THESIS

CHROMOSOME STUDIES OF *ALSTROEMERIA PELEGRINA* L.

Submitted by

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In partial fulfillment of the requirements

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WE HEREBY RECOMMEND THAT THE THESIS CHROMOSOME STUDIES OF *ALSTROEMERIA PELEGRINA* L. PREPARED UNDER OUR SUPERVISION BY JANICE L. STEPHENS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE.

Committee on Graduate Work

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ABSTRACT OF THESIS

CHROMOSOME STUDIES OF *ALSTROEMERIA PELEGRINA* L.

Karyotype analysis is one of the approaches to determine the parental species used for hybrid variety breeding. *Alstroemeria pelegrina* L. is considered to be one of the primary parents used in the development of many commercial cultivars of *Alstroemeria*. Somatic chromosomes were prepared from root tip cells using the acetocarmine squash method. Measurements from these cells were then used to develop a karyotype for *A. pelegrina* L..

The genome of *A. pelegrina* L. was shown to consist of two groups of four chromosomes each. The first group consists of metacentric or submetacentric chromosomes in which the smallest submetacentric is satellited. The second group consists of four acrocentric chromosomes in which chromosomes 5, 7 and 8 are satellited.

The small submetacentric satellited chromosome in the first group provides a marker chromosome which is unique to *A. pelegrina* L.. The presence of this chromosome in the karyotype of a cultivar would indicate that *A. pelegrina* L. was a parent of that cultivar.

Although *A. pelegrina* L. was shown to have a very similar karyotype for all the plants studied there were some differences between them. This indicates that chromosomal changes may be occurring within the species in individual plants resulting in karyotypic polymorphism..

A study of the anthers from immature buds at anaphase I showed 8-8 separation without any abnormality. The stages of diakinesis or metaphase I showed that chromosome pairing was normal with 8 bivalents.

Pollen viability varied from 44.7% in *A. pelegrina* L. 'rosea' to 80% in *A. pelegrina* L. 'alba'.

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CONTENTS

LIST OF FIGURES

LIST OF TABLES

Chapter 1

INTRODUCTION

Alstroemeria has become a popular cut flower in recent years with most of the cultivars having originated from polyploidization and hybridization of various parental species as well as from mutagen treatment (Broertjes and Verboom, 1974; Verboom, 1980).

Detailed studies of the chromosomes of species and cultivars will provide some information on the origin of the cultivars. Once the parental species are known and documented it will be possible to develop new varieties by a systematic and organized breeding program.

At the present time *Alstroemeria* growers in the United States are required to pay substantial royalties to the Dutch breeders which is assessed on the square footage in production. Plants are normally leased to the greenhouse owner from the breeder (Healy and Wilkins, 1981).

Since the pedigrees of most of the cultivars are known only to the original breeders one of the objectives of *Alstroemeria* cytology is the determination of the parental species used in cultivar development. Detailed karyotype analysis of wild species will provide the information on chromosomal constitution necessary to establish the probable parental species utilized for cultivar development in *Alstroemeria*.

Thus it is possible to produce our own unique cultivars for use in the United States through the detailed study of the karyotype of the species known to have been used as parents for cultivar production. Comparison of these karyotypes with those seen in commercial cultivars will provide breeders with the information needed

to begin a successful breeding program. This will result in substantial savings to local growers.

Although chromosome studies in the genus *Alstroemeria* were first reported in by Strasburger as early as 1882 they have been spasmodic and rather limited. More recent studies by Tsuchiya and Hang (1987, 1990) and Hang and Tsuchiya (1988) have contributed considerably to our knowledge and understanding of the chromosomes of several cultivars and species of *Alstroemeria*.

However, during their preliminary studies Tsuchiya and Hang (1990) found a possible karyotype polymorphism in some species.

It is important to study chromosomal constitution including karyotypic polymorphism in detail in each species in order to use it for analysis of the origin of various cultivars.

This research focuses on the analysis of karyotype of *A. pelegrina* L. including karyotypic polymorphism. This work also provides some information on meiotic chromosome behaviour and pollen fertility of several plants in this species.

Chapter 2

LITERATURE REVIEW

2.1 Background

Alstroemeria pelegrina was first mentioned in the writings of Father Fueillée in 1714. This Spanish botanist said that it was the favorite flower of the Incas. It is native to the coastal sand dunes of Chile and Peru and was found in the gardens of the Inca rulers of Peru. It is quite possible that this is the origin of the name Peruvian Lily. It was introduced to Kew gardens in 1753 (Robinson, 1963).

Carolus Linnaeus received seeds from his friend Alstroemer who had collected them from Father Fueillée in Peru. The genus was subsequently named in honor of Alstromer and first species received was named *Alstroemeria peregrina*. There is a description and illustration of this in Linnaeus' work (Linnaeus, 1762).

J. G. Baker (1888) indicated that the habitat of *A. Pelegrina* was "Hab. Chile, near Valparaiso etc." Uphof (1952) states that "apparently the center of distribution of the genus is Chile and adjacent territory. Some species have a wide distribution like *A. pelegrina* and *A. ligtu*".

The taxonomic placement of *Alstroemeria* is as follows:

Kingdom :	Plantae
Division:	Anthophyta
Class:	Monocotyledones
Super Order:	Liliiflorae
Order:	Liliales
Family:	Alstroemeriaceae
Genus:	Alstroemeria

Initially the alstroemeria were described by Father Feuillée under the name of *Hemerocallis* (Uphof, 1952). Herbert (1837) recognized 29 species of *Alstroemeria* which he included in the family *Amaryllidaceae* (Stinson, 1942). The genus remained in this family until Hutchinson (1934) recognized it as a genus in the family *Alstroemeriaceae* (Uphof, 1952).

By 1985 however Dahlgren *et al.* (1985) had further investigated the phylogeny of the genus and concluded that it should be classified as above.

The genus *Alstroemeria* is composed of herbaceous perennials with roots which have thickened to form rhizomes. These roots are modified to store nutrients and water and often contain a considerable amount of starch. The stems may be up to 4m long or so small that they are barely visible above the surface (*A. pygmaea*). The leaves are turned upside down, or resupinate, due to the twisting of the base. The leafbase is more or less sessile with the leaves being lanceolate to linear in shape.

The branches end in umbel-like inflorescences which may have from one (seldom, *A. pygmaea*) to 10 to 30 flowers (*A. aurantiaca*). The flowers are often subtended by green, leaf-like bracts which vary from very minute (*A. apertiflora*) to quite large (*A. caryophyllaea*). The brightly colored flowers are often spotted and are trimerous, epigynous, bisexual and actinomorphic or slightly zygomorphic. The lower segment of the inner cycle differs in shape and coloring from the other two members whereas the three segments of the outer cycle differ in shape from the three in the center.

There are 3+3 stamens which have narrow filaments and small oblong anthers which are attached by the base. The ovary is inversely conical and three celled. Each cell contains many superimposed ovules. The single style is filiform, the stigma being separated about half way down into three parts or lobes with the stigmatic surface being of the wet type. The fruit is a dehiscent, loculicidally 3-valved capsule and the seeds are globose (Dahlgren *et al.*, 1985; Uphof, 1952).

A. pelegrina has stems about 30cm or less high with about 30 thin ascending leaves which are 5cm or less long and 1.25cm or less wide. The flowers are usually

pink or white. There is a greater or lesser degree of marking with red or deep pink on the two erect perianth segments, the others being usually flushed with the same color (Bailey, 1947; Robinson, 1963). This can be seen in figure 2.1.

Many of the cultivars of *Alstroemeria* are polyploids, however there are some which are diploid (Tsuchiya and Hang,1987, 1990; Tsuchiya *et al.*, 1987; Hang and Tsuchiya, 1988,). Because of the lack of detailed information on the pedigree of many of these cultivars it is difficult to determine their true origin.

Examination of a total of 25 cultivars by Tsuchiya and others showed that only four were diploid (2n=2x=16) while eight cultivars were tetraploid (2n=4x=32) or near tetraploid (2n=4x-1=31, 2n=4x+1=33), twelve were triploid (2n=3x=24) and one was hypertriploid (2n=3x+1=25).

The four diploid cultivars 'Eureka', 'Zebra', 'Canaria' and 'Orchid' clearly show that the two genomes are not homologous, suggesting hybrid origin of two parents with different karyotypes (Hang and Tsuchiya, 1988). Three of the triploid cultivars contain two homologous or near homologous genomes and one different genome. The other triploid cultivars have three different genomes (Hang and Tsuchiya, 1988). Analysis of the tetraploid cultivar 'Luciana' suggests that it may contain two genomes from each of two different parents whereas 'Jubilee' which is also tetraploid probably has three sets of one genome and one set of another genome (Hang and Tsuchiya, 1988).

Brief analysis of twelve species (Tsuchiya and Hang, 1987; 1990) showed that they were all diploid (2n=2x=16). Meiosis generally showed eight bivalents at metaphase I and pollen fertility ranged form 80 to 98 percent.

2.2 Karyotype analysis

Taylor (1926) reported on several studies of *Alstroemeria* species by Strasburger and Guignard as well as on his own observations. Strasburger (1882) worked with

Figure 2.1: Examples of *A. pelegrina* 'rosea' and 'alba'.

A. chilensis Lood. and observed n=8 chromosomes in microspore development. His figures would seem to indicate little more than a slight difference in size between the chromosomes.

Guignard (1884) studied *A. pelegrina* and *A. psittacina* which both showed n=8 chromosomes. *A. pelegrina* chromosomes were found to exhibit both median and terminal fiber-attachment.

Taylor (1926) studied the species *A. braziliensis* and found that root tips showed 2n=16 chromosomes. These are of quite different sizes and he placed them into six classes. In the first class he lists the largest chromosome pair which "has the fiber attachment constriction near its center". The second pair he describes as long chromosomes with well developed short arms about 2-3 times as long as broad. Next there are three pairs of acrocentric chromosomes as well as one pair of acrocentrics with satellites. The seventh pair is similar to the second except that it is much shorter. The eighth pair has submedian fiber attachment but is much smaller than the first pair.

Whyte (1929) published a study of the chromosomes of *Alstroemeria* and *Bomarea*. He used both microtome sections and the smear method and found that the smear preparations provided a more accurate depiction of meiosis. Whyte (1929) studied the chromosomes of *A. aurantiaca*, *A. pulchella* and *A. haemantha* and concluded that they all agree with the account given by Taylor (1926) for *A. braziliensis*. The somatic chromosomes studied in root tip mitoses were then grouped into six classes.

There was a pair of large metacentric chromosomes, a large submetacentric pair with satellites, a pair of acrocentric chromosomes with satellites, three pairs of shorter acrocentric chromosomes, a small submetacentric pair and a small metacentric pair.

In observations of the meiotic divisions of pollen mother cells of *A. pulchella* a number of bivalent chromosome types were noted. These chromosome types were classified according to the shape of the bivalent pair during metaphase I.

Whyte's study (1929) of the eight pairs of chromosomes of *Alstroemeria* confirms the description given by Taylor (1925) as outlined above, the only additional observation being that there is a large distal satellite on the large submetatcentric pair of chromosomes.

Sato (1938) studied the karyotypes of several families within *Amaryllidaceae* including two species of *Alstroemeria, A. chilensis* and *A. pulchella*, using microtome sections.

A. chilensis has 16 chromosomes. Two pairs of long chromosomes with submedian constriction and six pairs of short chromosomes of which four pairs have subterminal constrictions and the remaining pairs have submedian ones. One pair of long chromosomes has a satellite on the short arm, one pair of short chromosomes has a satellite at the proximal end and another pair of short chromosomes also has a satellite at the distal end.

A. pulchella also has 16 chromosomes but these were generally found to be shorter than those in *A. chilensis*. The chromosome complement has one pair of long chromosomes with submedian constrictions, one pair of median chromosomes with subterminal constrictions and six pairs of short chromosomes of which four pairs have almost terminal constrictions, one pair has a subterminal constriction and the remaining one pair has a median one. Of these chromosomes one pair of short chromosomes with an extremely subterminal constriction has a satellite at its proximal end.

The pair of median chromosomes was also thought to exhibit a secondary constriction in the distal arms and thus be SAT-chromosomes. It is suggested that this pair of median chromosomes in *A. pulchella* may be derived by an inversion of the short arm with a satellite of the long chromosomes in *A. chilensis*.

The book "Chromosome Atlas of Flowering Plants" (Darlington and Wylie, 1955) indicates also that La Cour found the chromosome numbers for *A. ligtu, A. rosea* and *A. campaniflora* to be 2n=16.

Tsuchiya and Hang (1987, unpublished data) studied 11 species and 25 cultivars in the genus *Alstroemeria.* All 9 species had 2n=2x=16 chromosomes in agreement with the work outlined above. The 11 species studied were *A. aurantiaca, A. psittacina, A. pulchella, A. pelegrina, A. versicolor, A. haemantha, A. chilensis, A. caryophyllaea, A. ligtu, A. siera* and *A. hookeri.* There was however considerable differences noted in the karyotypes of these species with some showing their heterozygous nature with one or more non-homologous pairs. Meiosis was normal and high pollen fertility was noted.

Of the 25 cultivars recorded four were 2n=2x=16, twelve were 2n=3x=24, one was 2n=3x+1=25, six were 2n=4x=32, one was 2n=4x+1=33 and one was 2n=4x-1=31. Meiotic behaviour was abnormal in all cultivars and pollen was almost completely sterile except for the hypertriploid 'Orange Beauty' and most of the tetraploids which showed low pollen fertility.

Preliminary results of karyotype analysis of the diploid cultivars (Hang and Tsuchiya, 1988, Tsuchiya *et al.*, 1987) indicate the hybrid origin of the cultivars 'Orchid', 'Canaria', 'Eureka' and 'Zebra'.

The genomes of these diploid cultivars consist of two groups of four chromosomes:

1. A large metacentric or submetacentric, a medium-sized submetacentric or subtelocentric, a small metacentric or submetacentric and a subtelocentric all without satellites.

