DISSERTATION

CYTOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF *ALSTROEMERIA, LEONTOCHIR* AND *BOMAREA*

Submitted by

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WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY JANICE L. STEPHENS ENTITLED CYTOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF *ALSTROEMERIA, LEONTOCHIR* AND *BOMAREA* BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

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ABSTRACT OF DISSERTATION

CYTOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF *ALSTROEMERIA, LEONTOCHIR* AND *BOMAREA*

Isozyme characterization and cytological analysis were used to characterize and identify species and cultivars of *Alstroemeria, Leontochir* and *Bomarea*. Twenty four species, hybrids and color variants of *Alstroemeria,* two plants of *Leontochir ovallei* and one plant of *Bomarea* species, as well as 23 cultivars were available for isozyme characterization. Nine species of *Alstroemeria*, two *Leontochir ovallei*, one *Bomarea* species and 22 *Alstroemeria* cultivars were investigated to determine their karyotypes and the Giemsa banding patterns of their chromosomes.

Seven isozyme systems were identified which exhibited a high level of polymorphism among the species. A single technique was developed for the extraction of all of these enzymes. The enzyme systems investigated were phosphogluco-mutase, phosphogluco-isomerase, 6-phosphogluconate dehydrogenase, aspartate amino transferase, malic enzyme, esterase and leucine amino peptidase.

It was found that between 11 and 18 of the species and hybrids could be identified uniquely for each of the first six enzyme systems. The final system, leucine amino peptidase was tested on only 11 species and hybrids and 9 different patterns were identified. Using only 3 of the seven enzyme systems it was possible to uniquely identify all of the species and hybrids investigated.

When the 23 cultivars were analyzed, using the first six enzymes, a high level of variability was evident. This was expected due to the polyploid nature of most of the cultivars. The highest level of variability was found for esterase with which 20 of the 23 cultivars exhibited unique banding patterns. All of the cultivars could be uniquely characterized using a combination of only two of the six enzymes.

Very little is known about the parentage of most of the cultivars. Comparison of the banding patterns of the cultivars and the species allowed the identification of the possible parents of each of the cultivars studied.

Using both karyotype analysis and Giemsa banding it was possible to identify individual chromosomes within each species. The identification of a particular species could then be described in terms of the characteristics of each chromosome pair within the complement. The karyotype of all three genera was quite similar, particularly the distribution of acrocentric and the smaller metacentric and submetacentric chromosomes. However, *Alstroemeria* had $2n=2x=16$ chromosomes, whereas *Leontochir* and *Bomarea* had $2n=2x=18$ chromosomes. Characterization and identification of cultivars was also possible using these techniques. Each cultivar studied had a unique complement of chromosomes which clearly identified the cultivar.

Using these two approaches it is possible to uniquely identify the species and cultivars of all three genera. This information may now be used to determine the ppobable parentage of the cultivars. The present study also provides an approach which can be used to further the understanding of the relationships

between these three genera. When more species have been studied and their unique characteristics revealed, it will be possible to use this information to clarify the identity of the putative parents of the European cultivars.

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Chapter 1

INTRODUCTION

Alstroemeria have become very important as a cut flower in the last 15 years in the United States. They require lower temperatures for flowering and growth than many other greenhouse grown floral crops making them cheaper to produce and are prized for their long vase life. The cultivars currently grown in the United States for cut flowers were developed in the Netherlands and in England earlier this century. The parentage of the cultivars was never completely revealed by the breeders. In some instances one of the putative parents was named but rarely are two or more ancestral parents known.

There are over 60 species of Alstroemeria native to South America, generally from Peru, Argentina and Brazil. Some species are also known to be endemic to Mexico and Central America. The common name of these flowers is Peruvian Lily or Lily of the Incas since they were known to be grown by Inca rulers in their flower gardens. The first species was brought to England in 1753.

Since the parentage of the common commercially grown cultivars is not known, development of local cultivars in the U.S. has been less successful than that in Europe. American breeders are required to pay lease fees for the European plants that they grow as well as a royalty for every stem that they sell. This makes these cut flowers more expensive than they otherwise would be.

Identification of the species most likely involved in the breeding of the European cultivars would allow American breeders to better focus their breeding efforts. This would speed up development of cultivars for local growing conditions.

Two approaches were used in this study to provide breeders with the necessary information to identify breeding materials. The first approach (chapters 1-5) was to utilize the genetic polymorphisms known to exist within the enzyme systems of many plants, through the characterization of the isozyme banding patterns of these plants. The second approach (chapters 6-10) was to carry out an extensive cytological study of several species and cultivars in order to identify each of them with respect to the chromosome complement and the Giemsa banding patterns of each of the chromosomes or chromosome pairs.

Isozymes have been used in many instances to identify and characterize individual plants and species. They are able to provide information for the patenting of new cultivars and lines as well as to unravel the genetic relationships between plant species.

Using polyacrylamide gels the various isozymes of seven enzyme systems were separated and analysed to determine their distribution within each species of the genus *Alstroemeria*. Similar analyses of the related genera *Bomarea* and *Leontochir ovallei* were also conducted in order to compare the three genera and to provide information that will allow the level of relatedness of them to be determined.

Analyses of 23 commonly grown cultivars were also conducted in order to compare their isozyme banding patterns with those of the species. The bands present within each cultivar were then compared with the bands produced by each of the species. Where a high level of common bands were revealed between the cultivar and one of the species, it was proposed that the species may have been a possible parent for that cultivar. In some instances two or more species were identified as possible putative parents.

Most of the European cultivars were the result of hybridization and polyploidization of various species. Many of them were developed through the process of mutagenesis using x-rays.

This may have resulted in changes within the DNA which in turn changed the proteins produced by the plant. The precise identification of the cultivars is therefore not readily achieved.

The first section of this work reviews the results of isozyme analyses already completed for many horticultural and agronomic crops. The many uses of isozymes in determining genetic identity and relationships among species and genera are discussed.

The two main methods for separating isozymes and their relative merits and problems are addressed in order to justify the methodology chosen in this study which was to use polyacrylaminde gel as the separation medium.

The results obtained through this research provide information which allows for the characterization and identification of all the species and cultivars included in the study. The relationship of the banding patterns of the species to that of the cultivars allows some speculation as to the possible putative parents of the cultivars.

Although much work still remains, the results of this research provide the basis by which species and cultivars of *Alstroemeria* and the related genera *Leontochir* and *Bomarea* may be easily characterized and their relationships to one another determined.

The second part of this study provides further information which may aid in the identification of the species and cultivars. The conclusion to this work is therefore placed at the end of the second section and addresses the results of both parts of the research.

Chapter 2

LITERATURE REVIEW

The phenotype of a plant is determined by its morphology and its production of secondary compounds such as flavenoids and terpenoids. However, it is difficult to use this approach when establishing phylogenetic and systematic relationships since there is no accurate equation between phenotype and genotype.

Enzyme electrophoresis, on the other hand, provides data which is fundamentally different from morphological data and/or secondary chemistry. Differences in the electrophoretic mobility of the enzymes are usually the result of changes in the structural genes coding for polypeptides, thus electrophoretic differences between isozymes are a direct result of genetic differences.

There are also a number of disadvantages when using morphological characters as markers in plant genetic studies. There may be epistatic and or pleiotrotropic effects which limit the number of markers which can be assembled in a single stock. More importantly the recessive alleles of genes for morphological characters may be deleterious when homozygous. Most isozyme loci, however, have alleles which are co-dominantly inherited. They cause no deleterious effects through pleiotropy or recessiveness, and heterozygotes are easily distinguishable from homozygotes. Thus, isozymes have been widely used as markers for systematic, genetic and evolutionary studies.

Electrophoresis is a chromatographic technique for the separation of mixtures of ionic compounds. Gel electrophoresis combines elements of separation based on charge and gel filtration or separation based on size. Thus, the charged molecules migrate at a rate determined by the charge and the size of the protein as well as its shape. We can then use enzyme activity staining to visualize the separated bands. Many enzymes have a particular stain and incubation condition which can be used to visualize the position of that enzyme in the gel. Since isozymes are different molecular forms of an enzyme with the same substrate specificity (Markert and Moller, 1959), there may be more than one band visible in the gel. Multiple molecular forms of enzymes have been found by electrophoretic methods in almost every organism studied so far.

Each species may have a unique banding pattern with each band representing proteins of different mobility in a polarized electric field . The number of isozymes detectable depends on several factors such as the number of genes coding for the enzyme, the number of alleles of each gene which specify electrophoretically distinct polypeptides, the quarternary structure of the enzyme and the extent to which the subunits of polymeric enzymes can cross-multimerize as intergenic enzymes.

