregularities were attributed to inversions.

In Nigritella nigra (L.) Rchb. f. which had a usual diploid number of forty, Heusser (1938) reported finding in addition to the haploid number of twenty, also some pollen grains with nineteen and twenty-one chromosomes.

In Zeuxine sulcata Lindl., an apomictic species, Seshagiriah (1941) noted a very abnormal microspore formation. Sometimes the first division was completed, other times it never started; the second division was mostly never initiated and as a result the final products of meiosis were quite irregular, dyads, monads, triads and numerous micronuclei. Later

the whole pollinia usually broke down. Swamy (1946a) complemented the work of Seshagiriah on Zeuxine sulcata by finding irregularities at meiosis in both the pollen mother cells and the egg mother cells that caused most of the development to cease after the heterotypic division. The pollen was not entirely aborted, however, for he was able to successfully germinate some.

The reports of Kamemoto and Randolph (1949) and Kamemoto (1950, 1952) are among the most recent studies in meiosis of orchids. Among other things, the reduction divisions of the intergeneric hybrid of Laelia anceps Lindl. 'Stella' and Cattleya (Enid G.) 'Alba' were examined. The hybrid displayed a somatic chromosome number of forty as did both the parents; however, at metaphase I varying numbers of bivalents and univalents were noted. Such irregularities were associated with sterility in the hybrid. Other studies were carried on with the pairing configurations in various Cattleya, Laeliocattleya and Brassocattleya hybrids. At times the somatic chromosome numbers varied from the usual forty by one to three chromosomes in these hybrids; these numbers probably arose through abnormal reduction divisions in the parental plants. Apparently some gametes are still viable even though they display great differences in the chromosome number. In many intrageneric Cattleya hybrids, metaphase I pairing was relatively regular with about twenty bivalents, while in Laeliocattleya and Brassocattleya intergeneric hybrids, pairing at metaphase I was more variable. It is remarkable that among orchids even many "intergeneric" hybrids are relatively fertile.

Miduno (1954) analysed the hybrids between two species of Bletilla that differed in chromosome numbers. B. striata Rchb. f. ($n=16$) and B. formosana Schl. ($n=18$) were crossed; the hybrid at meiosis showed mostly

sixteen bivalents and two univalents. No trivalents were ever seen. At anaphase I bridges were often observed with the formation of a number of fragments. Consequently micropollen grains were found to be of fairly regular occurrence. At pollen division the chromosome numbers were found to be variable.

Storey (1952) reported the chromosome numbers of several species of Vanda and several of its hybrids. Those species in which the haploid numbers were determined, were found to exhibit no irregularities at microsporogenesis. The hybrids, in contrast, displayed a great deal of variation. Some of the diploids were seemingly quite regular and others showed a large number of abnormalities at meiosis; the triploids, of course, also were irregular at microspore formation.

Haploidy and Polyploidy

Secondary association of chromosomes has been occasionally seen in the reduction division of some orchids indicating a possible polyploid origin. Hagerup (1938, 1944) mentioned seeing it in both Orchis maculatus L. f. ericetorum Linton. and O. morio L. Miduno (1940) noted it in Bletilla striata Rchb. f. var. gebina Rchb. f., a form of a species that exhibited a great deal of irregularity at meiosis. His report is of interest because he also discusses a number of haploid plants that were derived from this otherwise normal diploid species. In the cross of Bletilla striata var. gebina x Eleorchis japonica F. Maekawa a number of the offspring were raised and grown to maturity. The plants could immediately be separated into two groups, those that bloomed and those that did not. Those that bloomed, were seen to favor the pistillate parent very strongly, and all proved to have a chromosome number of sixteen, exactly one-half the somatic number of Bletilla striata var.

gebina. The nonblooming plants all had a mitotic number of thirty-six, the sum of the haploid chromosome numbers of the parent plants, sixteen and twenty. The meiotic divisions in these haploid plants were examined; a variable amount of secondary pairing seemed to be present, but was not at all constant. At times there were as many as sixteen univalents seen at metaphase I. The separation at the heterotypic division resulted in distributions from 0-16 to 8-8 chromosomes. The results of this reduction division yielded pollen grains with from two chromosomes to those with more than thirty-two.

Haploid plants are thought to occur naturally in other genera of the Orchidaceae. Hagerup (1944, 1945, 1947) reported some very interesting cases of haploid embryos in several orchids, very probably arising through facultative parthenogenesis. Polyploid embryos were also of fairly common occurrence. At times, such embryos arose by both male nuclei fusing with the same egg; at other times more than one pollen tube entered the sac, liberating several sperm nuclei that occasionally united with the same egg. Polyploid plants of many of these orchids have often been found in nature, but a haploid has yet to be discovered.

The occurrence of polyploid species or forms of species seems to be quite common in the Orchidaceae. Table 1 is a compilation of species within the family in which polyploid varieties have been reported. It is probable here that in many cases the species lines have been drawn too inclusively and the cytology merely reveals the weaknesses of the taxonomy.

In addition to this reported intraspecific polyploidy, many species within a genus often appear to be polyploid or aneuploid derivatives of lower chromosome numbered plants, as shown by the studies of Vermeulen (1938) in Orchis and by the investigations of Hagerup (1944, 1945, 1947)

TABLE 1

ORCHID SPECIES HAVING POLYPLOID VARIETIES OR RACES

Species	Haploid No.	Diploid No.	Authority
<u>Gymnadenia odoratissima</u> Rich.	10	20	F & Z (1924)*
" "	20	40	Heusser (1938)
<u>G. conopea</u> (L.) R. Br.	8	-	Codat (1924)
" "	10	20	F & Z (1924)
" "	16	-	Strasburger (1888)
" "	20	40	Richardson (1938)
" "	20	-	Barber (1942)
" "	20	-	Afzelius (1943)
" "	-	40	Frahm-Leliveld (1941)
" "	-	40	Heusser (1938)
" "	40	80	Heusser (1938)
<u>Goodyera procera</u> Hook.	11	-	Afzelius (1943)
" "	-	42	Miduno (1939)
<u>Epipactis helleborine</u> (L.) Cr. Wats. & Coul.	10	-	Weijer (1952)
" "	20	-	Weijer (1952)
<u>Epidendrum nocturnum</u> Jacq.	20	-	Hoffmann (1930)
" "	-	c.80	Kamemoto (1950)
<u>Habenaria hyperborea</u> (L.) R. Br.	-	42	Humphrey (1943)
" "	-	42	Kamemoto (1950)
" "	-	63	Kamemoto (1950)
" "	42	-	Harmsen (1943)

*Fuchs and Ziegenspeck: most contemporary authorities disregard this work.

TABLE 1 Cont.

Species	Haploid No.	Diploid No.	Authority
<u>Laelia albida</u> Batem.	-	42	Kamemoto (1950)
" "	-	63	Kamemoto (1950)
<u>Laelia gouldiana</u> Rchb. f.	-	40	Kamemoto (1950)
" "	-	60	Kamemoto (1950)
<u>Nigritella nigra</u> (L.) Rchb. f.	19	-	Chiarugi (1929)
" "	20	40	Heusser (1938)
" "	c.30	c.60	Afzelius (1928)
" "	32	64	Afzelius (1932)
<u>N. rubra</u> Rich.	19	-	Chiarugi (1929)
" "	-	80	Heusser (1938)
<u>Orchis coriophorus</u> L.	10	20	F & Z (1924)
" "	19	38	Heusser (1938)
<u>O. incarnatus</u> L.	10	20	F & Z (1924)
" "	20	-	Hagerup (1938)
" "	-	40	Heusser (1938)
<u>O. latifolia</u> L. ex Fries	10	20	F & Z (1924)
" "	-	40	Eftimiu-Heim (1941)
" "	-	40	Maude (1939)
" "	40	-	Hagerup (1938)
" "	-	80	Vermeulen (1938)
" "	-	80	Maude (1939)
" "	-	80	Heusser (1938)
<u>O. maculata</u> L.	10	20	F & Z (1924)
" v. <u>meyeri</u> Rchb. f.	20	-	Hagerup (1938)
" "	20	-	Barber (1942)
" "	-	40	Love, A. & D. (1944)

TABLE 1 Cont.

Species	Haploid No.	Diploid No.	Authority
<u>O. maculata</u> L.	-	40	Vermeulen (1938)
"	-	40	Heusser (1938)
" v. <u>ericetorum</u> Linton	40	-	Hagerup (1938)
"	-	80	Heusser (1938)
<u>O. militaris</u> L.	10	20	F & Z (1924)
"	21	-	Hagerup (1938)
"	21	42	Heusser (1938)
<u>O. traunsteineri</u> Saut.	10	20	F & Z (1924)
"	-	40	Heusser (1938)
"	-	80	Heusser (1938)
" ssp. <u>russowii</u> Klin.	-	122	Vermeulen (1938)
<u>O. ustulata</u> L.	-	20	F & Z (1924)
"	21	-	Hagerup (1938)
"	21	42	Heusser (1938)
<u>Paphiopedilum insigne</u> (Wall.) Pfitz.	8-9	-	Suessenguth (1921)
"	c.12	-	Afzelius (1916)
"	12-13	-	Heitz (1926)
"	-	26	Mehlquist (1947)
"	-	26	Duncan (1947)
"	-	26	Kamemoto & Randolph (1949)
"	-	26	Duncan (1947)
" 'Ernestii'	-	26	Duncan (1947)
" 'Laura Kimball'	-	26	Mehlquist (1947)
" 'Royalty'	-	26	Mehlquist (1947)
" 'Sanderæ'	-	26	Mehlquist (1947)
" "	-	26	Duncan (1947)
" 'Sylhetense'	-	26	Mehlquist (1947)
" "	-	26	Duncan (1947)
" 'Tonbridgense'	-	26	Mehlquist (1947)
"	c.16	-	Hoffmann (1930)
"	16	-	Francini (1931)
" 'Harefield Hall'	-	39	Mehlquist (1947)
" "	-	39	Duncan & MacLeod (1948)
<u>Pecteilis radiata</u> Ratinisque	-	32	Miduno (1939)
(variegated form)	-	48	Miduno (1940b)

and Midumo (1938) in Epipactis.

Meiosis was investigated in naturally occurring triploid hybrids of Orchis species by Heslop-Harrison (1953). In the hybrids between the diploid O. fuchsii Druce ($2n = 40$) and the tetraploids, O. purpurella Steph. and O. praetermissa Druce ($2n = 80$) there was a regular formation of twenty bivalents and twenty univalents. The two tetraploids were considered to be amphidiploids with allosyndetic pairing, for they showed typical "diploid" behavior during microsporogenesis. The pairing configurations in the hybrids suggested that O. fuchsii was probably one of the progenitors of these two polyploid species.

Storey (1953) reported a highly interesting case in Vanda in which pentaploids were produced by mating two diploid clones. A diploid plant, V. Mevr. L. Velthuis G., that developed from an intersectional cross in the genus, was mated to V. coerulea Griff., also a diploid. The resulting progeny, V. Nora Potter G., were all found to be pentaploids. Microsporogenesis was studied in both parents and was found to be normal in V. coerulea, yet in V. Mevr. L. Velthuis G. it was found to be quite irregular. This irregularity is explained on the basis of the evolutionary divergence of the two genomes found in this plant, one from the spatulate-leaved group and the other from the terete-leaved section. Meiosis resulted in sporads with numerous micropollen grains, or sometimes only a pair of microspores and occasionally single giant pollen grains. On the basis of the chromosome numbers of the plants of V. Nora Potter G., it was theorized that only these giant gametes were functional and they combined with the usual haploid gametes from V. coerulea to form offspring of a pentaploidal nature.

Abnormalities in meiotic divisions often produce gametes with aneuploid

or polyploid chromosome numbers. These may in turn give rise to plants of an aneuploid or polyploid nature. Misdivisions in somatic tissue may likewise yield chimeras or single cells of differing genotypes. This phenomenon has been profusely illustrated in many plants; examples are not lacking in the Orchidaceae. In a cytological examination of the ripening ovary of Epidendrum ciliare L., Geitler (1940) detected a few tetraploid sectors. Miduno (1938) made a rather comprehensive study of the results of mixoploidy in the root tissue of Epipactis sayekiana Makino and Cephalanthera shizuoi F. Maekawa. Besides an increase in cell and nuclear size in the tetraploid cells, there was an apparent increase in the number of nucleoli.

Another interesting curiosity sometimes observed in orchids is the event of aneusomaty, a happening most often observed in the genus Paphiopedilum. This genus has remarkably large chromosomes, well constructed for cytological work. Probably the first to call attention to a small somatic variation in chromosome number in this genus was Francini (1934) in P. villosum (Lindl.) Pfitz. In fifty cells from the root tips of this species he was able to count the following numbers of chromosomes:

No. of chromosomes	19	21	23	24	25	26	27	28
No. of cells	1	1	1	3	4	33	4	3

He attributed this variation to misdivision of certain chromosomes, especially those with strong secondary constrictions. Duncan (1945) showed a weak variation of chromosome numbers in P. wardii Summerhayes from forty-one to forty-five. Through the construction of idiograms of the karyotypes he was able to show that there were twenty basic types of chromosomes and that three of these types might be present in numbers from three to six so that there was replication at the trisomic to hexasomic levels. In a later publication of Duncan and MacLeod (1950a) several

other species of Paphiopedilum were noted as showing the same phenomenon as found in P. wardii.

Karyotypes

The karyotypes of plants are very useful in predicting phylogenetic affinities of the species and may be helpful in predicting cross compatibilities and the ultimate fertility or sterility of the hybrids. If the chromosomes are large and have morphological dissimilarities, information concerning the size, position of primary and secondary constrictions, presence or absence of satellites, etc., may be supplied. Probably the most outstanding studies along these lines in the Orchidaceae have been done with the genus Paphiopedilum, a subject ideally suited for such investigations. The research was probably initiated by Francini (1934) when he gave rough idiograms of the chromosome complements of two species of the genus. The comprehensive works of Duncan and MacLeod (1948a and b, 1949a, b and c, 1950a and b) carried on this line of investigation. These studies of the chromosome numbers and morphology have contributed greatly to a clearer systematic understanding of the genus.

Cytological Investigations within the Genus Cymbidium

Cytological studies of the Cymbidium have been very meager. A few inquiries into the chromosome numbers of the species and some of the hybrids have been made: Suessenguth (1921), Hoffmann (1930), Sugiura (1936), Mehlquist (1952), Vacin (1954), Menninger (1954), Wimber (1954), Wimber and Herndlund (1955) and Wells (1956). Swamy reported the development of the female gametophyte and embryo (1942, 1946) and also of the male gamete in C. bicolor Lindl.

Nothing was reported here that deviated greatly from the norm found

in other orchids. Table 2 is a compilation of the chromosome counts that have been reported for Cymbidium species. Table 3 lists those numbers of the primary hybrids. Mehlquist (1952) and Vacin (1954) have published lists of the chromosome numbers of both the hybrids and the species; Wells (1956) lists the numbers for one hundred ten plants.

From studying these reports it becomes obvious that a few clones have been selected through the years by both commercial and amateur growers for use as parental plants. These clones in some cases, upon cytological examination, have turned out to be tetraploids. Through extensive use of these tetraploid plants as parents in combination with diploids, a large number of triploids have been raised. As is the case with most triploids, they have proven to be relatively infertile and have thus generally slowed rapid development along these polyploid lines. Most of the breeding programs today employ polyploid plants by generally mating tetraploids with diploids. With the making of such crosses the grower usually assures himself of a large number of plants with good quality flowers by current standards. Breeding on the diploid level, though still desirable for future developments, does not yield nearly the level of quality. To the grower this difference is highly important, for a plant that is nurtured through four to seven years with specialized care from seed to blooming plant is a large investment. If a high percentage of these plants at the time of bloom are undesirable, the loss to the grower is sizable, however, if through the use of polyploids this loss may be cut down, it can be of advantage to the amateur and commercial man alike.

TABLE 2

CHROMOSOME NUMBERS OF CYMBIDIUM SPECIES

Species	Haploid No.	Diploid No.	Authority
<u>C. aloifolium</u> Sw.	-	40	Mehlquist (1952)
<u>C. bicolor</u> Lindl.	20	-	Swamy (1941)
<u>C. eburneum</u> Lindl.	-	40	Mehlquist (1952)
<u>C. erythrostylum</u> Rolfe	-	40	"
<u>C. finlaysonianum</u> Lindl.	-	40	"
<u>C. giganteum</u> Wallich	-	40	"
<u>C. grandiflorum</u> Grif.	-	40	"
" 'Westonbirt'	-	40	"
<u>C. i'ansoni</u> Hort.	-	40	"
<u>C. insigne</u> Rolfe	-	40	"
" 'album'	-	40	"
" 'albens'	-	40	"
" 'rodochilum'	-	40	"
" 'Westonbirt'	-	40	"
<u>C. lowianum</u> Rehb. f.	9-10	-	Suessenguth (1921)
"	20	-	Hoffmann (1930)
"	-	40	Mehlquist (1952)
" v. <u>concolor</u> Rolfe	-	40	"
" 'Fir Grange'	-	40	"
" 'McBean's'	-	40	"
" 'Pitt's'	-	40	"
" 'St. Denis'	-	40	"
" 'Comte d'Hemptinne'	-	40	Vacin (1954)

TABLE 2 Cont.

