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A Chromosome Study of Chlorophytum Elatum

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A CHROMOSOME STUDY OF

CHLOROPHYTUM ELATUM

BY

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CHLOROPHYTUM ELATUM

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree. Acceptance of this thesis does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Advisor Date

Head, Botany Department Date

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INTRODUCTION

The spider plant, Chlorophytum elatum R. Br., a member of the family Liliaceae, is a native of South Africa. It has been propagated almost exclusively by vegetative means and it is now grown widely over the world as an ornamental plant. Many studies by other workers have revealed that with only two exceptions all species in the genus Chlorophytum have somatic chromosome numbers that are multiples of seven, ranging from 14 to 56 (c.f. Darlington and Wiley, 1955).

Although the chromosome number of Chlorophytum elatum has been reported to be 2N = 28 (Sato, 1942; Baldwin and Speese, 1951) information regarding its karyotype is not complete and meiotic behavior is still lacking. Storey (1968), after studying the mitotic phenomenon in roottip cells, suggested that this species might be an autotetraploid with a basic number X = 7. However, he failed to study the meiotic chromosomes which would be essential in order to verify his assumption.

The clone of Chlorophytum elatum, which has been maintained in the Botany Greenhouse, was noted to show some degree of sterility. Preliminary observations of the pollen indicated that about 25 percent of the grains were non-stainable and irregular in shape. This implied that either a change in chromosome number or an alteration of chromosome structure might occur in this particular clone such as was

observed in other genera by Carnahan and Hill (1962); Dhaliwal, Pollard, and Lorz (1962); Doughty (1936); Honma (1968); Kreft (1968); Sutton (1937); Thomas (1960); Upcott and LaCour (1936); Upcott (1937); and Whitaker (1935).

This study was undertaken to ascertain the karyotype of Chlorophytum elatum and to study the meiotic chromosome behavior. A karyotypic analysis supported by a study of the behavior of chromosomes during diakinesis and/or metaphase I was made to determine the ploidy of this clone. The study of the meiotic chromosome behavior at later stages of meiosis along with a karyotypic analysis was made to find an explanation for the clone's sterility.

LITERATURE REVIEW

The chromosome numbers in the genus Chlorophytum have been reported to vary from 14 to 56 and, with two exceptions, they were found to be multiples of 7 (c.f. Darlington and Wiley 1955) and Wet (1957).

Baldwin and Speese (1951) made an extensive collection of Chlorophytum spp. in West Africa. They found that 4 of the 5 species of Chlorophytum growing in Liberia had chromosome numbers of either 14 or 28. The results of their meiotic studies indicated that Chlorophytum orchidastrum and Chlorophytum viviparum had 14 pairs of chromosomes while Chlorophytum inornatum and Chlorophytum laxum, 7 pairs, indicating that the former two species were tetraploids the latter two diploids. Chlorophytum alismifolium (2N = 16), however, was considered to be a derived diploid since 6 bivalents plus a trivalent and a univalent were occasionally present at the first metaphase stage of microsporogenesis.

No information regarding the chromosome association in Chlorophytum elatum was found in the literature. However in a study of the mitotic phenomena in the storage roots of this species, Storey (1968) noted the occurrence of somatic groupings of 7 chromosomes in some cells in cortical tissue composed of an admixture of diploid and polyploid cells. He then suggested that the species might have originated as an autotetraploid with a basic number X = 7. Unfortunately, he was unable to verify his assumption about the chromosome

pairing relationship because none of the plants he worked with flowered during the time his study was in progress.

Information obtained from a karyotypic analysis might give some clue concerning the ploidy of a plant. D. Sato (1942), using paraffin technique, studied the somatic chromosomes of some species in Liliaceae and its allied families. He noted that the nucleus of Chlorophytum elatum var. variegatum contained 12 long, 8 medium, 4 short, and 4 satellite chromosomes. However, no comparable measurements of the chromosomes were available.

Early studies of karyotypes were usually made on microscopic specimens prepared by using the paraffin technique. The materials used were invariably metaphase cells of roottips.

Investigations into the types of chromosomes in Aucuba japonica, Meurman (1929), studied metaphase cells obtained from paraffin sections of roottips and anthers that were stained with iodine gentian violet. Camera lucida drawings of the chromosomes were made from suitable mitotic, metaphase cells and from these drawings total chromosome lengths were measured. From the data obtained he was able to classify the chromosomes into 8 groups of 4. This evidence, along with a high degree of quadrivalent configurations at metaphase I of meiosis, led him to conclude that this species was autotetraploid and not allotetraploid.

Darlington (1927), Derman (1931), Ellison (1937), Levan (1932, 1933), Tjebbes (1928), Upcott and LaCour (1936), and Upcott (1936), also applied the paraffin technique in chromosome studies similar to that used by Meurman (1929).

There are, however, several disadvantages in the use of the paraffin technique in chromosome studies. First, it is impossible to obtain a paraffin section showing all of the chromosomes in a cell at the same focal distance. Secondly, slides prepared in this way do not show chromosomes spread well enough, making individual chromosome observation very difficult. Thirdly, in many species the chromosomes prepared without pretreatment for shortening are too long and the ends too diffuse to allow accurate measurements to be made.

The disadvantages of the paraffin technique were corrected by Tjio and Levan (1950). They (c.f. Sharma and Sharma 1965) pretreated excised roottips from Agapanthus umbellatus with 8-oxyquinoline prior to fixation. This pretreatment caused mitotic arrest and chromosome contraction which aided greatly in obtaining well-spread, measurable chromosomes. In slide preparation the roottips were heated in a mixture of aceto-orcein and N HCl for staining and were then squashed in a drop of aceto-orcein. Chromosomes so prepared were found to be considerably shortened, well-spread and could all be found in the same plane.