Genetic analysis allows the distinction to be made between different forms of enzymes that are products of different alleles, i.e. allozymes, and those that are coded by different genes, i.e. isozymes. Therefore, it is possible to quantify exactly the number of genes included in the study, as well as the number of alleles at each loci.

The actual number of bands present from each gene is also affected by the active subunit composition of the enzyme. Species homozygous at the locus have only one band for the enzyme. If the species is heterozygous, two bands will be seen for monomeric enzymes, one for each allele in the chromosome compliment. If the enzyme is dimeric, however, three bands will be seen, one band for each of the homodimeric enzymes and an intermediate band for the heterodimeric enzyme. Similarly, for a tetrameric enzyme there will be five bands present as the two alleles can associate in five different conformations to construct the active enzyme.

In plants, a further factor to be considered is the localization of enzymes in subcellular compartments. Since the same catalytic reaction is required, these isozymes will be revealed as extra bands in the gel. For example, both plastids and cytosol contain enzymes for the reactions of the oxidative pentose phosphate pathway and of glycolysis (Gottleib, 1982). Generally there are two isozymes for each enzyme of glycolysis and the pentose phosphate pathway, at least three or four of aspartate aminotransferase, at least three of nicotinamide adenine dinucleotide-specific malate dehydrogenase and two or three alcohol dehydrogenases.

It is interesting to note that both conifers and the flowering plants, which diverged more than 100 million years ago, have the same number of isozymes of each enzyme. The tissue examined was green leaves in the flowering plants and the large female gametophyte in the seed of the conifers. There appears to have been a conservation of metabolic activities in different subcellular compartments as well as conservation of the same number of isozymes in these distinct tissues (Gottleib, 1982).

It should also be noted that plastid isozymes often exhibit much less variability than the cytosolic isozymes. There are fewer variants per species and the electrophoretic mobilities of all plastid isozymes are closely similar, always migrating to the same narrow region of the gel whereas those of the cytosolic isozymes are often grossly different (Gottleib, 1982).

As the organism develops from embryo to adult there is a differential expression of enzymes in different cell types, tissues and organs as each becomes necessary for different biochemical pathways. For instance, the catalase enzyme system in *Zea mays* is coded by three genes *Cat-1, Cat-2* and *Cat-3. Cat-1* is active during kernal development and then rapidly decreases in activity in the scutellum after seed germination. *Cat-2* increases in activity after seed germination, peaking at day 4 of seedling growth and then declines. Therefore, at day one after germination, only *Cat-1* is expressed, as growth proceeds *Cat-1* and *Cat-2* are simultaneously expressed and during later stages of seedling growth only *Cat-2* is observed. Differential rates of synthesis and degradation are responsible for the change in expression from *Cat-1* to *Cat-2* as development proceeds (McMillin, 1983).

It has also been demonstrated that many isozymes are tissue specific. Weeden (1984) revealed that, of 10 loci useful for distinguishing white seeded bean cultivars, only malic enzyme, rubisco, adenylate kinase, esterase and acid phosphatase exhibited differences in activity or presence of bands when different tissues were used. Aconitase 1 and aconitase 3 were found to be present in *Annona cherimola* flowers only, and not in leaf extracts (Ellstrand and Lee, 1987). Similarly, specific esterase isozymes were only present in embryo-scutellum and immature endosperm but not in leaf tissue of barley (Hvid and Nielsen, 1977). Hart (1983) includes information on the tissue location of many of the isozymes of *Triticum aestivuum* cv Chinese Spring as does Brown (1983) in his discussion of barley. Tissue specificity of thirteen enzymes in rice has been determined by Second (1982) and Second and Trouslot (1980). A number of papers by Tanksley, Rick and others indicates the tissue specificity of many enzyme loci of tomato (Rick, 1983). Examples of tissue specificity may also be found for tobacco, petunia, eucalyptus, potatoes and peas.

Another factor which affects the appearance of different isozymes may be the environmental growing conditions of the plant. Mineral nutrition, cold temperature hardening and disease infection may all influence the intensity of isozyme activity or the appearance of different molecular forms (Pierce and Brewbaker, 1973). However, Nielsen (1985) concluded that environment was not very important.

It is clear, therefore, that great care must be taken to collect samples for isozyme analysis that are from comparable tissues of a similar developmental stage. Additionally, clear separation of isozyme bands is a cumulative result of proper sample size, extraction buffer, gel composition and pH, electrolyte, electrical current and stain technique. Obviously, some error is often introduced with these variables (Pierce and Brewbaker, 1973).

Evolution of Isozymes

Multiple forms of an enzyme may provide important genetic variation on which selection can operate, thus causing divergence between isozymes. The degree of divergence may be evaluated if we have sequence information on the genes coding the proteins, the physical and kinetic properties of the isozymes and a complete knowledge of the temporal and spatial expression of each isozyme. The DNA sequence comparison permits an absolute measure of the genetic change between the two isozymes while the other properties allow an estimation of the functional divergence. Although we often have little or no knowledge of the coding gene sequence, the structural and kinetic characteristics of isozymes as well as developmental and tissue distribution are more easily obtained (Weeden, 1983).

Multiple forms of an enzyme can be produceded by alteration of the translated polypeptide. These conformational isozymes merely represent different structural configurations of the same polypeptide, e.g. malate dehydrogenase and enolase. Condensation of an enzyme into groups of two or more may produce a series of aggregational isozymes, e.g. glutamic dehydrogenase, glyceraldehyde and 3-phosphate dehydrogenase. Post-translational modification of the proteins will also result in the formation of isozymes (Weeden, 1983).

An important factor in producing multiple enzymes is the process of gene duplication which is then followed by divergence between the loci. This will often result in a zymogram phenotype that is similar to that produced by heterozygous individuals. However, each gamete will contain one copy of each locus instead of only one of the two alleles as in a true heterozygote. Duplication may occur in a number of different ways. The process of unequal crossing over between homologous chromosomes will lead to a tandem duplication of genetic material on one pair of chromosomes. Chromosomal rearrangements may produce inverted or non-adjacent duplications. Gene amplification can result in duplicate segments of genetic material on non-homologous chromosomes or a series of reciprocal translocations between non-homologous chromosomes may also result in gene duplication. Polyploidy is an important source of gene duplication in vascular plants and the result is that every gene in the plant will be duplicated.

The most important source of isozymes in the plant cell seems to have arisen through the transfer of the genomes of the prokaryotic symbionts; in other words, when the cyanobacteria and bacteria became a part of the plant cell to form the chloroplast and the mitochondria. The products of these genes have retained their specificity for the organelle and enzymes originally possessed by both the endosymbiont and the host cell have become organelle and cytosolic isozymes respectively (Weeden, 1983).

Given that various isozymes may have arisen through one of these methods, what is the evolutionary potential of isozymes? Evolution of an enzyme is generally a slow process of mutation followed by selection or random drift. Isozyme evolution differs from this description in that the presence of a second form of the enzyme in the organism may influence the evolutionary opportunities available to the first. The potential for isozymes to differentiate and develop new functions depends to a large extent on the manner by which they are generated. Thus, the likelihood for divergence between conformational isozymes is very different from that of isozymes coded by duplicated loci.

Isozymes with identical structural genes, that is, those due to post-translational modifications, conformational changes or aggregational variations, may exhibit altered isoelectric points, temperature stablility differences or different kinetic properties. However, since these gene products presumably fill some essential role in cellular metabolism, they are probably not able to acquire new functions while still maintaining an original role and evolution at the level of structural gene would be very slow.

Allozymic forms of an enzyme can differ in their amino acid sequence as well as in their modifications and should display greater variation than the previous group of isozymes. The divergence of these isozymes is still constrained by the metabolic role they fill in the cell. Any change that impairs the normal function of an allozyme will be selected against unless that function is of minor importance to cellular metabolism or the change provides the organism with additional capabilities that are of greater use than those lost. Major alterations in the active or regulatory sites, sub-unit binding regions or controlling elements for gene expression are rarely beneficial to an organism already adapted to its environment. Thus, major changes are still not expected at the allozyme level (Weeden, 1983).

The greatest potential for isozyme evolution is found in those isozymes coded by separate loci. Gene duplications produce identical copies which presumably are both actively transcribed. Often, only one of these duplicate loci is necessary to fulfill the cell's need for the product, permitting the second locus to collect 'forbidden' mutations. Such isozyme systems might exhibit differences in specificity, in regulatory controls or in developmental expression without reducing the fitness of an individual. Variation in certain isozymes may be important in a plant's capacity to survive in a variety of environments (Shaw and Prasad, 1970). Circumstantial evidence indicates that polymorphisms may play a role in the increased adaptability of an organism, as shown by the correlation of polymorphism patterns with patterns of environmental variation (Jain, 1969; Markert and Moller, 1959). Sing and Brewer (1971) showed that greater protein diversity was found in widely adapted *Triticum* species than in the more narrowly adapted *Aegilops* relatives. Similarly, Arulsekar and Bringhurst (1983) indicate that octaploid *Fragraria,* which occupy a much broader ecological range in California than the diploids, have an increased isozyme variability than the diploids.