CHROMOSOME NUMBERS OF CYMBIDIUM SPECIES

Species	Haploid No.	Diploid No.	Authority
<u>C. Parishii</u> Rchb. f. v. <u>Sanderæ</u> Hort.	-	40	Mehlquist (1952)
<u>C. pumilum</u> Rolfe	-	40	"
" 'albo-marginalis'	-	40	"
<u>C. schroderi</u> Rolfe	-	40	"
<u>C. sinense</u> Willd.	-	40	Sugiura (1936)
<u>C. tracyanum</u> Hort.	-	40	Mehlquist (1952)

TABLE 3

CHROMOSOME NUMBERS OF CYMBIDIUM PRIMARY HYBRIDS

Hybrid	Parentage	Diploid No.	Authority
Albanense	erythrostylum x insigne	40	Mehlquist (1952)
Ceres 'F. J. Hanbury'	i'ansoni x insigne	40	Vacin (1954)
" 'Girrahween'	" " "	40	Wells (1956)
Coningsbyanum 'Brockhurst'	insigne x grandiflorum	40	Mehlquist (1952)
" "	" " "	40	Vacin (1954)
Doris	insigne x tracyanum	40	Mehlquist (1952)
Eburneo-lowianum	eburneum x lowianum	40	"
" 'concolor'	" " "	40	"
Gattonense	lowianum x tracyanum	40	"
Gottianum 'Westonbirt'	eburneum x insigne	40	"
Lowio-grandiflorum	lowianum x grandiflorum	40	"
" 'Westonbirt'	" " "	40	"
Minuet	pumilum x insigne	40	"
Pauwelsii	insigne x lowianum	40	Hoffmann (1930)
"	" " "	40	Mehlquist (1952)
" 'Magnificum'	" " "	40	"
" 'Brockhurst'	" " "	40	"
" 'Comte d'Hemptinne'	" " "	80	"
" " "	" " "	80	Vacin (1954)

TABLE 3 Cont.

Hybrid	Parentage	Diploid No.	Authority
Rosefieldense	parishii x i'ansoni	40	Mehlquist (1952)
Wiganianum	eburneum x tracyanum	40	"

MATERIALS AND METHODS

The material used in the present study was obtained from plants found in the writer's own collection and in the collections of various amateur and commercial growers in the southern California area. The species at the time of flowering were checked against the original descriptions whenever possible, so that cases of misidentification could be eliminated. Correct identification of the F_1 hybrids presented an entirely different problem. The primary hybridizations between many of the species have without a doubt been made several times, often with different clones of the same species. Owing to the natural genotypic variance from clone to clone and to the heterozygous genotypes possessed by the individual clones, these hybrids are often quite diverse in phenotype. Positive identification of such clones is more difficult. In named clones of certain primary hybrids, more certainty could be attached to the labeling; in others, individual histories as far as known could be checked. And in some only general intermediate appearance could be compared. Any or all of these criteria for confirmation of the identity of primary hybrids are really insufficient, for in the early days of orchid hybridizing when most of these crosses were made, accurate records of crosses were often not kept.

Buds for meiotic studies were collected in most species and hybrids when they were about to break through the modified sheathing leaves on the spike. Fixations were usually made on warm days between the hours of 10 A.M. and 4 P.M. The hybrid, C. Minuet G., was an exception, for meiosis did not occur until the buds were out and well separated. The

material was fixed in 1:3 acetic-alcohol overnight before examination, and could be stored successfully for periods up to a year in 70% alcohol.

For the examination of somatic chromosomes root tips were cut from plants on warm, sunny days, quartered with a razor blade, placed in ice-water at 0° C. for one to three hours and fixed in 1:3 acetic-alcohol overnight prior to examination.

The meiosis in the pollinia were studied after staining with iron-aceto-carmine. Small bits of the pollinia were teased out with a dissecting needle and placed on a slide with a small drop of iron-aceto-carmine. Additional iron was worked into the aceto-carmine with an iron needle until the stain was a dark reddish-purple; the bits of the pollinia were then squashed using a flattened needle and the cover slip applied. The slide was heated over a steam bath for about thirty seconds and removed; a very gentle pressure was brought to bear upon the cover slip through blotting paper and the slide was then examined. The steam bath seemed to be superior to heating over an alcohol lamp because a more even temperature could be maintained.

Mitotic phases in the root tips were examined after staining with aceto-lacmoid according to the procedure outlined by Wimber (in press). The stain was prepared by dissolving one gram dry lacmoid in fifty cc. hot glacial acetic acid, allowing to cool for five minutes and filtering. This stock solution was thereupon diluted 50-70% with water, a few drops at a time in a watch glass when ready to use. Lacmoid is not a certified stain, therefore samples from different sources did not give equal results. That obtained from Mathison, Coleman and Bell (batch nos. 421014 and 450412) proved quite satisfactory. The quartered root tips

were macerated in 10-20% hydrochloric acid for five to fifteen minutes. The tip of one of the root tip sections (1-2 mm.) was removed, placed on a slide with a drop of the diluted aceto-lacmoid and squashed using a flattened needle. The cover slip was applied, the liquid under it was briefly brought to near the boiling point over an alcohol lamp, and a vigorous pressure was brought to bear upon the cover slip through blotting paper. If any bubbles appeared a minute amount of stain was applied to the edge of the cover slip and they were gently worked out.

The pollen grains were examined using the following technique. The pollinia were removed from mature or near mature flowers, cut into small bits and placed in a small amount of water in a test tube. The tube was then placed in a steam bath for at least five minutes. This procedure softened the pollinia so that it could be easily squashed. Bits of the pollinia were removed from the water and squashed in a small drop of lactol-phenol cotton blue by using a flattened needle. The cover slips were applied, ringed with fingernail polish, allowed to ripen for several days and then examined.

TAXONOMY

The genus Cymbidium was founded by Swartz (1800) and encompassed a wide range of forms including three or four species of both West Indian and South African origin. The nomenclatorial history of the genus has taken a rather devious path since the beginning; the recording of all but the most salient points shall be omitted here, for it lies beyond the scope of this work. The boundaries of the genus remained unchanged until 1832 when Lindley allayed some of the confusion; he admitted, however, that the genus was probably composed of several genera, the characters of which were rather vague and could not be further determined until a greater number of representative species were known. In the years following while the Botanical Register was being published, Lindley was able to remove many of the previous errors when new specimens were examined. Reichenbach reduced the genus to its more natural limits and Bentham and Hooker (1883), about 75 years ago, more or less delimited the genus as it stands today.

The essential features of Cymbidium as stated by Holttum (1953) are:

Terrestrial or epiphytic plants, with short or somewhat elongated pseudobulbs, each bearing a small number of leaves; in most cases the whole pseudobulb covered with the closely overlapping sheathing bases of the leaves; leaves rather long and narrow, erect or curved, rarely stalked, thick or thin, never plicate; inflorescence a raceme from near base of pseudobulb, erect or pendulous, usually long; flowers large or fairly large; sepals and petals about equal and free, usually spreading; lip 3-lobed, the side-lobes erect and close to the sides of the column, the central part of the lip between them with two longitudinal keels; column rather long; pollinia two, cleft, joined by a common caudicle and seated directly on the broad disc.

The genus consists of between 50 and 60 species dispersed over the eastern Asia-Pacific area. Members of the genus may be found in the Himalayas from Nepal, Sikkim and Assam through Burma and on into Indo-China. Mainland representatives of the genus may also be picked up in the bordering areas of China and Malaya. It is well represented in the East Indies and the Philippine area and can be found as far north as the island of Honshu in Japan, as far east as the Solomon Islands and as far south as the 35° parallel in eastern Australia.

The taxonomic relationships within the genus are still rather vague, although there is reason to believe that the species could be placed in natural groupings fairly readily with some future study.

In 1849 Blume founded the genus Cyperorchis based on one species, C. elegans Blume. The basic difference between this genus and Cymbidium rests on the connivence of the perianth segments; the flowers, therefore, do not fully expand as those of Cymbidium. Since that time some authors have recognized the genus and some have disregarded it, stating that the differences are too trivial. In horticultural practice the tendency has been to include all those forms of Cyperorchis under the genus Cymbidium.

There appear to be three, more or less related species in Madagascar which have been related to Cymbidium at one time or another. In 1918 Rolfe proposed the genus Cymbidiella to accommodate those species which had up to that time been carried under Cymbidium. The distinguishing characteristics which seem to separate this genus from Cymbidium are the strongly three lobed labellum and the paniculate inflorescence that is sometimes present. Horticulturally these species, too, are carried under the genus Cymbidium.

Morphologically the genus Grammatophyllum appears to be closely related to Cymbidium, for here the most noticeable difference is in the way the pollinia are carried on separate upgrowths, whereas in Cymbidium they are seated directly on the disc.

Two other genera usually placed in the same tribe with Cymbidium are Dipodium and Porphyroglottis. These are of minor horticultural interest and shall not be discussed here.

HYBRIDIZATION

In the past seventy-five years seemingly unrestricted crossings have been made in the genus Cymbidium by commercial firms and amateur hybridizers. The criterion that seemed to govern the use of certain species was size -- the smaller the flower, the less desirable it was. As a result the large flowered species, found most prolifically in the Himalayas, were the major contributors to the gene pools of our modern hybrids. To be sure a few of the smaller flowered species were utilized in the matings, but these were for the most part rather unpopular with hybridizers. Table 4 gives the species of Cymbidium that have been employed in crosses through 1955 as recorded in Sanders' Complete List of Orchid Hybrids. The species are enumerated according to their importance, as rated by the number of hybrids that have been made with each; the area of distribution is also mentioned for each. It will be noted that two of the species sometimes placed in Cyperorchis seem to cross quite readily with many of the others. Except for the crossing of Cyperorchis with Cymbidium no other intergeneric cross has been recorded by Sanders except one, and a strong case of invalidity could probably be built up against it with a little study. Sanders registered Phaiocymbidium chardwarensense as a cross of Cymbidium giganteum Wallich and Phaius grandifolius Lour. These species were placed in different subtribes by Schlechter (1926) and are quite distantly related morphologically. Pfitzer (1906) commented on this alleged hybrid: "Again, some few years ago the "Gardeners' Chronicle" reported that a hybrid between Phaius and Cymbidium was exhibited at one of the Society's

meetings, but this supposed hybrid I consider to be a similar case to that of Zygopetalum mackaii Hook. For it is said that the plant looked in every respect like a strong growing Phaius."

The Orchid Review of November 1922 recorded a possible cross of Grammatophyllum and Cymbidium. It was stated that Grammatophyllum papuanum J. J. Smith pollinated by Cymbidium insigne Rolfe produced viable seeds and that some seedlings were being raised. No further mention of this asserted bigeneric cross was made.

TABLE 4
NUMBER OF RECORDED CROSSES WITH EACH CYMBIDIUM
SPECIES THROUGH 1955

Species	No. Crosses	Distribution
<u>C. insigne</u> Rolfe	72	Annam
<u>C. lowianum</u> Rchb. f.	55	Burma, Assam
<u>C. tracyanum</u> Hort.	37	Burma, Siam
<u>C. erythrostylum</u> Rolfe	35	Annam
<u>C. grandiflorum</u> Griff.	34	Nepal, Sikkim
<u>C. i'ansoni</u> Hort.	30	Burma, Annam
<u>C. eburneum</u> Lindl.	23	N. India
<u>C. giganteum</u> Wallich	13	N. India
<u>C. parishii</u> Rchb. f.	10	Burma
<u>C. mastersii</u> Griff. ex Lindl.*	5	N. India
<u>C. pumilum</u> Rolfe	4	China
<u>C. tigrinum</u> Parish ex Hook.	4	Burma
<u>C. devonianum</u> Paxt.	4	Assam
<u>C. aloifolium</u> Sw.	2	Burma
<u>C. finlaysonianum</u> Lindl.	2	Malaya
<u>C. elegans</u> Lindl.*	2	Nepal
<u>C. pendulum</u> Sw.	2	India
<u>C. schroderi</u> Rolfe	2	Annam
<u>C. longifolium</u> D. Don	1	Nepal

*Sometimes placed in Cyperorchis

CYTOLOGY

Karyology

The modern hybrid *Cymbidiums* have all stemmed from what are now considered to be twenty species. Eight of these have played a major role in producing most of the modern hybrids and the remaining twelve have been of very minor importance, entering into the gene pools but rarely. The chromosome numbers of the eight major species have been determined in the present investigation, four of the minor species have been studied and nine other species that have never had a recorded hybrid were also investigated. Table 5a is a listing of the species of *Cymbidium* that have been studied in the present problem. The diploid chromosome numbers all proved to be forty. The species that are sometimes placed in *Cyperorchis* are considered to be in the genus *Cymbidium* here.

Morphologically the chromosomes are too small to allow detailed studies of their structure. Two idiograms were made from photographs (figs. 1 and 2) of *C. grandiflorum* Griff. and *C. lowianum* Rchb. f., but accurate representation of the individual chromosomes was impossible owing to their small size. The final idiograms showed little more than a gradual transition from small to large chromosomes (fig. 3). The karyotypes of the whole group seemed to be very similar. In no case were any markedly singular chromosomes present. There was seemingly in every instance a diploid complement of forty chromosomes that look alike at metaphase.

Table 5b lists two species of Grammatophyllum which also have a somatic chromosome number of forty. Here, although the cells are approximately the same size as are found in Cymbidium, the chromosomes are considerably smaller as shown by the chromosomes of G. scriptum Blume (fig. 4) when compared to the chromosomes of C. devonianum Paxt. and C. iridifolium A. Cunn. (figs. 5 and 6); all of these plates are reproduced at the same magnification. Although the size of the chromosomes differed from cell to cell within a single root, apparently varying as the volume of the cell, the size difference from cell to cell within a root or from species to species, never was as great as found here in Grammatophyllum when comparing with Cymbidium.

An attempt was made to establish the chromosome number of a related form from Madagascar, Cymbidiella rhodochila (Rolfe) Rolfe. Cytologically it proved to be very difficult and no definite number could be determined. Yet its karyotype was distinctly different from anything so far seen in this group (figs. 7 and 8). There were without a doubt four pair of extremely large chromosomes and also a large number of much smaller pairs. No success was attained in resolving the number of small chromosomes. The quantity of these seemed to vary greatly from cell to cell. There appeared to be either a number of small chromosomes with extremely heavy primary constrictions at which point there was often a breakage while squashing, or there was a large quantity of very small chromosomes that were often associated during the somatic mitoses. No final conclusion was reached; unfortunately suitable root tips were rather uncommon on the material at hand.

TABLE 5a

DIPLOID CHROMOSOME NUMBERS OF CYMBIDIUM SPECIES DETERMINED
IN THIS STUDY

Species	2n
<u>C. cochleare</u> Lindl.*	40
<u>C. devonianum</u> Paxt.	40
<u>C. eburneum</u> Lindl.	40
<u>C. ensifolium</u> Sw.	40
<u>C. erythrostylum</u> Rolfe	40
<u>C. forrestii</u> Rolfe	40
<u>C. giganteum</u> Wallich	40
<u>C. grandiflorum</u> Griff.	40
<u>C. havsoni</u> Hort.	40
<u>C. insigne</u> Rolfe	40
<u>C. iridifolium</u> A. Cunn.	40
<u>C. kanran</u> Makino	40
<u>C. lancifolium</u> Hook.	40
<u>C. lowianum</u> Rehb. f.	40
<u>C. mastersii</u> Griff. ex Lindl.*	40
<u>C. pumilum</u> Rolfe	40
<u>C. schroderi</u> Rolfe	40
<u>C. simonsianum</u> King and Pantl.	40
<u>C. sinense</u> Willd.	40
<u>C. tracyanum</u> Hort.	40
<u>C. whiteae</u> King and Pantl.	40

*Sometimes placed in Cyperorchis.

TABLE 5b

DIPLOID CHROMOSOME NUMBERS OF GRAMMATOPHYLLUM SPECIES
DETERMINED IN THIS STUDY

Species	2n
<u>G. scriptum</u> Blume	40
<u>G. speciosum</u> Blume	40

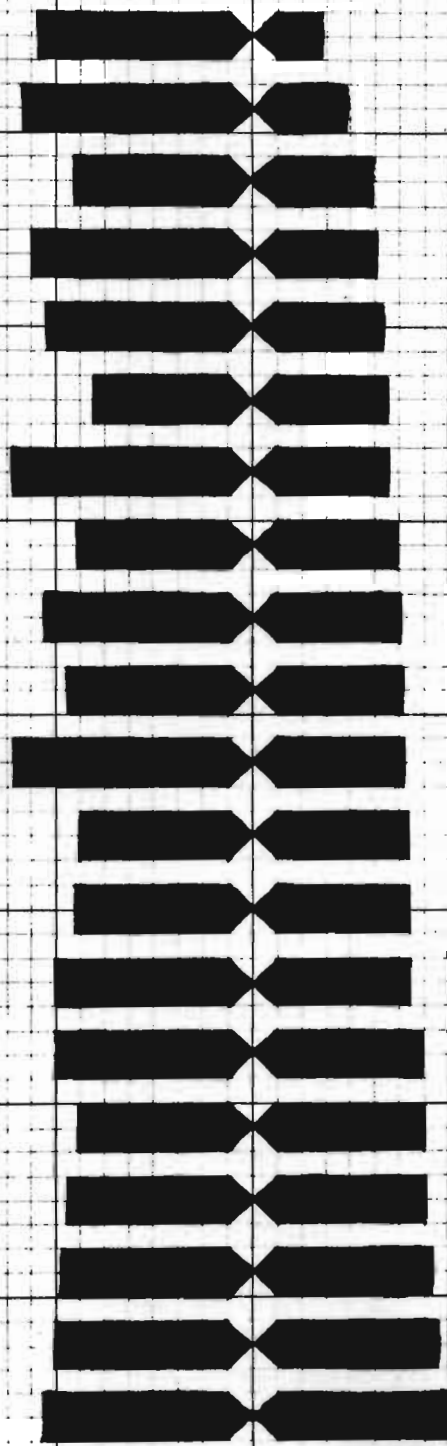


FIG. 1. CHROMOSOMES FROM ROOT
TIP OF CYMBIDIUM GRANDIFLORUM.
X 2000

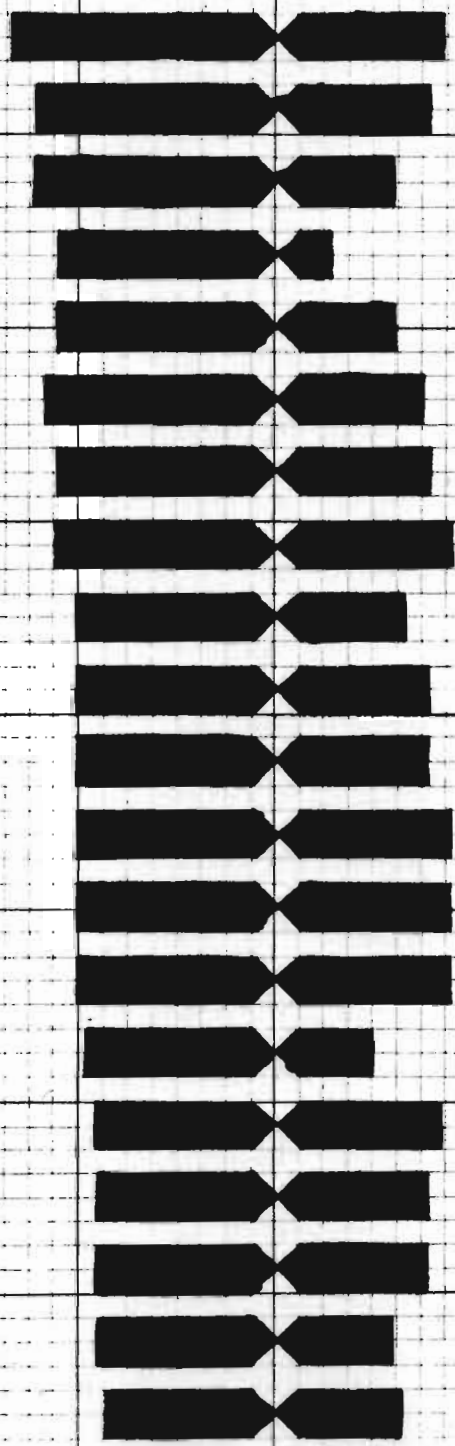


FIG. 2. CHROMOSOMES FROM ROOT
TIP OF CYMBIDIUM LOWIANUM.
X 2000 PHASE CONTRAST

FIG. 3. IDIOPHASES OF THE CHROMOSOMES OF
CYMBIDIUM GRANDIFLORUM AND C. LOWIANUM
TAKEN FROM FIGURES 1 AND 2.