Somatic chromosomes of some genera of Compositae were classified by Huziwara (1962) using the squash technique described by Tjio and

Levan (1950). He used over-all length, positions of the centromeres, and secondary constrictions to identify the chromosomes. In comparing chromosome sizes, "relative lengths" were used in idiogram construction and karyotypic analysis. These "relative lengths" represented the ratios of the length of each individual chromosome to the length of the longest chromosome of the same cell.

Bhattachryya and Jenkins (1961) made a karyotype study of Secale cereale "L. Dakold" using cold water for pretreatment. Several measurements of each chromosome using an ocular screw micrometer, were taken from 10 metaphase cells and the mean values adopted. They measured the length of the long arm and short arm in each of the chromosomes and added these two measurements together to obtain a total chromosome length. An arm index ratio was calculated by dividing long arm length into short arm length. For idiogram construction and karyotypic analysis the chromosomes were paired according to similarities in total length and arm index ratios.

Using a colchicine pretreatment which contracted the chromosomes and aided in chromosome spreading, Yim (1963) obtained measurable chromosomes of Pinus rigida with the squash method. He made measurements from photomicrographs of the cells, using an engineer's divider. The chromosome arm, if curved, was measured along a series of straight lines tangent to the arc described by the arm. The measurements included the total length, long arm length, and short arm length for

each chromosome. The measured values used in idiogram construction were values expressed as percent values against the whole length of the longest chromosome in a 2N complement.

Marenah and Holden (1967) in their studies of the Avena karyotype used bromonaphthalene for pretreatment before fixing and squashing the cytological material. The two arms of each chromosome were measured and from these measurements an arm index ratio was calculated for every chromosome. According to the arm ratio index, the chromosomes were classified and divided into 4 groups, namely, satellited, median, sub-median, and sub-terminal. The chromosomes within each group were arranged in descending order according to total length.

Karyotypic analysis on various plants has also been done by Thomas (1960), Ross and Miller (1961), Natarajan and Simak (1962), Tanaka and Shimotomai (1963), Shindo and Kamemoto (1965), Tanaka (1967), Raj and Seethoiah (1969), Saini and Davis (1970), and Ourecky (1970). They followed procedures essentially the same as that described by Tjio and Levan (1950) with modifications to make the procedure more suited to their materials.

Chromosome aberrations in Chlorophytum have been reported only by Thomas (1960) when he observed that some of the somatic cells of Chlorophytum heynei (2N = 14) showed a heterozygous translocation. Instances of heterozygous inversions have been found in many species of Liliaceae and its related families but to date none have been reported for the genera Chlorophytum.

Upcott (1937) made an extensive meiotic study in Tulipa. She found that nearly all species and varieties examined were heterozygous for at least one inversion. The presence of chromatin bridges and corresponding fragments at the anaphase I stage (AI) and/or anaphase II stage (AII) was the cytological evidence of the presence of inversions. Nine of eleven diploids and all the triploids studied were structurally heterozygous for at least one inversion.

Frankel (1937) found inversions present in five of twenty-seven species of the genera Fritillaria that he studied. In Fritillaria dasyphylla, inversions were present but chromosome pairing was normal. There was, however, a high frequency of chromatin bridges and/or fragments at the AI stage. Frankel observed inversion loops at pachytene and acentric fragments associated with loop chromatids in one daughter complement at AI and considered them to be evidences that these bridges and/or fragments were the result of a heterozygous paracentric inversion. He stated that the univalents and/or fragments were usually excluded from the daughter nuclei. At the tetrad stage they become either micronuclei or separate cells called microcytes.

Mensinkai (1939) made a cytogenetic study of Allium. Of the 17 species studied, he found 6 to be inversion heterozygotes. Bridges and fragments, evidence of inversion heterozygosity, were observed in more than 10 percent of the pollen mother cells of two other species. However they were found in less than 10 percent of the pollen mother cells of the remaining two species that contained inversions. He considered the presence of micronuclei in tetrads to be due to lagging chromosomes and/or fragments.

Inversions have also been investigated in genera outside the family Liliaceae. Flagg (1958) in his investigations of mutation and translocation found a chromatin bridge with a fragment present in Rhoeo discolor. He considered this to be cytological evidence of a cross-over within a heterozygous paracentric inversion.

Bridges with fragments were also found in barley [Holm (1960), Smith (1935), Bhaduri and Sharma (1949), and Das (1955)]. Holm obtained a negative correlation between the pollen fertility and the presence of chromatin bridges and fragments.

Carnahan and Hill (1962) studied a hexaploid clone of Festuca arundinaceae (2N = 42). They found bridges and/or fragments present at AI and considered these due to a pairing of chromosomes heterozygous for an inversion. They also found a highly significant correlation coefficient of +0.37 between the percent of AI cells with bridges and/or fragments versus the percent of tetrads with micronuclei.

In his investigations into the meiotic behavior of a barley stock known to have an inversion, Kreft (1968) observed several abnormal configurations at both AI and AII. Some of these abnormalities were 1 bridge and 1 fragment, 1 bridge only, 1 fragment only, 2 bridges and 2 fragments, 1 bridge and 2 fragments, and large lagging fragments. It was noted that as the percentage of abnormal configurations increased the degree of plant fertility decreased.