Since duplicated loci may result in a drain on the cell's energy reserves with little in the way of benefit to the cell, the situation will resolve itself by either silencing one of the loci or by their divergence until their roles are no longer redundant. Ultimately, in the latter case, the loci will produce independent enzymes. It is most likely, however, that one of the loci becomes silenced so that the organism expresses only one form of the enzyme (Weeden, 1983).

It has also been proposed that heterosis may be a factor in isozyme evolution. Hybrid bands which appear in F_1 samples may be associated with superior performance or efficiency of an enzyme system. Heterozygotes may combine alleles for active but unstable enzymes forms with those specifying stable but inactive forms thus producing a hybrid enzyme that is both stable and active (Pierce and Brewbaker, 1973).

This is not the case for isozymes originating from a symbiotic or endosymbiotic relationship. These enzymes were initially adapted to distinct metabolic environments and these differences are often maintained in the symbiotic complex. Since these enzymes are so specialized they have very little effect on one another and continue to evolve independently of each other. Diploid and polyploid plants (such as ferns) exhibit more isozymes of this type than of those produced by gene duplication. The difference in the evolutionary fate of each type of isozyme is thus a reasonable explanation for this disparity. The organelle-specific isozymes are maintained because their function cannot easily be replaced by other isozymes in the cell. In contrast, isozyme systems generated by duplication or polyploidy will usually degenerate to a single locus system.

Characterization using isozymes

The use of isozymes for cultivar identification and as tools for unravelling the genetic relationships between plant species has been widely investigated in the last thirty years. Isozymes may be separated by gel electrophoresis, using either starch or polyacrylamide as the gelling agent.

Most analyses of plant isozymes has been carried out on starch gels although polyacrylamide is now often used. Starch gels are used in a horizontal apparatus which can be kept cold on ice trays. The materials are inexpensive and non-toxic and preparation of samples is very simple. Gels may also be sliced horizontally allowing for staining of 3-4 enzyme systems from a single run. A major disadvantage of starch gels, however, is that starch from different sources or different batches may give varying results for both rate of migration and resolution. There may also be impurities present in some batches which interact with the enzymes being studied (Shaw and Prasad, 1970). Starch frequently lacks clarity and this, combined with the relatively poor resolution, may make it difficult to distinguish between allozymes or isozymes with small migrational distance differences (Suurs et al., 1989).

Polyacrylamide gels are very transparent and give excellent resolution with sharper bands. These gels may be prepared with various concentrations resulting in different pore sizes, and it is possible to have pore size gradients and a discontinuous pH within a single gel. The disadvantages of using polyacrylamide, however, are its expense and the fact that it is a neurotoxin if ingested. These problems are minimized through careful handling of both the monomer and polymer of polyacrylamide and by using very thin (<1mm) vertical gel slabs. The ability of polyacrylamide gels to separate isozymes with only small differences in migrational capacities and thus delineate different cultivars or species outweighs the problems that may be incurred through its use.

Screening plants for isozyme banding patterns is a non-destructive process which is relatively inexpensive and can rapidly screen large numbers of plants. Only small amounts of plant tissue are needed and this may be from leaves, roots, flowers, pollen, seeds or callus. Plants may also be screened at the seedling stage and undesirable genotypes can be eliminated early in the programs, resulting in considerable savings of time and money.

Some of the applications of isozyme data have been: 1) screening variability in plant populations, 2) identifying sexual and somatic hybrids, 3) reducing the number of backcross generations in a backcrossing program, 4) using isozymes as markers for a breeding program,

5) detecting genetic diversity in plant populations (Moore and Collins, 1983) and 6) construction of linkage maps for plant chromosomes (Tanksley and Rick, 1980).

The most frequent use of isozymes in recent years has been for cultivar identification. In many instances cultivars may be morphologically indistinguishable yet have growth traits or physiological characteristics which make each more desirable for certain situations. If the zymogram fingerprint is known for each cultivar then it becomes a simple matter to ensure that the correct one is chosen for the research or breeding program or for sale to growers.

Suurs et al. (1989) found that by using young growing leaves of *Lycopersicon* or *Solanum* species clear consistent results could be found for several enzymes using polyacrylamide gels. Tanksley and Rick (1980) separated hybrid progeny isozymes of *Lycopersicon* and *Solanum* parents on starch gels. *L. esculantum* primary trisomics were used as female parents in crosses with *Solanum pennellii* accession LA716, which is homozygous for the variant allele Aps-2^{r4}. Trisomic F_1 progeny were selected and one month old seedlings from F_2 seeds of each trisomic type were assayed for Aps-2 genotypes. The authors were then able to locate a number of isozymic loci in the linkage map of tomato. They found that the distribution of isozymic genes was non-random and that as many as 31% of enzymic genes characterized may be of a tandemly duplicate nature. Now that the map location of these isozymic genes are known they can be used as biochemical markers to map other genes - isozymic, morphological, physiological, phytopathological, or otherwise. Precise information about the segregation of individual genes, provided by isozyme analysis enables us to determine the degree of genetic differentiation between taxa, to determine abnormal segregation and to compare recombination rates (Tanksley and Rick, 1980).

In order to utilize wild germplasm as a source of genetic variability for domesticated species, it is necessary to eliminate undesirable wild genes, which are incorporated into the cultivars along with desirable traits. As discussed by Tanksley and Rick (1980), isozymic loci may be used as markers of chromosome segments to screen the backcross progeny extensively for recurrent parent isozyme genotypes. If multiple locus selection is used, the return to the recurrent parent genotype will be achieved more quickly. When the genes to be introgressed are recessive or a combination of dominant and recessive, F_2 selection coupled with isozyme analysis may be effective.

When tight linkages exist between a desirable gene and an isozymic gene, the isozymic gene could be used as a tag. This would allow for the selection of the desirable trait without direct scoring, which may be difficult and time consuming. Examples of this application found in tomato are the linkage between the acid phosphatase locus (*Aps-1*) and the nematode resistance ene (*Mi*) (Rick and Forbes, 1974), the linkage between the peroxidase locus (*Prx-2*) and the male sterility gene (*Ms-10*), and the linkage between Pgi-1 and Adh-2 genes and the genetic elements for cold tolerance (Vallejos and Tanksley, 1988).

Primary trisomics are available in diploid potatoes and have been used to locate several genes on chromosomes. Since the commercial potato, *Solanum tuberosum* L., is a tetraploid and the diploid Solanum species are often self-incompatible, there are few chromosome markers known in *Solanum*. However, because of their co-dominant expression, isozyme loci can be used as chromosome markers (Quiros and McHale, 1985). Utilization of allelic isozymes as markers of loci in close association with genes for given traits may facilitate the choice of parents and/or the selection of progeny based on isozyme phenotype (Oliver and Martinez-Zapater, 1985). Quiros and McHale (1985) presented inheritance data for six isozymic loci, *Prx-2, Prx-3, Prx-5, Mdh-1, Pgi-1* and *Sdh-1*. They concluded that *Prx-3, Prx-5, Pgi-1* and *Sdh-1* can be used as genetic markers for potatoes and its related species.

The use of isozyme patterns for precise identification of strawberry cultivars in patent disclosures was reported by Bringhurst et al. (1981). They used three isozyme systems, PGI (see Table 2.1 for abbreviations), LAP and PGM to separate and identify 22 strawberry cultivars. Six different PGI patterns were revealed with two being unique to specific cultivars, another shared by two cultivars and the rest being spread throughout the remaining cultivars. For PGM, five banding patterns were studied with one being unique to a specific cultivar. There were four different LAP banding patterns with two unique to specific cultivars and the remaining two distributed among the other cultivars. Using any two of these enzyme systems enabled the unique classification of at least four cultivars whereas the use of all three systems enabled the unique classification of 12 of the 22 cultivars, with each of the others being indistinguishable from one other cultivar. These pairs could possibly be classified separately if another enzyme system were included.

Nehra et al. (1991b) also used the same isozymes as markers for strawberry cultivars grown in both tissue culture and in the greenhouse. They showed that these isozymes remained stable with the age of the plants as well as in both tissue culture and greenhouse growing conditions. They also found five different banding patterns for PGM but none of these were the same as those found by Bringhurst et al. (1981) and were in a different part of the gel. The three PGI

Table 2.1. Abbreviations used to designate enzymes.