CYMBIDIUM GRANDIFLORUM



CYMBIDIUM LOWIANUM

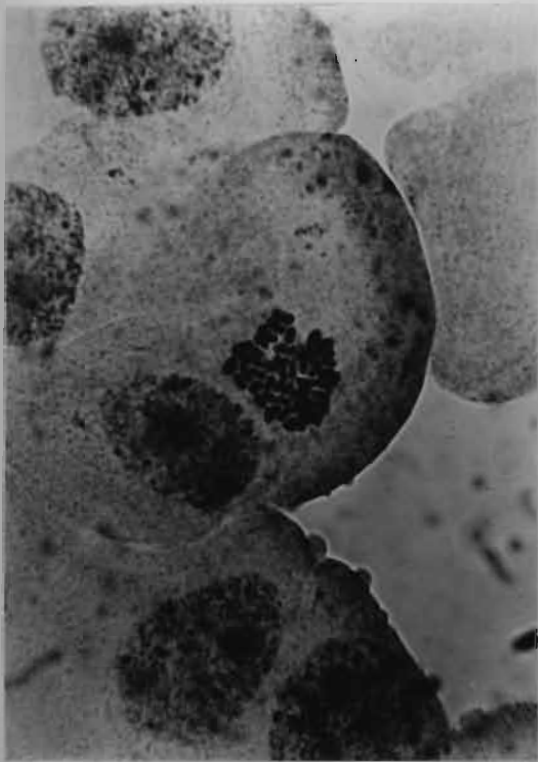


FIG. 4. CHROMOSOMES FROM ROOT
TIP OF GRAMMATOPHYLLUM SCRIPTUM.
x 1300

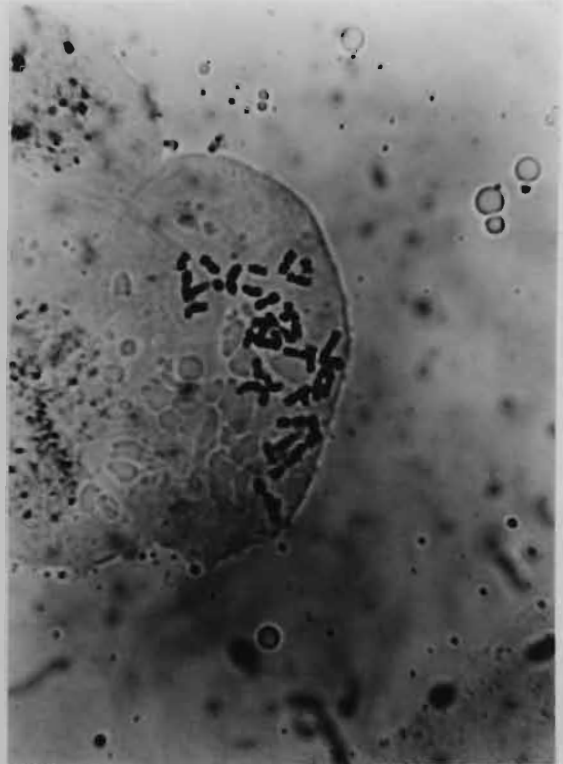


FIG. 5. CHROMOSOMES FROM ROOT
TIP OF CYMBIDIUM DEVONIANUM.
x 1300

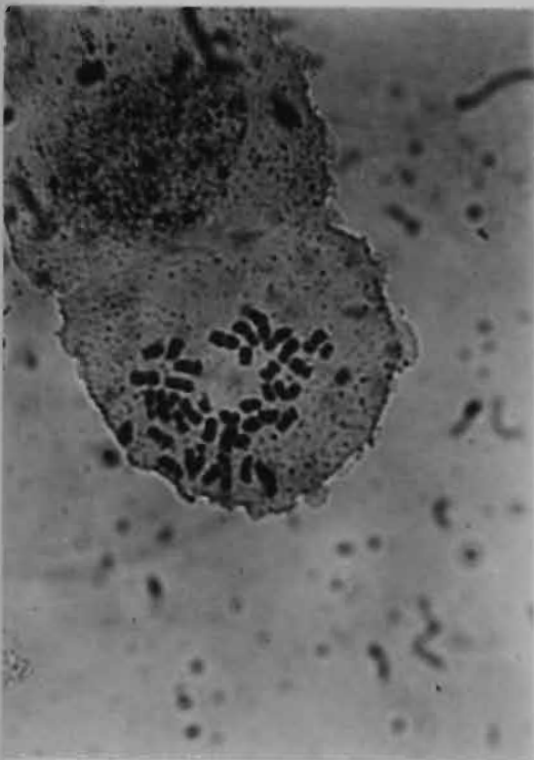


FIG. 6. CHROMOSOMES FROM ROOT
TIP OF CYMBIDIUM IRIDIFOLIUM.
x 1300

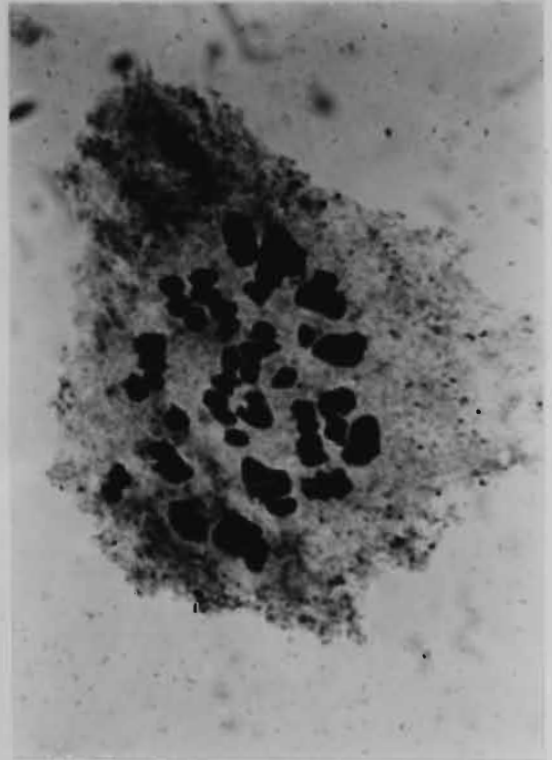


FIG. 7. CHROMOSOMES FROM ROOT
TIP OF CYMBIDIELLA RHODOCHILA.
x 1300

Meiosis in the Species

Unfortunately many species of Cymbidium that were at one time quite common in cultivation have retreated before the many hybrid forms and are now approaching rarity. The only two that remain in any numbers are C. lowianum and C. tracyanum Hort. These two species generally bloom freely under cultivation, whereas many of the other species are more exacting in their cultural requirements. Because of this rarity, some of the species are represented in this investigation only once and unfortunately some not at all.

In the species, meiosis occurred more or less simultaneously throughout the entire pollinia; however, there seemed to be a definite developmental gradient, with some portions of the pollinia somewhat advanced over others. For example, in some pollinia telophase I, metaphase II and telophase II could be found. A few sections through the pollinia of C. tracyanum and C. lowianum which were stained with safranin and fast green disclosed that divisions were usually initiated in the interior of the mass of pollen mother cells and proceeded toward the periphery. The pollinia is divided into four parts with two series of two parts each. The juncture of the two parts of each pollinium was generally the last area to begin division. This lag in the initiation of the divisions facilitated study, for two or three stages of meiosis could be examined in one pollinium.

The stages of prophase were generally without merit for the purposes of determining pairing abnormalities and chiasma frequency. Metaphase I considerations were limited to a tabulation of the number of univalents present that were not on the metaphase plate. At telophase I, univalents, bridges and fragments were recorded. At times the interphase between the

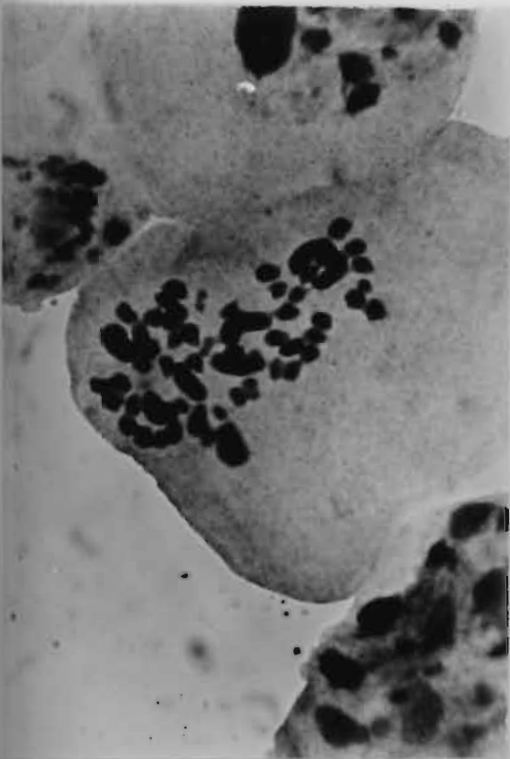


FIG. 8. CHROMOSOMES FROM ROOT TIP OF CYMBIDIELLA RHODOCHILA. x 1300

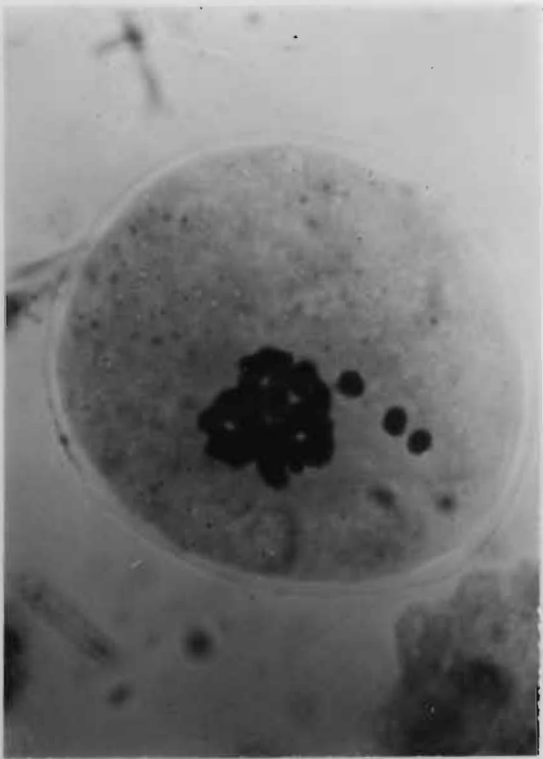


FIG. 9. MICROSPOROGENESIS, METAPHASE I SHOWING 3 UNIVALENTS. CYMBIDIUM LOWIANUM VAR. CONCOLOR. x 1300

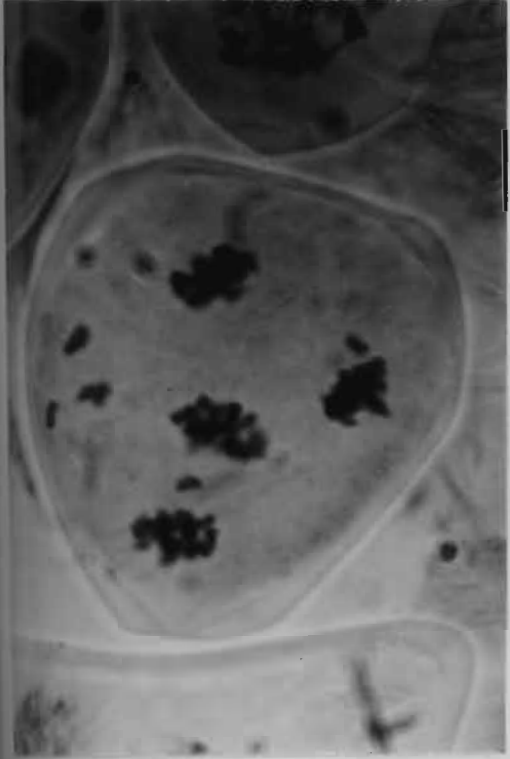


FIG. 10. MICROSPOROGENESIS, TELOPHASE II SHOWING UNIVALENTS OR FRAGMENTS. CYMBIDIUM LOWIANUM x 1300

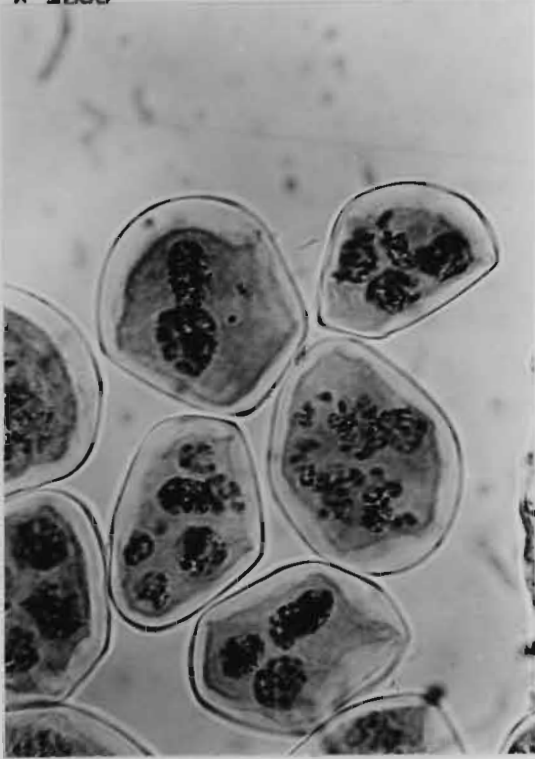


FIG. 11. AFTER MICROSPOROGENESIS, NUMEROUS MICRONUCLEI VISIBLE. CYMBIDIUM LOWIANUM VAR. CONCOLOR. x 560

heterotypic and the homotypic divisions was rather pronounced and the nuclei would lapse into a state with diffuse chromatin; at other times, telophase I was followed directly with metaphase II. When a distinct interphase was encountered, micronuclei and bridges that sometimes remained intact were noted. At metaphase II univalents not on the plate and fragments were counted. Anaphase-telophase II at times showed bridges, univalents and fragments, and these were tabulated. The distinction between fragments and univalents in some phases was not easily made, therefore they are not listed separately in the following analyses. The gones were also examined and any micronuclei or remaining bridges were listed. The study of the pollen tetrads included the recording of any micropollen grains. In the meiotic study of the species and the primary and more advanced hybrids, 200 pollen mother cells or their compound derivatives were examined in each phase that was located in sufficient quantity.

One clone each of C. schroderi Rolfe, C. erythrostylum Rolfe, C. grandiflorum and C. i'ansoni Hort. was examined in this investigation. Two clones of C. tracyanum, C. pumilum Rolfe and C. insigne Rolfe were studied and six different varieties of C. lowianum were explored. Of the C. lowianum clones there were four different plants of C. lowianum var. concolor Rolfe represented. It is thought that these were divisions of one original clone, for their characteristics were essentially similar and there are no definite records of this plant having been imported from the wild state more than once.

Tables 6 and 7 are compilations of the meiotic behaviors of these species except C. i'ansoni. Unfortunately only a mature pollinia was available for study in this species; out of 200 compound pollen grains

there was no observed micropollen. A perusal of these tables shows that some of the clones examined were quite regular in their reduction division cycles, whereas others sometimes displayed large numbers of anomalies. C. schroderi, C. erythrostylum, C. grandiflorum, C. tracyanum and most of the clones of C. lowianum were basically normal in microsporogenesis, whereas clones of the species C. insigne and C. lowianum var. concolor showed varying amounts of abnormal behavior during reduction division.

Tables 6 and 7 show that the reduction divisions in C. lowianum var. concolor, in contrast to the other clones of C. lowianum, were rather irregular. Univalents and/or fragments were fairly common at metaphase I and telophase II (figs. 9 and 10). The final results in tetrads and compound pollen grains showed micronuclei and micropollen far in excess of anything shown in the other clones of C. lowianum (fig. 11).