2. Four acrocentric chromosomes of different sizes with very small short arms with or without satellites.

The six cultivars which showed the triploid chromosome number of 2n=3x=24 have different karyotypes although they all had 3 large metcentric or submetacentric chromosomes in common. The chromosome complements in the triploids ranged from three non-homologous genomes in the cultivars 'Yellow King' and 'Mona Lisa' to two homologous and one different genome in the cultivars 'King Cardinal', 'Appelbloesem' and 'Pink Triumph'. The cultivar 'Rosita' has a more complex karyotype. Its principal feature however is the presence of a small subtelocentric chromosome with a distal satellite on the short arm, which is not found in any of the other cultivars. The hypertriploid cultivar 'Orange Beauty' has one extra telocentric chromosome. The remaining chromosomes comprise two homologous genomes and a third genome which contains another special chromosome, the smallest subtelocentric with a tiny satellite on the short arm.

The tetraploid cultivars 'Luciana' (2n=4x-1=31) and 'Jubilee' (2n=4x=32) exhibit very different chromosome constitiutions. 'Luciana' probably consists of two different parental species or biotypes with approximately 12 pairs of chromosomes that seem to be homologous or almost homologous. 'Jubilee' has a chromosome complement which can be divided into two groups of two genomes each. Ten pairs were homologous or near homologous in chromosome size and shape. This cultivar also contained a unique chromosome, a medium sized submetacentric carrying a tiny satellite on the short arm. This chromosome is different from the medium sized submetacentric SAT-chromosome found in the cultivars 'Orange Beauty' and 'Rosita'.

Tsuchiya *et al.* (1987) found similar genomic arrangements in 10 other cultivars studied, with one group of 4 metacentric and/or submetacentric chromosomes of various sizes and a second group of four acrocentrics with tiny short arms with or without a satellite.

Studies of *A. ligtu* hybrids (Tsuchiya *et al.*, 1987) however show a considerable difference from that of other cultivars. The main differences are (1) a total of 10

chromosomes are metacentric, submetacentric or subtelocentric, rather than the 8 seen in other cultivars, (2) two submetacentric pairs of medium size carry satellites of different sizes on their short arms and (3) depending on the cell observed, one or two of the smallest acrocentric chromosomes showed a tertiary constriction at the distal segment of the long arm. Meiotic pairing in *ligtu* hybrids was almost normal with 8 bivalents in most of the sporocytes. This general karyotype of *ligtu* hybrids was also noted by Rustanius *et al.* (1990).

A summary of the information available on the chromosome numbers and karyotypes of various species and cultivars of the genus *Alstroemeria* is presented in Tables 2.1 and 2.2.

Table 2.1: Chromosome number and karyotype of species of genus *Alstroemeria*

Author	Species	Chromosome number	Karyotype
Strasburger, 1882	*A. chilensis*	$n = 8$	
Guignard, 1884	*A. pelegrina*	$n = 8$	Metacentric, telocentric
1891	*A. psittacina*	$n = 8$	
Taylor, 1926	*A. braziliensis*	$2n = 16$	2 large metacentric 2 submetacentric 6 acrocentric 2 acrocentric with satellites 2 small submetacentric 2 subtelocentric
Whyte, 1929	*A. aurantiaca* *A. pulchella* *A. haemantha*	$2n = 16$	2 large metacentric 2 submetacentric with distal satellites 2 small acrocentrics with proximal satellites 6 acrocentrics of equal length 2 small metacentrics 2 small subtelocentrics
Sato, 1938	*A. chilensis*	$2n = 16$	2 large metacentrics 4 small submetacentrics 2 small acrocentrics with proximal satellites 2 small acrocentrics with distal satellites 4 small acrocentrics

Table 2.1: Chromosome number and karyotype of species of genus *Alstroemeria* (continued)

Author	Species	Chromosome number	Karyotype
Sato,1938	*A. pulchella*	$2n = 16$	2 large submetacentrics 2 metacentrics with distal satellites 8 acrocentrics 2 acrocentrics with proximal satellites 2 subtelocentrics.
Goodspeed, 1940	*A. 'ligtu type'*	$2n = 32$	
La Cour, 1945	*A. ligtu*	$2n = 16$	
	A. rosea	$2n = 16$	
	A. campaniflora	$2n = 16$	
Tsuchiya and Hang 1990	*A. aurantiaca*	$2n = 2x = 16$	
	A. psittacina	$2n = 2x = 16$	
	A. pulchella	$2n = 2x = 16$	
	A. pelegrina	$2n = 2x = 16$	
	A. versicolor	$2n = 2x = 16$	
	A. haemantha	$2n = 2x = 16$	
	A. chilensis	$2n = 2x = 16$	
	A. caryophyllaea	$2n = 2x = 16$	
	A. hookeri	$2n = 2x = 16$	

Table 2.2: Chromosome number and karyotype of cultivars of genus *Alstroemeria*

Author	Cultivar	Chromosome number	Karyotype
Broertjes &	*A. 'Orchid'*	$2n = 2x = 16$	
Verboom, 1974	*A. 'Beauty'*	$2n = 2x = 16$	
	A. 'Edison'	$2n = 2x = 16$	
	A. 'Starosa'	$2n = 2x = 16$	
	A. 'Regina'	$2n = 3x = 24$	
Tsuchiya and Hang, 1987,	*A. 'Eureka'*	$2n = 2x = 16$	2 large metacentric or submetacentric
Tsuchiya *et al.*, 1987	*A. 'Zebra'*	$2n = 2x = 16$	2 medium submetacentric or subtelocentric
Hang and Tsuchiya, 1988	*A. 'Canaria'*	$2n = 2x = 16$	2 small metacentric or submetacentric
	A. 'Orchid'	$2n = 2x = 16$	2 subtelocentric without satellites 8 acrocentric with or without satellites.
	12 triploid cultivars	$2n = 3x = 24$	All triploids have three large metacentrics or submetacentrics in common. 'Rosita' includes a small subtelocentric with a distal satellite.
	A. 'Orange Beauty'	$2n = 3x + 1 = 25$	
	A. 'Luciana'	$2n = 4x - 1 = 31$	Includes one extra telocentric chromosome. The smallest subtelocentric in the genome has a satellite.
	6 tetraploid cultivars	$2n = 4x = 32$	'Jubilee' includes a medium submetacentric with satellite.
	A. 'Rosario'	$2n = 4x + 1 = 33$	
	A. litu hybrids	$2n = 2x = 16$	8 metacentric, submetacentric or subtelocentric 4 medium submetacentric with satellites, 4 acrocentrics with or without a tertiary constriction at the distal segment of the long arm

Chapter 3

MATERIALS AND METHODS

The plants of *A. pelegrina* L. used in this study are listed in Table 3.1 along with their source and in which part of this experiment they were used.

Table 3.1: *A. pelegrina* L. used in this study

Plant	Acquisition number	Color	Origin	Experiment
1	89H-7-8	rosea	Dr. S. Hirao, Japan	Mitosis
2	87H-10-4B	alba	Hortus Botanicus Gotoburgensis	Mitosis
3	87H-2-12	rosea	Dr. S. Hirao, Japan	Mitosis
4	87H-5-1		Mr. Salmon, Avon, England	Mitosis
5	87H-10-13		Hortus Botanicus Gotoburgensis	Mitosis
6	88H-3-1		Dr. R. Wygnanki, Chile	Meiosis
7	87H-10-2	alba	Hortus Botanicus Gotoburgensis	Meiosis
8	87H-2-4	rosea	Dr. S. Hirao, Japan	Meiosis

The plants were grown in a 1:1:1 mixture of peat, perlite and soil or a 2:2:1 mixture of perlite, sand and gravel to facilitate the collection of root tips for the study of mitotic chromosomes. No artificial lighting was used during the growing period and the temperatures ranged from approximately $11^{o}C$ at night to $17^{o}C$ during the day. Shading with 30% shade cloth was also provided.

3.1 Mitosis

Root tips were collected from September, 1989 until May, 1990 between 3 and 4 p.m. The plants were gently removed from the pots and white, actively growing root tips were cut and placed into vials of distilled water. The vials were then placed

into containers of ice and water ($1°C$) and stored in a refrigerator for 18 hours. A pretreatment time of less than 18 hours resulted in nicely constricted chromosomes but they were difficult to separate. The slightly longer pretreatment resulted in well separated chromosomes with clearly visible satellites.

After pretreatment, the root tips were transferred to different vials containing a 3:1 mixture of 95% ethanol:glacial acetic acid for fixation. The vials were then left at room temperature for 24 hours.

The fixed roots were then transferred to a solution of 0.7% carmine in 45% acetic acid (acetocarmine). Preparations were made using Tsuchiya's modified acetocarmine squash method (Tsuchiya, 1971). If squashes were attempted within 24 hours after staining it was difficult to work with the root tips and many broken cells were found. Trial and error determined that the root tips should remain in the acetocarmine for one week at which time chromosomes were darkly stained and squashes were still easy to prepare. Reasonable squashes could be attained up to one month after initially placing the root tips into the acetocarmine.

For each plant a minimum of 20 cells was examined to determine the chromosome number. A representative cell from each plant was then chosen to conduct a karyotype analysis. Chromosomes in this cell were well separated and secondary constrictions were visible. These cells were then photographed with a Carl Zeiss Photomicroscope II. Upon printing a total magnification of X1,200 was achieved. The slides were made permanent by applying several drops of a 10:1 mixture of 45% acetic acid and glycerol to the edge of the coverslip.

The chromosomes of the photographed cells were arranged into two groups and numbered from one to sixteen. The first group contained eight chromosomes which appeared to be metacentric, submetacentric or subtelocentric of different sizes. The second group contained eight acrocentric chromosomes. Three or four pairs of chromosomes within the complement had satellites. The length of all chromosomes,

excluding the satellites, was then measured and chromosomes in each of the two groups were arranged in order of descending size and renumbered from one to sixteen. Homologous pairs were then determined based on centromeric position, length of chromosomes and the presence or absence of satellites. These chromosomes could then be arranged to form a kayryogram.

Averages of the paired chromosomes were then determined for total length, relative length, index and ratio. A genome karyotype could then be determined for each plant studied and the relative values compared between genomes. Chromosome lengths cannot be used as a means of comparison since these lengths will vary due to degree of chromosome contraction at various mitotic stages, variations in chemical agents, the duration of pre-treatment or the squash technique itself. The relative length, however, is determined by dividing the absolute chromosome length of a particular chromosome by the sum total of all the chromosome lengths in the complement. The relative length of each chromosome is therefore expressed as a percentage of the total sum (Tjio and Hagberg, 1951).

Centromeric location was then calculated as the ratio of the long arm to short arm as described by Levan *et al.* (1964). The nomenclature system of Levan *et al.* was then followed to determine if the chromosomes were median, submedian, subterminal or terminal. The more common alternate terms metacentric, submetacentric, subtelocentric or acrocentric were utilized because they enjoy wider acceptability and understanding.

Karyotype analysis allows us to describe the genome of an organism with respect to chromosome number, length of chromosomes, position of centromere and size and presence or absence of the nucleolar organizing region. This analysis is usually conducted when the chromosomes are in mitotic metaphase.

In order to provide coherence between studies presented by different authors it is necessary to use a single, widely accepted nomenclature system. For this reason

the nomenclature for centromeric position proposed by Levan *et al.* (1964) has been utilized.

Centromeric location can be calculated as either the difference (d) = 1 - s or as a ratio (r) = l/s where l is the length of the long arm and s is the length of the short arm (Figure 3.1).

Assuming that a chromosome consists of ten arbitrary units the variation in centromeric position is from median to terminal. One half of a chromosome is then subdivided into the regions indicated in Table 3.2.

The term telocentric would thus equate to the terminal point T of the system of Levan *et al.* (1964), while acrocentric refers to those chromosomes termed t in which the ratio of long to short arm is greater than 7.0. Metacentric is synonymous with the m region, including the M point. Thus chromosomes with arm ratios between 1.0 and 1.7 belong in this group, including isochromosomes and other chromosomes with equal-sized arms. Similarly submetacentric corresponds with the term sm and subtelocentric with the term st. Subtelocentric is a term which has been popularly used and is thus maintained even though strictly speaking the st region is in fact subacrocentric.

This situation is represented in figure 3.2 in which the characteristic chromosome type of each of the four regions is drawn. The use of the abbreviated terms M, m etc. makes descriptions of chromosomes simpler and more accurate.

Another feature of chromosomes which is described by the karyotype is the presence or absence of secondary constrictions or nucleolar organizer regions. The region of the chromosome distal to the secondary constriction is referred to as a satellite.