Enzyme	Abbreviation
Aldolase	ALD
Aspartate amino transferase	AAT
Diaphorase	DIAP
Esterase	EST
Fumarase	FUM
Glucose phosphate dehydrogenase	GPD
Glucose -6-phosphate dehydrogenase	G-6-PD
Hexokinase	HK
Isocitrate dehydrogenase	IDH
Isocitrate dehydrogenase	ICD
Leucine amino peptidase	LAP
Malate dehydrogenase	MDH
Malic enzyme	ME
Peptidase	PEP
Phosphogluco-isomerase	PGI
Phosphogluco-mutase	PGM
Shikimic acid dehydrogenase	SDH
Tetrazolium Oxidase	TO
Triose phosphate isomerase	TPI

patterns were the same as three of the six identified by Bringhurst et al (1981), although again they were in different parts of the gel. The chloroplastic PGI isozyme was located at an R_f (distance migrated relative to a dye front) of about 0.68 by Nehra et al. but was located at 0.5 by Bringhurst et al (1981). There was also a great difference between the LAP isozymes reported by the two groups. The great variation in isozyme patterns reflects the complex, octaploid nature of strawberry as well as the huge number of different cultivars available. The changed position of the bands in the gel may also be a result of the use of starch gels by Bringhurst et al (1991). as compared to the polyacrylamide gels used by Nehra et al (1991a, 1991b).

A study of nuclear DNA content and isozyme variation in *Fragaria X ananassa* callus cultures (Nehra et al. 1991b) demonstrated that isozyme banding patterns for EST, LAP, PGI and PGM did not change with the polyploid and aneuploid changes that occurred in the aging callus cultures.

Ellstrand and Lee (1987) highlighted the importance of isozyme markers for cultivar identification. *Annona cherimola* (cherimoya or custard apple) cultivars are frequently misidentified since cultivars are characterized primarily by fruit shape and fruit surface characteristics. These characteristics may vary considerably from fruit to fruit on the same tree, and fruits may not appear on grafted material for up to five years. The authors found that of 17 loci investigated, 12 averaged 3.1 alleles per polymorphic loci in leaves of grafted scion wood. Among the 15 cherimoya cultivars analyzed, no two shared the same multilocus profile. The probable parents of the variety `Bonita' were also identified using this information, and the identity of a mislabelled cultivar was established.

Pascual et al. (1993) used isozymes as genetic markers to characterize seven Spanish cherimoya cultivars and to compare these to the Californian cultivars. They showed that the Spanish cultivars were easily separated using combinations of only two enzyme systems, AAT and IDH, AAT and TPI or TPI and PGM. Two of the cultivars were found to be identical. The Spanish cultivars also differed from the more diverse Californian cultivars.

Using seed or seedling tissue, Weeden (1984) revealed 10 loci which were useful in distinguishing cultivars of white seeded beans. Of 90 cultivars studied, 52 were uniquely identified by their allozyme fingerprint. The remaining 38 could be subdivided into 14 sets, the majority of which consisted of only two cultivars each. These sets may be further subdivided as additional loci are screened. Several cultivars were found to possess rare alleles by which they could be identified without resorting to the entire genotype. These alleles can be used as genetic markers for crosses involving these lines and as a possible means for tracing of particular traits in an F_2.

Nielsen (1980) was able to identify all 35 expected genotypes in tetraploid ryegrass (*Lolium spp.*) segregating for four alleles at a *Pgi* enzyme locus. This system could thus be used as a tool to separate hybrids and self-pollinations in a breeding program of tetraploid ryegrass. The varieties could then be distinguished by the differences in allele frequency.

Wolfe (1976) used isozyme patterns for a number of different enzyme systems to study over 60 varieties of wine and table grapes. He demonstrated that varieties which are morphologically indistinguishable may be separated by their isozyme patterns for two or more enzyme systems. Conversely, however, he noted that some varieties, that are morphologically distinct, were not separable based on the four enzyme systems used. Obviously, it is important to consider many varying factors when trying to distinguish varieties.

Three white, table grape cultivars and a bud sport of one of them were separated by Schwennesen et al. (1982) based solely on the enzyme catechol oxidase. Other enzymes examined showed little or no difference among cultivars.

Loukas et al. (1983) used pollen of grape cultivars to study the inheritance of nine polymorphic isozyme systems encoded by 10 loci. Segregation patterns in the F_1 progenies of crosses made by hand pollination indicated that allozymes belonging to the same enzyme system are probably controlled by a single loci. The systems studied were PEP, GPD, ALD, PGM, TO, ICD, FUM, G-6-PD, and PGI. Analysis of the inheritance of these allozymes showed a very high degree of heterozygosity, consistent with the hypothesis that these cultivars are hybrids of genetically distinct cultivars or individuals. Further examination of grape isozymes may allow the identification of the parental cultivars as well as provide information on the history of cultivars and details of human intervention in the cultivation of grape species.

Genetic analysis of 11 allozyme polymorphisms was carried out by Weeden et al. (1988) on the progeny of "Cayuga White" x "Aurore", two complex interspecific grape hybrids. Their work confirmed the single gene inheritance of GPI-C, PGM-C and LAP-1 proposed by Loukas et al. (1983) and also extended to 17 the number of polymorphic isozyme loci defined for *Vitis*. Segregation at most of the isozyme loci gave simple Mendellian ratios in the progeny examined, with MDH-2 providing the only exception.

It has been assumed that with their relatively high chromosome number (2n=38), most grape species are of polyploid origin (Weeden et al., 1988). However, the results of the study by Weeden et al. (1988) for AAT-1, AAT-2 and/or AAT-3 did not indicate that locus duplication due to polyploidy had occurred. Only two isozymes of both PGM and PGI, one cytosolic and one plastid, were identified, consistent with the expected number of a typical diploid plant. These results indicate that if grapes are of polyploid derivation then considerable diploidization and loss of duplicate loci expression has occurred. These researchers suggest that the isozyme loci studied may be used as molecular markers for the development of a linkage map for *Vitis* as well as to tag genes controlling commercially important characters.

Torres et al. (1978b, 1982) used isozymes to study the genetic control of AAT, PGI and PGM in *Citrus* and it near relatives. Species relationships in *Citrus* with its two subgenera, *Citrus* and *Papeda*, has been difficult to ascertain. Researchers have often proposed conflicting taxonomies for this important economic crop. A further problem for breeders has been to distinguish asexual nucellar seedlings from zygotic seedlings, and this generally could not be ascertained until fruiting begins five or more years after seedling development. Torres et al. (1978b) used isozyme patterns to overcome some of these problems. Using AAT-1, AAT-2, PGI and PGM they were able to identify a unique combination of genotypes for each species. They also noted that almost all the individual biotypes within a species have the same genotypes. Leaves from seedlings only one to two months old produce zymograms identical to those of older, fruiting individuals of the same biotype. It is therefore possible to use the isozyme markers to distinguish nucellar seedlings from those of zygotic origin very early in the breeding program.

Torres et al. (1982) investigated six other gene loci (*Mdh-1, Mdh-2, Lap, Hk, Idh* and *ME*) of Citrus in a continuation of the work and confirmed that the cultivars within a species were identical and that most of their genotypes were heterozygous. This suggests that each of the nine species of cultivated citrus are from a single prototype, several through hybridization, and that the cultivars within each species arose non-sexually as sports.

Working with avocado, Torres (1978a) was able to characterize several cultivars and demonstrated that isozyme analysis will be useful in the study of the evolution and relationship of the three avocado races, as well as for cultivar adaptability and rootstock identification.

Gene dosage effects due to a change in the copy number of the coding genes results in alterations of isozyme phenotypes. Thus, in a heterozygous triploid the activity of the allozyme from the parent contributing the 2n gamete should be twice as great as that contributed by the other parent (Tsaftaris et al., 1981) or that both alleles of a heterozygous parent would be expressed in the triploid. Chyi and Weeden (1984) used this approach to determine which parent contributed the 2n gamete in the formation of triploid apple cultivars. Of all the systems analyzed, 6-PGD was the most informative and the authors concluded that the parent contributing the 2n gamete in both the triploid cultivars examined was the female.

Using six enzyme systems, Weeden and Lamb (1985) were able to characterize fifty-four apple cultivars. Cultivar verification is usually not possible until fruit is produced on trees that are several years old. This may result in considerable loss of time and money if an incorrect cultivar is sold to growers. Some morphological characteristics may be used to distinguish seedlings, however, isozyme analysis offers a possible alternative method for cultivar identification. Of the six enzyme systems studied (6PGD, AAT, IDH, PGI, TPI and DIAP), 6PGD and AAT were found to be the most useful in discriminating among cultivars. Within cultivar polymorphism did not exist in the isozyme systems investigated but there was considerable variation between cultivars.