A clone of C. insigne showed the most irregularity of any species yet examined. A glance at the tables shows that univalents appeared in about 25% of the cells at metaphase I; at telophase I bridges were found in about 12% of the pollen mother cells and univalents and/or fragments in about 35%. At the sporad stage 35% of the groups of four nuclei had no micronuclei and were otherwise normal in appearance. Another clone of C. insigne from which pollen was obtained had a much higher level of regularity, for there were 85% of the pollen tetrads without micropollen grains.

Meiosis in the Primary Hybrids

Altogether a total of forty clones were examined from twelve different primary crosses. Unfortunately there was little hope of tracing the

TABLE 6

CHROMOSOME SEPARATION DURING MICROSPOROGENESIS

IN CYMBIDIUM SPECIES*

Species	Clone	No. PMC's with a given no. of:							% PMC's with:	
		Univalents and/or fragments			Bridges			Univalents and/ or Fragments	Bridges	
		Division I			Division II					
1	2	3	1	2	3	Bridges				
<u>C. schroderi</u>	#1	3	-	-	1	-	-	-	1.0	0.0
<u>C. erythrostylum</u>	#1	1	-	-	2	-	-	-	0.7	0.0
<u>C. grandiflorum</u>	#1	17	-	-	5	2	-	5	6.0	2.5
<u>C. tracyanum</u>	#1	3	-	-				-	0.7	0.0
<u>C. insigne</u>	#1	97	15	7				38	24.2	19.0
<u>C. pumilum</u>	#2	-	-	-	1	-	-	-	0.0	0.0
"	#3				9	-	-	-	2.2	0.0
<u>C. lowianum</u>	#1	5	-	-	2	-	-	-	1.7	0.0
"	#2				2	-	-	-	1.0	0.0
"	'Pitt's'	-	-	-	4	-	-	-	1.0	0.0
"	'Comte d'Hemptime'	-	-	-				-	0.0	
"	v. <u>concolor</u>	28	20	1	25	18	-	-	23.0	0.0

*Where possible 200 PMC's were examined at each phase of meiosis.

TABLE 7

NUMBER OF SPORADS WITH MICRONUCLEI
IN CYMBIDIUM SPECIES

Species	Clone	Sporads with micronuclei					Total number sporads examined
		Number				%	
		1	2	3	4		
<u>C. schroderi</u>	#1	3	-	-	-	0.7	400
<u>C. erythrostylum</u>	#1	1	-	-	-	0.0	400
<u>C. grandiflorum</u>	#1	11	3	-	-	1.7	800
<u>C. tracyanum</u>	#1	-	-	-	-	0.0	200
"	#2	-	-	-	-	0.0	200
<u>C. insigne</u>	#1	157	58	9	6	57.5	400
"	#2	27	-	-	-	13.5	200
<u>C. pumilum</u>	#1	-	-	-	-	0.0	200
"	#2	1	1	-	-	0.5	400
"	#3	-	-	-	-	0.0	400
<u>C. lowianum</u>	#1	2	-	-	-	1.0	200
"	#2	-	-	-	-	0.0	200
"	'Pitt's'	1	1	-	-	1.0	200
"	'Moore'	-	-	-	-	0.0	200
"	'Comte d'Hemptime'	-	-	-	-	0.0	200
"	v. <u>concolor</u> (#1)	41	9	-	4	27.0	200
"	" (#2)	41	9	-	6	14.0	400
"	" (#3)	44	24	6	-	37.0	200

exact ancestry of many of these plants; therefore, it is not known whether some of the plants of the same cross name were siblings or not. Without a doubt a number of these crosses have been remade several times using different clones of the same species.

Figure 12 shows diagrammatically the species and the primary hybrids of those species that have been examined. It would seem from studying this figure that some species have been more popular with hybridists than others. In actuality, if C. i'ansoni, C. devonianum and C. pumilum were removed from the chart, and the hybridizing history of each one of the remaining species was examined, it would be found that all possible primary hybrids between these species have been recorded save the one between C. grandiflorum and C. erythrostylum.

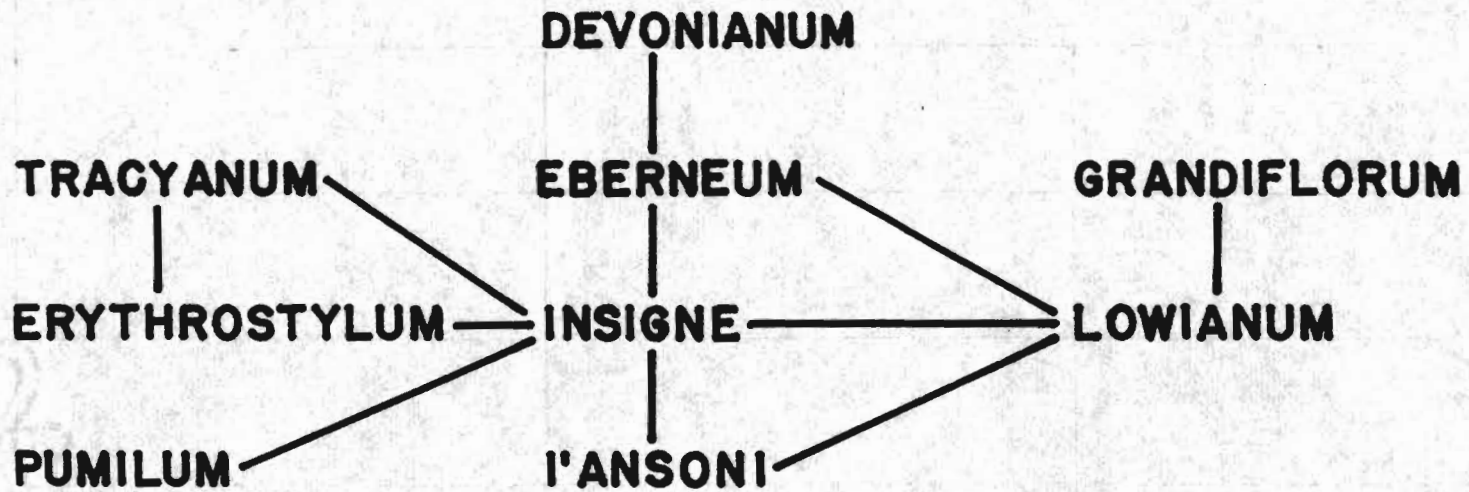


Fig. 12. A diagrammatic representation of the crosses examined in this study.

Cymbidium Lotta G.
(C. i'ansoni x C. lowianum)

Tables 8 and 9 are compilations of the data taken from the meiotic stages that were found in the primary hybrids. The tables show that between 24% and 28% of the sporads of this cross contained micronuclei. Occasional bridges were found at telophase I and interphase implying the probability of the presence of at least one inversion.

Cymbidium Gottianum G.
(C. eburneum x C. insigne)

In all, three clones of C. Gottianum G. were examined cytologically. They were extraordinarily regular in their reduction division, even more so than a number of the species. In almost every phase examined, a total of 199 to 200 cells out of 200 exhibited no anomalies.

Cymbidium Pauwelsii G.
(C. insigne x C. lowianum)

A total of eight clones of C. Pauwelsii G. were considered as representatives of this hybridization. Some of the final figures in tables 8 and 9 are somewhat incongruous. For example, in clone #3 in interphase and telophase, out of 200 cells, 107 and 98 respectively were without univalents or fragments (figs. 13 and 14), whereas the gones showed no micronuclei. There was a considerable amount of variation from clone to clone in respect to the anomalies present, with some seemingly very regular, and others exhibiting large numbers of univalents and bridges.

Buds were taken serially from one raceme of C. Pauwelsii G. (#3) and the tetrad stages were examined. Table 10 lists the results. It will be noted that different buds showed a great variation in the number of micronuclei that were present. Of the five buds studied the sporads that contained

micronuclei ranged from about 14% to 40%.

C. (Pauwelsii G.) 'Comte d'Hemptinne' is a singular case here, for it is the only tetraploid primary hybrid known in this group. It was not exceedingly normal at microsporogenesis. Bridges were detected at both telophase I and telophase II (figs. 16 and 17), and univalents were not uncommon (fig. 18).

Cymbidium Doris G.
(*C. insigne* x *C. tracyanum*)

In tables 8 and 9 are shown the results of the investigation of five clones of *C. Doris G.* Clones #1 through #4 were relatively consistent at meiosis and showed few micronuclei at the sporad stage. Clone #5, however, displayed a large number of univalents and/or fragments at metaphase II and telophase II. The tetrads and pollen showed a comparatively lower number of micronuclei and micropollen grains respectively.

Cymbidium Ceres G.
(*C. i'ansoni* x *C. insigne*)

Here again there is a rather extensive range of irregularity. In clone #1 at telophase I and interphase, univalents and/or fragments were quite common (figs. 19 and 20). Clone #2 displayed a higher degree of normality and *C. (Ceres G.) 'F. J. Hanbury'* was even higher. During some phases bridges were observed (figs. 21 and 22).

Cymbidium Eburneo-lowianum G.
(*C. eburneum* x *C. lowianum*)

Two clones only of *C. Eburneo-lowianum G.* were examined. Here, as in *C. Gottianum G.*, the number of abnormalities was very low. Only occasional univalents were observed (fig. 23), with most of the division cycles very regular (figs. 24 and 25). It is of interest to note that *C. (Eburneo-lowianum G.) 'Concolor'* has as one parent *C. lowianum v.*

concolor, one of the varieties of this species that was unusually irregular in its reduction division.

Cymbidium Lowio-grandiflorum G.
(C. lowianum x C. grandiflorum)

Meiotic divisions were only discovered in one clone, C. (Lowio-grandiflorum G.) 'Westonbirt'. Pollen grains were observed in both this clone and C. (Lowio-grandiflorum G.) 'Fred Jackson'. Meiosis was extremely regular and few micropollen grains were discovered.

Cymbidium Coningsbyanum G.
(C. grandiflorum x C. insigne)

In the two clones examined, as shown in tables 8 and 9, the reduction cycles were relatively regular. Only an occasional bridge was discovered in C. (Coningsbyanum G.) 'Brockhurst'.

Cymbidium Hanburyanum G.
(C. erythrostylum x C. tracyanum)

Here, three plants of this cross were investigated. Clone #1 appeared to be quite regular during microsporogenesis, whereas clone #2 and C. (Hanburyanum G.) 'Magnificum' were less exact during the divisions. Bridges were sometimes present at telophase II and univalents appeared occasionally at metaphase I.

Throughout this investigation unreduced cells were encountered only rarely, and their frequency was generally too low to appear in the cell counts. Figure 26 shows a dyad. Cell wall formation usually does not occur until after meiosis is over, yet here wall formation is beginning. Whether this is a cell that has yet to undergo the homotypic division or whether the chromosomes have divided and restitution nuclei have been formed is unknown. The probable assumption here, is that each nucleus contains the diploid chromosome number of forty.

Cymbidium Albanense G.
(C. erythrostylum x C. insigne)

Only one clone of this primary hybrid was studied, and from this plant only the tetrad and pollen stages were obtained in sufficient numbers to include as statistical evidence. About 12% of the gones contained micronuclei and 9% of the compound pollen grains contained micro-pollen.

Cymbidium Jean Brummitt G.
(C. devonianum x C. eburneum)

Only one plant was considered and unfortunately none of the meiotic stages were found. Thus, only the pollen was observed. Here out of 200 sporads, 26% had micronuclei.

Cymbidium Minuet G.
(C. insigne x C. pumilum)

This group of primary hybrids proved to be the most interesting of all. Six different plants were studied. When meiotic divisions were encountered, they were so irregular that it was often difficult to tell the stage. They were, in fact, found to behave so abnormally that no data could be taken to show the numbers of univalents, micronuclei or bridges. Figures 27 through 32 illustrate these phenomena. Figure 27 shows a cell at metaphase I, whereas figures 28 and 29 show pollen mother cells at undeterminable stages. Figures 30 and 31 are photographs of cells that should be at the tetrad stage, yet only show innumerable pieces of chromatin material. Finally, figure 32 is an illustration of pollen division, but instead of the normal quartet of cells of equal size, there are cell walls surrounding eight nuclei, each with different numbers of chromosomes.

TABLE 8

CHROMOSOME SEPARATION DURING MICROSPOROGENESIS
IN CYMBIDIUM PRIMARY HYBRIDS

Hybrid	Clone	No. PMC's with a given no. of:						% PMC's with:		
		Univalents and/or fragments Division I			Univalents and/or fragments Division II			Bridges	Univalents and/or Fragments	Bridges
		1	2	3	1	2	3			
C. Lotta G.	#1	23	6	2	31	5	-	10	19.2	2.5
C. Gottianum G.	#1	-	-	-	-	-	-	-	0.0	0.0
"	#2	-	-	-	-	-	-	-	0.0	0.0
C. Pauwelsii G.	#1	27	5	-	38	31	2	-	25.7	0.0
"	#2	-	-	-	16	4	-	-	10.0	0.0
"	#3	128	26	-	-	-	-	41	38.5	10.2
"	#4	13	-	-	27	1	1	-	10.5	0.0
"	#5	9	-	-	-	-	-	-	4.5	0.0
"	'Comte d' Hemptinne'	-	-	-	41	25	5	10	17.7	2.5
C. Doris G.	#1	-	-	-	7	2	-	-	4.5	0.0
"	#2	2	-	-	-	-	-	-	1.0	-
"	#3	6	3	-	2	2	-	1	3.2	0.0
"	#4	2	-	-	-	-	-	-	1.0	-
"	#5	-	-	-	89	37	4	1	32.5	0.0
C. Ceres G.	#1	183	51	33	-	-	-	-	44.5	0.0
"	#2	-	-	-	69	43	16	12	32.0	3.0
"	#3	41	2	-	-	-	-	-	21.5	-
"	'F.J.Hanbury'	-	-	-	21	4	2	-	14.0	0.0
C. Eburneo-lowianum G.	#1	6	1	-	13	2	-	3	5.5	1.5
"	'Concolor'	15	6	2	-	-	-	1	5.7	0.5
C. Lowio-grandiflorum G.	'Westonbirt'	3	-	-	2	1	-	-	1.0	0.0
C. Coningsbyanum G.	#1	15	5	-	-	-	-	1	10.0	0.5
"	'Brockhurst'	2	-	-	7	5	-	5	3.5	2.5

TABLE 8 Cont.

Hybrid	Clone	No. PMC's with a given no. of:						% PMC's with:		
		Univalents and/or fragments			Univalents and/or			Bridges	Bridges	
		Division I			Division II					
1	2	3	1	2	3	Fragments				
C. Hanburyanum G.	#1	1	-	-	6	-	-	-	1.7	0.0
"	#2				29	3	-	2	15.5	1.0
"	'Magnificum'	15	6	1	35	5	-	6	15.5	3.0

TABLE 9

NUMBER OF SPORADS WITH MICRONUCLEI IN
CYMBIDIUM PRIMARY HYBRIDS

Hybrid	Clone	Sporads with micronuclei				%	Total number sporads examined
		Number					
		1	2	3	4		
C. Lotta G.	#1	37	10	2	-	24.5	200
"	#2	29	8	-	-	28.5	200
C. Gottianum G.	#1	1	-	-	-	0.5	200
"	#2	1	-	-	-	0.5	200
"	#3	-	-	-	-	0.0	200
C. Pauwelsii G.	#2	13	2	-	-	7.0	200
"	#3	89	19	-	-	27.0	400
"	#4	-	-	-	-	0.0	200
"	#5	16	-	-	-	8.0	200
"	#6	123	61	8	5	51.7	400
"	'Magnificum'	10	6	-	-	8.0	200
"	'Comte d'Hemptinne'	13	-	-	-	6.5	200
Doris G.	#1	4	-	-	-	2.0	200
"	#2	2	-	-	-	1.0	200
"	#3	1	-	-	-	0.5	200
"	#4	8	-	-	-	4.0	200
"	#5	21	7	-	-	14.0	200
"	#6	30	-	-	-	15.0	200
C. Ceres G.	#1	71	15	8	4	49.0	200
"	#2	29	4	1	-	17.0	200
"	#3	44	6	1	1	26.0	200
"	#4	16	1	-	-	8.5	200
"	'F. J. Hanbury'	18	-	1	-	9.0	200
C. Eburneo-lowianum G.	#1	6	-	-	-	1.5	400
"	'Concolor'	7	2	-	-	4.5	200

TABLE 9 Cont.

Hybrid	Clone	Sporads with micronuclei				%	Total number sporads examined
		Number					
		1	2	3	4		
C. Lowio-grandiflorum G.	'Fred Jackson'	6	-	-	-	3.0	200
"	'Westonbirt'	7	-	-	-	1.7	400
C. Albanense G.	#1	38	3	-	-	10.2	200
C. Coningsbyanum G.	#1	13	-	-	-	6.5	200
"	'Brockhurst'	28	3	-	-	7.7	400
C. Hanburyanum G.	#1	-	-	-	-	0.0	200
"	#2	35	7	1	-	26.5	200
"	'Magnificum'	28	2	-	-	15.0	200

TABLE 10

NUMBER OF SPORADS WITH MICRONUCLEI IN *C. PAUWELSII* G. [#3]
 FROM DIFFERENT FLOWERS OF THE SAME RACEME*

1	Number micronuclei			%
	2	3	4	
45	23	3	4	37.5
60	21	-	-	40.5
18	9	-	-	13.5
28	8	1	-	18.5
45	12	1	-	29.0

*200 sporads were examined from each flower

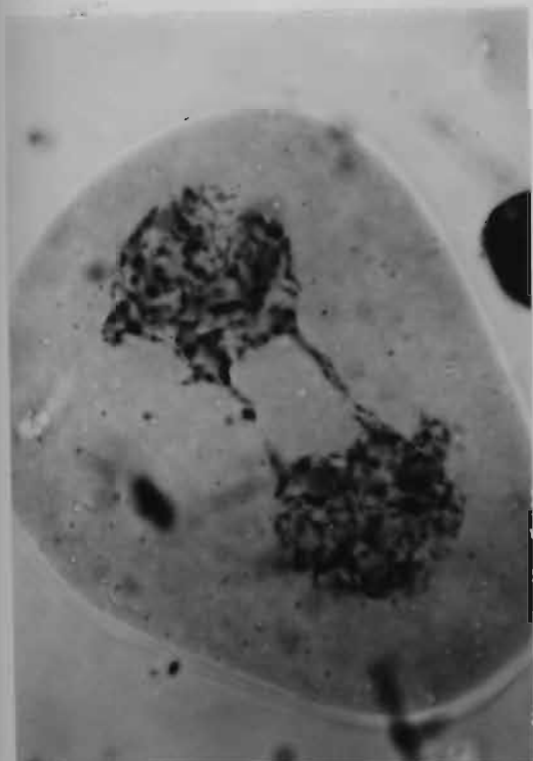


FIG. 13. MICROSPOROGENESIS, INTER-PHASE SHOWING TWO BRIDGES. $\times 1300$ CYMBIDIUM PAUWELSII G. (#3).