There have been many other studies investigating inversions in plants: Dhaliwah, Pollard, and Lorz (1962); Doughty (1936); Honma (1968); Ising (1960); Jenkins and Thomas (1939); Matsuura (1950); Morgan (1950); Muntzing (1934 and 1935); Richardson (1936); Smith (1935); Sutton (1937); and Upcott (1936).

MATERIALS AND METHODS

Individuals from a single clone of Chlorophytum elatum R. Br. were used in this investigation. It was supplied through the courtesy of the Hong Kong Baptist College.

Chlorophytum elatum has white, thickened often tuberous roots. The many dark green leaves are fasciculate, relatively short and broad (30 centimeters long, 10 to 17 millimeters), long pointed, and narrowed into petiole-like bases. It produces stolons (Figure 1-A) and has whitish flowers (Figure 1-B), in a long open raceme, 20 millimeters across, rotating with narrow separate segments. The seeds (Figure 1-D) are black, flattened or disc-like and are borne in acutely 3-angled capsules (Figure 1-C).

For the somatic chromosome study, plants were repotted once every month to initiate new root growth. Healthy roottips 1-2 centimeters long, were collected at noon and placed in a vial containing 0.4 percent aqueous colchicine solution. The roottips were aspirated for 30 minutes to ensure good penetration of the colchicine solution into the tissue, then put into a refrigerator at 5^{o} C. Four hours later the roottips were transferred into freshly prepared Carnoy's fixative (a mixture of 1 part glacial acetic and 3 parts absolute alcohol) and stored in a refrigerator at 5^{o} C. for 48 hours or longer. The roottips were then cleaned with a small camel hair brush, hydrolyzed in N HCl at 60^{o} C. for 9 minutes, and returned to Carnoy's fluid

Figure 1. (A) Chlorophytum elatum plant showing vegetatively reproductive stolons (B) a flower (C) a capsule (D) seeds.

for 10 minutes before the microscopic slides were made. In preparing slides, the aceto-orcein squash technique as described by Darlington and LaCour (1960) was applied.

Measurements for idiogram construction and karyotypic analysis were made from 20 cells showing well-spread metaphase chromosomes. These measurements included long arm length, short arm length, and total length of each of the chromosomes in a cell. In order to minimize errors three measurements for each item were made and a mean was calculated to represent the actual length. All measurements were made under an oil immersion objective lens using a filar micrometer eyepiece attached to the microscope.

Arm indices were calculated for each chromosome in a cell by dividing the long arm length into the short length. The chromosomes were then grouped according to the presence or absence of satellites and the position of the centromere.

For meiotic chromosome studies, flower buds 2-4 millimeters long, were collected between noon and 3:00 P. M. and fixed immediately in freshly prepared Carnoy's fixative for 48 hours. They were then transferred to 70 percent ethanol and stored at 5^{o} C.

Microscope slides for the meiotic study were made using the propionic-carmine squash technique according to Darlington and LaCour (1960).

RESULTS

The somatic chromosome number of this clone was found to be 2N = 28. This number agrees with the findings of Sato (1942) and Storey (1968). Figure 2 shows a cell at mitotic metaphase prepared from a roottip of the plant.

In studying the karyotype it was assumed that chromosome contraction, affected by the pretreatment of colchicine, would be proportional to its length regardless of the colchicine concentration or the duration of mitotic arrest. Thus the 2 longest chromosomes in one cell would be homologous with the 2 longest chromosomes of another cell.

The validity of this assumption was tested statistically using the principle of correlation. A scatter diagram was constructed by plotting the mean length of the 5 shortest chromosomes against the length of the longest chromosome in the same cell. Figure 3 is a scatter diagram showing a positive relationship between the 2 categories of chromosomes. A significant correlation coefficient of $r = +0.675$ then was calculated indicating that chromosomal contraction was proportional to length.

The averages of chromosome lengths, long arm lengths, short arm lengths, and arm ratios for each individual chromosome are presented in Table 1. The lengths of the chromosomes measured, under the

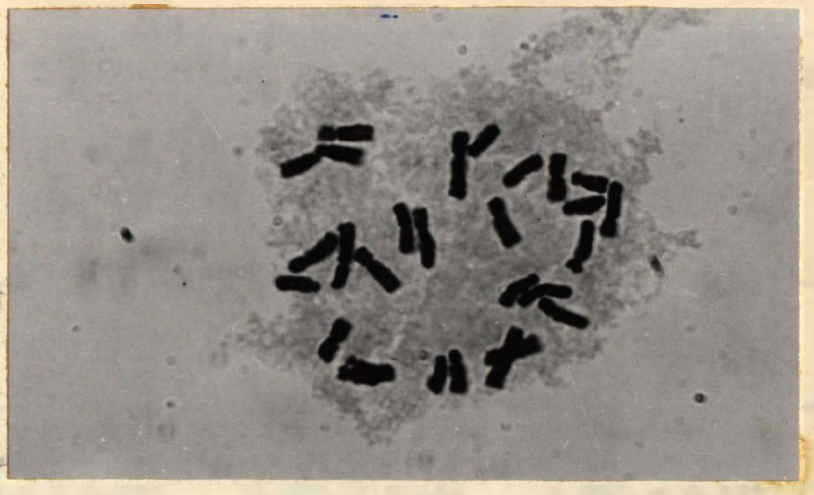

Figure 2. A mitotic metaphase cell of Chlorophytum elatum showing 28 chromosomes, X1200.