Samimy and Cummins (1992) used isozymes of six enzyme systems (ADH, PGI, PGM, 6PGD, MDH and PER) to distinguish 13 apple rootstocks. PGM and 6PGD together were sufficient for complete identification of all the rootstocks. It is essential that the orchardist be able to select a true-to-type rootstock cultivar since this is what will determine the relative tree growth rate, ultimate size, anchorage, precocity, productivity and response to hazards of the soil environment. The identification of cultivars based on isozyme data is time and cost effective as compared to identification based on morphological characters and physiological responses.

Isozyme polymorphisms were used by De Wald et al. (1988) to characterize pineapple cultivars. Using PGM and PER, unique identification of 15 of the 27 cultivars examined was possible, and, in most instances the members of a horticultural group differed in genotype at one or more of the isozyme loci. Cultivars of common origin were found to have identical banding patterns, and the origin of cultivars thought to have originated via mutation was supported or refuted on the basis of their isozyme genotypes.

Using the same five loci for two enzyme systems, De Wald et al. (1992) were able to conduct taxonomic studies of *Ananas* (pineapple) genus to determine the genetic basis of the observed banding patterns. It was found that the isozymes were useful in characterizing and classifying plant introductions as well as *Ananas* species. Although pineapple is generally considered to be self-incompatible, these isozyme studies confirmed that the cultivar Cambray exhibited self-compatibility, thus showing that not all domestic pineapple cultivars are obligately self-incompatible.

Isozyme markers have been identified in *Salvia columbariae* (Hashemi et al. 1993) in order to assist the breeding program of this new oilseed crop.

Suiter (1988) used isozymes to investigate the suspected polyploid origin of the diploid A genome species of the genus *Gossypium*. The results he obtained were consistent with the hypothesis which suggests that at least two ploidy amplification events occurred during the evolution of the genus. For many morphological markers and within the enzyme systems PGM and TPI of the allotetraploid AD genome of *Gossypium*, a loss of duplicate gene expression has been observed, indicating a pattern of diploidization.

Kennedy and Thompson (1991) identified nine enzyme systems exhibiting polymorphism among *Ipomeae batatas* L. (sweetpotato) cultivars. They were able to identify each of the nine cultivars studied using five of the nine enzyme systems, and proposed that this variability will provide a method for precisely identifying cultivars for patent purposes.

Reyes and Collins (1992) used isozyme analysis to try to determine the relationship between *Ipomeae batatas* and *I. trifida*. They concluded that *I. trifida* may be one of the ancestors of *I. batatas* although other species must also be investigated.

Isozyme analysis was found to be generally useful for identifying raspberry cultivars (Cousineau and Donnelly, 1992) although other techniques such as mini-satellite DNA analysis may also be needed to separate isozymically similar cultivars. However, Cousineau et al. (1993) suggest that isozyme markers may be useful to accelerate gene introgression from related species, as well as for the evaluation of wild populations for genetic variability, the tagging of traits, and the development of a linkage map.

Guayule is a rubber producing shrub that is a potential new industrial crop for arid and semi-arid regions. It is important that a distinction can be made in polyploids between progenies derived from fertilized eggs and those produced from maternal tissue through the process of facultative apomixis. Since only one morphological marker (flower color) has been identified, biochemical markers and their link to desirable traits need to be established in order to screen seedlings. Several enzyme systems have been investigated (Hashemi and Estilai, 1992) which show promise in solving these problems.,

The identification of kiwifruit cultivars and clones is possible through morphological features of flowers or fruits (Messina et al., 1991). Identification of cultivars before flowering is very important for mass propagation and to ensure that mix-ups do not occur in manipulating plant stocks. They found that AAT, PGI and PGM showed polymorphism among cultivars and this was used to confirm that cultivars present in Europe are composed of different clones, and are not always representative of the standard cultivars supposed to have been propagated and exported from New Zealand and/or France.

Beaver and Iezzoni (1993) suggest that, based on isozyme studies, sour cherry, *(Prunus cerasus* L.) which is a tetraploid (2n=4x=32), could have arisen from sexual polyploidization via an unreduced gamete from sweet cherry (*P. avium* L., 2n=2x=16). They also determined that sour cherry is probably an allotetraploid with ground cherry (*P. fructicosa* Pall.) as the other progenitor species.

As early as 1967, Hart and Bhatia reported characteristic enzyme patterns for *Nicotiana* species but intraspecific isozyme variation was much less than that between species. Torres et al. (1978b) used isozyme polymorphism to demonstrate that each species of *Citrus* had a combination of genotypes that was distinct from all the others whereas all cultivars within a species had almost no variation.

Tomato species were found to range from almost complete monomorphy for the tested loci, APS, EST,GOT, and PBX (Rick, 1983) to highly polymorphic. Interestingly, *Lycopersicon pimpenellifolium*, a completely self-fertile species was polymorphic at all loci; however regional differentiation resulted in fixation of different alleles. *L. hirsutum* was shown to have two populations which were self-compatible and monomorphic as well as a self-incompatible population which had up to 72% polymorphy. The authors suggested that in both these instances the species were originally highly variable but as they spread variability was reduced. The most polymorphic species are *L. peruvianum* and *L. chilcense* which are both strictly self-incompatible.

In marked contrast, McLeod et al. (1983) found that the level of polymorphism in *Capsicum* species ranged from only 3.8 to 38.4% with the domesticated taxa being less polymorphic than the wild taxa. However, there was very little variability found between populations within a taxon. There was also a low level of heterozygosity in all taxa, and this, together with the low levels of polymorphism, are probably the result of the ability of peppers to facultatively self-fertilize.

Diploid, annual species of *Mendicago turbinata* and *M. trunculata* showed no variation among plants within each accession (Quiros, 1983). Annual *Mendicago* species are strict selfers, which explains the lack of polymorphism within accessions. All the plants were homozygous for a fixed allele. There were differences, however, between these species as well as between *M. soleirollii* and *M. tornata* (Quiros et al., 1980) and between *M. murex* and *M. turbinata*.

Very little genetic variation was found within populations of *Cucumis melo* (Dane, 1983) with the proportion of polymorphic loci ranging from 0 to 0.27. Similarly, there was little variability in the peroxidase isozyme patterns from many of the other *Cucumis* species although some intraspecific differences were found in APS and GOT isozymes. Using peroxidase isozymes, she grouped the *Cucumis* species into four groups which differed from each other while species within groups were more similar. *Cucurbita* species also showed distinct isoenzyme patterns for several enzyme systems that allow them to be uniquely characterized (Dane, 1983).

At this time there is no information available on the enzyme systems in *Alstroemeria*. In fact, as far as can be ascertained, no genetic markers have been developed for either Alstroemeria species or cultivars. Alstroemeria species are found throughout a wide range in South America, mainly in Brazil and Peru. Their habitat ranges from sandy coastal areas to dry deserts to high mountains. Each species is particularly adapted to its own ecological niche. Alstroemeria are outcrossing plants but there are strong incompatibilities between many of the species. This suggests that there would be marked interspecific differences in both morphology and biochemical markers, whereas intraspecifically there may be much less variation.

Isozyme analysis has been utilized by many researchers since 1959 to identify cultivars and species, to determine phylogenetic relationships and to estimate genetic distances. Since this information is almost completely lacking in *Alstroemeria* species at this time, investigation of isozyme polymorphism may provide data necessary for the successful breeding of new *Alstroemeria* cultivars in the United States.

Chapter 3

MATERIALS AND METHODS

A total of 47 species and cultivars have been examined (Table 3.1). These plants were all container grown in a greenhouse under natural daylength with temperatures ranging from 10°C in winter to 28°C in summer. Leaf samples were collected from young, vegetative shoots using the top 3 to 5 fully opened and resupinate leaves. Samples were taken on at least 4 different occasions and 3 to 6 electrophoretic runs were made for each enzyme from each collection time.

There is a great deal of confusion at this time about the identity of many *Alstroemeria* species. Accordingly, the alternate names sometimes used in the literature have been included (Table 3.1). The designation used in this report for each species is the one provided by the original source of the plant or seed. Included in the species examined were two color forms of *A. pelegrina*, two forms of *A. aurantiaca*, and specimens of both *A. psittacina* and *A. pulchella*. The latter two species are considered to be different names for the same species (Hannibal, 1941; Stinson, 1942; Robinson, 1963). Likewise, *A. chilensis* is most likely a form of *A. ligtu*. Since *A. haemantha* crosses easily with other species (Robinson, 1963) it is probable that the plants studied were actually hybrids.