Idiograms were developed based upon the long arm and short arm ratios, relative lengths and centromeric location. Since the actual length of the chromosomes has been affected by experimental artifacts, as discussed above, comparison between chromosome complements of different cells on the basis of absolute size are

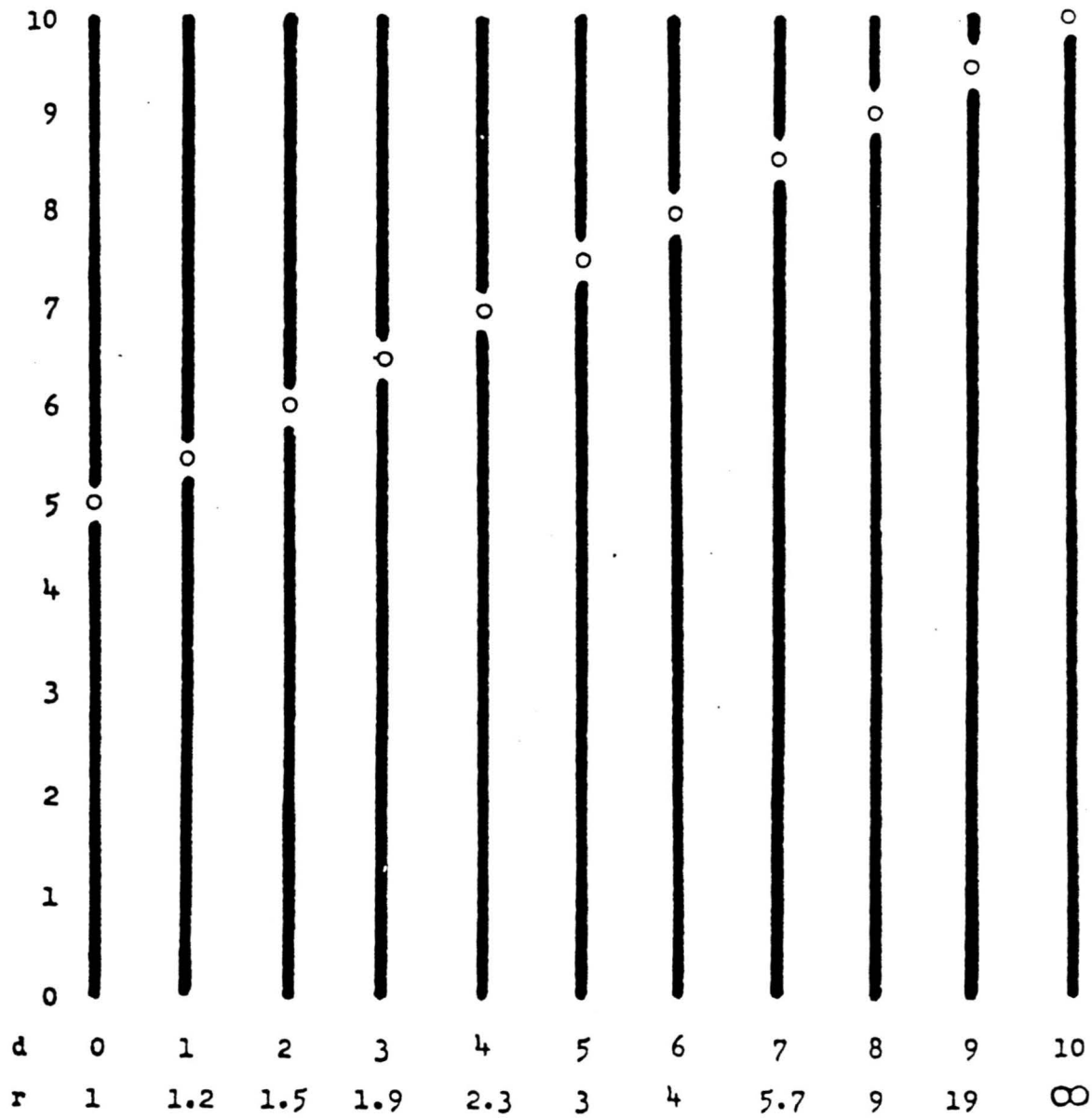

Figure 3.1: The range of variation in centromeric position (from Levan *et al.*, 1964)

Table 3.2: Centromere position and chromosome description (from Levan *et al.*, 1964).

Term	Location	d value	r value	Alternate term
M	median point	0.0	1.0	metacentric
m	median region	0.0-2.5	1.0-1.7	metacentric
sm	submedian region	2.5-5.0	1.7-3.0	submetacentric
st	subterminal region	5.0-7.5	3.0-7.0	subtelocentric
t	terminal region	7.5-10.0	7.0-∞	acrocentric
T	terminal point	10.0	∞	telocentric

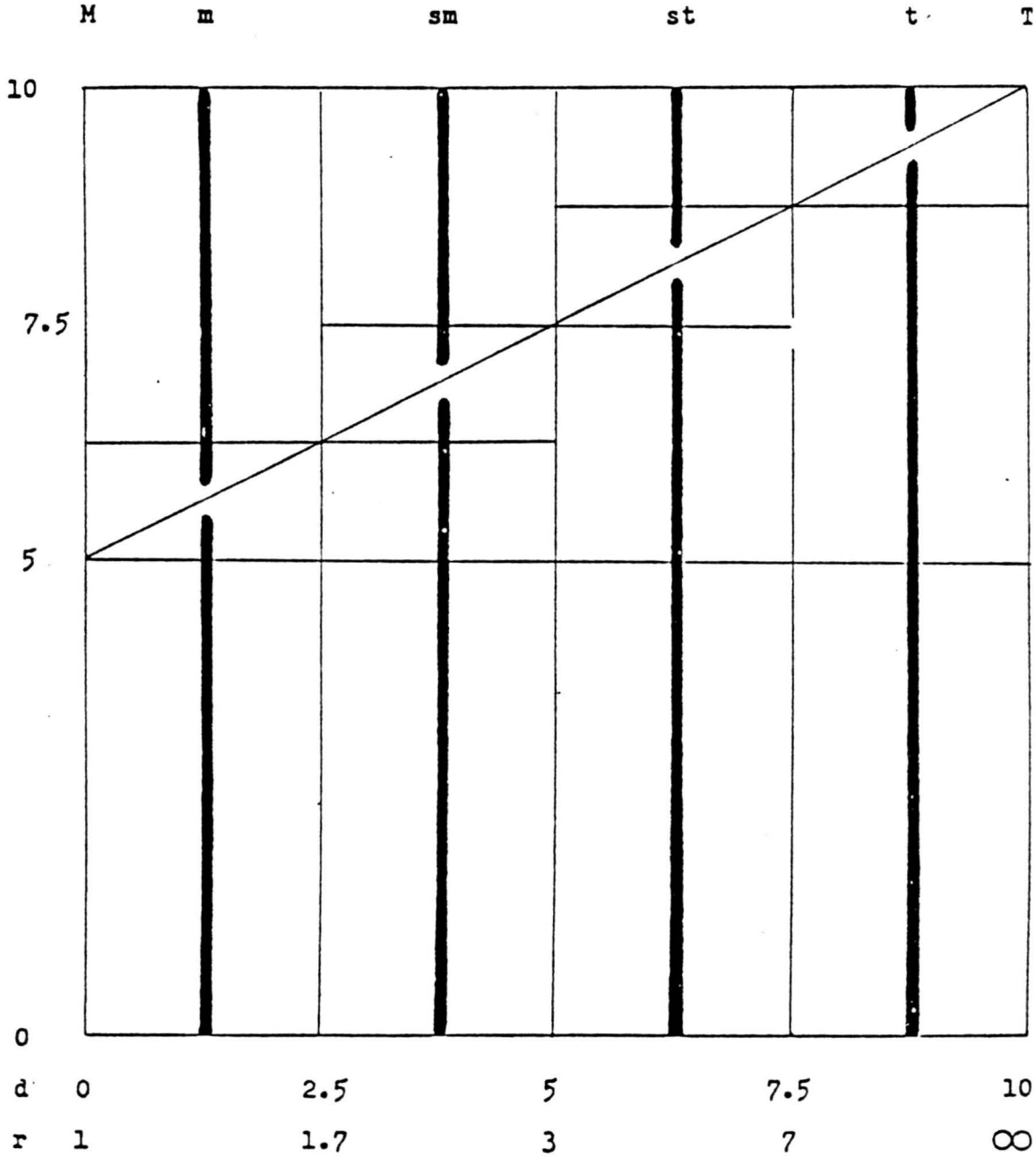

Figure 1.2: The range in variation in centromeric position divided into four equal regions: m, sm, st and t; each region represented by one characteristic chromosome. (from Levan *et al.*, 1964)

subject to error. In order to overcome these experimental variations the measurements of the chromosome pairs were made relative to one another by multiplying the long and short arm measurements by a conversion factor. The respective conversion factors were calculated by dividing the relative (new) total genome length, which was set at 100 units for each species, by the actual (old) total genome length. Once these conversions were made an average length for each arm of each pair was then determined. An idiogram could then be drawn using these adjusted measurements (Schlarbaum and Tsuchiya, 1984).

3.2 Meiosis

Meiotic chromosomes were studied using microsporocytes in anthers from immature buds. Anthers were removed in the morning and placed into a fixative of three parts 95% ethanol to one part glacial acetic acid. A bud size of approximately 4mm in diameter with anthers of 4mm in length was found to correlate with the appropriate meiotic stages of diakinesis and metaphase I. After fixing for 1 hour the anthers were studied to determine their meiotic stage. Once the correct stage was present all other anthers were collected immediately and fixed for 24 hours. The anthers were then transferred to acetocarmine stain and left for 2 weeks. Preparations were made by removing a thin cross section from the anther and gently squashing it in a 45% solution of acetic acid after gently heating.

Chromosome configurations were analyzed by studying a minimum of 30 microsporocytes at metaphase I. A representative cell was then photographed using the Carl Zeiss Photomicroscope II. Slide were again made permanent by applying drops of 10:1 45% acetic acid to glycerol around the coverslip. A total magnification of X1200 was achieved at printing.

3.3 Pollen Viability

Pollen viability was estimated from whole anthers collected at 10.00a.m. Only mature, dehisced anthers with pollen evident were collected. Staining was carried out in the greenhouse. The anther was removed with a pair of forceps and shaken onto a clean, dry glass slide. Acetocarmine (0.2%) stain was then dropped in a circle around the pollen and a coverslip was put into place. The slide was then taken to the laboratory and quickly passed through a flame to facilitate staining. Approximately one thousand pollen grains per slide were counted to determine relative viability. A cell was considered viable if it was plump and well stained. Non-viable cells were empty, shrunken and unstained. Anthers of *A. pelegrina 'rosea'* and *A. pelegrina 'alba'* were each collected on two separate occasions. After counting, pictures were taken and were printed at X400 magnification.

Chapter 4

RESULTS

4.1 Mitotic Analysis of *Alstroemeria pelegrina* L.

Several plants of *A. pelegrina* were studied with two objectives in mind. The first was to determine the general karyotype for the species and the second was to ascertain whether there are some variations between different plants within the species.

All of the plants of *A. pelegrina* studied have a chromosome number of $2n = 2x = 16$. These chromosomes can be divided into two groups. The first group contains four pairs of metacentric, submetacentric or subtelocentric chromosomes of different sizes. The smallest pair exhibits polymorphism with respect to the presence of satellites. The second group contains the remaining four pairs all of which are acrocentric. In most instances three of these pairs were satellited.

PLANT NUMBER 1: *A. pelegrina* 'rosea' 89H-7-8

The measurements presented in Table 4.1 were made from the cell shown in Figure 4.1. This cell was representative of other cells observed and photographed. Table 4.2 was then constructed from Table 4.1 and was used to describe the karyotype of this plant.

This plant exhibited the typical karyotype found in this species. The first four pairs of chromosomes numbered from one to eight form a group of metacentric or submetacentric pairs. The largest chromosome pair of this group was more than twice the length of the next largest chromosomes in the complement. The small submetacentric pair, pair number 4, carried small satellites on their short arms.

Figure 4.1: Somatic metaphase chromosomes of Plant number 1. Chromosomes are numbered as in table 4.1. X1200

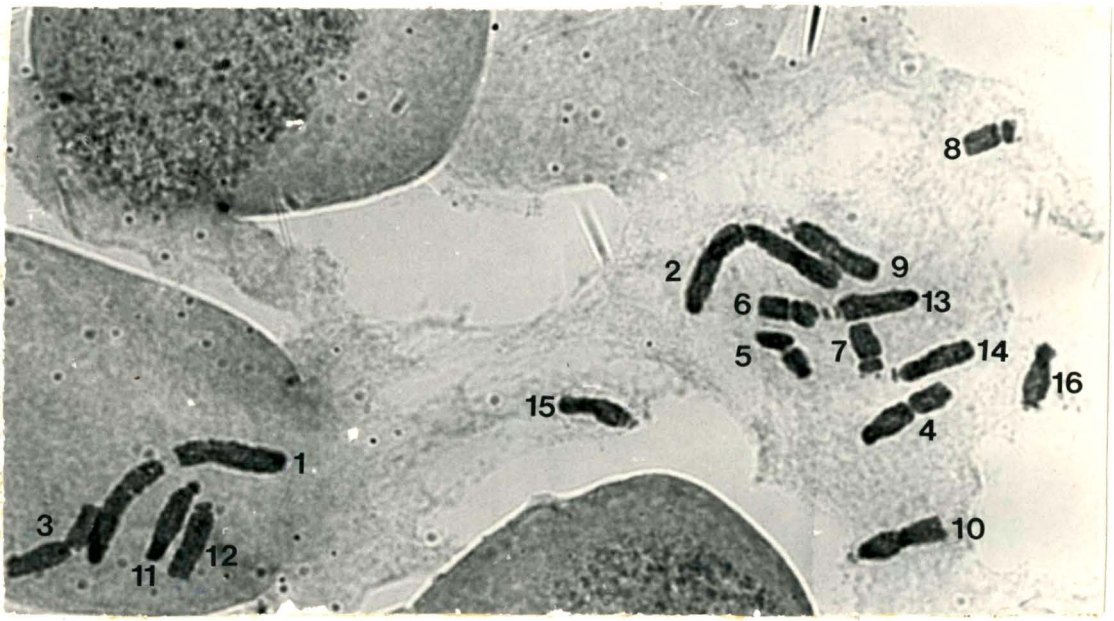
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Table 4.1: Somatic chromosomes of Plant number 1.

Chromosome	L[a] μ	S[b] μ	SAT μ	Total[c] μ	Relative length	Index S/L	Ratio L/S	Centromere position[d]
1	14.2	12.3		29.5	15.5	0.87	1.1	m
2	12.3	11.3		23.6	13.8	0.92	1.1	m
3	7.5	5.2		12.7	7.4	0.69	1.4	m
4	6.3	4.8		11.1	6.5	0.76	1.3	m
5	4.2	3.3		7.5	4.4	0.86	1.3	m
6	3.3	3.2		6.5	3.8	0.88	1.03	m
7	4.2	1.7	0.4	5.9	3.4	0.40	2.5	sm
8	4.2	1.7	0.4	5.9	3.4	0.40	0.5	sm
9	10.0	0.6	0.6	10.6	6.2	0.06	16.7	t
10	9.6	0.6		10.2	6.0	0.06	16.0	t
11	9.2	0.8		10.0	5.8	0.09	11.5	t
12	7.5	0.8		8.3	4.8	0.11	9.4	t
13	8.3	0.4	0.6	8.7	5.1	0.05	20.8	t
14	8.3	0.4	0.6	8.7	5.1	0.05	20.8	t
15	7.5	0.6	0.4	8.1	4.7	0.08	12.5	t
16	6.7	0.4	0.4	7.1	4.2	0.06	16.8	t

[a]L – Long arm.