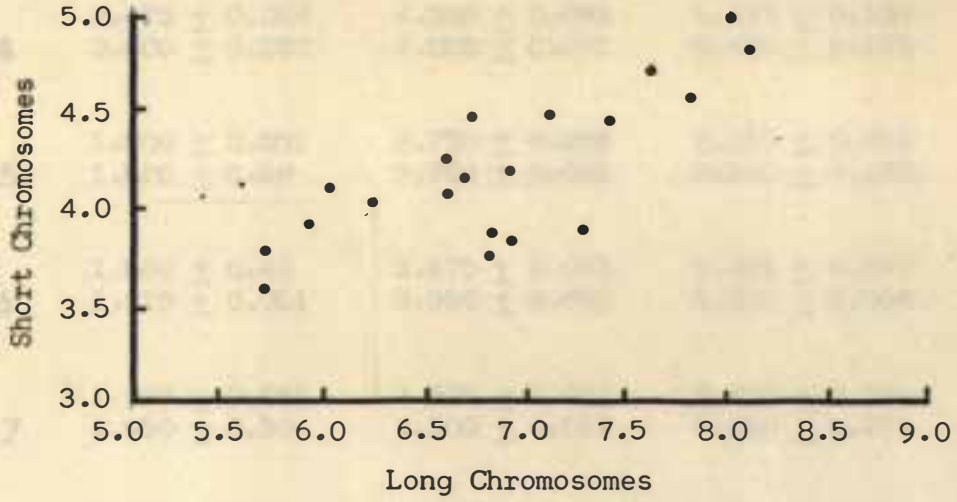

Figure 3. A scatter diagram of co-variation of long and short chromosomes of Chlorophytum elatum.

TABLE I

ARM LENGTHS, CHROMOSOME LENGTHS, AND ARM RATIOS

OF CHLOROPHYTUM ELATUM

No.	Short Arm	Long Arm	Total	Arm Ratio
1	2.400 ± 0.071	3.900 ± 0.052	6.300 ± 0.140	0.6153
	2.350 ± 0.053	3.875 ± 0.064	6.225 ± 0.115	0.5949
2	2.400 ± 0.045	2.475 ± 0.026	4.875 ± 0.045	0.9696
	2.325 ± 0.042	2.325 ± 0.045	4.650 ± 0.50	1.0000
3	2.250 ± 0.054	2.325 ± 0.071	4.575 ± 0.073	0.9677
	2.200 ± 0.028	2.325 ± 0.057	4.525 ± 0.069	0.9462
4	2.475 ± 0.084	4.200 ± 0.093	6.675 ± 0.134	0.5892
	2.400 ± 0.087	4.050 ± 0.082	6.450 ± 0.132	0.5925
5	1.800 ± 0.051	3.750 ± 0.039	5.550 ± 0.093	0.4800
	1.800 ± 0.69	3.700 ± 0.053	5.500 ± 0.128	0.4864
6	1.800 ± 0.42	3.675 ± 0.071	5.475 ± 0.077	0.4897
	1.825 ± 0.051	3.550 ± 0.092	5.375 ± 0.064	0.5140
7	1.875 ± 0.061	3.525 ± 0.033	5.400 ± 0.044	0.5319
	1.950 ± 0.30	3.500 ± 0.063	5.450 ± 0.079	0.5571
8	2.025 ± 0.061	3.150 ± 0.092	5.175 ± 0.058	0.6428
	1.875 ± 0.041	3.225 ± 0.063	5.100 ± 0.053	0.5813

TABLE I (CONCLUDED)

No.	Short Arm	Long Arm	Total	Arm Ratio
9	1.125 ± 0.052	4.275 ± 0.085	5.400 ± 0.057	0.2631
	1.150 ± 0.049	4.150 ± 0.067	5.300 ± 0.045	0.2771
10	1.425 ± 0.054	3.375 ± 0.092	4.800 ± 0.051	0.4222
	1.400 ± 0.044	3.300 ± 0.064	4.700 ± 0.057	0.4242
11	0.900 ± 0.072	3.450 ± 0.059	4.350 ± 0.076	0.2608
	0.825 ± 0.068	3.500 ± 0.048	4.325 ± 0.072	0.2357
12	1.275 ± 0.057	3.000 ± 0.086	4.275 ± 0.128	0.4250
	1.250 ± 0.053	2.950 ± 0.048	4.200 ± 0.114	0.4237
13	1.125 ± 0.055	3.000 ± 0.025	4.125 ± 0.052	0.3750
	1.125 ± 0.060	2.925 ± 0.053	4.050 ± 0.055	0.3846
14	1.200 ± 0.036	2.775 ± 0.026	3.975 ± 0.025	0.4324
	1.125 ± 0.037	2.700 ± 0.045	3.825 ± 0.041	0.4166

conditions described, varied from 3.8μ to 6.6μ. It appears that these chromosomes form 14 pairs and none of the pairs were detected to be heteromorphic according to pairing t-tests.

Based upon the location of the centromere and the presence or absence of satellites, the somatic chromosomes of this plant can be further classed into 4 groups; satellite group, median group, sub-median group, and sub-terminal group.

Satellited Group (SAT).

Two chromosomes are included in this group and are numbered as Chromosome 1 in Table 1. This chromosome pair was 6.262μ long and submetacentric therefore may be consistently distinguished from the rest of the chromosomes.

Median Group (M).

Chromosomes having their arm ratios between 0.90 and 1.00 were placed in this category. The chromosomes of this group had mean arm ratios ranging from 0.95 for Chromosome 3 to 0.98 for Chromosome 2 (Table 1). The total lengths for the chromosomes in this group varied from 4.525μ to 4.875μ. The differences in lengths and arm ratios of the chromosomes in this group were not large enough to ascertain which were homologous pairs. In all cells measured, the arm ratios of the M group chromosomes were very distinct from those of the SM group and ST group thereby making the M chromosomes an identifiable group.