Gel formation

Polyacrylamide gels 0.8mm thick were formed using a multiple gel pouring stand from Idea Scientific to constuct separating gels. This apparatus allowed the preparation of up to 15 separating gels at a time. Each 10 x 10 cm plate sandwich requires about 6ml of solution to fill to the desired height. The stock solutions listed in Tables 3.2 and 3.3 (Wetter and Dyck, 1983) were made and stored in the refrigerator except as indicated. A fresh preparation of 28mg ammonium persulfate in 20ml of dH_2O was made each time gels were prepared.

The gel plates were set up in the multiple gel pouring stand as follows. The stand was laid on its back to an almost horizontal position. A spacer was placed on each side of the base of the stand to maintain a space between the bottom of the glass plates and the stand.

Table 3.1: Abbreviations, source, origin and synonyms for alstroemeria species and cultivars

Species or cultivar	Synonym	Source	Origin	Abbrev.
A. angustifolia ssp angusifolia		Fred Meyer	Chile	An
A. aurantiaca Don - yellow flower	*A. aurea* *A. lutea*	Unknown	Chile	A
A. aurantiaca Don - orange flower		Unknown	Chile	At
Bomarea species		Miyake, Japan	Chile	B
A. caryophyllae		Dr. G. Pisen Utah	Chile	C
A. chilensis Cree	*A. ligtu ssp ligtu*	U.K.	Chile	Ch
A. diluta ssp diluta Bayer		Fred Meyer, California	Chile	D
A. exserens Meyer		"	Chile	E
A. garaventae Bayer		"	Chile	G
A. haemantha Ruiz and Pavon	*A. ligtu ssp simsii*	U.K.	Chile	H
A. hookeri ssp hookeri Loddiges	*A. pulchra* *A. rosea*	Fred Meyer	Chile	Ho
A. ligtu hybrid		Holland	Chile	Lh
A. ligtu ssp simsii Sprengel	*A. pulchella* var pilosa	Fred Meyer	Chile	Li
A. ligtu `Miyake spotless'		"	Chile	Lm

Table 3.1: Abbreviations, source, origin and synonyms for alstroemeria species and cultivars *continued*

Species or cultivar	Synonym	Source	Origin	Abbrev.
Leontochir ovallei		Miyake nursery, Japan	Chile	Lo
A. ligtu L. *species*	?A. chilensis	Chile	Chile	Ls
A. magenta Bayer		Fred Meyer	Chile	M
A. magnifica ssp maxima Philipi		"	Chile	Mm
A. pelegrina L. *alba*		Hortus Bot. Gotoburgensis	Chile	Pa
A. pelegrina L. *rosea*		Japan	Chile	Pr
A. psittacina Lehm	*A. pulchella* *A. braziliensis*	Jardin Alpin-Commun de Meyrin, France	Brazil	Ps
A. pulchella L.	*A. psittacina*	Univ. Bot Garden, Dresden, Germany	Brazil	Pu
A. sierrae	*A. magnifica ssp magnifica*	Parigo, England	Chile	S
A. versicolor Ruiz & Pavon		Wülfinghoff, Netherlands	Chile	V

Table 3.1: Abbreviations, source, origin and synonyms for alstroemeria species and cultivars *continued*

Cultivar	Abbrev.
\`Arizo'	a
\`Eureka'	b
\`Carmen'	c
\`Mona Lisa'	d
\`Neva'	e
\`Pink Triumph'	f
\`Rosita'	g
\`Orange Beauty'	h
\`Rosario'	i
\`Orchid'	j
\`Appelbloesem'	k
\`Orego'	l
\`Regina'	m
\`Red Surprise'	n
\`Alnba'	o
\`King Cardinal'	p
\`Luciana'	q
\`Jubilee'	r
\`Texas'	s
\`Yellow King'	t
\`Canaria'	u
\`Marina'	v
\`Monika'	w

Table 3.2: Separating Gel Ingredients

Solution Number	Ingredients	Volume for 12 Gels	
		6.3%	5.4%
1 10ml	36.6g Tris, 48ml 1N HCl, made up to 100ml with dH_20		10ml
2	30g acrylamide, 0.74g Bis acrylamide, made up to 100ml with dH_2O	17ml	14ml
3	28ml ammonium persulfate per 20ml dH_2O (Make fresh each time)	40ml	40ml
	Water	16ml	16ml
	TEMED	20µl	20µl

Table 3.3 Stacking Gel Ingredients

Solution Number	Ingredients	Volume for 4.3% solution ml
4	5.7g Tris, 0.08ml of TEMED, add enough H_3PO_4 to bring to pH of 6.9. Bring to 100ml with dH_2O	0.5
5	10g acrylamide, 2.5g Bisacrylamide made up to 100ml with dH_2O	1.5
6	4mg ribovflavin made to 100ml dH_2O (stable for up to 2 weeks)	0.5
7	40g sucrose made to 100ml with dH_2O	2.0

A few drops of water were then placed on the back of the stand and a glass plate (10 x 10cm) was placed onto this. The water drops are to prevent any gel from getting in between the gel sandwiches or between the last sandwich and the stand.

Side spacers were placed on either side of the gel plate, extending slightly above the gel top. A second glass plate was then placed in position over the spacers but positioned about 2mm higher than the first to make sample application easier. A few drops of water were again placed on the second glass plate and the next glass plate was placed in position. This is the beginning of the next gel sandwich and procedures outlined above were then repeated until the desired number of sandwiches were prepared.

When the final sandwich was ready the bottom spacers were removed, water drops were put onto the plate surface and a heavy plastic plate was put into position on top of all the sandwiches. A large cork and a rubber band were used to hold all of the plates in place. The entire apparatus was then sealed by the application of silly putty. The silly putty was rolled into a "string" about 30cm long and placed around the inside edge of the pouring frame. It was then pushed into place, being careful to seal into the corners extremely well so that no leakage could occur. The frame was then placed upright and marked at about 1cm below the bottom of the comb depth to provide a fill mark for pouring the separating gel.

Ingredients for the separating gel were mixed to make either 6.3% or 5.4% gels (Table 3.2). The gels were poured immediately and all gas bubbles were removed by gently tipping and tapping the apparatus on the bench top. The top of the gels was then covered with primary butanol saturated with dH_2O, and left to polymerize for about one hour. The butanol above the polymerized separating gel was then poured off and blotted with "Kim-Wipes". The gel surface was then washed a minimum of 20 times with dH_2O, blotting between each wash to remove all traces of the butanol.

At this point the sets of glass plates with the gel in between, can be separated from one another using a razor blade and/or can be stored in the refrigerator for up to 2 weeks before use inside a "ziploc" bag.

When ready to use, a gel sandwich was removed from the bag and placed into the electrophoresis equipment. The sandwich was slipped into the slot of the upper reservoir and positioned with the gel top just barely above the slot. Clips were used to attach the gel to each side of the holder, being careful to position the clip over the spacer without dislodging it. This avoids bending the glass.

The upper reservoir was then tipped so that the gel was in a horizontal position and the top of the slot was sealed with 1.5% molten agarose, both at the front and the back. The gel was then returned to the vertical running position and the sides checked for any other gaps. A few drops of agarose were added to close any possible gaps.

A 4.2% stacking gel was then prepared by mixing 0.5ml of solution 4, 1.5ml of solution 5, 0.5ml of solution 6 and 2ml of solution 7 (Table 3.3) for each two separating gels. The stacking gel solution was then added to the top of the separating gel with a pasteur pipette and the comb was inserted. The gel slab was then placed under a fluorescent light source to polymerize the stacking gel for approximately 1/2 - 1 hour. The appropriate electrode buffer (Table 3.4) was then added to correct levels in both the anode and cathode compartments of the electrophoresis cell taking care to remove any trapped bubbles in the lower chamber. The comb was then carefully removed and the wells were washed with the buffer, using a Hamilton syringe.

Extraction

The first three to five fully opened and resupinate leaves were collected from young vegetative shoots of alstroemeria plants (Table 3.1) for a total of approximately 0.5g. Polytron homogenization was performed in an ice bath for 20 to 30 seconds using 1.5ml of extraction buffer (Table 3.4) per 0.5g of fresh leaves.

Two other methods of extraction - manual glass homogenization and mortar and pestle - were also used and the results compared with polytron homogenization to determine which method gave the clearest and most stable results. On the basis of these comparisons, polytron homogenization was chosen as the most appropriate method of extraction.

The homogenate was then centrifuged in a Baxter Scientific Biofuge 17R centrifuge at 11,068g for 30 minutes at 4°C. The supernatant was divided into 24µl aliquots to which 2µl of bromophenol blue tracking dye was added and then stored at -70°C for up to 4 weeks until further use.