[b]S – Short arm.

[c]Satellite not included in measurement.

[d]Classification according to Levan *et al.* (1964).

Table 4.2: Somatic chromosome pairs of Plant number 1.

Chromosome pair	L[a] μ	S[b] μ	SAT μ	Total[c] μ	Relative length	Index S/L	Ratio L/S	Centromere position[d]
1	13.3	11.8		25.1	29.3	0.89	1.1	m
2	6.9	5.0		11.9	13.8	0.72	1.4	m
3	3.7	3.2		7.0	8.2	0.87	1.2	m
4	4.2	1.7	0.4	5.9	6.9	0.4	2.5	sm
5	9.8	0.6	0.6	10.4	12.1	0.06	16.3	t
6	8.3	0.8		9.1	10.6	0.06	10.4	t
7	8.3	0.4	0.6	8.7	10.1	0.05	20.8	t
8	7.1	0.5	0.4	7.6	8.9	0.07	14.2	t

[a]L – Long arm.

[b]S – Short arm.

[c]Satellite not included in measurement.

[d]Classification according to Levan *et al.* (1964).

The second group of chromosomes in this complement consisted of four pairs of acrocentric chromosomes numbered 5 to 8 (Table 4.2). Three of these pairs, numbers 5, 7 and 8 were satellited. The satellites of pair numbers 5 and 8 were about the same length as the short arms whereas the chromosomes of pair number 7 had satellites which were much larger than the short arms. The acrocentric chromosome pair number 6 is not satellited but the short arms were slightly larger than those of the other acrocentric chromosomes.

Pair 1: This pair of chromosomes had an average total length 25.1μ . The relative length of 29.3 percent was very similar to that seen in the other plants studied. The chromosomes are metacentric with the primary constriction in the median region.

Pair 2: The average total length of this pair was 11.9μ and the relative length is 13.8 percent. These metacentric chromosomes had the primary constriction in the median region.

Pair 3: These metacentric chromosomes had an average total length of 7.0μ and a relative length of 8.2 percent. The primary constriction was in the median region.

Pair 4: The smallest pair of chromosomes in this group was sub-metacentric with an average total length of 5.9μ and a relative length of 6.8 percent. Each chromosome in the pair carried a satellite which was about 1/4 the length of the short arm.

Pair 5: The average total length of this pair of chromosomes was 10.4μ with a relative length of 12.2 percent. This was the longest chromosome pair of the second group of chromosomes. The unique feature of this pair was that only the longest chromosome of the pair (number 9) was satellited. This satellite was about the same length as the short arm.

Pair 6: As in the other plants studied this pair of acrocentric chromosomes was not satellited. The average total length was 9.1μ with a relative length of 10.6 percent.

Pair 7: This pair of acrocentric chromosomes had an average length of 8.7μ and a relative length of 10.2 percent. Both chromosomes in the pair were satellited with the satellite being larger than the tiny short arm.

Pair 8: The chromosomes of this pair had an average total length of 7.6μ and a relative length of 8.9 percent. Both of these acrocentric chromosomes had a satellite which was about the same length as the short arm.

PLANT NUMBER 2: *A. pelegrina* 'alba' 87H-10-4B

Measurements were made from the photograph shown in Figure 4.2 of a single cell of this plant. Other cells were also measured to be sure that this was a representative sample for this plant. Chromosome number 2 was broken in this cell. Other cells were examined to ascertain that this chromosome was metacentric. The appearance of the two arms as separate telocentric chromosomes was an artifact due to breakage during slide preparation.

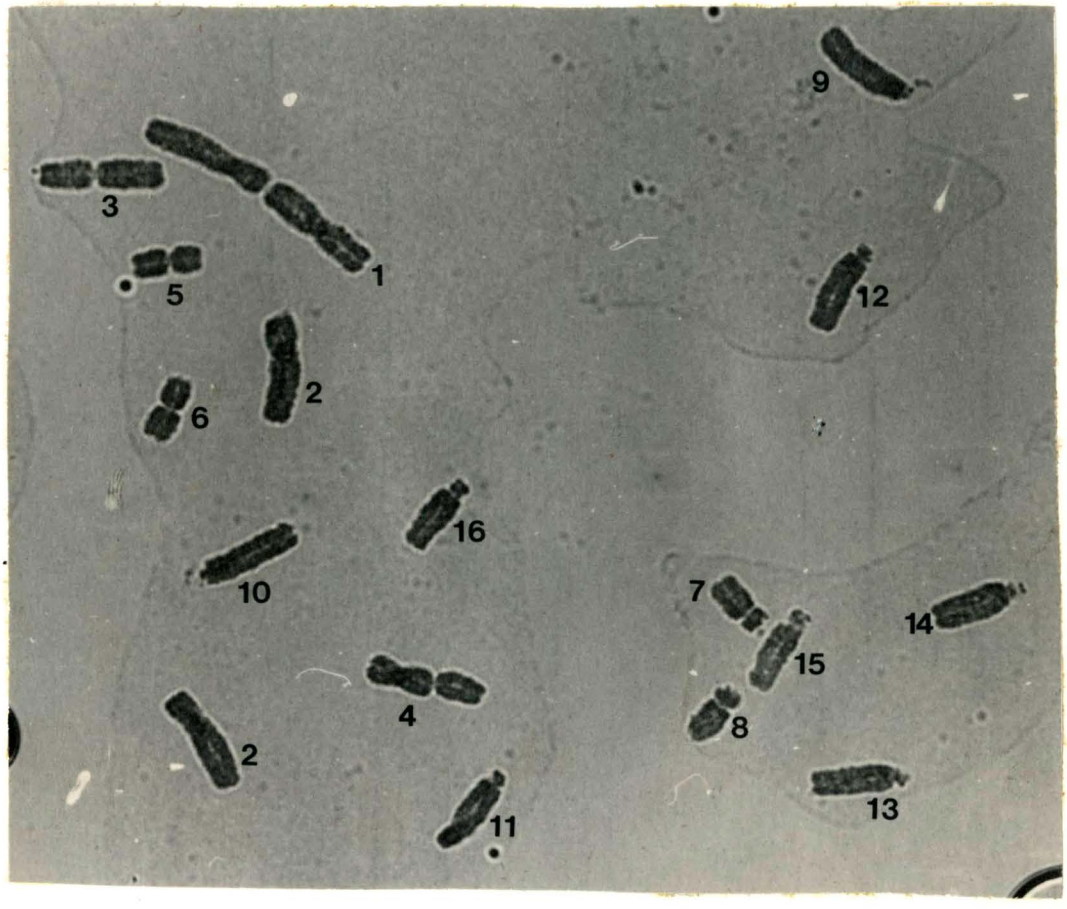
The results of these measurements as well as the presence or absence of satellites, the relative length, chromosome index and centromere position are presented in Table 4.3.

This plant also exhibited the typical karyotype found in this species as described for Plant 1.

Table 4.4 was compiled from table 4.3 and shows the average values of measurements for each pair of chromosomes. These measurements were used to describe the karyotype of this plant.

Pair 1: This was the longest pair of chromosomes in the complement with an average total length of 26.9μ and a relative length of 28.6 percent. This pair was classed as metacentric with the primary constriction in the median region. This

Figure 4.2: Somatic metaphase chromosomes of Plant number 2. Chromosomes are numbered as in table 4.3. X1200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constriction was very close to the median point as shown by the ratio (r) of long to short arm lengths of 1.04.

Pair 2: The average total length of this pair of chromosomes was 13.4μ or about half the length of the longest pair. This pair had a relative length of 14.3 percent. These chromosomes were metacentric with the primary constriction in the median region.

Pair 3: This pair had an average total length of 7.15μ and a relative length within the complement of 7.6 percent. The chromosomes of this pair were also metacentric with the primary constriction in the median region.

Pair 4: The chromosomes of this pair had an average total length of 6.5μ and a relative length of 7.0 percent. The chromosomes had the primary constriction in the submedian region and were thus classified as submetacentric. Both members of this pair also had small satellites which were about 1/3 the length of the short arm.

Pair 5: This was the longest pair of the group of acrocentric chromosomes found in the complement, although pairs 6 and 7 are also of very similar length. Pair 5 had an average total length of 11.9μ and a relative length of 12.6 percent. The short arm was very tiny, with the index of short to long arm being only 0.05. This pair of chromosomes carried satellites which were about the same length as the short arm of the chromosomes.

Pair 6: This pair had an average total length of 10.7μ and a relative length of 11.4 percent. The short arm was considerably larger than those in the other acrocentric chromosomes with an index of 0.14. There were no satellites found on this pair of chromosomes.

Pair 7: The seventh pair of chromosomes in the complement was also satellited. The short arm was very tiny with an index of 0.07 and the satellites were about the same length as the short arm. This pair of chromosomes had an average total length of 9.4μ and a relative length of 10.0 percent.

Table 4.3: Somatic metaphase chromosomes of Plant number 2.

Chromosome	L[a] μ	S[b] μ	SAT μ	Total[c] μ	Relative length	Index S/L	Ratio L/S	Centromere position[d]
1	15	13.8		28.8	15.3	0.92	1.09	m
2	12.5	12.5		25.0	13.3	1.0	1.0	M
3	7.7	5.8		13.5	7.2	0.75	1.3	m
4	7.5	5.8		13.3	7.1	0.77	1.3	m
5	4.2	3.3		7.5	4.0	0.79	1.3	m
6	3.5	3.3		6.8	3.6	0.94	1.06	m
7	4.8	1.7	0.4	6.5	3.5	0.35	2.8	sm
8	4.8	1.7	0.6	6.5	3.5	0.35	2.8	sm
9	11.3	0.6	0.6	11.9	6.3	0.05	18.8	t
10	11.3	0.6	0.6	11.9	6.3	0.05	18.8	t
11	9.6	1.3		10.9	5.8	0.14	7.4	t
12	9.2	1.3		10.5	5.6	0.14	7.1	t
13	8.8	0.6	0.4	9.4	5.0	0.07	14.7	t
14	8.8	0.6	0.4	9.4	5.0	0.07	14.7	t
15	7.5	0.6	0.4	8.1	4.3	0.08	12.5	t
16	7.5	0.3	0.8	7.8	4.1	0.04	25.0	t

[a]L – Long arm.

[b]S – Short arm.

[c]Satellite not included in measurement.

[d]Classification according to Levan *et al.* (1964).

Table 4.4: Somatic metaphase chromosome pairs of Plant number 2.

Chromosome pair	L[a] μ	S[b] μ	SAT μ	Total[c] μ	Relative length	Index S/L	Ratio L/S	Centromere position[d]
1	13.7	13.2		26.9	28.6	0.95	1.04	m
2	7.6	5.8		13.4	14.3	0.76	4.2	m
3	3.8	3.3		7.1	7.6	0.86	1.15	m
4	4.8	1.7	0.5	6.5	7.0	0.35	2.82	sm
5	11.3	0.6	0.6	11.9	12.6	0.05	18.8	t
6	9.4	1.3		10.7	11.4	0.14	7.2	t
7	8.8	0.6	0.4	9.4	10.0	0.07	14.7	t
8	7.5	0.4	0.6	7.9	8.4	0.06	18.7	t

[a]L – Long arm.

[b]S – Short arm.

[c]Satellite not included in measurement.

[d]Classification according to Levan *et al.* (1964).

Pair 8: The shortest chromosomes in this group have an average total length of 7.9μ and a relative length of 8.4 percent. The short arm was again very tiny with an index of 0.06. Both chromosomes of this pair were satellited with the satellites being larger than the short arms.

PLANT NUMBER 3: *A. pelegrina* 'rosea' 87H-2-12

Measurements were made from a single representative cell of this plant, which was determined after viewing a number of preparations. The cell used is shown in Figure 4.3.

Table 4.5 was then constructed to show the length of the chromosomes, the centromere position, the presence or absence of satellites, the relative length and the chromosome index.

The karyotype of this plant was then described using the average values of measurements made for each pair of chromosomes as shown in Table 4.6.

The karyotype of this specimen was similar to that of plants number 1 and 2 except the chromosomes of pair number 6 were subtelocentric.

Pair 1: The longest pair of chromosomes in the complement had an average total length 28.7μ and a relative length of 28.7 percent. The primary constriction is in the median region and the chromosome pair is thus classed as metacentric.

Pair 2: This pair of chromosomes had an average total length of 15.2μ and a relative length of 15.2 percent. The chromsomes were metacentric with the primary constriction in the median region.

Pair 3: The average total length of this pair was 8.0μ with a relative length of 8.0 percent. Again the chromosomes were classed as metacentric with the primary constriction in the median region.