Sub-Median Group (SM).

Chromosomes having arm ratios between 0.45 and 0.85 were placed in this group and as noted in Table 1, there were 5 pairs of sub-median chromosomes identified. These chromosome pairs were designated Chromosome 4 through Chromosome 8 and their arm ratios ranged from 0.48 to 0.61. Morphologically the SM group had within it 2 distinct chromosome pairs; Chromosome 4 and Chromosome 7. Slight morphological differences among the other chromosomes of the group made it impossible to positively identify homologous pairs.

Sub-Terminal Group (ST).

Chromosomes with arm ratios between 0.22 and 0.44 were placed in the sub-terminal group. Twelve chromosomes constituted the sub-terminal group and included Chromosomes 9 through 14. Although the arm ratios of Chromosome 9 and Chromosome 10 are almost identical, they are easily distinguishable on the basis of total length. Differences in total chromosome lengths also eliminates error in ascertaining pairing relationships in the otherwise morphologically similar ST pairs 10, 12, and 14. In considering total length alone, Chromosome pair 13 is very similar to Chromosome pairs 12 and 14 but the arm ratio values make Chromosome 13 a separate and distinct pair.

From the information in Table 1 an idiogram (Figure 4-A) was constructed to linearly arrange the karyotype of this clone of Chlorophytum elatum consisting of 14 pairs of chromosomes as shown in (Figure 4-B). Total length and arm ratio criteria, as shown in Table 1, rule out the presence of 7 groups of 4 chromosomes each.

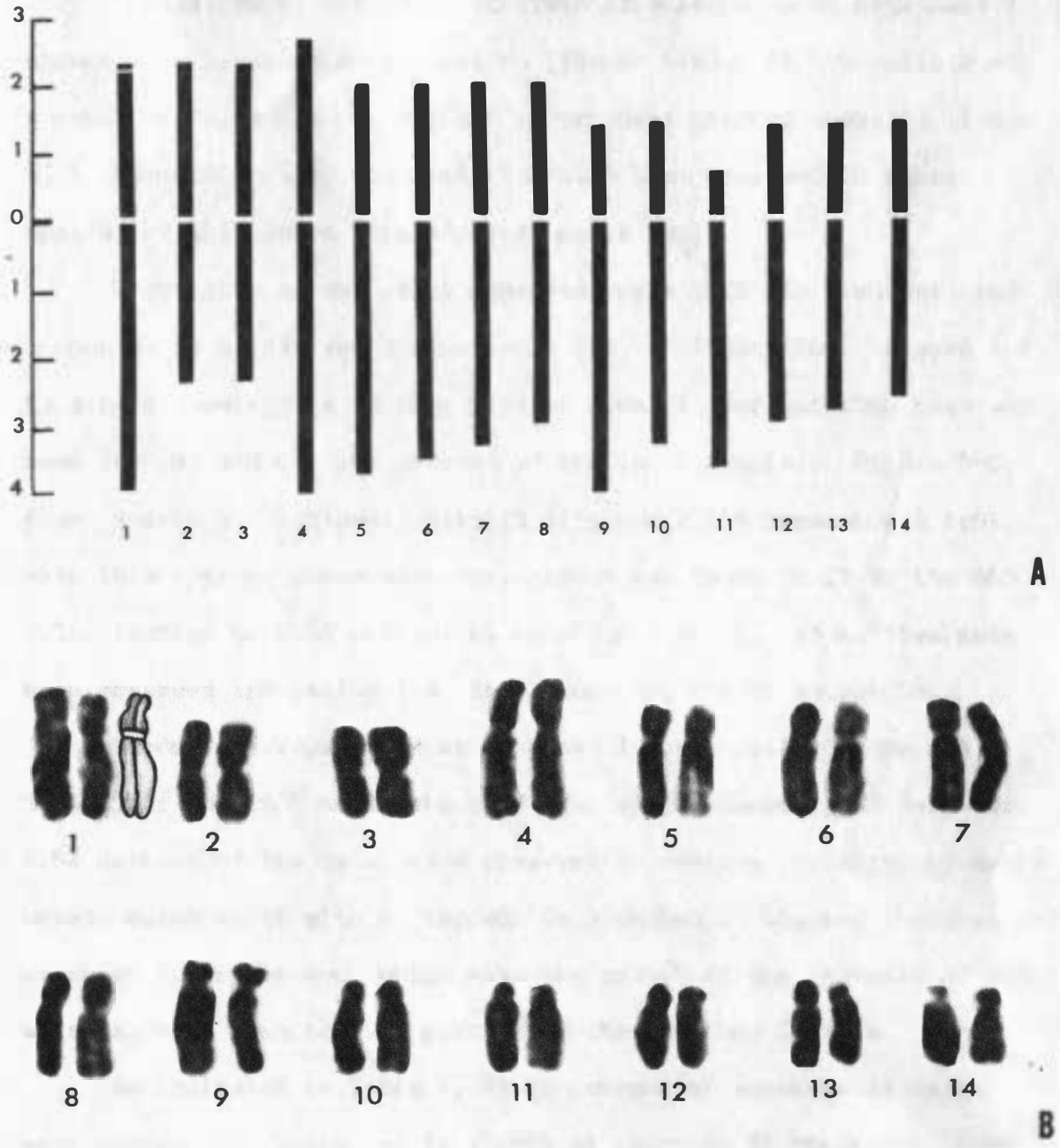

Figure 4. The idiogram and karyotype of Chlorophytum elatum (A) the idiogram (B) the karyotype X3500.