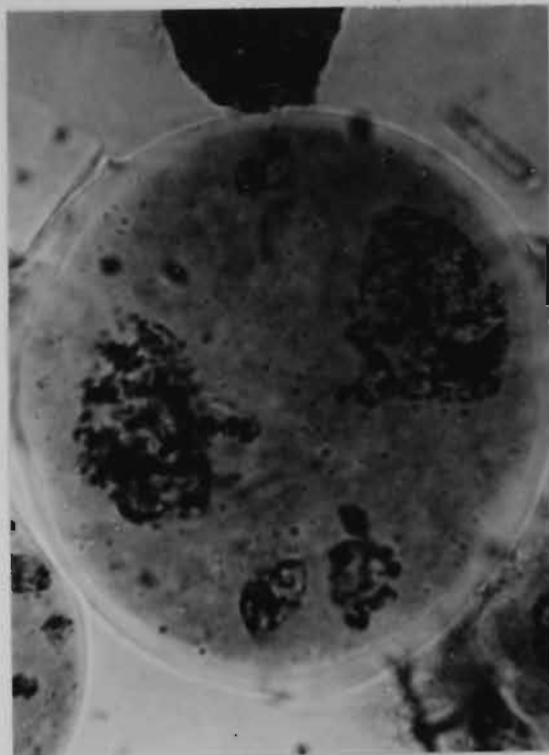


FIG. 14. MICROSPOROGENESIS, INTER-PHASE SHOWING MICRONUCLEI. $\times 1300$ CYMBIDIUM PAUWELSII G. (#3).

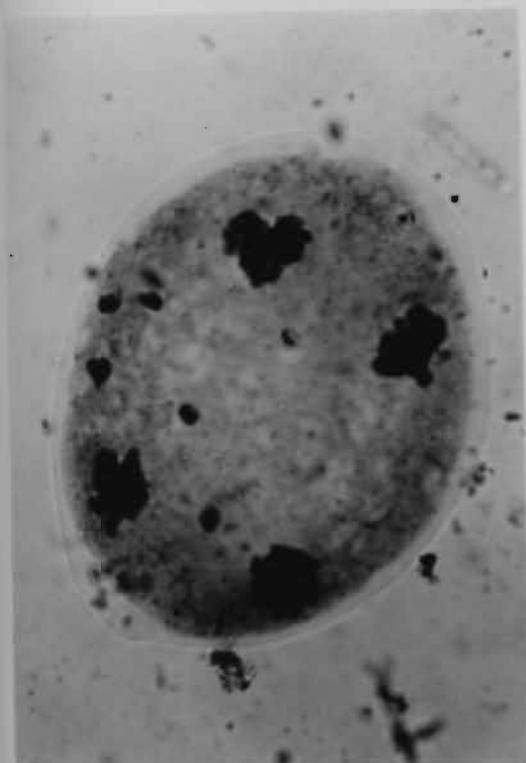


FIG. 15. MICROSPOROGENESIS, TELO-PHASE II SHOWING UNIVALENTS. $\times 1300$ CYMBIDIUM PAUWELSII G. (#3).

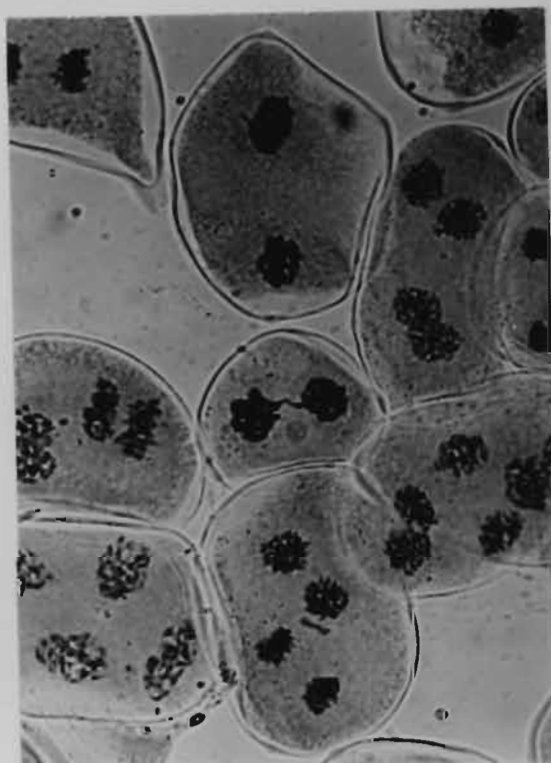


FIG. 16. MICROSPOROGENESIS, TELO-PHASE I SHOWING BRIDGE. CYMBIDIUM (PAUWELSII G.) 'COMTE D'EMPTINNE' $\times 560$

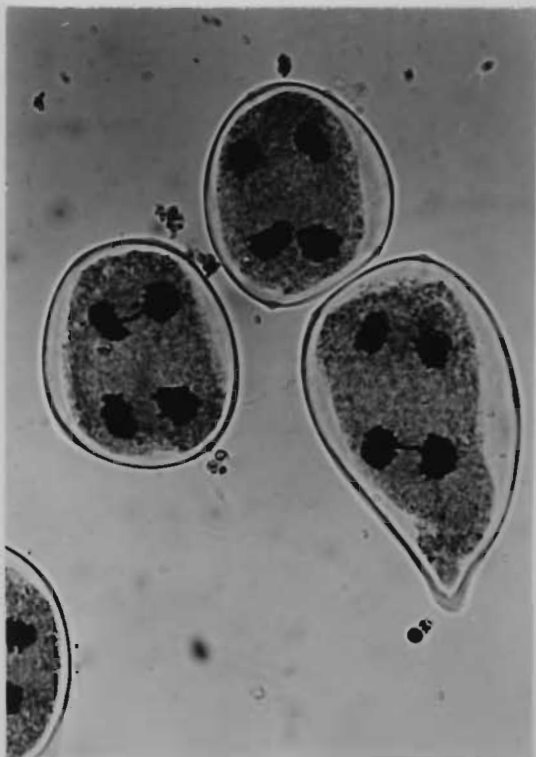


FIG. 17. MICROSPOROGENESIS, TELOPHASE II SHOWING BRIDGES. x 560
CYMBIDIUM (PAUWELSII G.) 'COMTE D'
HEMPTINNE'.

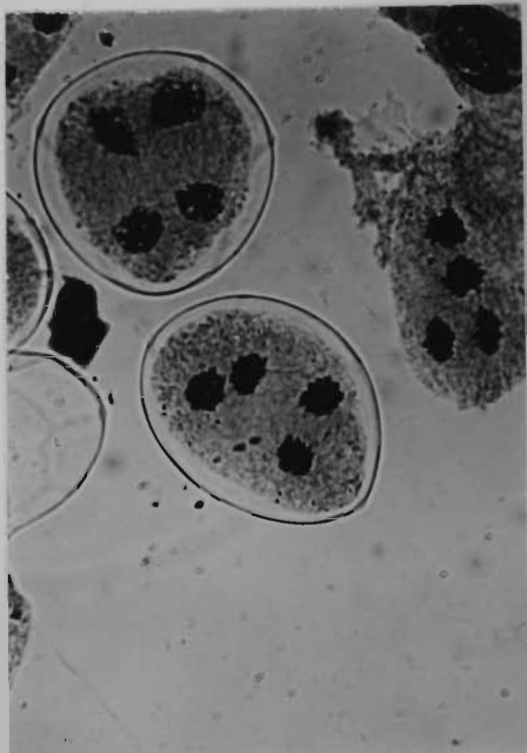


FIG. 18. MICROSPOROGENESIS, TELOPHASE II SHOWING UNIVALENTS. x 560
CYMBIDIUM (PAUWELSII G.) 'COMTE D'
HEMPTINNE'.



FIG. 19. MICROSPOROGENESIS, TELOPHASE I AND INTERPHASE SHOWING UNIVALENTS AND MICRONUCLEI. CYMBIDIUM CERES G. (#1). x 350

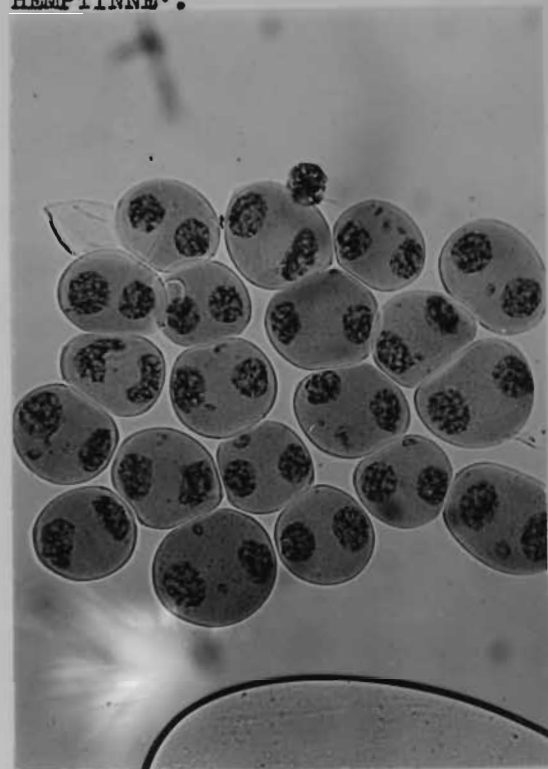


FIG. 20. MICROSPOROGENESIS, INTERPHASE SHOWING MICRONUCLEI. x 350
CYMBIDIUM CERES G. (#1).

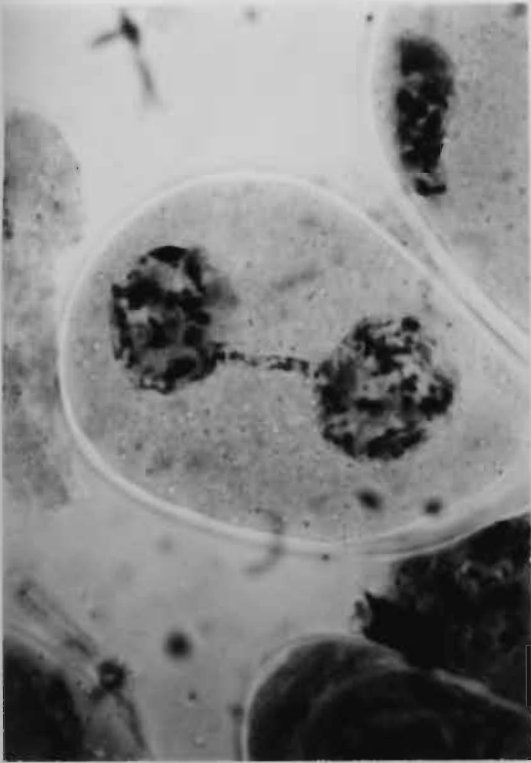


FIG. 21. MICROSPOROGENESIS, INTER-PHASE SHOWING BRIDGE. CYMBIDIUM CERES G. (#1). x 1300

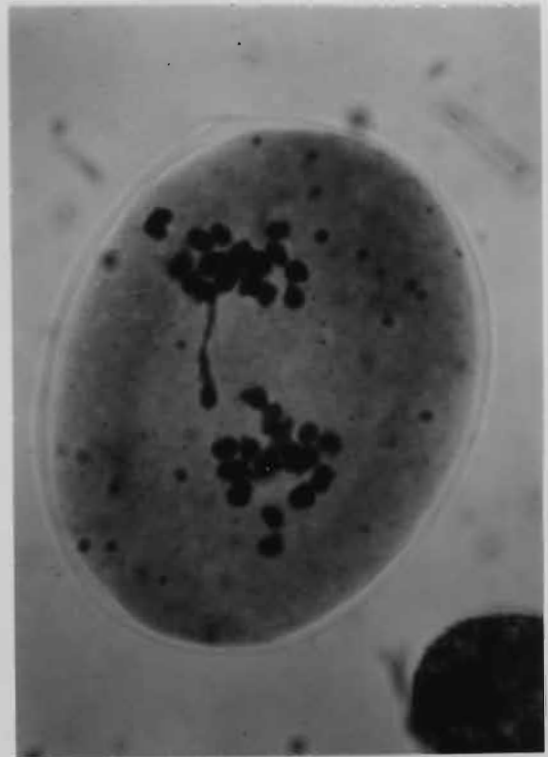


FIG. 22. MICROSPOROGENESIS, TELO-PHASE I SHOWING BRIDGE. CYMBIDIUM CERES G. (#1). x 1300

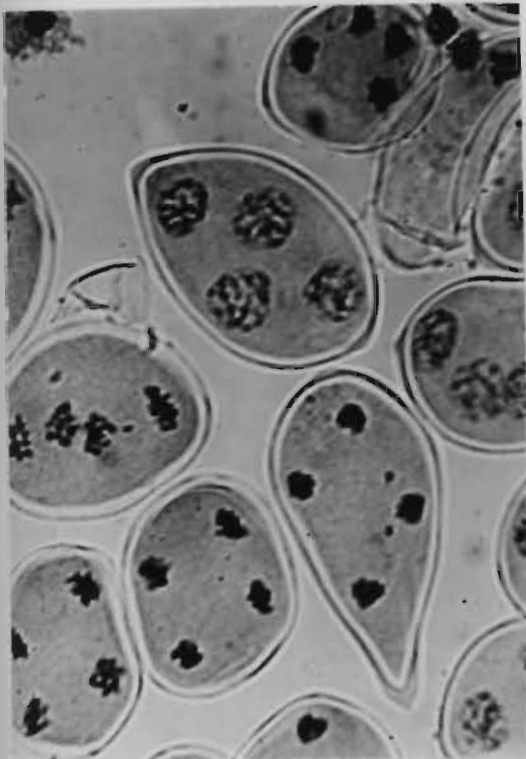


FIG. 23. MICROSPOROGENESIS, TELO-PHASE II SHOWING UNIVALENTS. x 560 CYMBIDIUM EBERNEO-LOWIANUM G. (#1).

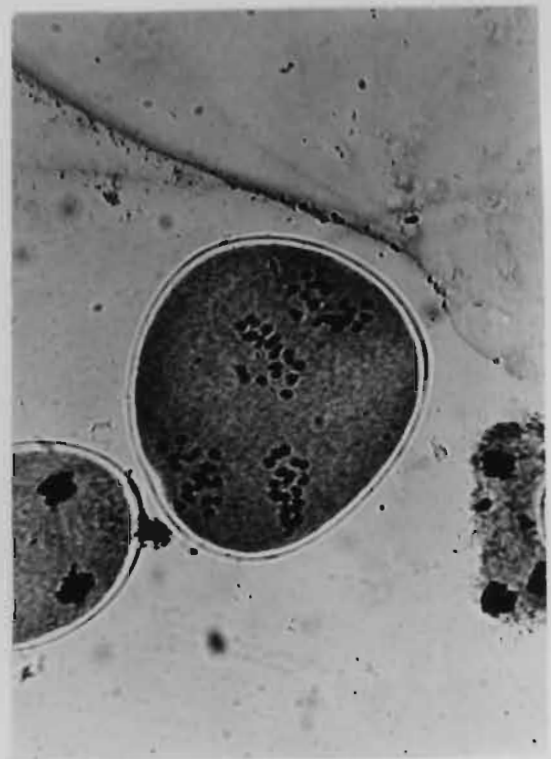


FIG. 24. MICROSPOROGENESIS, TELO-PHASE II, NORMAL DIVISION. x 560 CYMBIDIUM EBERNEO-LOWIANUM G. (#1).

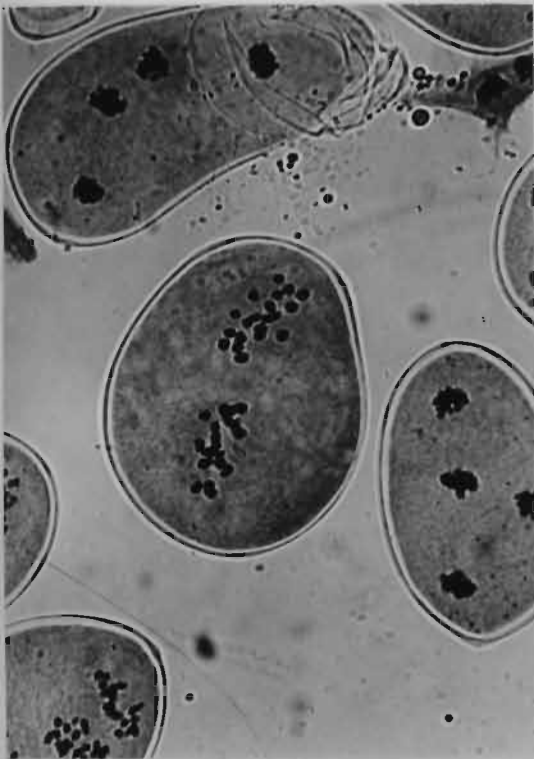


FIG. 25. MICROSPOROGENESIS, META-PHASE II AND TELOPHASE II. $\times 560$ CYMBIDIUM EBERNEO-LOWIANUM G. (#1).



FIG. 26. AFTER MICROSPOROGENESIS, 2 TETRADS AND 1 DYAD VISIBLE. $\times 560$ CYMBIDIUM HANBURYANUM G. (#1).