Pair 4: As in the previous plant this chromosome pair was submetacentric with the primary constriction in the submedian region. The average total length was 6.7μ with a relative length of 6.4 percent. There was a small satellite visible on each

Figure 4.3: Somatic metaphase chromosomes of Plant number 3. Chromosomes are numbered as in table 4.5. X1200

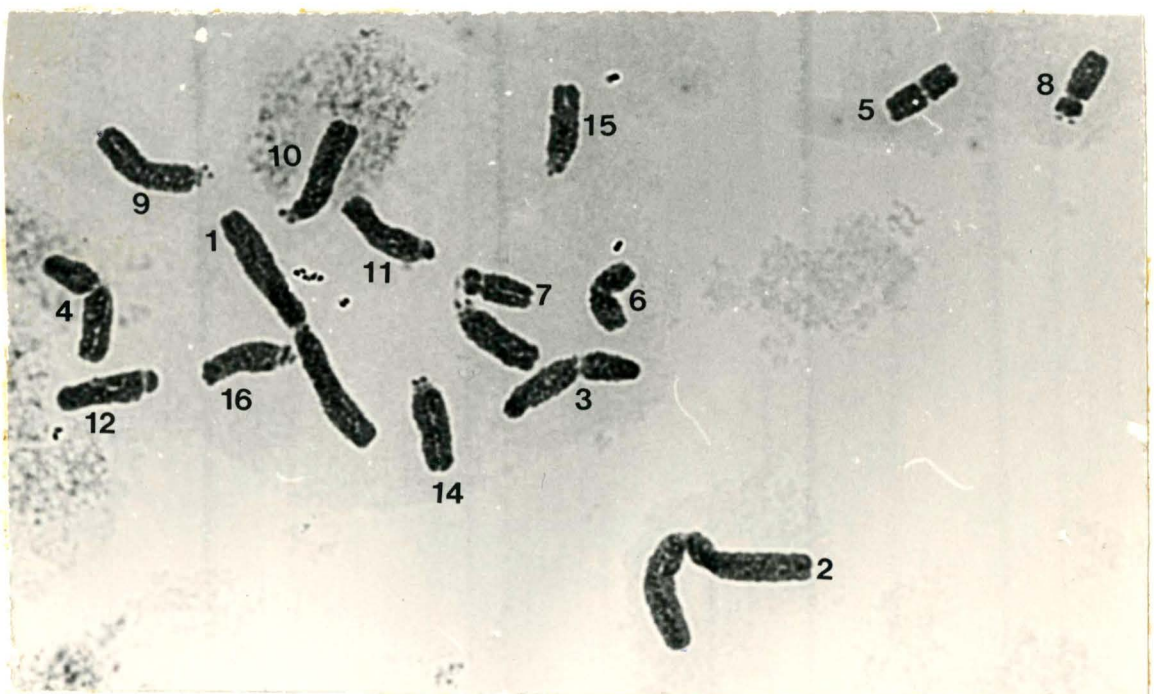
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Table 4.5: Somatic metaphase chromosomes of Plant number 3.

Chromosome	L[a] μ	S[b] μ	SAT μ	Total[c] μ	Relative length	Index S/L	Ratio L/S	Centromere position[d]
1	15.0	14.2		29.2	14.6	0.95	1.06	m
2	15.0	13.3		28.3	14.1	0.89	1.1	m
3	9.2	6.3		15.5	7.7	0.68	1.5	m
4	8.3	6.7		15.0	7.5	0.81	1.2	m
5	4.2	3.8		8.0	4.0	0.9	1.1	m
6	4.2	3.8		8.0	4.0	0.9	1.1	m
7	5.0	1.7	0.4	6.7	3.3	0.34	2.9	sm
8	5.0	1.7	0.4	6.7	3.3	0.34	2.9	sm
9	11.7	0.4	0.6	12.1	6.0	0.03	29.2	t
10	11.3	0.4	0.8	11.7	5.8	0.03	28.3	t
11	9.6	1.7		11.3	5.6	0.18	5.6	st
12	9.6	1.3		10.0	5.0	0.14	7.4	t
13	9.2	0.6	0.8	10.0	5.0	0.06	15.3	t
14	9.2	0.6	0.6	10.0	5.0	0.06	15.3	t
15	8.3	0.8	0.4	9.1	4.5	0.10	14.4	t
16	8.3	0.6	0.4	8.9	4.4	0.07	13.8	t

[a]L – Long arm.

[b]S – Short arm.

[c]Satellite not included in measurement.

[d]Classification according to Levan *et al.* (1964).

Table 4.6: Somatic metaphase chromosome pairs of Plant number 3.

Chromosome pair	L[a] μ	S[b] μ	SAT μ	Total[c] μ	Relative length	Index S/L	Ratio L/S	Centromere position[d]
1	15.0	13.7		28.7	28.7	0.96	1.09	m
2	8.7	6.5		15.2	15.2	0.74	1.3	m
3	4.2	3.8		8.0	8.0	0.9	1.1	m
4	5.0	1.7	0.4	6.7	6.4	0.34	2.9	sm
5	11.5	0.4	0.7	11.9	11.8	0.03	28.7	t
6	9.6	1.5		11.1	10.6	0.16	6.4	st
7	9.2	0.6	0.7	9.8	10.0	0.06	15.3	t
8	8.3	0.7	0.4	9.0	8.7	0.08	11.8	t

[a]L – Long arm.

[b]S – Short arm.

[c]Satellite not included in measurement.

[d]Classification according to Levan *et al.* (1964).

chromosome in the pair which had a length of about 1/4 the length of the short arm.

Pair 5: The longest pair in the second group of chromosomes in the complement had an average total length of 11.9 μ and a relative length of 11.8 percent. The chromosomes were acrocentric with the primary constriction in the terminal region. The short arms were very tiny with an index of 0.03. Each of the chromosomes was satellited with the satellite being slightly larger than the short arm.

Pair 6: This pair of chromosomes was unique to this plant as it was subtelocentric rather than acrocentric as found in the other plants examined. Several different cells were photographed and measured to determine that this was in fact a characteristic of this plant and not an artifact. Since the chromosomes were very close to being categorized as acrocentric (r = 6.4 compared to r = 7.0) and visually appeared to be acrocentric, they are left within this second group of chromosomes. This also allows this karyotype to be compared to that of the other plants examined. The average total length of this pair of chromosomes is 11.1μ with a relative length of 10.6 percent. These chromosomes were not satellited.

Pair 7: This pair of acrocentric chromosomes had an average total length of 9.8μ and an relative length of 10.0 percent. Both members of the pair were satellited with the satellites being slightly longer than the short arms. The short arms were again very small with an index of 0.06.

Pair 8: The eighth pair of chromosomes in this plant was only slightly smaller than the seventh pair with an average total length of 9.0μ and a relative length of 8.7 percent. The short arm however was slightly larger in this pair than in pair 7, with an index of 0.08. The satellites found on this pair of chromosomes were smaller than the short arms.

PLANT NUMBER 4: *A. pelegrina* 87H-5-1

A single representative cell of this plant is presented in Figure 4.4. This was used to make measurements of total length, relative length, index and position of the primary constriction. These results are shown in Table 4.7.

An average measurement for each pair of chromosomes was then made and the results of these measurements are shown in Table 4.8. This table was then used to describe the karyotype of this plant.

Pair 1: This longest pair of chromosomes had an average total length of 25.5μ and a relative length of 29.4 percent. These chromosomes were metacentric with the primary constriction in the median region.

Pair 2: These metacentric chromosomes had the primary constriction in the median region. They had an average total length of 11.7μ and constituted 13.5 percent of the total length of the complement.

Pair 3: This pair of chromosomes was also metacentric with the primary constriction in the median region. It had an average total length 7.0μ and a relative length of 8.1 percent.

Pair 4: A number of cells were examined and photographed until it could be conclusively established that this pair of chromosomes was unique to this plant. The uniqueness was due to the fact that only one of this pair of chromosomes was satellited. As in the other plants this pair was submetacentric and its average total length is 6.35μ. The relative length of this pair was 7.4 percent. The satellite that was present was about 1/4 the length of the short arm.

Pair 5: The average total length of this pair of chromosomes was 10.5μ which constitutes a relative length of 12.1 percent. Both the chromosomes of this pair were satellited with the satellite being longer than the short arm. The short arm of these acrocentric chromosomes was very tiny with an index of 0.04.

Figure 4.4: Somatic metaphase chromosomes of Plant number 4. Chromosomes are as numbered in table 4.7. X1200

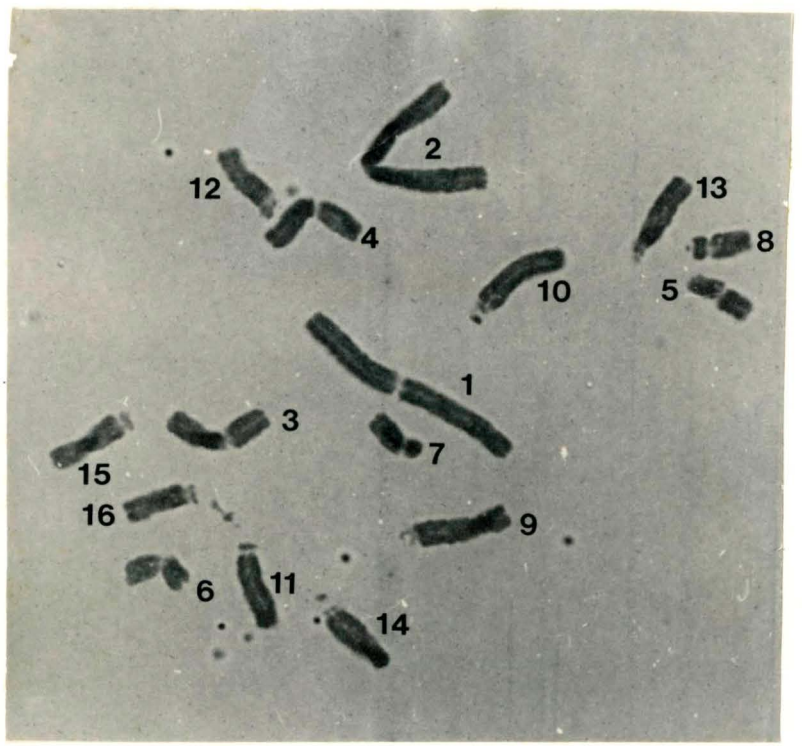
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Table 4.7: Somatic metaphase chromosomes of Plant number 4.

Chromosome	L[a] μ	S[b] μ	SAT μ	Total[c] μ	Relative length	Index S/L	Ratio L/S	Centromere position[d]
1	13.0	12.5		25.5	14.7	0.96	1.04	m
2	13.0	12.5		25.5	14.7	0.96	1.04	m
3	7.0	5.0		12.0	6.9	0.71	1.4	m
4	7.0	4.5		11.5	6.6	0.64	1.6	m
5	4.0	3.5		7.5	4.3	0.87	1.1	m
6	3.5	3.0		6.5	3.8	0.86	1.2	m
7	4.7	2.0		6.7	3.9	0.43	2.4	sm
8	4.5	1.5	0.3	6.0	3.5	0.33	2.6	sm
9	10.5	0.5	0.7	11.0	6.3	0.05	21.0	t
10	9.5	0.5	0.5	10.0	5.8	0.05	9.0	t
11	9.5	0.7		10.2	5.9	0.12	13.6	t
12	8.5	1.0		9.5	5.5	0.123	8.3	t
13	8.5	0.5	1.0	9.0	5.2	0.06	17.0	t
14	7.0	0.7	1.0	7.7	4.4	0.1	10	t
15	7.0	0.3	0.7	7.3	4.2	0.04	23.3	t
16	6.5	0.7	0.3	7.2	4.2	0.1	9.3	t

[a]L – Long arm.

[b]S – Short arm.

[c]Satellite not included in measurement.

[d]Classification according to Levan *et al.* (1964).

Table 4.8: Somatic metaphase chromosome pairs of Plant 4.

Chromosome pair	L[a] μ	S[b] μ	SAT μ	Total[c] μ	Relative length	Index S/L	Ratio L/S	Centromere position[d]
1	13.0	12.5		25.5	29.4	0.96	1.04	m
2	7.0	4.7		11.7	13.5	0.68	1.5	m
3	3.7	3.3		7.0	8.1	0.87	1.1	m
4	4.6	1.7	0.3	6.3	7.4	0.38	2.7	sm
5	10.0	0.5	0.6	10.5	12.1	0.05	20.0	t
6	9.0	0.8		9.8	11.4	0.09	11.3	t
7	7.7	0.6	1.0	8.3	9.6	0.07	2.8	t
8	6.7	0.5	0.5	7.2	8.4	0.07	13.4	t

[a]L – Long arm.

[b]S – Short arm.

[c]Satellite not included in measurement.

[d]Classification according to Levan *et al.* (1964).

Pair 6: The next pair of acrocentric chromosomes in this group had an average total length of 9.8μ and a relative length of 11.4 percent. The short arms of this pair are larger than those of the other acrocentrics within this group. These chromosomes were not satellited.

Pair 7: Both the chromosomes in this pair of acrocentrics had satellites. The short arm was again very tiny with the satellites being longer than the short arm. The average total length of these chromosomes was 8.3μ with a relative length of 9.6 percent.

Pair 8: The smallest chromosomes of this group had an average total length of 7.2μ and a relative length of 8.4 percent. This pair was acrocentric with both chromosomes carrying a satellite.

PLANT NUMBER 5: *A. pelegrina* 87H-10-13

Figure 4.5 shows a representative cell of this plant. Measurements from this cell are listed in Table 4.9, along with the other definitive features of these chromosomes.

Table 4.10 was compiled from table 4.9 and gives the average values of measurements for each pair of chromosomes. It is this table which is used to describe the karyotype of this plant.

Pair 1: This was the only pair of metacentric chromosomes in which the centromere was at the median point. This pair had an average total length of 30 μ and a relative length of 14.2 percent.

Pair 2: The average total length of this pair of metacentric chromosomes was 14.5μ with a relative length of 13.8 percent. The centromere was located in the median region.

Pair 3: The third pair of metacentric chromosomes had an average total length of 8.0μ and a relative length of 7.6 percent.

Pair 4: The submetacentric chromosomes of this pair had an average total length of 7.8μ and a relative length of 7.3 percent. It is interesting to note that

Figure 4.5: Somatic metaphase chromosomes of Plant number 5. Chromosomes numbered as in table 4.9. X1200

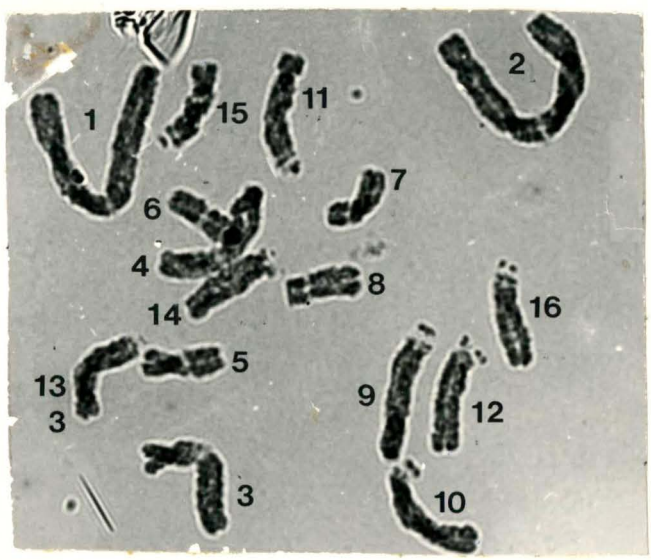

1
15
11
2
7
6
4
8
14
16
5
13
3
9
12
3
10

Table 4.9: Somatic metaphase chromosomes of Plant 5.