A majority of the cells observed at diakinesis or metaphase I showed a 14 bivalent configuration (Figure 5-A). Of 665 cells observed, 642 or 96.54 percent had chromosomes pairing normally (Table 2). Association into bivalents has also been observed in other species of this genus (Baldwin and Speese 1951).

A fraction of the cells observed had a 13II (13 bivalent) and 1 open II or a 13II and 2 univalents (2I) configuration. Figure 5-B is a cell showing the 13 II's plus an open II configuration that was seen in 6 of 665 or 0.90 percent of the cells studied. Figure 5-C shows a cell at diakinesis with 13 II's and 2 I's present. A cell with this type of chromosome arrangement was found in 17 of the 665 cells studied or 2.56 percent as shown in Table 2. No multivalents were observed indicating that this clone was not an autoploid.

Chromosome separation at anaphase I was normal (Figure 6-A) in 1525 of the 1565 cells observed (Table 2). However, 24 cells or 1.54 percent of the total were observed to contain a single chromatin bridge accompanied with a fragment (Figure 6-B). Lagging chromosomes as shown in Figure 6-C, which were the result of the presence of univalents, were seen in 1.02 percent of the anaphase I cells.

As indicated in Table 2, 97.28 percent of anaphase II cells were normal and Figure 7-A is a cell at anaphase II showing a clean separation of chromatin material. No anaphase II cells contained a bridge, however 12 of 496 or 2.42 percent of the anaphase II cells did

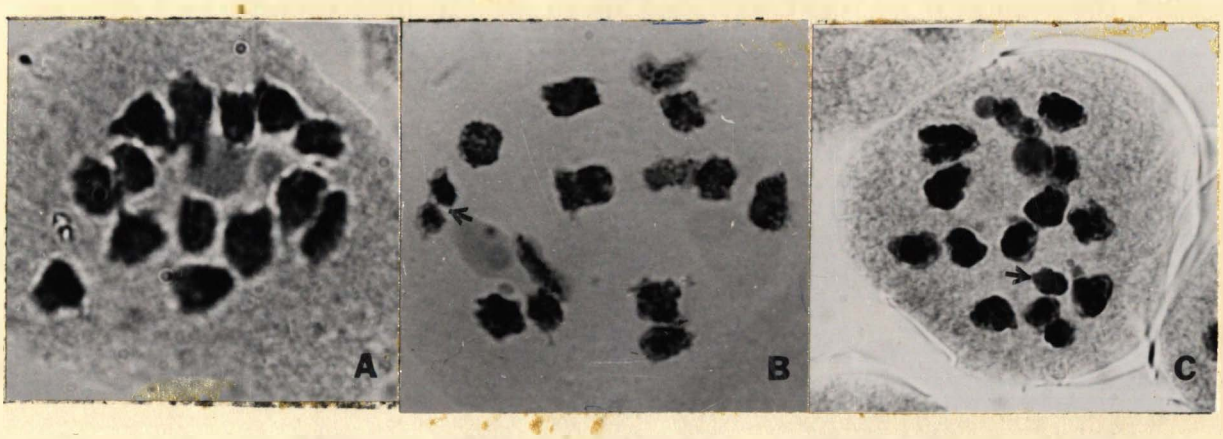

Figure 5. A cell at diakinesis (A) with 14 bivalents X1400 (B) with 13 bivalents and 1 open bivalent (indicated by arrow) X1400 (C) with 13 bivalents and 2 univalents (indicated by arrow) X1400.

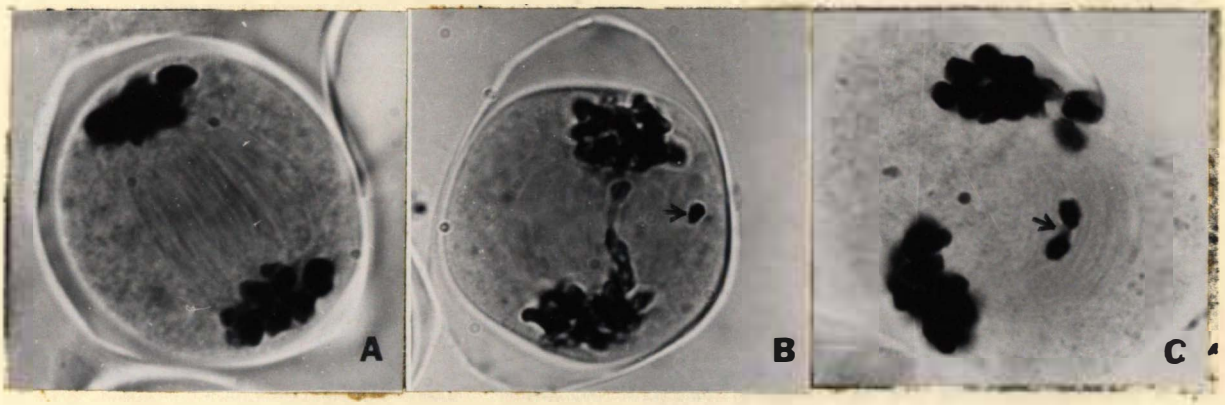

Figure 6. A cell at anaphase I showing (A) normal separation of chromosomes X1200 (B) a bridge and a fragment (indicated by arrow) X1200 (C) with lagging chromosome (indicated by arrow) X1100.