Eighteen to twenty microliters of sample were loaded into the wells of the polyacrylamide gel using a micropipette and special tips that were able to fit within the 0.78mm wide wells. Samples were kept on ice once removed from the freezer, and electrode buffers were ice-cold.

Table 3.4: Buffers

1. Phosphate solution A (0.2M)	Sodium phosphate monobasic $NaH_2PO_4.H_2O$ 27.8g/L
2. Phosphate solution B (0.2M)	Sodium phosphate dibasic $Na_2HPO_4.7H_2O$ 53.6g/L
3. 1.0M Phosphate buffer	
4. Lithium Borate	1.6g $LiOH.H_2O$, 11.6g Boric acid (anhydrous free acid). Dissolve and adjust pH to 8.3. Make to 1 liter.
5. Tris-glycine	6.5g Trizma base, 28.8g glycine. Dissolve and adjust pH 8.3. Make up to 1 liter. Dilute 1:10 for use as an electrode buffer.
6. Extraction buffer sucrose, (wt/vol)	0.2M Tris HCl pH8.5, 1M 8mg/ml DTT, 10% PVP

Electrophoresis

Electrophoresis was carried out in an Idea Scientific mini-slab apparatus in a cold room at 3±1°C. A voltage of 150V and current of 20mA was used to stack all the sample to the bottom of each well. After 10 minutes the voltage was increased to 400V and 50mA for about 60 to 100 minutes. When the tracking dye had migrated to within 0.5 cm of the bottom of the gel, electrophoresis was stopped.

The gel sandwich was then removed from the apparatus and the glass plates were separated. The gel which adheres to one plate, was gently loosened. Disposable vinyl gloves were used to handle the gels at all times. The stacking gel was removed and the upper right hand corner of the gel was notched. The gel was then slipped into the appropriate stain and incubated until bands appeared. The isozyme staining solutions and approximate staining times and temperatures are in Table 3.5.

When staining was judged to be complete, the solution was discarded and the gel was rinsed 3 times in tap water or once in 10% acetic acid and twice in tap water, with the first rinse being treated as hazardous waste. The gels were fixed for 30 minutes in a solution of 20% ethanol, 10% glycerol before drying.

After staining, the gels were photographed against a frosted glass light box with a Nikkon 35mm camera using Kodak Pan Plus X 135 (125 ASA) Black and White film and the appropriate filters.

Gels were dried overnight on a gel drying frame, between two layers of Ultraclear cellophane (Idea Scientific #1080).

The position of each band on the gel was expressed as a ratio of the distance from the top of the gel to the center of the band relative to the distance the dye fromt travelled (R_f). These values provide information about the relative position of bands within and between the lanes. Since it is improbable that every gel is prepared exactly the same each time, i.e. slight differences in volume measurements, temperature variation, gelling time variation etc., the R_f values are not precisely reproducible. The pattern of bands produced for each specimen for each stain, however, remain the same each time the electrophorsis is carried out.

Wherever possible, samples were collected at different times and two or three electrophoretic runs were made for each stain. In the case of materials provided as leaf tissue samples only, at least 2 runs were made for each stain.

Table 3.5: Staining Solutions and Incubation Conditions

Stain (Reference)	Ingredients for 100ml of solution		Gel Conc	Electrode Buffer	Incubation Conditions
ESTERASE (Arulsekar & Parfitt, 1986)	Fast Blue RR Salt	40mg	6%	Tris-glycine	30°C in dark for 15-30 minutes
	Dissolve in 40ml of DI water				
	Phosphate solution A	50ml			
	Phosphate solution B	10ml			
	0.1% α-napthylacetate	2ml			
ASPARTATE AMINO TRANSFERASE (NSSL)	Fast Blue BB salt	100mg	6%	Tris-glycine	37°C in dark for 30 minutes
	Substrate solution	100ml			
	Substrate solution				
	α-ketoglutaric acid	36.5mg			
	L-aspartic acid	133mg			
	PVP-40	500mg			
	EDTA	50mg			
	Na_2HPO_4	1.42g			
	DI water	100ml			
6-PHOSPHOGLUCONATE DEHYDROGENASE (Soltis, et al., 1983)	1.0M Tris HCl buffer pH 8.0	10ml	6%	Tris-glycine	37°C in dark for 30 minutes
	DI water	90ml			
	6-phosphogluconic acid	40mg			
	1.0M $MgCl_2$	2ml			
	NADP	10mg			
	MTT	10mg			
	PMS	2mg			
LEUCINE AMINOPEPTIDASE (Soltis, et al., 1983)	L-leucine-β-napthyl amide (free base)	20mg	6%	Lithium borate	37°C in dark for 30 minutes
	Dissolve in N'-N-dimethyl formamide	5ml			
	1.0M Phosphate buffer pH 6.0	10ml			
	DI water	90ml			
	Fast Black K salt	50mg			

Table 3.5: Staining Solutions and Incubation Conditions *Continued*

Stain Incubation (Reference)	Ingredients for 100ml of solution		Conc	Gel Buffer	Electrode Conditions
PHOSPHO-GLUCO MUTASE (Soltis, et al., 1983)	1.0M Tris HCl buffer pH 8.0	10ml	6%	Lithium borate	37°C in dark for 30 minutes
	DI water	90ml			
	Glucose-1-phosphate disodium salt	50mg			
	Glucose-6-phosphate dehydrogenase	40unit			
	NADP	10mg			
	MTT	10mg			
	PMS	2mg			
PHOSPHO-GLUCO MUTASE ISOMERASE (Soltis, et al., 1983)	1.0M Tris HCl buffer pH 8.0	10ml	6%	Lithium borate	37°C in dark for 30 minutes
	DI water	90ml			
	1.0M $MgCl_2$	1ml			
	Fructose-6-phosphate, disodium salt	30mg			
	Glucose-6-phospate dehdrogenase	40unit			
	NADP	10mg			
	MTT	20mg			
	PMS	2mg			
MALIC ENZYME (Soltis, et al., 1983)	1.0M Tris HCl buffer pH 8.0	10ml	5%	Lithium borate	37°C in dark for 30 minutes
	DI water	80ml			
	2.0M DL-malic acid (add NaOH pellets to pH 8.0)	10ml			
	1.0M $MgCl_2$	2ml			
	NADP	20mg			
	MTT	20mg			
	PMS	2mg			

Chapter 4
RESULTS

Several extraction buffers and extraction techniques were compared to determine the optimum method to use with Alstroemeria. Extraction buffers containing 2-mercaptoethanol and/or sodium metabisulfite were found to destroy some enzyme systems and so were not suitable for use. The best extraction buffer was modified from one developed by Nehra et al (1991) and contained 10% PVP as an antioxidant as well as 8mg/ml DTT to prevent the action of the phenolic compounds present in the cells. Since one objective of this study was to provide a method by which Alstroemeria species and cultivars may be easily identified, simplicity of sample preparation was considered vital. For this reason, it was imperative that a single extraction buffer be identified that would allow investigation of several different enzyme systems. It is likely that further modification of the extraction buffer may give good results for other enzyme systems not included in this study.

A comparison of twenty four different preparation methods was made. The tissue was ground in the buffer in a mortar and pestle for either 30 seconds or 1.5 minutes; or it was ground in a glass homogenizer for either 1 or 4 minutes; or it was ground in a Kinetamatic polytron homogenizer (Brinkmann Instruments) for either 15 or 30 seconds. A portion of each sample was then centrifuged while the remainder was placed on ice in the refrigerator, to separate solids from the supernatant. One half of the supernatant of each of these samples was then frozen at -70°C the other half was kept on ice in the refrigerator.

Although each of the three grinding techniques resulted in bands at the same positions in the gel, there was a marked difference in the quality of the bands produced after staining for esterase (Fig.4.1). Samples prepared using mortar and pestle grinding had more oxidation occurring which resulted in streaking and blurring of the bands. The most anodal band was often difficult to see. Glass homogenization resulted in very distinct bands with no streaking or blurring, however, the most anodal band was faint compared to the two more cathodal bands. Following polytron homogenization all three bands were clearly visible in the gel with very little streaking or blurring. The uncentrifuged samples had more streaking within the gel for both mortar and pestle and polytron homogenization preparations. Whether the sample had been frozen or was used

Figure 4.1. Comparison of preparation methods - Esterase stain

Lanes 1-8: Glass homogenization

Lanes 9-16: Mortar and pestle

Lanes 17-24: Polytron homogenization

Odd numbered lanes were centrifuged

Even numbered lanes were not centrifuged

Lanes 1, 2, 5, 6, 9, 10, 13, 14, 17, 18, 21, 22 - Fresh preparations

Lanes 3, 4, 7, 8, 11, 12, 15, 16, 19, 20, 23, 24 - Frozen preparations fresh had no effect on the quality of the results, even for samples kept on ice for up to three days. The length of the grinding times had little effect on the clarity of results for either glass homogenization or mortar and pestle preparation. For the polytron homogenization the longer grinding time produced some streaking of the gels, particularly for samples which were kept on ice and not frozen.