Chromosome	L[a] μ	S[b] μ	SAT μ	Total[c] μ	Relative length	Index S/L	Ratio L/S	Centromere position[d]
1	15.0	15.0		30.0	14.2	1.0	1.0	M
2	15.0	15.0		30.0	14.2	1.0	1.0	M
3	8.3	6.7		15.0	7.7	0.8	1.2	m
4	8.3	5.8		14.1	6.7	0.7	1.4	m
5	4.2	3.8		8.0	3.8	0.9	1.1	m
6	4.2	3.8		8.0	3.8	0.9	1.1	m
7	5.8	2.3		8.1	3.8	0.4	2.5	sm
8	5.6	1.9		7.5	3.5	0.3	2.9	sm
9	12.9	0.3	0.7	13.2	6.2	0.02	43.0	t
10	12.5	0.7	0.8	13.2	6.2	0.06	17.8	t
11	11.7	0.4	0.8	12.1	5.7	0.03	29.0	t
12	11.5	0.7	0.07	12.2	5.7	0.06	16.4	t
13	10.8	0.4	0.2	11.2	5.3	0.04	27.0	t
14	9.6	0.4	0.2	10.0	4.7	0.04	24.0	t
15	9.2	0.4	0.6	9.6	4.5	0.04	23.0	t
16	9.2	0.4	0.6	9.6	4.5	0.04	23.0	t

[a]L – Long arm.

[b]S – Short arm.

[c]Satellite not included in measurement.

[d]Classification according to Levan *et al.* (1964).

Table 4.10: Somatic metaphase chromosome pairs of Plant number 5.

Chromosome pair	L[a] μ	S[b] μ	SAT μ	Total[c] μ	Relative length	Index S/L	Ratio L/S	Centromere position[d]
1	15.0	15.0		30.0	28.4	1.0	1.0	M
2	8.3	6.2		14.5	13.8	0.75	1.33	m
3	4.2	3.8		8.0	7.6	0.9	1.1	m
4	5.7	2.1		7.8	7.3	0.36	2.78	sm
5	12.7	0.5	0.7	13.2	12.4	0.04	25.0	t
6	11.6	0.5	0.7	12.1	11.4	0.05	20.0	t
7	10.2	0.4	0.2	10.6	10.0	0.04	15.0	t
8	9.2	0.4	0.7	9.6	9.0	0.04	25.0	t

[a]L – Long arm.

[b]S – Short arm.

[c]Satellite not included in measurement.

[d]Classification according to Levan *et al.* (1964).

a large number of cells were examined and it was determined that there were no satellites on this pair of chromosomes. This is contrary to all the other plants studied.

Pair 5: The average total length of this pair of acrocentric chromosomes was 13.2μ. They had a relative length of 12.4 percent and each chromosome in the pair had a satellite. The satellite in both cases was slightly longer than the tiny short arm.

Pair 6: This pair of acrocentric chromosomes had an average total length of 12.1μ and relative length of 11.4 percent. It should be noted that satellites were also present on both chromosomes of this pair whereas they were not found in any other plants. Careful analysis of all possible cells verified that this pair did indeed carry satellites. The short arm was somewhat shorter than that seen on the same pair of chromosomes in the other plants and the satellites were a little larger than the short arms.

Pair 7: The relative length of this pair of chromosomes was 10.0 percent. The average total length was 10.6μ. These acrocentric chromosomes had a small short arm and each carried a very tiny satellite which was only half the length of the short arm.

Pair 8: These acrocentric chromosomes also had a small short arm but the satellites they carried were almost twice the length of the short arm. The average total length was 9.6μ and the relative length was 9.0 percent.

4.2 Meiosis in *A. pelegrina* L.

Meiosis was studied in pollen mother cells (microsporocytes) in *A. pelegrina* anthers collected from immature flower buds. These buds were about 4 mm in diameter. The anthers were 4 mm long and approximately 2 mm in diameter when the required stages of diakinesis and metaphase I were present. A minimum of thirty pollen mother cells were observed in each of the three plants studied.

At both diakinesis or metaphase I stages chromosome pairing was normal with 8 bivalents. The chromosomes formed either open rings or rod bivalents. In all cells studied there was one or two pairs which formed only a very loose association at metaphase I. Photomicrographs of pollen mother cells at metaphase I are shown in Figures 4.6-4.8.

Cursory analysis of approximately ten cells per plant which were at anaphase I did not reveal the presence of any abnormalities such as bridges. All cells studied showed 8-8 separation.

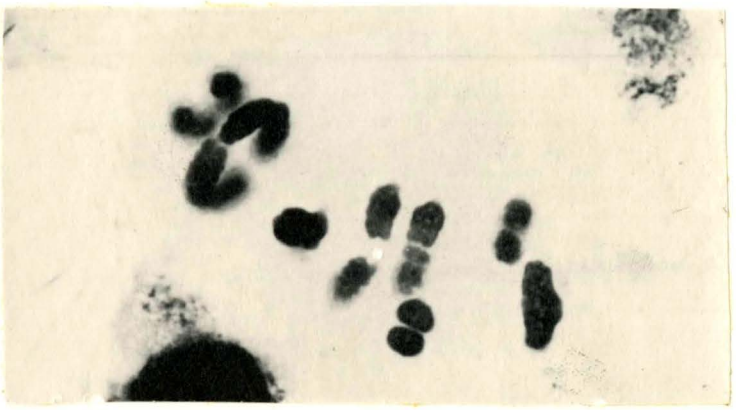
4.3 Pollen fertility of *A. pelegrina* L.

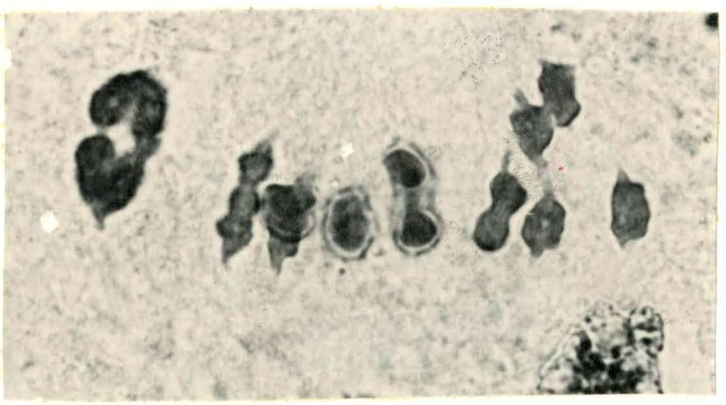
Pollen was collected from newly ripened anthers on two or three separate occasions from each of the two flower colors 'rosea' and 'alba'. Pollen was determined to be viable if it readily stained with the acetocarmine stain. It was easy to see non-viable pollen grains as they were shrunken and contained no color at all. This can be clearly observed in Figure 4.9. Table 4.11 shows the results obtained when approximately 500 to 1000 pollen grains were counted per slide.

Figure 4.6: Meiotic metaphase I observed in a pollen mother cell of Plant number 6 showing eight bivalents. One pair shows loose pairing or precocious separation.

Figure 4.7: Meiotic metaphase I observed in a pollen mother cell of Plant number 7 showing eight bivalents. The two pairs at the right end show loose pairing or precocious separation.

Figure 4.8: Meiotic metaphase I observed in a pollen mother cell of Plant number 8 showing eight bivalents. One pair shows loose pairing or precocious separation.





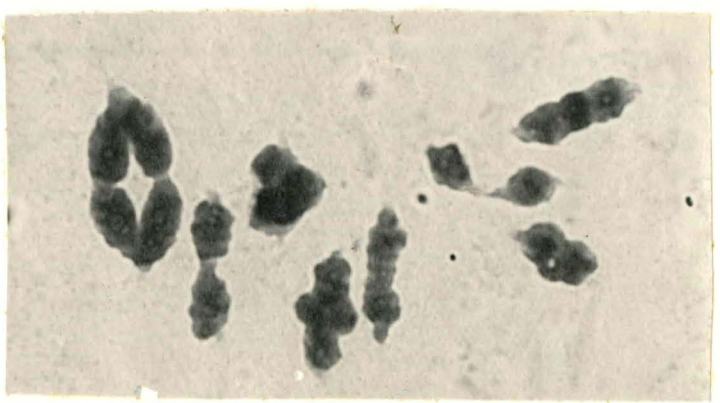
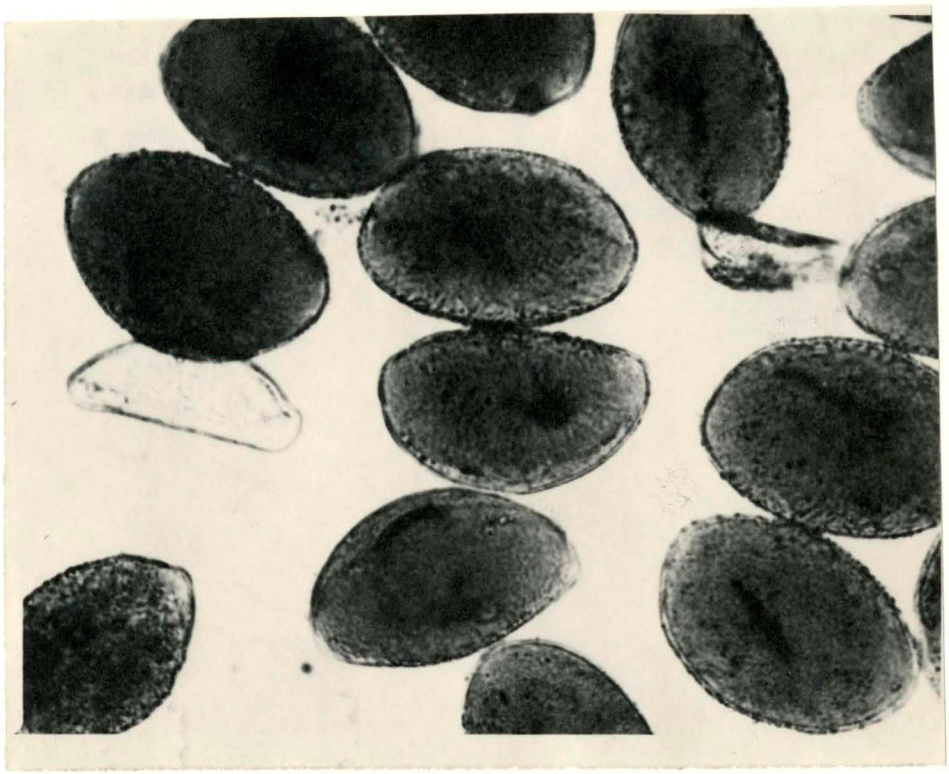


Figure 4.9: Pollen grains of *A. pelegrina* (a)'rosea' (Plant number 8) (b)'alba' (Plant number 7) X400



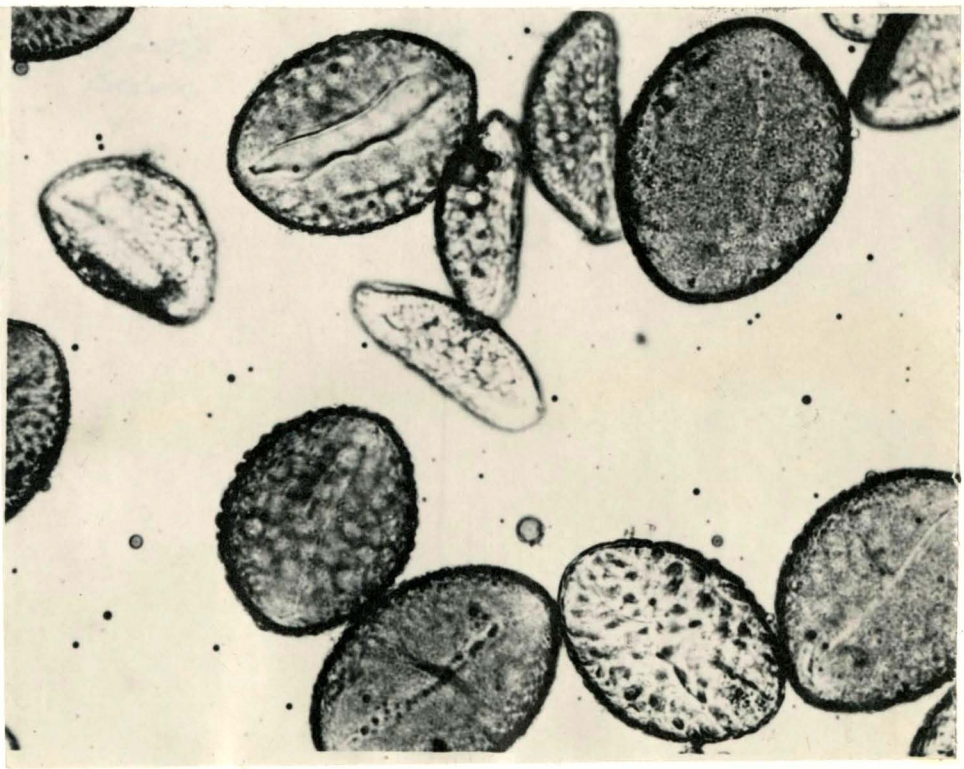


Table 4.11: Pollen fertility for *A. pelegrina* 'rosea' and 'alba'

	'Rosea'			'Alba'		
Replicate	Viable	Abortive	Total	Viable	Abortive	Total
1	527	503	1030	835	197	1032
2	338	520	858	812	346	1158
3	246	351	597			
% Viable	44.7			75.2		

Chapter 5

DISCUSSION

In order to determine a general karyotype for the species *A. pelegrina* an average index was calculated for each pair of chromosomes from all the plants studied. To average the values obtained for each plant it was necessary to adjust the measurements to account for experimental artifacts. A conversion factor was calculated for each plant and the measurements for that plant were then multiplied by the conversion factor (Schlarbaum and Tsuchiya, 1984). The resultant adjusted values (Table 5.1) were then comparable to one another and could be used to calculate an average length for each pair as well as the average values for the long arm, the short arm and the index . An average value for each pair of the complement was then calculated and is shown in Table 5.2. These values were then used to plot the genomic diagram shown in Figure 5.1, using the percent of total chromosome length in the genome (relative length) for the axis of the abscissa (x-axis) and short arm:long arm ratio (chromosome index) for the axis of the ordinates (y-axis) (Tjio and Hagberg, 1951).