FIG. 27. MICROSPOROGENESIS, META-PHASE I SHOWING UNIVALENTS. $\times 560$ CYMBIDIUM MINUET G. (#1).



FIG. 28. MICROSPOROGENESIS, UNDETERMINABLE STAGE. CYMBIDIUM MINUET G. (#1). $\times 560$

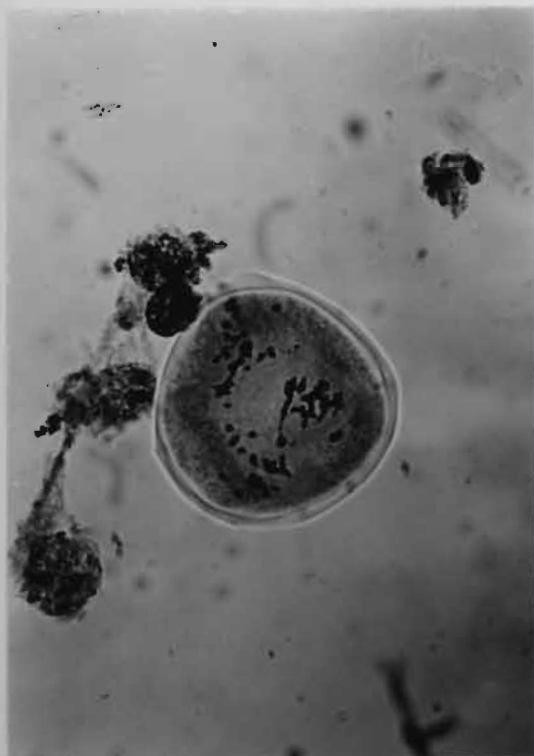


FIG. 29. MICROSPOROGENESIS, UNDETERMINABLE STAGE. CYMBIDIUM MINUET G. (#2). x 560



FIG. 30. AFTER MICROSPOROGENESIS, "TETRAD" STAGE SHOWING NUMEROUS NUCLEI. CYMBIDIUM MINUET G. (#1). x 560

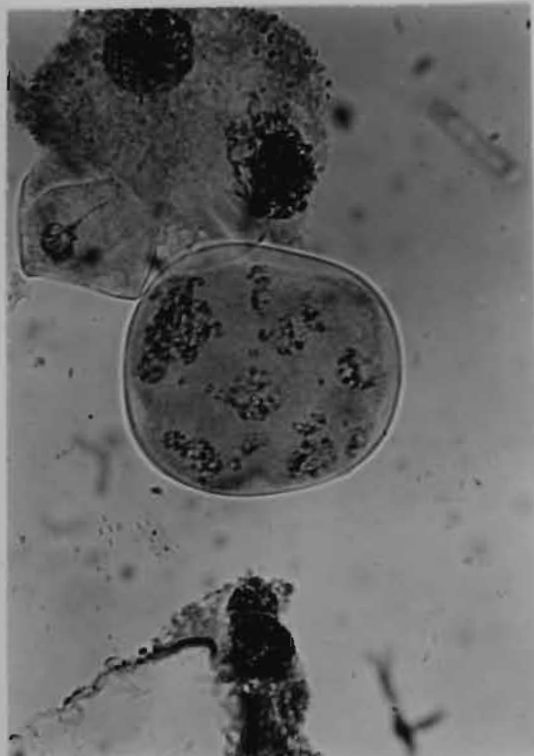


FIG. 31. AFTER MICROSPOROGENESIS, "TETRAD" STAGE SHOWING NUMEROUS NUCLEI. CYMBIDIUM MINUET G. (#2). x 560



FIG. 32. COMPOUND POLLEN GRAIN AT POLLEN DIVISION. CYMBIDIUM MINUET G. (#2). x 560

Meiosis in the Complex Hybrids

Only a very superficial examination of some of the more complex hybrids was attempted. Representative plants were taken from the four known ploidy levels, diploid, triploid, tetraploid and pentaploid. Meiotic stages were examined only in the triploids, whereas sporads were observed in all four ploidy groups. Tables 11 and 12 show these results.

It is of interest to note that all the diploids save one, had few micronuclei at the sporad stage. The high number of micronuclei possessed by the diploid, *C.* (Adelma G.) 'Shangri-la' seems rather incongruous in this system.

The triploids examined showed 38% to 57% of the sporads without micronuclei, and a correspondingly high number of univalents and/or fragments (figs. 33, 34, 35 and 36). Generally speaking the number of irregularities at meiosis was greater here than in any of the primary hybrids except *C.* Minuet G. These triploids are representatives of crosses of tetraploids with diploids. In producing these hybrids, three different tetraploid plants have been employed.

In contrast to the large numbers of micronuclei in the triploids, the tetraploids possessed fewer signs of abnormalities, with *C.* (Alexanderi G.) 'Westonbirt' showing far more micronuclei at the sporad stage than *C.* (Babylon G.) 'Carpentier'.

In addition to the usual diploid hybrids, two hybrids of *C.* pumilum were examined also. One clone of *C.* Pumander G. (*C.* Louis Sander G. x *C.* pumilum) and two of *C.* Sweetheart G. (*C.* Alexanderi G. x *C.* pumilum) showed pollen sporads that appeared to be as abnormal as *C.* Minuet G.

TABLE 11

CHROMOSOME SEPARATION DURING MICROSPOROGENESIS IN
TRIPLOID CYMBIDIUM HYBRIDS

Hybrid	Clone	No. PMC's with a given no. of:										% PMC's with:			
		Univalents and/or fragments Division I					Univalents and/or fragments Division II					Bridges	Univalents and/or Fragments	Bridges	
		1	2	3	4	5	1	2	3	4	5				
C. Bodmin Moor G.	#1	54	24	20	9	5								56.0	
C. Fantasia G.	#1	75	36	20	19	5						25		38.7	12.5
C. Olympus G.	'Rex'	51	43	27	30	18								84.5	
C. Peri G.	#10	54	41	30	4	2								65.5	
C. York G.	#1						65	30	18	3	3	-		59.5	

TABLE 12

NUMBER OF SPORADS WITH MICRONUCLEI IN
CYMBIDIUM HYBRIDS

Hybrid	Clone	Sporads with micronuclei				%	Total number sporads examined
		Number					
		1	2	3	4		
(2n)							
C. Adelma G.	'Shangri-la'	59	32	9	3	51.5	200
C. Cassandra G.	#1	8	-	-	-	4.0	200
C. Claudona G.	#1	9	3	-	-	6.0	200
C. Plover G.	'Fuchsia'	9	-	-	-	4.5	200
(3n)							
C. Crescendo G.	#1	52	38	3	2	47.5	200
C. Dorchester G.	'Alpha'	66	48	1	1	58.0	200
C. Olympus G.	'Rex'	72	11	2	-	42.5	200
C. York G.	#1	85	13	5	1	61.0	200
(4n)							
C. Alexanderi G.	'Westonbirt'	59	1	1	-	15.2	400
C. Babylon G.	'Carpentier'	2	-	-	-	1.0	200
(5n)							
C. Flamingo G.	'Nobilior'	73	60	21	9	81.5	200

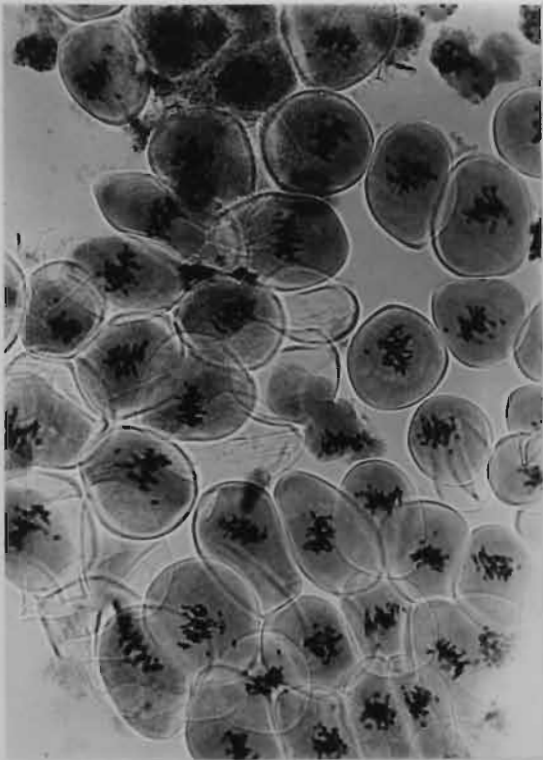


FIG. 33. MICROSPOROGENESIS, META-
PHASE I SHOWING UNIVALENTS. CYMBIDIUM
(OLYMPUS G.) 'REX' (3n). x 350

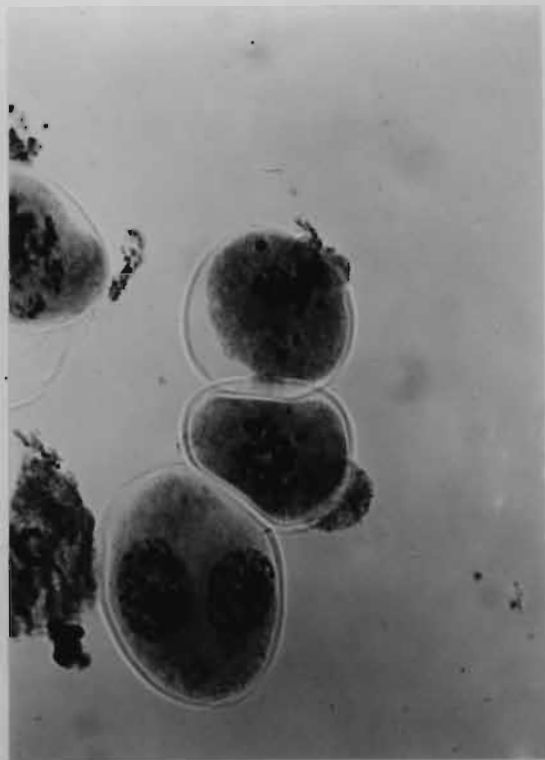


FIG. 34. MICROSPOROGENESIS, META-
PHASE I AND INTERPHASE SHOWING UNI-
VALENTS. CYMBIDIUM FANTASIA G. (#1)
(3n). x 560

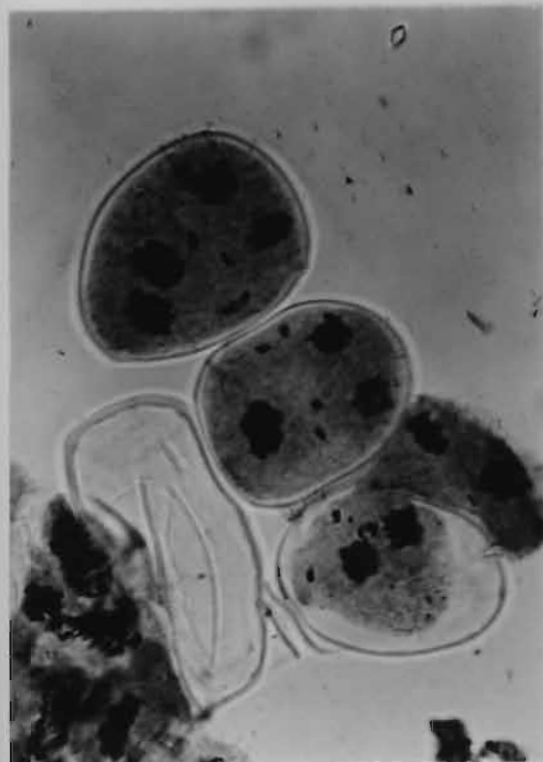


FIG. 35. MICROSPOROGENESIS, TELO-
PHASE II SHOWING UNIVALENTS.
CYMBIDIUM YORK G. (#1). x 560

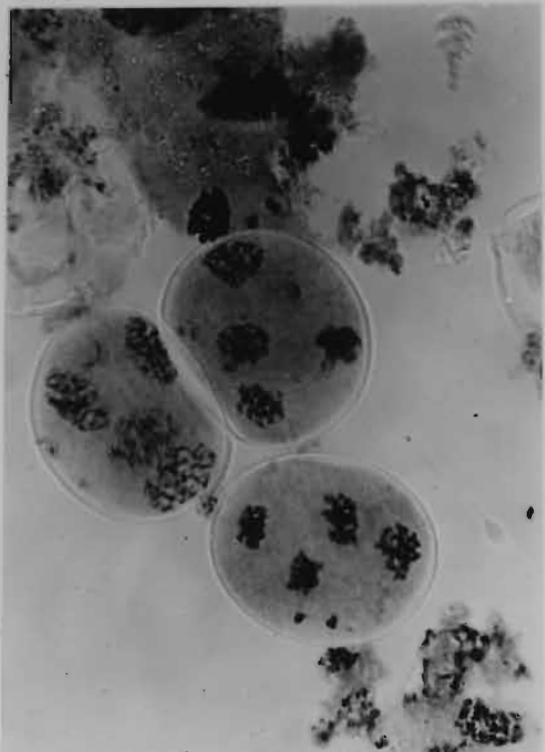


FIG. 36. MICROSPOROGENESIS, TELO-
PHASE II SHOWING UNIVALENTS.
CYMBIDIUM YORK G. (#1). x 560

DISCUSSION

Resemblances of the Karyotypes

Since all of the species of Cymbidium that have been examined in this study and by other authors have had a complement of forty small chromosomes which look very much alike at metaphase, it was impossible to diagnose morphologically the similarities and differences of each karyotype. The cytological characters of the genus appear to be fairly uniform. Even in the closely related genus, Cyperorchis, the cellular characteristics are such that it is indistinguishable from Cymbidium. However, the more distantly related genus Grammatophyllum varies in some degrees from Cymbidium. The staining qualities of the cells are different and the chromosome size is smaller, even though the same number is present. It is not known whether this genus will hybridize with Cymbidium. On the basis of chromosome size and cytological properties, it might be theorized that such a cross is not possible. Chromosome size differences, however, have not always been an insurmountable barrier in other plant groups. For example, in Crepis, Togby (1943) found that two species, C. neglecta L. and C. fuliginosa Sibth. et Sm., with size differences in their chromosomes, were able to cross and that the size differences were maintained in the hybrid. If there are enough homologies between the genomes of representatives of the two genera, Cymbidium and Grammatophyllum, and there are no internal physiological isolating barriers, the two should cross. If such a cross is possible the introduction of new genes into the hybrid Cymbidium group would

lead to a vast number of possibilities not seen before.

The cytological observations of Cymbidiella rhodochila seem to uphold Rolfe's separation of this genus from Cymbidium. The karyotype is extremely different from anything seen in Cymbidium. It is highly doubtful whether this plant would cross with any member of the Cymbidium alliance. Several commercial firms have tried to cross this species and another of the same group, Cymbidiella humblotii (Rolfe) Rolfe, with Cymbidiums and have been unsuccessful (written and oral communication).

Fertility in the Species

As a general rule, the species studied in this investigation have had a normal meiotic cycle. There were two exceptions.

The great amount of irregularity that was found in one clone of C. insigne is unexplained. Several theories might be advanced in order to interpret these results. It is significant that at the pollen grain stage, one of the clones of C. insigne was without a large number of irregularities whereas the other had numerous micropollen grains. These two clones were grown under somewhat different environmental conditions. If anything, the plant showing a high degree of abnormalities was grown under the more optimum nutritional level, for fewer deficiency symptoms were observed. The other clone, grown in Australia, displayed much leaf die-back, a symptom generally attributed to some nutrient deficiency or toxic condition of the compost, yet this clone showed few micropollen grains. It is possible that temperature affected the abnormal plant adversely, for during the period that the raceme was undergoing meiosis, several cold nights were experienced with the temperature almost reaching the freezing point. The Australian-grown plant was not subjected to such rigorous temperature conditions. Of course, it is possible that the

plant showing the abnormalities at meiosis was not a true species, but a naturally occurring hybrid form found in the wild or an artificially produced hybrid, yet it closely resembled the published description of this species.

C. lowianum var. concolor was the other plant that exhibited a disturbed microsporogenesis. It is not known with certainty whether the plants in cultivation under this name are of one clone or not. When first described by Rolfe (1891), he commented: "It is a fine plant with four racemes, which has appeared with Mr. Charles Eastweed, Lane House Nursery, Luddenden, Manchester. A single flower also came from Liverpool Horticultural Co. without any note of its origin." The varietal names "mandaianum" and "aureum" also appear in the literature and refer to forms closely resembling C. lowianum var. concolor. Thus, one plant of this genetic variant may have been introduced, or several plants closely resembling each other may have been imported. There is little hope of tracing the exact origin of the plants. Genetically the "concolor" character, which in this plant is apparently a suppression of all the anthoxanthin and anthocyanin flower pigments in the perianth segments, is synonymous with the "album" or "albens" form of C. insigne and with the normal form of C. eburneum. Thus, C. lowianum var. concolor crossed with C. insigne var. album reportedly yields only plants of C. Pauwelsii G. bearing flowers that are of a "concolor" or "albino" nature; and crossed with C. eburneum, produces progeny that are also "concolors."

Origin of Polyploidy

The meiotic irregularities in these plants of C. lowianum var. concolor are rather mystifying, for they were all exhibited even when

grown under a variety of environments. The logical conclusion, therefore, is that they are the result of an innate state, either a partial nonhomology of the genome sets or a genetically controlled condition.