TABLE II

CHROMOSOME CONFIGURATIONS OBSERVED AT VARIOUS MEIOTIC STAGES IN POLLEN MOTHER CELLS OF A CLONE OF CHLOROPHYTUM ELATUM

Diakinesis or Metaphase I

	14II	13II + 1II (open)	13II + 2I	Total
Number of Cells	642	6	17	665
Percentage	96.54	0.90	2.56	100

Anaphase I

	Normal Cells	Cells with Bridge Fragment	Cells with Lagging Chromosome	Total
Number of Cells	1525	24	16	1565
Percentage	97.44	1.54	1.02	100

TABLE II (CONCLUDED)

Anaphase II

	Normal Cells	Cells with Fragments	Total
Number of Cells	484	12	496
Percentage	97.58	2.42	100

Tetrads

	Normal Tetrads	Tetrads with Micronuclei	Total
Number of Cells	672	19	691
Percentage	97.25	2.75	100

contain fragments as shown in Figure 7-B. These fragments remained at the equatorial planes because they lacked centromeres.

At telophase II, of 691 tetrads observed, 672 or 97.25 percent were normal. Figure 7-C shows a tetrad with no micronuclei while Figure 7-D is a tetrad with a micronuclei present. Pollen grains developed from the tetrads with micronuclei would probably have been non-viable.

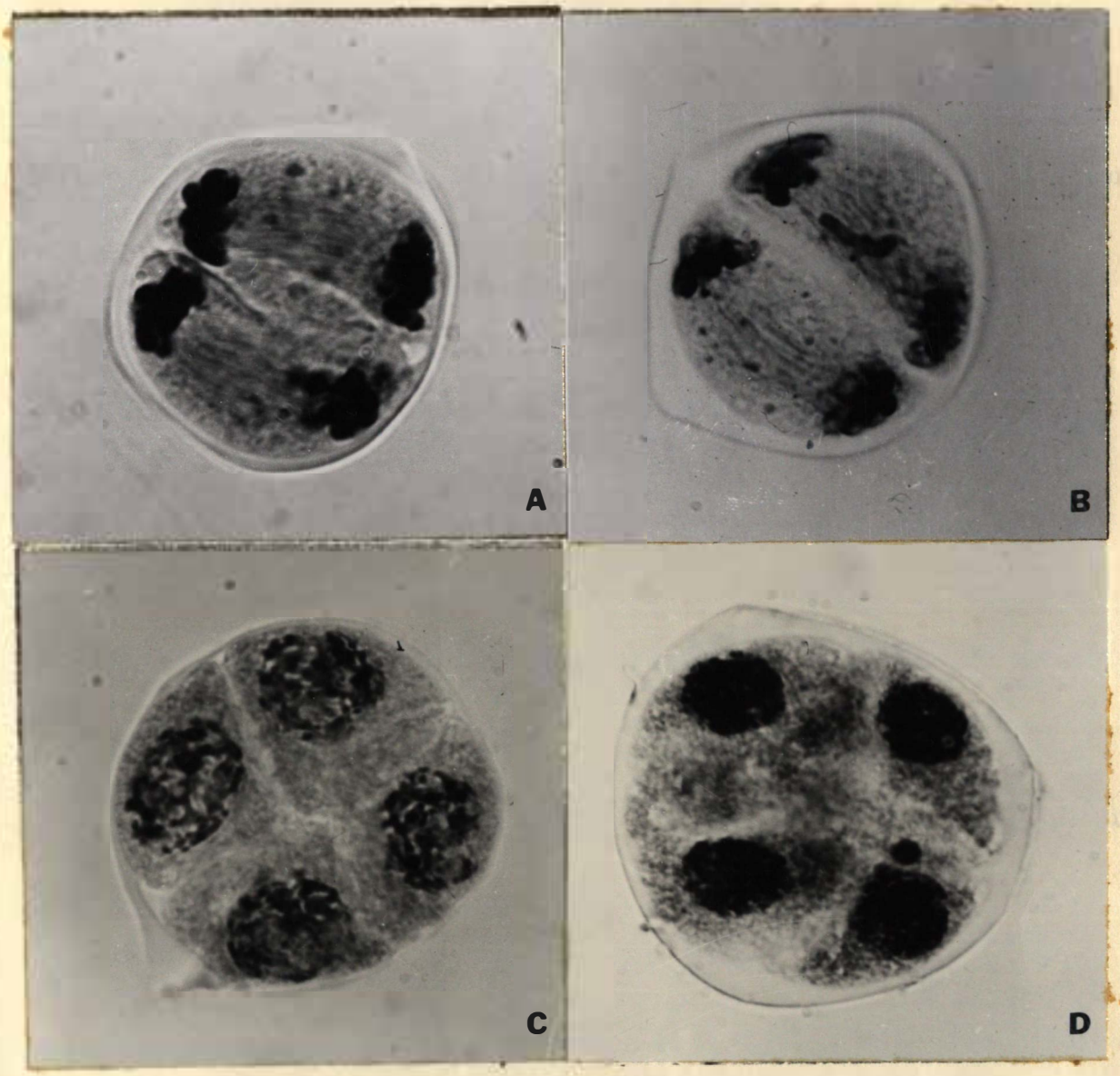

Figure 7. A cell showing (A) normal chromosome separation at anaphase II X1100 (B) fragment at anaphase II X1100 (C) no micronuclei at the tetrod state X1300 (D) micronuclei at the tetrad stage X1300.