A single cell (Figure 4.3) was selected from all of the plants studied to construct the karyogram shown in Figure 5.2. The chromosomes were placed into two groups based on the position of the primary constriction. They were then organized in order of descending length within each group.

The information shown in Table 5.2 was then used to construct the idiogram shown in Figure 5.3. This idiogram is thus a representation of the general appearance of the genome of *A. pelegrina*.

Table 5.1: Adjusted measurements (μ) for each chromosome arm in each of the plants of *Alstroemeria pelegrina*.

	Chromosome Pairs															
Specimen	1		2		3		4		5		6		7		8	
	L	S	L	S	L	S	L	S	L	S	L	S	L	S	L	S
Plant 1	7.7	6.8	4.0	2.9	2.2	1.9	2.4	1.0	5.7	0.3	4.8	0.5	4.8	0.2	4.1	0.3
Plant 2	7.3	7.0	4.0	3.1	2.0	1.7	2.5	0.9	6.0	0.3	5.0	0.7	4.7	0.3	4.0	0.2
Plant 3	7.5	6.9	4.3	3.3	2.2	1.9	2.4	0.9	5.8	0.2	4.8	0.7	4.6	0.3	4.2	0.2
Plant 4	7.5	7.2	4.1	2.8	2.2	1.9	2.7	1.0	5.8	0.3	5.2	0.5	4.5	0.3	3.9	0.3
Plant 5	7.0	7.0	3.9	2.9	2.0	1.8	2.7	1.0	6.0	0.2	5.4	0.2	4.8	0.2	4.3	0.2

Table 5.2: Average adjusted measurements for each chromosome pair in the complement of *Alstroemeria pelegrina*.

Chromosome pair	L[a] μ	S[b] μ	SAT μ	Total[c] μ	Relative length	Index S/L
1	7.3	6.9		14.2	28.6	0.95
2	4.0	2.9		7.0	14.1	0.73
3	2.1	1.8		3.9	7.8	0.88
4	2.5	0.9	0.2	3.5	7.5	0.37
5	6.2	0.3	0.3	6.5	13.1	0.05
6	5.0	0.4		5.4	10.9	0.08
7	4.6	0.2	0.4	4.8	9.7	0.04
8	4.1	0.3	0.2	4.4	8.6	0.07

[a]L - Long arm.

[b]S - Short arm.

[c]Satellite not included in measurement.

Figure 5.1: Diagrammatic presentation of the genome of *Alstroemeria pelegrina*.

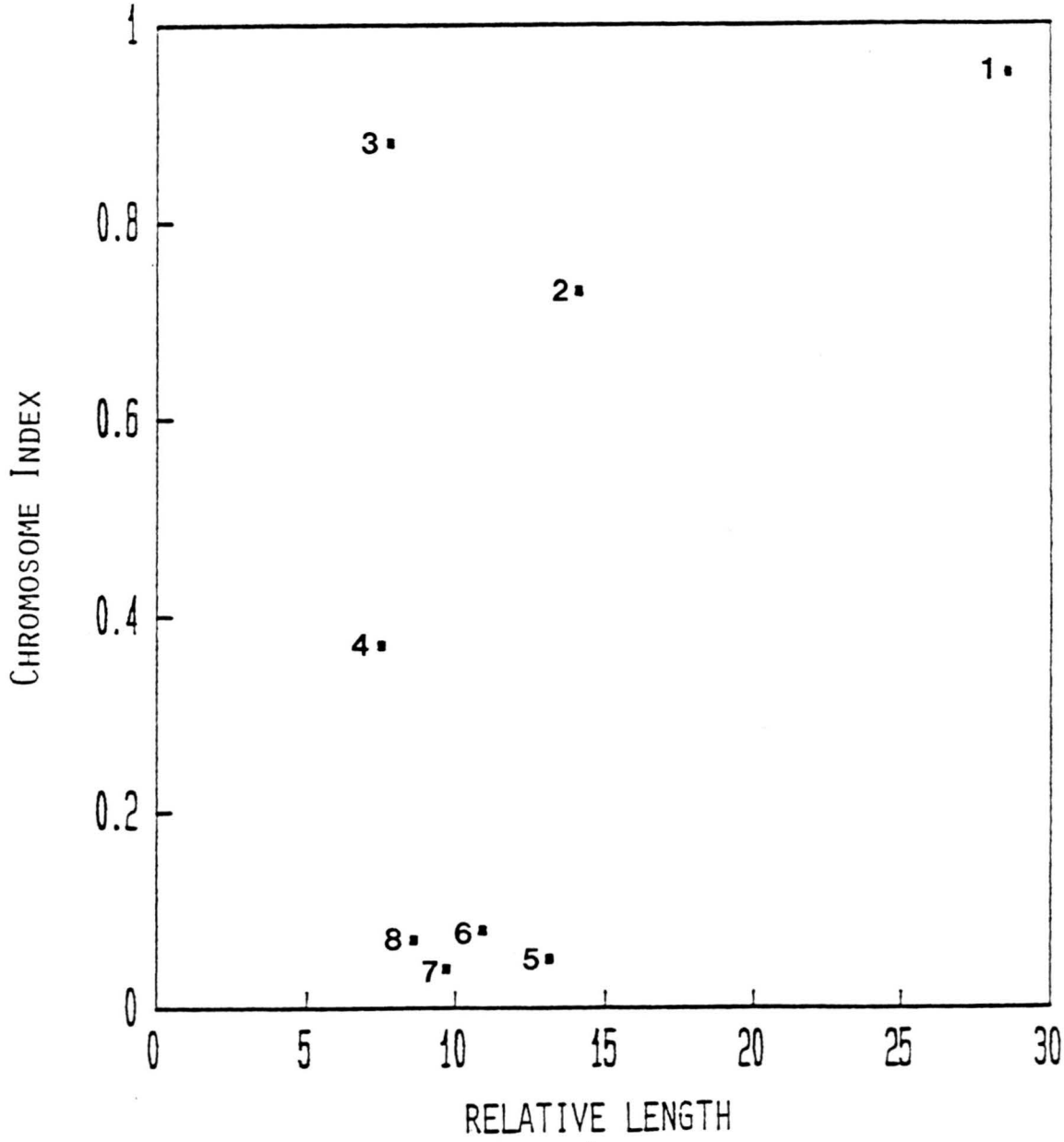

1
0.8
0.6
0.4
0.2
0
CHROMOSOME INDEX
1
3
2
4
8
6
7
5
0
5
10
15
20
25
30
RELATIVE LENGTH

Figure 5.2: The chromosomes of *A. pelegrina* (Plant number 3) from a representative cell of the species. The chromosomes are arranged into two groups based on position of the centromere. They are then arranged within the groups in order of descending length. X2000

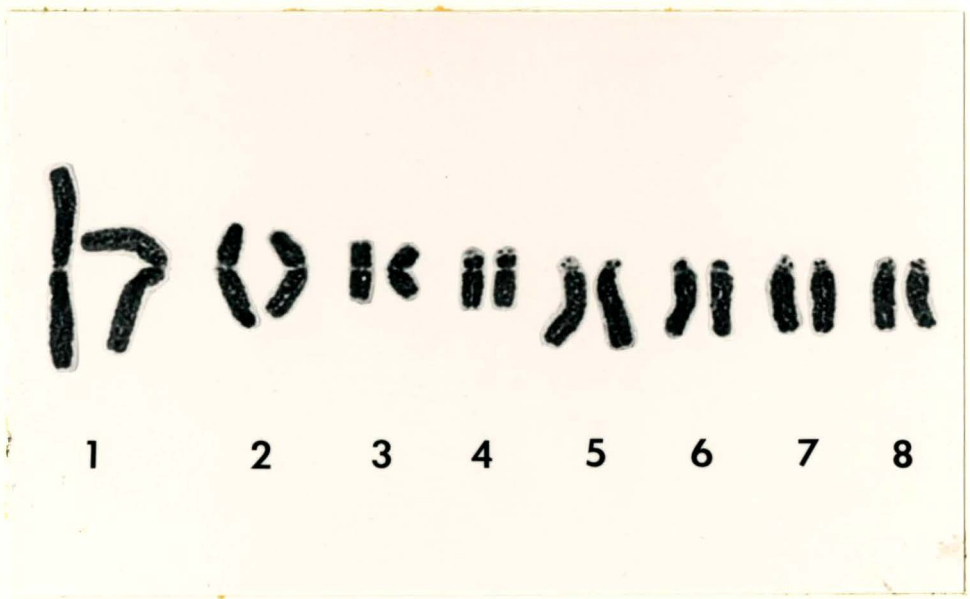
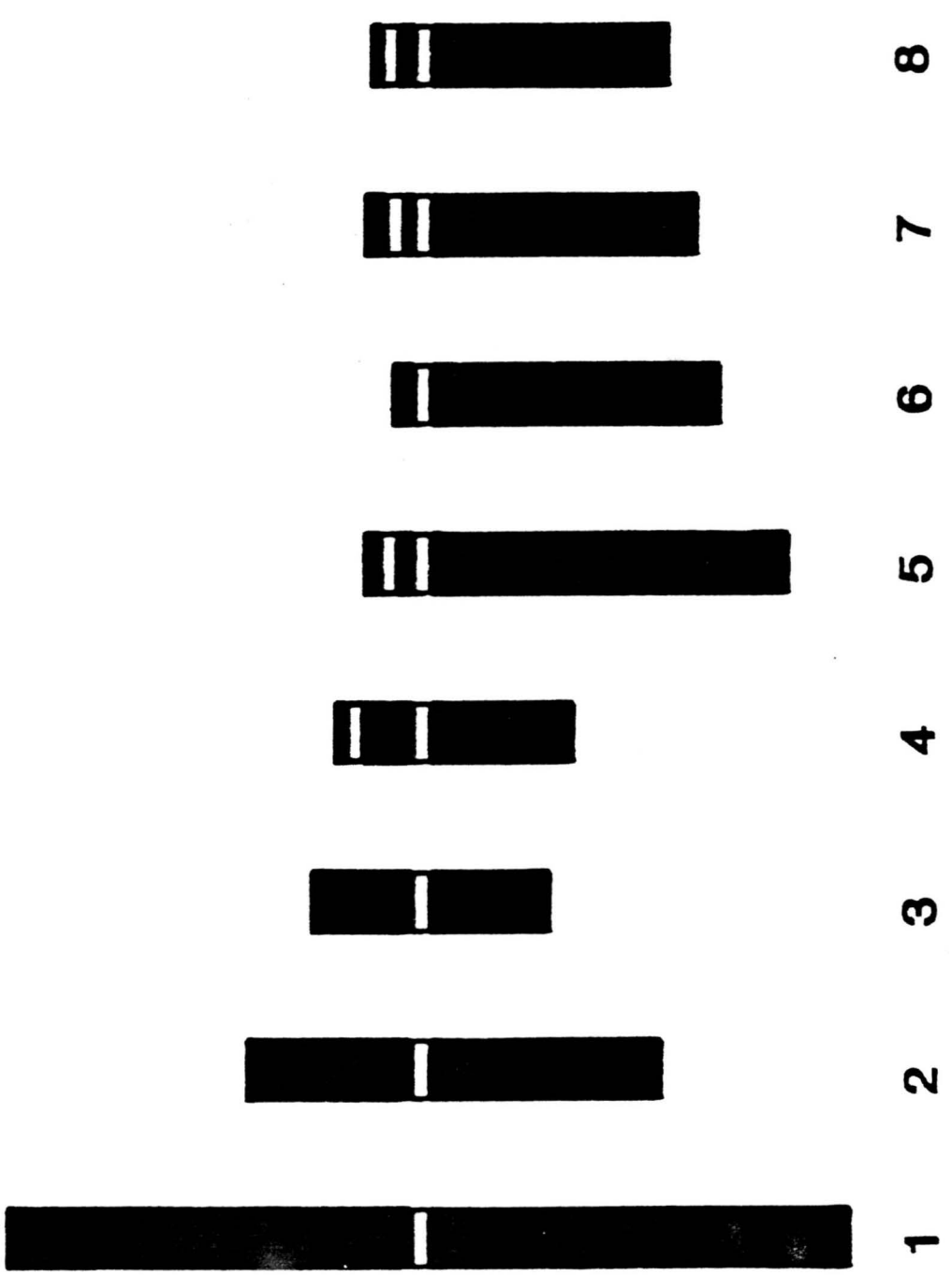

1 2 3 4 5 6 7 8

Figure 5.3: Idiogram of the genome of *Alstroemeria pelegrina* L.


8
7
6
5
4
3
2
1

The chromosome pairs found in all the complements studied were easy to separate into two groups. The acrocentric chromosomes were distinctive and there were no doubts as to which chromosomes belonged in which group. Within the first group the chromosomes were also easy to identify and place in the correct order due to the large size differences between the metacentric chromosomes. The smallest chromosome pair of this group, while only being slightly smaller in total length than metacentric pair number 3, was easy to distinguish due to the different shape and the presence of the satellites in most of the cases.

The second group of acrocentric chromosomes, on the other hand, are difficult to distinguish both under the microscope and in the photomicrograph. Careful measurements were necessary to determine the order of these chromosomes within the group. Chromosomes belonging to pair number 6 were easy to identify due to the longer short arm and the lack of satellites in plants number 1 through number 4. In plant number 5 however the acrocentric chromosomes would only be identified through measurement. Tsuchiya and Hang (1990, unpublished data) indicated that the Giemsa banding technique provided useful information on the identification of four pairs of acrocentric chromosomes.

Although the karyotype of all the plants studied was very similar there were some differences among them. The primary difference was the presence or absence of satellites on several pairs of chromosomes.