A further sidelight on this variety is encountered when a cytological study of some of its progeny is made. In combination with the species, *C. eburneum*, the primary hybrid, *C. (Eburneo-lowianum G.) 'Concolor'*, was produced. It is a diploid, but surprisingly enough has yielded several polyploid offspring. *C. (Alexanderi G.) 'Westonbirt'* [*C. (Eburneo-lowianum G.) 'Concolor'* x *C. insigne 'Westonbirt'*] is probably its most famous offspring for it is a tetraploid and has contributed greatly as a parent to the present day assemblage of *Cymbidiums*. Recently two triploids have been found that presumably have no polyploid ancestors, but each has as a direct parent, *C. (Eburneo-lowianum G.) 'Concolor'*. These are *C. (Minivet G.) 'Maxine'* [*C. (Eburneo-lowianum G.) 'Concolor'* x *C. Castor G.*] and *C. Miriam G. #1* [*C. (Eburneo-lowianum G.) 'Concolor'* x *C. Midas G.*]. It may be that *C. (Eburneo-lowianum G.) 'Concolor'* has functioned in the same unique way in all of these cases and furnished an unreduced gamete. An examination of the meiotic division of *C. (Eburneo-lowianum G.) 'Concolor'*, however, shows that it is usually quite regular and at the pollen stage few micropollen grains or dyads are encountered. Megasporogenesis was not studied in any of these plants; the results could prove to be different from that found in microsporogenesis. Unfortunately only one plant of this clone was examined and it was grown under very favorable conditions. It may be that when favored by different environmental conditions, the regular meiotic cycle would break down to a certain degree and some unreduced pollen grains would be produced. As shown in this study with *C. Pauwelsii G. #1*, the results of microsporogenesis may be different even upon the same raceme. The buds mature

serially, thus, each one may be subjected to different environmental influences of temperature, light, water and nutrition. It would appear that the variations in meiosis from bud to bud on the raceme may be a result of a varying environmental state. In other plants numerous studies have been conducted along these lines. In Gilia, Grant (1952) recorded a variation in chromosome pairing in F_1 hybrids according to whether they were growing in soil or pure sand. In raising F_2 generations of these hybrids, allotetraploid derivatives were more commonly derived from the starved parental plants than from the more luxuriantly grown hybrids. In Oenothera Zürn (1937) found a significant difference in chromosome pairing in plants that were examined before and after nutrients were added to their growing medium. Temperature also has been found to play an important role in determining the regularity of divisions in many plants. For example, Randolph (1932) was able to produce polyploid corn plants by subjecting seedlings to high temperatures. Aborted pollen grains and giant pollen grains were formed after heat shocks had been given to mature plants.

It may be that at certain environmental levels, *C. (Eburneo-lowianum G.) 'Concolor'* tends to form unreduced gametes more easily than under other conditions. It seems strange that *C. lowianum* var. concolor, one of the few species which has an abnormal reduction behavior, has given rise to a primary hybrid that has figured in the background of three known polyploids and which under favorable environmental conditions does not show a great number of irregularities at meiosis. The time is ripe for a detailed study of meiosis in several clones of hybrid *Cymbidiums* under varying conditions of light, heat, water and nutrition; more definite conclusions may then be reached. If certain environmental

conditions could be found that seem to favor nonreduction of gametes, a powerful tool would be within the grasp of the orchid hybridizer.

Fertility of the Hybrids

The F₁ Hybrids

The degree of crossability within the orchid family, as in other plant groups, varies greatly. Polygeneric crossings have been made between many genera with apparently little loss of fertility (Kamemoto, 1950). For example, the intergeneric hybrids between Cattleya, Laelia and Brassavola appear to maintain a fairly high fertility level. Many other intergeneric hybrids have been made with this Cattleya alliance. Some display a lowered fertility level, but yet not so low as to preclude the further use of the hybrids as parents. Sophranitis when crossed with certain members of this alliance seems to yield hybrids which are somewhat fertile, whereas in other hybridizations the progeny seem to be almost entirely sterile. Epidendrums cross with this group quite readily, but secondary hybridizations have never been recorded in Sanders' List of Orchid Hybrids. The same is true for the genera of Diacrium, Leptotes and Schomburgkia. Whether hybridizations were attempted with these intergeneric hybrids or whether the further use of them did not excite the fancy of the amateur or commercial hybridizers is not known.

In Cymbidiums, within the limited number of species that have been hybridized, the fertility of the hybrids has apparently remained at a relatively high level with some few exceptions. Some of the hybrids such as C. Gottianum G., C. Eburneo-lowianum G. and C. Lowio-grandiflorum G. were exceptionally regular at meiosis. This indicates a striking homology between the genomes of the two species that were mated to produce the hybrids. In others such as in C. Pauwelsii G., C. Ceres G. and C. Lotta G.

the reduction divisions were not so regular, with univalents, fragments and bridges showing up fairly regularly, showing that an evolutionary divergence of the genomes has occurred. And in other hybrids as *C. Doris* G. and *C. Hanburyanum* G., some of the plants showed a number of irregularities while others were almost without them.

The range of variability in some of the hybrid groups such as *C. Pauwelsii* G. or *C. Doris* G. might stem from several factors. Firstly, the plants might be misnamed. Secondly, the innate heterozygosity of the parental species may have yielded hybrids of a genetic constitution that varied enough to produce forms with just such extremes in meiosis. Thirdly, without a doubt the plants are not all siblings, thus, the assumption may be made that species of slightly different genetic constitutions have produced these hybrids. The result is as might be expected, variable. And lastly, even if the hybrids are of a fairly uniform hereditary background, the environment may have played a major role in visibly altering the reduction division cycles.

Yet, the irregularities in these hybrids were not great enough to reduce greatly the fertility of any of them. A sterility problem has never been encountered in any diploid *Cymbidium* hybrid except in one small group. Consequently it would appear that in the eight or ten Himalayan species that have been hybridized extensively, the chromosome sets are strikingly similar, with only a few inversions and translocations breaking up the homologies.

C. (Pauwelsii G.) '*Comte d'Hemptinne*' is a unique plant in this study for it is the only known tetraploid primary hybrid in the *Cymbidium* assemblage. Several theories might be advanced to account for this singular condition. 1. A post-fertilization doubling of the chromosomes

may have occurred. 2. A tetraploid may have been produced by a fusion of two unreduced gametes. 3. Polyspermaty may have resulted in the formation of a zygote with a tetraploid chromosome number as reported in Orchis and others by Hagerup (1944, 1947). Probably the most likely method of origin is the first one mentioned above. Either the first or second possibility would result in an individual having two genomes from each parent, while in the latter theory an individual would be formed having three genomes from the staminate parent. In general aspect, this clone shows enough intermediacy between the two species to safely eliminate the last hypothesis. The meiotic divisions show a number of univalents and/or fragments at metaphase II and telophase II; several bridges were also observed. This indicates that there were a number of polyvalents formed during prophase and that even though each chromosome probably had an identical homolog with which to pair, the homologies between the genomes of the two species was very great. Thus, with the formation of polyvalents, some meiotic irregularities would be expected with an ultimate formation of gametes often of a slightly unbalanced nature. These gametes are probably still viable and capable of fertilization.

The Pumilum Hybrids

The assemblage of C. pumilum hybrids is unique within the entire group of Cymbidiums, for here a sterility barrier is encountered that has been surmounted but rarely. The meiotic cycle in the primary hybrids, C. Minuet G., is almost entirely disrupted. At the end of microsporogenesis the chromosomes are generally strewn over the cell in a disorganized pattern. Compound "tetrad" pollen grains are often formed with a multitude of grains, none of which are of equal size. Attempts to hybridize representatives of this cross have met with almost complete failure. The Missouri Botanic

Garden has been successful in obtaining a few seedlings from a cross and the author has produced two seedlings by using *C. Minuet G.* as a staminate parent with *C. Windsor G.*

In this study the two other *C. pumilum* hybrids examined, *C. Pumander G.* and *C. Sweetheart G.*, displayed compound pollen "tetrads" that were equally distinctive as in *C. Minuet G.*, for they too, showed numerous grains of unequal sizes. From outward appearances the sterility barrier here would be as great as in *C. Minuet G.* However, an amateur hybridizer has obtained numerous seeds in a reciprocal cross using a clone of *C. Sweetheart G.* Thus, it would appear that certain crosses or certain clones of some crosses are more fertile than others. So even though there is a strong sterility barrier between *C. pumilum* and some of the orchids that are found in the so-called Himalayan Cymbidium belt, it is not so strong that it is impossible to surmount. If a tetraploid plant could be produced by the use of colchicine, the fertility would probably increase to a reasonable level and aid the hybridizer tremendously.

From observation of the meiotic irregularities that are found in *C. Minuet G.*, it would appear that there has been a strong divergence of the genomes in *C. pumilum* and *C. insigne* and its allies.

The Complex Hybrids

As a general rule the fertility of the more complex diploid hybrids is adequate to satisfy any demand for seed production that is made on them. The same is true of the tetraploids. Triploids may be used for parents, though oftentimes it is more difficult to induce a capsule to stay on a full term of nine months than when diploids or tetraploids are used exclusively. When triploids are mated to either diploids or tetraploids, a series of aneuploid progeny may often be expected, as reported by Wimber and

Hernlund (1955). Triploid-triploid crosses are more difficult to make. Apparently when triploids are used as parents, a fewer number of seeds are produced and as a result the capsules often do not ripen, but turn yellow and drop prematurely. This would indicate that an auxin or some other substance may be produced by the seeds that allows the capsule to mature properly; when only a few seeds are present not enough of the substance may be manufactured to allow maturation of the capsule. Such a mechanism would have a definite survival value to the diploid plants, for capsules that were insufficiently fertilized would fail to mature and thus, a drain on the stored food material of the plant would be prevented.

The Tetrad and Evolution

In other Angiosperms each nucleus of a tetrad formed during meiosis is an individual physiologic unit and as such must contain the hereditary material necessary for development of a microspore and then the further development of a microgametophyte or pollen tube. If such genetic material is absent the development of the microgametophyte may be halted at one of two levels. The microspore may not be functionally able to mature and thus abort, or the initiation of a pollen tube and further growth of it might not be physiologically possible. Therefore, the genic material necessary for maturity of the microspore and the formation of a functional microgametophyte may not necessarily be the same. If such assumptions are correct, then it might be theoretically possible to have nuclei formed possessing the necessary genetic material for successful germination and yet deficient in the hereditary material essential for maturation of the pollen grain. In orchids it would seem that the first hurdle toward the formation of a microgametophyte is removed, for the peculiar compound tetrad pollen grains found here are very unique; grains that are formed

with duplicate or deficient genetic properties and which would abort in other plants, are able to survive by the nuclear interaction of their partners. Barber (1942) reports micropollen grains surviving in the "tetrad" of Anacamptis pyramidalis Rich. Tuschajakova (1929) found that in Listera ovata (L.) R. Br. ($n = 17$) micropollen grains were often found and out of 500 microspores examined at the pollen division, 5 or 6 had 16 chromosomes and the same number of hyperploid grains ($n = 18$) were encountered. She postulated the occurrence of aneuploid forms containing diploid numbers ranging from 32 to 36. In 1933 Richardson confirmed this hypothesis by finding plants with 32, 34, 35 and 36 chromosomes and Hagerup (1947) later increased this range to 38. Midumo (1940a) found that even when the reduction division was drastically altered as in a haploid of Bletilla striata Rchb. f. var. gebina Rchb. f., there was no necrosis of the resulting nuclei and each "tetrad" matured. In Cymbidiums as shown by the studies of Wimber and Hernlund (1955), many of the unbalanced gametes produced by triploids are viable and capable of fertilization, but here the complicating factor of polyploidy must be assessed. Several aneuploid plants in Cattleyas and Laelias have been discovered by Kamemoto (1950), apparently a result of the fusion of gametes of an unbalanced nature. A search through the literature brings to light a large number of naturally occurring hypo- or hyperploid orchids.

Thus, if the genetic qualities for maturation of the microspores and the successful germination of the pollen grains are separable, in Angiosperms with physiologically individual pollen grains, the mechanism of microspore formation in orchids may provide for a source of genetic variability not present in most other seed plants; there might ultimately be a survival and germination of more genetically abnormal pollen grains than found elsewhere.

This hypothesis has a number of drawbacks. If the chromosome series found in the orchid family is any criterion, it is very probable that most of the orchids are polyploids, for until recently no definite diploid number was known below twenty. Dodson (1956) reported a diploid number of ten in Oncidium pusillum (L.) Rchb. f. The known diploid numbers in this family probably average around forty. If this suspected polyploidy of most orchids is true, then in most species there are a number of duplications of genic material scattered throughout the genomes. Such duplications would aid in the survival and germination of duplication-deficient pollen grains. Thus, this probable polyploidy of most orchids may mask the effects of the nuclear interactions in the tetrad.

Parallel cases of united tetrad pollen grains in other Angiosperms are rare. Erdtman (1945) gives a list of plants in which compound pollen grains have been found. They adhere in varying degrees, with some plants showing compound grains that are easily separated and others as in the orchids and asclepiads that are very firmly attached. Little has been said concerning the survival of duplication-deficient microspores in these plants. Levan (1942) in reporting the occurrence of a gene in Petunia that causes adherence of the pollen in tetrads, mentioned that one or more of the grains in an individual tetrad may abort.

Probably one of the foremost evidences that would indicate whether the nuclear interaction of the pollen grains is significant in increasing the genetic variability, would be the occurrence of aneuploid series or variants within a group. In orchids such aneuploid series do occur but whether one of the causal mechanisms can be pinpointed as this nuclear interaction or whether other factors such as polyploidy and lack of endosperm can explain the entire variability is unknown.

The time is not ripe for the confirmation or denial of this hypothesis,

for the necessary material with which to work has not yet been found. It can merely be stated that this nuclear interaction of the pollen grains working hand in hand with the specificity of the insect pollinators, the lack of endosperm and with the enormous number of seeds that each pollination produces, may account in part for the rapid evolution that apparently has been occurring in the orchids.

The Species Problem in Cymbidiums

As a general rule in most plant families, members of different genera cannot be crossed and if they can, the resulting hybrid is completely sterile. The Orchidaceae has long been a violator of this rule for there are a number of so-called genera that are interfertile as evidenced by the Cattleya - Laelia - Brassavola - Sophranitis complex and the Oncidium - Odontoglossum - Miltonia - Cochlioda group. These genera have in many cases been separated on seemingly trivial characters, which may have seemed real enough at the time of the founding of the genus, but which have broken down in the interim. Ofttimes these genera are connected by a complete series of intergrading forms which can neither be placed with safety in one genus nor the other. Thus, it would seem that in some tribes of the orchid family, the species is more natural than the genus.

Such a statement cannot be made for the genus Cymbidium, as evidenced by the intergeneric crosses that have been made with the genus. Only two have been recorded and one, with Phaius, can probably be discarded as a false hybrid; the other, with Cyperorchis, is qualified with the probability that this group with connivent perianth segments should not be considered as a separate genus. Thus, as shown by the breeding behavior, the Cymbidium genus seems to be a natural grouping with no known interfertile relatives.

The species situation in Cymbidium is obscure. How effectively the species are separated can only be judged by their behavior under cultivation, for herbarium material is very meagre and no field study has yet been attempted. By studying the meiotic cycle of many of the F_1 hybrids that have been artificially produced, it is obvious that a cross in nature would produce an abundance of seed and form hybrids that would be quite fertile. Therefore, it would seem that among most of the species that have been studied, no internal barriers are present that would prevent fertilization or bar seed production in the hybrid. Speciation among the part of the genus that has been studied, has been accompanied by very little chromosomal repatterning. Other isolating barriers must then be searched for.

The distribution of the species is very little known; only vague outlines of their distributions can be drawn. It is thought that there is some sympatry, at least an overlapping in a few areas. In observing the species under cultivation it becomes obvious that there are definite environmental preferences. So even for those species that may be sympatric, an ecologic isolation probably exists. Here, too, they may very well bloom at different times of the year. Probably the most effective isolating mechanisms are created by the pollinators. The pollinators of this genus, insect or otherwise, are largely unknown. The various shapes, colors and odors that the species of Cymbidium exhibit indicate that probably a wide range of insects undertake these operations and for the most part are fairly specific. Yet, if the records of many early importations are examined as often found in the Orchid Review, several so-called natural hybrids have been imported. Six such hybrid Cymbidiums have been recorded in Sanders' Complete List of Orchid Hybrids. The parentage of these alleged hybrids is usually formulated after the two

hypothetical parents are crossed and the progeny examined. Therefore, there is a rather strong possibility that occasional natural hybrids or hybrid swarms in nature are produced as a result of a breakdown of the isolating mechanisms.

SUMMARY

1. The chromosome numbers of twenty-one species of Cymbidium were determined, ten of which had not been reported before. The diploid numbers were found to be forty in all species. The small size of the chromosomes made it impossible to easily demonstrate morphological similarities and differences of the karyotypes.
2. The karyotype of Cymbidiella rhodochila separates it sharply from the genus Cymbidium.
3. Two species of Grammatophyllum were also found to have forty somatic chromosomes, though smaller than in Cymbidium.
4. Meiosis was examined in seven species and was found to be fairly regular in all except one clone of both C. insigne and C. lowianum var. concolor.
5. C. (Eburneo-lowianum G.) 'Concolor', a diploid, was found to have been the parent of three polyploid clones. The suggestion is made that the abnormal reduction divisions observed in C. lowianum var. concolor may have contributed in some way to the ability of C. (Eburneo-lowianum G.) 'Concolor' to produce unreduced gametes.
6. Microsporogenesis may produce different results on the same raceme, for a study of one clone of C. Pauwelsii G. showed varying numbers of micropollen grains in different buds. The suggestion is made that the environment may influence these final products.
7. The primary hybrids between many species of Cymbidiums from the Himalayan area are generally very fertile, indicating that speciation

in this group has involved very little chromosomal repatterning.

8. The hybridization of C. insigne and C. pumilum produces a relatively sterile F_1 hybrid, C. Minuet G. Meiosis in this primary hybrid is almost totally disrupted, pointing to a wide divergence of the genomes of these two species. Two other hybrids of C. pumilum show equal irregularities in the pollen.

9. Triploid hybrids show large numbers of abnormalities at microsporogenesis, but judging from known breeding behaviors of many triploids, the disruption at meiosis is not so great as to entirely preclude their use as parents.

10. The hypothesis is advanced that the compound pollen grain may be an important contributing factor to the rapid evolution of the orchids. Duplication-deficient pollen grains are able to mature in orchids, whereas they often would abort in other plants; these grains may be an important source of genetic variability not present in most plant groups.