DISCUSSION

In determining the nature of ploidy in Allium spp., Mensinkai (1939) observed 14 bivalents but no quadrivalents in the pollen mother cells of Allium Bidwelliae S. Wats (2N = 28) and considered the species to be an allotetroploid with a basic number X = 7. Levan (1933) identified Allium Schoenoprosum var. sibricum as an autotetraploid because of the 4-chromosome associations he observed in meiosis. Upcott (1937) also found the formation of quadrivalent chromosomes in autotetraploid Tulipa spp. and associations no higher than bivalents in allotetraploid species of Tulipa. A similar conclusion was made by Emerson and Beadle (1930) and Randolph (1935) in maize and in studies of rice (Chen and Po (1957) and sorghum (Ross and Chen 1962).

Since no more than two chromosomes associated together at meiosis and since no more than two chromosomes having the same predominant satellite were observed at mitosis in this study, autotetraploidy in Chlorophytum elatum, as proposed by Storey (1968), appears to be ruled out. If the basic number X = 7, as proposed by Wet (1957) and Yamazaki (1936) for the genus Chlorophytum, is accepted then Chlorophytum elatum should be considered to be either amphidiploid or allotetraploid.

The occurrence of a single chromatin bridge with an acentric fragment is an indication of the presence of a heterozygous paracentric inversion in the pollen mother cells of Chlorophytum elatum.

A chromatin bridge accompanied with an acentric fragment will occur at anaphase I as a result of a single crossover within the inverted segment. The frequency with which such crossovers take place depends upon the length of the inverted segment, its location in the chromosome, and the crossover characteristics of the individual. Doughty (1936), Honma (1960), Ising (1969), Flagg (1958), Frankel (1937), and Matsuura (1950) arrived at this conclusion as to the origin of the single chromatin bridge plus a fragment at anaphase I.

It is quite evident that the chromatids involved in the crossover within the inversion loop will be rearranged genetically. The acentric fragment will be lost, having no capacity for movement at anaphase and the bridge will be broken by the stress of anaphase movement or by the cell wall cutting across it. Frankel (1937), Sutton (1937), and Upcott (1937) concluded that micronuclei present in tetrads were the result of the presence of these acentric fragments. Hill (1962) found a significant correlation between the presence of bridges in the first anaphase cells and micronuclei at the tetrad stage.

It would be expected that breakage of the dicentric chromatin bridge and loss of the acentric fragment would lead to inviability in the haploid cells arising from meiosis thereby affecting plant fertility. Studies by Muntzing (1935), Holm (1960) and Kreft (1968) found a direct relationship between reduced pollen fertility, the number of tetrads with micronuclei, and the presence of chromatin

bridges and/or fragments. Similar results were obtained by Henderson et al. (1949) and Yao et al. (1958) in their studies of intervarietal crosses of rice.

Since the observed occurrence of open bivalents (6 of 665 cells) and of univalents (17 of 665 cells) was very low the univalents and open bivalents observed at diakinesis may be due to environmental factors. The univalents may pass to one pole without dividing at the first division of meiosis and divide normally at the second; but there is a strong tendency for them to lag at the first division. At times they may divide at the first division and then lag at the second. In either case, the lagging chromosomes usually are not included in the nuclei resulting from meiosis, but appear as micronuclei in the quartet of spores (c.f. Burnham 1962).

The chances for the accumulation of chromosome aberrances in plants reproducing vegetatively are much higher than in those plants reproducing sexually. Sexually reproducing plants require a union of a viable sperm and egg. Abberant chromosomes that cause sperm, egg, and/or seed mortality will not be carried on because the resulting generation will not be produced or at least will not reach maturity. The abberant chromosomes can be carried on, more aberrations may occur and accumulate in vegetatively reproducing plants. This is the reason why Upcott (1937) found such a high frequency of bridges with fragments in her studies of sterile Tulipa clones that were reproducing vegetatively.

The determination of polyploidy is significant to both the evolutionist and the taxonomist. The greater portion of our knowledge of polyploidy, as an evolutionary mechanism, has come from a comparison of natural polyploids with their supposed diploid relatives (c.f. Swanson 1961). Burham (1962) stated that it was now possible, in the laboratory, to resynthesize polyploid species from their suspected diploid parents, thus providing critical proof of the hypothesis that hybridization between species, followed by chromosome doubling, has taken place time and again to form new species. The knowledge of whether a plant, in this instance Chlorophytum elatum, is auto or alloploid aids in finding the plant's origin and tracing its phylogenetic lines.

SUMMARY

Cytological investigations were made to ascertain the ploidy and the cause of sterility in a clone of the spider plant, Chlorophytum elatum R. Br.

The somatic chromosome study showed that this plant had a karyotype consisting of 1 satellited pair, 2 median pairs, 5 sub-median pairs, and 6 sub-terminal pairs of chromosomes. There were not groups of 4 chromosomes each but rather 14 chromosome pairs indicating the clone was not autoploid. This was further supported by meiotic studies in which no multivalents but 14 bivalents were observed. Since X = 7 is considered to be the basic number for the genus Chlorophytum, it may be concluded that this clone was an allotetraploid.

Meiotic irregularities such as a chromatin bridge with a corresponding fragment and in other cases lagging chromosomes were observed at anaphase I, indicating the presence of a heterozygous paracentric inversion in one chromosome pair. The lagging chromosomes were the result of chromosomes which occasionally did not pair and this lack of pairing may be merely due to an environmental factor. Chromosome abnormalities such as fragments and micronuclei were observed at second meiotic division and were the result of the inversions and unpaired chromosomes found in the first meiotic division. An anaphase I aberration frequency of 2.56 percent contributed to the 24.08 percent pollen sterility that was observed.

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