Three of the plants studied had satellites on both submetacentric chromosomes 7 and 8 in pair number 4. In plant number 4 however a satellite was only observed on one of these chromosomes, usually chromosome number 8.

There are two possibilities to account for this anomaly. It is possible that the satellite was lost due to structural change in either this plant or one of its parents. The other possibility is that one of the parent plants carried no satellites on either of these chromosomes. When a gamete from one of these plants forms an embryo

with a gamete containing a satellited member from pair number 4, the resultant plant would contain a pair in which only one chromosome has a satellite.

In fact plant number 5 had no satellites on either chromosome of pair number 4. Every possible cell of this plant was examined to locate chromosomes 7 and 8. No satellite was ever found on either of these chromosomes. Figure 4.5 shows a single cell from this plant. Satellites can clearly by seen, as expected, on the acrocentric chromosomes, however, no satellites are present on chromosome pair number 4.

Tsuchiya and Hang (unpublished) also studied a plant of *A. pelegrina* in which they did not find any satellites on either chromosome of pair number 4.

Another interesting variation in the karyotype of *A. pelegrina* was seen in plant number 4. In this instance only one chromosome of pair number 5 had a satellite. Again all possible cells were carefully analyzed and it was determined that this pair did indeed have only one satellited member.

In the case of plant number 3 it should be noted that in the second group of chromosomes, pair number 6 is not acrocentric as in all plants but is subtelocentric. In order to determine what the difference is between this pair in plant number three and that found in the other plants, the measured values for each chromosome pair was converted to an adjusted value, as described to form the idiogram. These adjusted measurements are presented in Table 5.1.

Examination of the r-values of pair number 6 of each plant indicate that plant three has an average r-value of 6.4. This would classify this pair of chromosomes as subtelocentric in the classification system of Levan *et al.* (1964). However, when the two chromosomes of this pair are considered individually one is subtelocentric with an r-value of 5.6 whereas the other is acrocentric with an r-value of 7.4. All of the other chromosome numbers 11 and 12 had r-values greater than 7.0 which placed them in the acrocentric group. Plant number 2, however, had values very close to the dividing point at 7.4 and 7.1.

Since only one chromosome was actually classified as subtelocentric it is most likely that the results are due to experimental artifact or to structural changes such as duplication of a section of the chromosome, inversion or translocation.

Observations of plant number 3 in mitosis did not provide any indications to explain why the short arms of these two chromosomes varied. Two methods could be employed to explain this difference. The first is Giemsa banding and the second is observation of meiosis.

Giemsa banding reveals many of the details of chromosome structure. A comparison of the bands revealed in different plants and in homologous chromosomes would provide some clues as to what has taken place in the aberrant chromosome as shown by Tsuchiya and Hang (unpublished) in *A. aurantiaca* and *A. ligtu.* If a duplication had occurred in this short arm there might be a matching repeat of G-bands visible upon staining.

Study of the meiosis of this plant might show whether chromosomes 11 and 12 are entirely homologous if the segment with the structural aberration was large enough to effect pairing during synapsis.

Plant number 5 had a number of variations from the general karyotype. The first is the absence of satellites on pair number 4 which was discussed earlier. The second variation is found on pair number 6. In this plant satellites are present on both chromosomes of this pair whereas they are not found on the other plants studied. The average length of the short arms of these chromosomes in plant number 5 are less than half the length of the corresponding arms in the other plants (Table 5.1). The satellites are slightly longer than the short arms.

The origin of these chromosomes is difficult to explain. The total length of the short arm and the satellite is about the same as the short arms of the other plants. Thus it is possible that a secondary constriction developed on chromosomes 11 and 12 of plant number 5.

The second possibility is that a reciprocal translocation may have occurred between the short arms of pair number 4 and pair number 6. Reciprocal translocation occurs when two chromosomes are broken and then mutually exchange blocks of chromatin. The two new chromosomes will function normally in somatic cell division if each possesses a single centromere (Swanson, Merz and Young, 1981).

A reciprocal translocation between chromosomes of pair numbers 4 and 6 would then result in a change in the shape of both chromosomes. If a short piece of the chromosomes in pair number 4 along with the satellite was exchanged with a non-satellited segment of the chromosome in the pair number 6 the resultant genome would include non-satellited chromosomes in pair number 4 and satellited chromosomes in pair number 6.

Comparison of the karyotype of *A. pelegrina* with other species studied reveals many of the similarities between all of these species. Many of the species had a common chromsosome constitution consisting of two groups of four chromosomes in their complements, as does *A. pelegrina.* The first group contains four pairs of metacentric, submetacentric or subtelocentric chromosomes. The second group contains four pairs of acrocentric chromosomes with one to four pairs carrying satellites. The other species which have been shown to have this general karyotype are *A. braziliensis* (Taylor, 1926), *A. aurantiaca* (White, 1929; Tsuchiya and Hang, unpublished), *A.pulchella* (White, 1929; Sato, 1938; Tsuchiya and Hang, unpublished), *A. haemantha* (White, 1929; Tsuchiya and Hang, unpublished), *A. chilensis* (Sato, 1938; Tsuchiya and Hang unpublished), *A. versicolor*, *A. caryophyllaea* and *A. hookeri* (Tsuchiya and Hang, unpublished).

A deviation from this general karyotype was described for *A. ligtu* species and hybrids. In this case the first group consists of five chromosomes, including two satellited chromsomes and the second group has only three chromosomes which may be satellited (Rustanius, 1986; Tsuchiya *et al.*, 1987; Tsuchiya and Hang, unpublished).

The major difference between *A. pelegrina* and the other species which have been described by other authors is the presence of satellites on the sub- metacentric chromosome pair number 4. This is unique to this species and has not been documented as occurring in any other species. It is this characteristic which may allow us to speculate on which of the many hybrid cultivars of *Alstroemeria* had *A. pelegrina* as a parent.

A. pelegrina has long been considered to be one of the primary parents of the modern cultivars. The other reputed sources for these cultivated types are *A. aurantiaca* and *A. ligtu.*

Reproduction in alstroemeria utilizes a cross pollination system that would actually facilitate interspecific hybridization. The flowers of alstroemeria are generally large and open, with an accessible stigma with a wet surface, facilitating wind pollination mechanisms. The stigmatic surface also remains receptive long after anthesis occurs within that flower (Uphof, 1940).

Very few names of the parental species for the alstroemeria cultivars developed have been released. Two new varieties exhibited in the 1960's by John Goemans, 'Parigo's Pride' and 'Parigo's Charm', were identified as having three different species in their pedigree. The last one of these species was *A. aurantiaca* but the other details of the breeding program were withheld (Goemans, 1962).

The new alstroemeria variety 'Alsaan' released in 1983 was the result of crossing *A. aurantiaca* with the cultivar 'Orange Beauty' (Lin and Molnar, 1983).

A.'Regina' has been identified as originating from *A. pelegrina* and *A. aurantiaca* and was bred by Van Staaveren (Heins and Wilkins, 1979).

Errey (1962) indicated that the hybridizing of yellow *A. aurantiaca* with both *A. haemantha* and *A. ligtu* was begun in Australia in 1933 and resulted in better quality flowers with a wider color range from straw to deep flame scarlet.

The major problem in identifying the ancestral parents of the modern cultivars is that many of them have been developed through polyploidization and mutation

breeding as well as hybridization. Many of the diploid and polyploid cultivars were also further treated with various doses of radiation to produce yet another group of new cultivars (Tables 5.3 and 5.4).

In spite of the obvious difficulties when trying to interpret the karyotypes of many of the cultivars, it may be possible to make some assumptions based on the occurrence of specific marker chromosomes. In this study, it was shown that the presence of satellites on the sub-metacentric chromosome pair number 4 could be used as a marker to determine if *A. pelegrina* was used as an original parent for a particular cultivar under consideration.

Hang and Tsuchiya (1988) reported that the triploid cultivar "Rosita" contains a small sub-telocentric chromosome which has a small distal satellite. Similarly the hypertriploid cultivar "Orange Beauty" has a small subtelocentric chromosome which also has a small distal satellite. The tetraploid cultivar "Jubilee" contains a medium sub-metacentric chromosome with a small distal satellite. It would thus seem highly likely that these unique chromosomes indicate that *A. pelegrina* may have been one of the parental species of these cultivars.

Meiosis in all plants of *A. pelegrina* studied was rather regular with a slight indication of abnormal pairing or other meiotic aberrations. With this in mind it is very puzzling to note that pollen fertility is rather low. Tsuchiya and Hang (1990) also indicated that a pollen fertility of only 37 percent was found in this species. They found that meiosis of *A.pelegrina* produced some plants with eight bivalent pairs whereas in other plants sporocytes with 7II and 2I were observed. This indicates that there are perhaps some differences between individual plants.

Table 5.3: Cultivars developed through mutation breeding (from Micke *et al.*, 1985).

Name of new variety	Place and date of release (or approval)	Kind and date of mutagenic treatment (treated variety. line, clone...) or mutant crosses	Main improved attributes of variety
Yellow Tiger	The Netherlands, 1970	X-rays, 1967 (Orchid flower)	Yellow flower color with striking black stripes, original variety white with some yellow
Zebra stazeb	The Netherlands, 1975	X-rays, 1968 (Orchid flower)	Heavily striped flower
Zenith	The Netherlands, 1972	X-rays, 1972 (Carmen)	Orange-red flower color
Canaria	The Netherlands, 1970	X-rays,1967 (Orchid flower	Yellow flower color, original variety white height with some yellow
Capitol	The Netherlands, 1977	X-rays, 1972 (Carmen)	Salmon-pink flower color
Fanfare	The Netherlands, 1977	X-rays, 1972 (Carmen)	Red flower color
Harlequin	The Netherlands, 1973	X-rays, 1970 (Parigo's Charm)	Orange-yellow flower color
Harmony stabrons	The Netherlands, 1972	X-rays, 1968 (Regina)	Bronze flower color
Red Sunset	The Netherlands, 1979	X-rays, 1975 actively growing rhizomes	Improved flower color extended duration of flowering period

Table 5.3: Cultivars developed through mutation breeding (from Micke *et al.*, 1985) (continued).

Name of new variety	Place and date of release (or approval)	Kind and date of mutagenic treatment (treated variety. line, clone...) or mutant crosses	Main improved attributes of variety
Result	The Netherlands, 1977	X-rays, 1972 (carmen)	Bright red flower color
Rosali staliro	The Netherlands, 1975	X-rays, 1971 (Starosa)	Pink flower color
Rosita staroza	The Netherlands,1972	X-rays, 1968 (Regina)	Pink flower color
Trident	The Netherlands, 1977	X-rays, 1972 (Carmen)	Pink flower color
Valiant	The Netherlands, 1977	X-rays, 1972) (Carmen)	Light red flower color
White Wings	The Netherlands, 1971	X-rays, 300-400 R 1967, plant with stolons (Orchid flower)	White, except ears
Appelbloesem	The Netherlands,1979	X-rays 500 rad, 1977 (King Cardinal)	Pink flower color with two red dots
Atlas	The Netherlands, 1984	X-rays 500 rad growing rhizomes, 1978 (Red Sunset)	Salmon pink flower color, better winter production

Table 5.4: Cultivars developed through mutation breeding (from Mutation Breeding Newsletter, 1988)

Name of new variety	Place and date of release (or approval)	Kind and date of mutagenic treatment (treated variety. line, clone...) or mutant crosses	Main improved attributes of variety
Lilac Glory	The Netherlands, 1979	X-rays 400 rad growing rhizomes, 1973 (Rosario)	Purple flower color, rest of genotype unchanged
Jacqueline	The Netherlands, 1979	X-rays 400 rad growing rhizomes 1973 (Rosario)	Very successful cultivar with smaller but darker pink flower
Purple Joy	The Netherlands, 1979	X-rays 400 rad growing rhizomes, 1973 (Carmen)	Dark purple-red flower color short stems
La Paz	The Netherlands, 1984	X-rays 350 rad growing rhizomes,1981	Dark yellow flower color
Pink Panther	The Netherlands, 1984	X-rays 500 rad growing rhizomes, 1973 (Rosario)	Longer stems but Longer stems but somewhat smaller flowers
Patricia	The Netherlands, 1978	X-rays 500 rad, 1978 (Pink Triumph)	Improved flower color, reduced stem length
Pink Tiger	The Netherlands, 1983	X-rays 350 rad, 1979 (Pink Panther)	More heavily striped striped than original cultivar, earlier flowering under winter conditions

Chapter 6

SUMMARY AND CONCLUSIONS

The objectives of this work were to study the karyotype of *A. pelegrina* in detail, to describe any variations of this karyotype in individual plants and to provide information which will enable us to ultimately develop new cultivars.

The *A. pelegrina* species used in this study originated as seed from four different sources. They were all found to have a somatic chromosome number of $2n = 2x = 16$. These chromosomes were placed into two groups. The first group contained four pairs of metacentric or submetacentric chromosomes and the pairs were numbered from 1 to 4. The second group contained four pairs of acrocentric chromosomes which were numbered from 5 to 8. There were four pairs of satellited chromosomes in the complement, usually pairs 4, 5, 7 and 8.

Chromosome pair number 4 is unique to *A. pelegrina*. This small submetacentric pair was found to be polymorphic with regard to the presence of satellites. Three plants had satellites on both chromosomes of the pair, one plant had satellites on only one chromosome and one plant had no satellites. The presence of this satellited chromosome could be used as a marker chromosome to identify cultivars in which *A. pelegrina* has been used as a parent.

A general karyotype for all plants of *A. pelegrina* has been established. This karyotype is applicable to all specimens of *A. pelegrina* regardless of their origin. Individual plants exhibit minor variations in the karyotype. The marker chromosome pair number four can be used to identify cultivars of which *A. pelegrina* is

an ancestor and, in fact, several commercial cultivars are identified which have this unique chromosome present in their genome.

Meiosis in both *A. pelegrina* 'rosea' and *A. pelegrina* 'alba' was normal with eight bivalents formed in each case.

Pollen fertility varied from approximately 45% in 'rosea' to 75% in 'alba'.

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