11. The genus Cymbidium seems to be a natural grouping. The species in cultivation are generally well defined although intermediate forms have been imported from the wild in the past, indicating that the isolating mechanisms in nature are not final and natural hybrids are occasionally formed.

ACKNOWLEDGMENTS

Many persons have had a hand, in both small and large ways, in the production of the present dissertation. For personal counsel during the entire course of this study Drs. Lee Lenz and Verne Grant deserve first mention and receive my heartfelt thanks. Materials have been supplied by numerous people and organizations without which this work would not have been possible. Among those that have donated materials are Mr. Robert Casamajor, Mr. Arno Bowers, Mr. and Mrs. Menninger, Mr. Charles M. Hill and Jerry and Don Sherman. Mr. Kermit Hernlund of the Dos Pueblos Orchid Company deserves special mention for his sage advice and willing cooperation.

Particular thanks are due to Mr. Samuel B. Mosher, who by his generous fellowship grants, has enabled me to continue through three years of graduate study to the completion of this dissertation.

LITERATURE CITED

- Afzelius, K. 1916. Zur Embryosackentwicklung der Orchideen. Sv. bot. Tidsskr. 10: 183-227.
- _____. 1928. Die Embryobildung bei Nigritella nigra. Sv. bot. Tidsskr. 22: 82-91.
- _____. 1932. Zur Kenntnis der Fortpflanzungsverhältnisse und Chromosomenzahlen bei bei Nigritella nigra. Sv. bot. Tidsskr. 26: 365-369.
- _____. 1943. Zytologische Beobachtungen an einigen Orchidaceen. Sv. bot. Tidsskr. 37: 266-276.
- Ames, Oaks. 1946. The evolution of the orchid flower. Amer. Orch. Soc. Bull. 14: 355-360.
- Baranow, P. 1915. Recherches sur le developpement du sac embryonnaire chez les Spiranthes australis Lindl. et Serapias pseudocordigera Morie. Bull. Soc. Imp. Nat. Moscou. N. S. 29: 74-92.
- _____. 1918. Contributions a l'etude de l'embryologie des Orchidees. Jour. soc. bot. Russie. 2: 20-29.
- _____. 1925a. Contribution a l'etude de l'embryologie des Orchidees. II. Herminium Monorchis R. Br. (Ref. Bot. Centralbl. N. F. 7: 266).
- _____. 1925b. Sur la reduction de la generation feminine sexuelle dans la famille des Orchidees. Bull. Univ. Asie Centrale Taschkent 10: 181-195. (Russian with French summary).
- Belling, J. 1924. Detachment of chromosomes in Cypripedium acaule. Bot. Gaz. 78: 458-460.
- Bentham, G. and J. D. Hooker. 1883. Genera Plantarum. III. London.
- Barber, H. N. 1942. The pollen-grain division in the Orchidaceae. Jour. of Genetics 43: 97-103.
- Blume, K. L. 1849. (Description of Cyperorchis) Mus. Bot. Lugd. Bat. 1: 48.
- Chiarugi, A. 1929. Diploidismo con anfimissa e tetraploidismo con apomissa in una medesima specie: 'Nigritella nigra'. Boll. Soc. Ital. Biol. Sper. 4: 659-61.

- Chodat, R. 1924. La caryocinese et la reduction chromatique observees sur le vivant. C. R. Soc. Phys. et Hist. Nat. Geneve 41: 96-99.
- Dodson, C. H. 1956. Cytological studies in the genus Oncidium. M.A. thesis. Claremont College.
- Duncan, R. E. 1945. Production of variable aneuploid numbers of chromosomes within the root tips of Paphiopedilum Wardii. Amer. Jour. Bot. 32: 506-509.
- _____. 1947. The hybrid lady slipper. Orch. Digest. Sept.-Oct.: 199-207.
- Duncan, R. E. and R. MacLeod. 1948a. Chromosomes of the Brachypetalums. Amer. Orch. Soc. Bull. 17: 170-174.
- _____. 1948b. Chromosomes of the Insigne complex of Lady-slippers. Amer. Orch. Soc. Bull. 17: 424-429.
- Duncan, R. E. and R. MacLeod. 1949a. The chromosomes of the continental species of Paphiopedilum with solid green leaves. Amer. Orch. Soc. Bull. 18: 84-89.
- _____. 1949b. The chromosomes of some of the Polyantha. Amer. Orch. Soc. Bull. 18: 159-163.
- _____. 1949c. The chromosomes of the species of Cochlopetalum Hallier. Amer. Orch. Soc. Bull. 18: 573-576.
- _____. 1950a. The chromosomes of Eremantha Tesselata. Amer. Orch. Soc. Bull. 19: 137-142.
- _____. 1950b. The chromosomes of Paphiopedilum Sublaeve. Amer. Orch. Soc. Bull. 9: 489-492.
- Eftimiu-Heim, P. 1941. Recherches sur le noyau des Orchidees. Le Botaniste 31: 65-110.
- Erdtman, G. 1945. Pollen morphology and plant taxonomy V. On the occurrence of tetrads and dyads. Sv. bot. Tidsskr. 39: 286-297.
- Frahm-Leliveld, J. A. 1941. Some remarks on the formation of the pollinia in Gymnadenia conopea (L.) R. Br. Naturwetenshap. Tijdschr. Nederland-Indie 101: 242-244.
- Francini, E. 1931. Recherche embriologique e cariologica sul genere "Cypripedium". Nuovo Giorn. Bot. Ital. N.S. 38: 154-212.
- _____. 1932. Un reperto cariologico nella F₂ di x Paphiopedilum Leeanum. Nuovo Georn. Bot. Ital. N. S. 39: 251-253.
- _____. 1934. Ibridazione interspecifica nel genere "Paphiopedilum". Nuovo Giorn. Bot. Ital. N. S. 41: 189-237.

- Fuchs, A. and G. Ziegenspeck. 1924. Aus der Monographie der Orchis Traunsteineri Saut. Kap. III. Entwicklungsgeschichte einiger deutscher Orchideen. Bot. Arch. 5: 120-132.
- Geitler, L. 1940. Kernwachstrum und Kernbau bei zwei Blütenpflanzen. Chromosoma 1: 474-485.
- Grant, V. 1952. Cytogenetics of the hybrid Gilia millefoliata x achilleaefolia. I. Variations in meiosis and polyploidy rate as affected by nutritional and genetic conditions. Chromosoma 5: 372-390.
- Guignard, L. 1883. Recherches sur le developpement de l'anthere et du pollen des Orchidees. Ann. des Sci. natur. 6: 26-45.
- Hagerup, O. 1938. Studies on the significance of polyploidy, II. Orchis. Hereditas 24: 258-264.
- _____. 1944. On fertilisation, polyploidy and haploidy in Orchis maculatus L. Sens. Lat. Dansk Botanisk Arkiv 11, no. 5.
- _____. 1945. Facultative parthenogenesis and haploidy in Epipactis latifolia. Det. Kgl. Danske Vidensk. Selskab. Biologiske Meddelelser. 19, no. 11.
- _____. 1947. The spontaneous formation of haploid, polyploid and aneuploid embryos in some orchids. Det. Kgl. Danske Vidensk. Selskab. Biologiske Meddelelser. 20, no. 9.
- Harmsen, L. 1943. Studies on the cytology of arctic plants. Meddelelserom Grønland 131: 1-15.
- Heitz, E. 1926. Der Nachweis der Chromosomen. Vergleichende Studien über ihre Zahl, Grosse und Form im Pflanzenreiche. Zeit. f. Bot. 18: 625-681.
- Heslop-Harrison, J. 1953. Microsporogenesis in some triploid dactylor-chid hybrids. Ann. Bot. N.S. 17: 539-549.
- Heusser, C. 1938. Chromosomenverhältnisse bei schweizerischen bastionen Orchideen. Ber. Schweiz. Bot. Ges. 48: 562-605.
- Hildebrand, F. 1863. Die Fruchtbildung der Orchideen, ein Beweis für die doppelte Wirkung des Pollen. Bot. Zeit. 21.
- Hoffmann, K. 1930. Beiträge zur Cytologie der Orchidaceen. Planta 10: 523-595.
- Holttum, R. E. 1953. A revised flora of Malaya. Vol. I. Orchids of Malaya. Singapore.
- Humphrey, L. M. 1934. The somatic chromosomes of Cypripedium hirsutum and 6 species of the genus Habenaria. Amer. Nat. 68: 184-186.

- Johansen, D. A. 1950. Plant embryology. *Chronica Botanica*.
- Kamemoto, H. 1950. Polyploidy in Cattleyas. *Amer. Orch. Soc. Bull.* 19: 366-373.
- _____. 1952. Further studies of polyploid Cattleyas. *Bull. Pacific Orch. Soc. of Hawaii* 10: 141-148.
- Kamemoto, H. and L. F. Randolph. 1949. Chromosomes of the Cattleya tribe. *Amer. Orch. Soc. Bull.* 18: 366-369.
- Leven, A. 1942. A gene for the remaining in tetrads of ripe pollen in Petunia. *Hereditas* 28: 429-435.
- Lindley, J. 1832. *Genera et species Orch.* London.
- Löve, A. and D. 1944. Cytotaxonomical studies on boreal plants. III. Some new chromosome numbers of scandinavian plants. *Ark. Bot.* 31: 1-23.
- McQuade, H. A. 1949. The cytology of Paphiopedilum Maudiae Hort. *Ann. Mo. Bot. Gard.* 36: 433-472.
- Maude, P. F. 1939. The Merton catalogue. A list of the chromosome numbers of species of British flowering plants. *New Phytol.* 38: 1-31.
- Mehlquist, G. A. L. 1947. Polyploidy in the genus Paphiopedilum Pfitz. (Cypripedium Hort.), and its practical implications. *Mo. Bot. Gard. Bull.* 35: 211-228.
- _____. 1952. Chromosome numbers in the genus Cymbidium. *Cym. Soc. News.* 7, Jan., 17-24.
- Menninger, E. D. 1954. Tetraploid Cymbidiums. *Amer. Orch. Soc. Bull.* 23: 158-61.
- Miduno, T. 1938. Chromosomenstudien an Orchidazeen. I. Karyotyp und Mixoploidie bei Cephalanthera und Epipactis. *Cytologia* 8: 505-514.
- _____. 1939. Chromosomenstudien an Orchidazeen II. Somatische Chromosomenzahlen einiger Orchideen. *Cytologia* 9: 447-451.
- Miduno, T. 1940a. Chromosomenstudien an Orchidazeen. III. Über das Vorkommen von haploiden bei Bletilla striata Rchb. f. var. gebina Rchb. f. *Cytologia* 11: 156-177.
- _____. 1940b. Chromosomenstudien an Orchidazeen. IV. *Cytologia* 11: 179-185.
- _____. 1954. Chromosomenstudien an Orchidazeen. V. *Cytologia* 19: 239-248.

- Müller, K. 1847. Beiträge zur Entwicklungsgeschichte des Pflanzenembryo. Bot. Ztg. 5.
- Pace, L. 1907. Fertilization in Cypripedium. Bot. Gaz. 44: 353-374.
- _____. 1909. The gametophytes of Calopogon. Bot. Gaz. 48: 126-137.
- _____. 1914. Two species of Gyrostachys. Baylor Univ. Bull. 17.
- Pfitzer, E. 1880. Beobachtungen über Bau und Entwicklung der Orchideen. 5. Zur Embryoentwicklung und Keimung der Orchideen. Verh. Nat.-Med. Ver. Heidelberg N. F. 2: 23-30.
- _____. 1906. Hybridisation and the systematic arrangement of orchids. Rep. 3 internat. Confer. in Genetics. Ed. by Rev. W. Wilks, 218-221.
- Prosina, M. N. 1930. Über die von Cypripedium-Typus abweichende Embryosackentwicklung von Cypripedium guttatum Sw. Zeitschr. Wiss. Biol. Planta 12: 532-544.
- Randolph, L. F. 1932. Some effects of high temperature on polyploidy and other variations in Maize. Proc. Nat. Acad. Sci. 18: 222-229.
- Richardson, M. M. 1938. The chromosomes of some British orchids. Proc. Univ. Durham Phil. Soc. 9: 135-140.
- Rolfe, R. A. 1891. (Description Cymbidium lowianum var. concolor) Gard. Chron. II: 187.
- _____. 1918. (Description of Cymbidiella) Orch. Rev. 26: 58.
- Sander's complete list of orchid hybrids. St. Albans. 1946.
- Sander's list of orchid hybrids, addendum. 1946-1947 - 1948. St. Albans. 1949.
- Sander's list of orchid hybrids, addendum. 1949 - 1950 - 1951. St. Albans. 1952.
- Sander's list of orchid hybrids, addendum. 1952 - 1953 - 1954. St. Albans. 1955.
- Schlechter, R. 1926. Das System der Orchidaceen. Notizbl. Bot. Gart. u. Mus. Berlin-Dahlem 9: 563-591.
- Schnarf, K. 1929. Embryologie der Angiospermen. Berlin.
- _____. 1931. Vergleichende Embryologie der Angiospermen. Berlin.
- Seshagiriah, K. N. 1941. Morphological studies in Orchidaceae I. Zeuxine sulcata Lindl. Jour. Ind. Bot. Soc. 20: 357-365.
- Sharp, L. W. 1912. The orchid embryo sac. Bot. Gaz. 54: 372-385.

- Storey, W. B. 1952. Chromosome numbers of some Vanda species and hybrids. Amer. Orch. Soc. Bull. 21: 801-806.
- _____. 1953. The pentaploid origin of Vanda Nora Potter. Bull. Pac. Orch. Soc. of Hawaii 11, no. 1: 17-25.
- Strasburger, E. 1888. Über Kern und Zellteilung im Pflanzenreich, nebst Anhang über Befruchtung. 239-244. Jena.
- Suessenguth, K. 1921. Beiträge zur Frage des systematischen Anschlusses der Monokotylen. Beih. Bot. Zbl. 38: 1-79.
- Sugiura, T. 1936. A list of chromosome numbers in Angiospermous plants. II. Proc. Imp. Acad. 12: 144-146.
- Swamy, B. G. L. 1941. Development of the male gamete in Cymbidium bicolor Lindl. Proc. India Acad. Sci. 14: 454-460.
- _____. 1942a. Morphological studies in three species of Vanda. Curr. Sci. 11: 285.
- _____. 1942b. Female gametophyte and Embryogeny in Cymbidium bicolor Lindl. Proc. India Acad. Sci. 15: 194-201.
- _____. 1943a. Embryology of Orchidaceae. Curr. Sci. 12: 13-17.
- _____. 1943b. Gametogenesis and embryogeny of Eulophea epidendreae Fischer. Proc. Nat. Inst. Sci. India 9: 59-65.
- _____. 1944. The embryo sac and embryo of Satyrium nepalense Don. Jour. Ind. Bot. Soc. 23: 66-70.
- _____. 1945. Embryo sac and fertilization in Cypripedium spectabile. Bot. Gaz. 107: 291-295.
- _____. 1946a. The embryology of Zeuxine sulcata Lindl. New Phytol. 45: 132-136.
- _____. 1946b. Embryology of Habenaria. Proc. Nat. Inst. Sci. India 12: 413-426.
- _____. 1946c. Some notes on the embryo of Cymbidium bicolor Lindl. Curr. Sci. 15: 139-140.
- _____. 1947a. On the life history of Vanilla planifolia. Bot. Gaz., 108: 449-456.
- _____. 1947b. On the suspensor types in orchids and their significance. Amer. Jour. Bot. 34: Suppl. 12a.
- _____. 1948a. Agamospermy in Spiranthes cernua. Lloydia 11: 149-162.
- _____. 1948b. Embryology of Epidendrum prisimatocarpum. Bull. Torrey Bot. Club 75: 245-249.

- Swamy, B. G. L. 1949a. Embryological studies in the Orchidaceae I. Gametophytes. Amer. Mid. Nat. 41: 184-201.
- _____. 1949b. Embryological studies in Orchidaceae II. Embryogeny. Amer. Mid. Nat. 41: 202-232.
- Swartz, O. 1800. (Description of Cymbidium) K. Vet. Acad. Stockh. Nya. Handl. XX.
- Tobgy, H. A. 1943. A cytological study of Crepis fuliginosa, C. neglecta and their F₁ hybrid and its bearing on the mechanism of phylogenetic reduction in chromosome number. Jour. Genet. 45: 67-111.
- Traub, M. 1879. Notes sur l'embryogenie des quelques Orchidees. Verh. Akad. Amsterdam, Natuurk. 19: 1-50.
- Tuschnjakova, M. 1929. Embryologische und zytologische Beobachtungen über Listera ovata. Planta 7: 29-44.
- Vacin, E. F. 1954. A treatise on the hybridization of the Orchidaceae. Hawaii Orch. Soc. Bull. 6: no. 1, 3-20.
- Vermeulen, P. 1938. Chromosomes in Orchis. Chron. Bot. 4: 107-108.
- Weijer, J. 1952. Colour differences in Epipactis helleborine and selection of the genetical varieties by environment. Genetica 26: 1-32.
- Wells, E. 1956. A tabulation of 110 chromosome counts of award and other choice cymbidiums. Amer. Orch. Soc. Bull. 25: 175-178.
- Wimber, D. E. 1954. Note on Cymbidium Flamingo 'Nobilior'. Cym. Soc. News 9, no. 4, 16-17.
- _____. In press. An improved acetic-lacmoid squash technic for orchids. Stain Tech.
- Wimber, D. E. and K. Herlund. 1955. Aneuploid chromosome numbers in Cymbidiums. Amer. Orch. Soc. Bull. 24: 743-745.
- Woodard, J. W. 1951. Some chromosome numbers in Phalaenopsis. Amer. Orch. Soc. Bull. 20: 256-258.
- Zürn, K. 1937. Untersuchungen zur physiologie der Meiosis. IX. Die Bedeutung der Plastiden für den Ablauf der Meiosis. Jb. wiss. Bot. 85: 706-731.