Based on these results all samples were prepared with the polytron homogenizer for 20 seconds, centrifuged at 4°C for 30 minutes at 11,500g, and the supernatant was divided into aliquots and frozen at -70°C until needed. Although it is not necessary to centrifuge the samples in order to produce good results, it was found that some preparations were very dense and centrifuging was quicker than waiting for the preparations to separate by gravity.

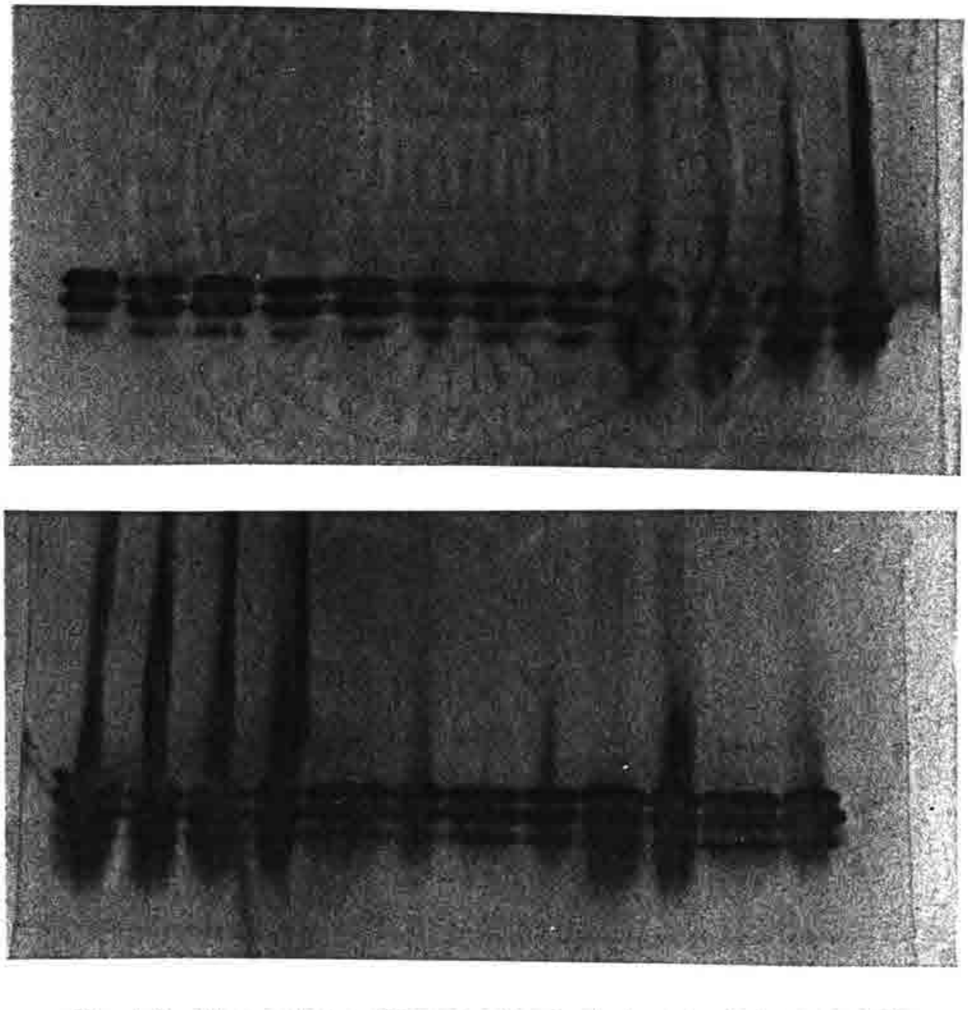


Fig. 4.1. Comparison of preparation techniques - Esterase stain.

If rapid, preliminary results are required, however, the samples could be ground in a glass homogenizer or polytron homogenizer and the supernatant loaded directly into the gel.

A number of different enzyme systems were studied for Alstroemeria species. Some were found to give consistent results using the preparations method outlined above while others gave inconsistent or no results. There were no usable results for glutamate dehydrogenase, alcohol dehydrogenase, malate dehydrogenase, or acid phosphatase despite trying several different staining solutions and techniques. It is probable that good results might be obtainable for these enzymes if a different extraction technique were used. Since part of the objectives for this study was to find a simple, easy method for species identification, identifying several additional extraction procedures for individual enzyme systems was inappropriate.

Inconsistent results were obtained for leucine amino peptidase and peroxidase. In both cases clear results were obtained from samples collected in January and February of 1993 but at other times only indistinct or no bands could be identified. This would suggest that there may be a strong time of year or growth stage effect on the expression of these enzymes.

The enzyme systems which exhibited clear results at all times throughout the year were aspartate amino transferase (AAT), esterase (EST), malic enzyme (ME), 6-phosphogluconate dehydrogenase (6-PGD), phosphogluco-mutase (PGM) and phosphogluco-isomerase (PGI). There were some instances, most notably with AAT and EST systems, when extra faint bands were sometimes seen. Only those bands which were present consistently throughout the year were included in the analysis and characterization of each species and cultivar.

SPECIES

There were a number of species for which more than one individual plant were available for analysis. Due to problems in the greenhouse in previous years some of the plants may have been divisions of a single plant whereas others originated from quite different sources. For all species, except *A. versicolor*, no variation was found in the isozyme banding patterns for the seven enzyme systems studied among the various plants of a single species. Since *A. versicolor* has been shown to have a highly polymorphic chromosome karyotype (Tsuchiya and Hang, 1989) the variation in isozymes present within each plant was not unexpected.

Gels were subjected to electrophoresis and stained for each enzyme system a minimum of six times, with some enzymes being examined up to 14 times. The first twelve species and hybrids listed on each diagrammatic representation were included in all of the electrophoretic runs. The next twelve species and hybrids became available at later dates and were tested from two to four times for each enzyme system.

Phosphogluco-mutase (Fig.4.2)

There were two distinct zones of activity for this enzyme, one around the Rf value of about Rf 0.64 and second at the Rf value of 0.80. Either one or two bands were found in each zone for all the species studied. The same banding pattern was found for both color variants of *A. aurantiaca* with two cathodal bands at Rf 0.64 and 0.66 and two anodal bands at Rf 0.76 and 0.80. Similarly both color variants of *A. pelegrina* had only a single isozyme band at Rf 0.64. *A. ligtu sp., A. chilensis, A. garaventae, A. diluta ssp diluta, A. ligtu `miyake spotless',A. hookeri ssp hookeri,* and *A. ligtu simsii* all had the same two isozyme band locations at Rf 0.64 and 0.79. *A. angustifolia ssp angustifolia* and *A. magenta* had the same isozyme banding patterns, as did *A. haemantha* and *A. exserens*. The other nine alstroemeria species and hybrids as well as *Leontochir ovallei* and *Bomarea sp.* all had unique isozyme banding patterns. The six plants of *A. versicolor* exhibited some slight variability in the position of the two anodal bands , with some plants having only a single band at Rf 0.71 but not at Rf 0.80.

Phosphogluco-isomerase (Fig.4.3)

Gels stained for PGI have two distinct zones of banding, one between Rf 0.58 and 0.70 and the other between Rf 0.30 and 0.52. Three different allozymes are present in the more anodal region of the gel , with one of these allozymes being present for each of the 22 species, color variants or hybrids. A fourth allozyme, at Rf 0.58, was present only in *A. angustifolia ssp angustifolia* and *Leontochir ovallei*. A much higher level of variability was found in the cathodal series of allozymes. There may be from two up to six allozymes for each of the alstroemeria species, color variants and hybrids. The bands in this region of the gel are possibly due to the activity of two different isozymes, one around Rf 0.40 and the second at about Rf 0.49. The most cathodal isozyme had three different alleles, with each species or hybrid having either one or two alleles. The isozyme centered at Rf 0.49 had seven alleles with each species or hybrid

Figure 4.2a. Phosphogluco-mutase - species preparations

From left to right: Ps*, Ps, H, H, H, C, A, At, Ch, Lh, Ls, Ls, Ps, Pa, Pr

Ls, Li, Mm, Lm, E, A, Ho, M, An, D, G, Ls

Ls, V, V, V, V, V, V, Pu, Pu, Pu, Ps, Lo, Lo, B, Ls

*Abbreviations from Table 2.1

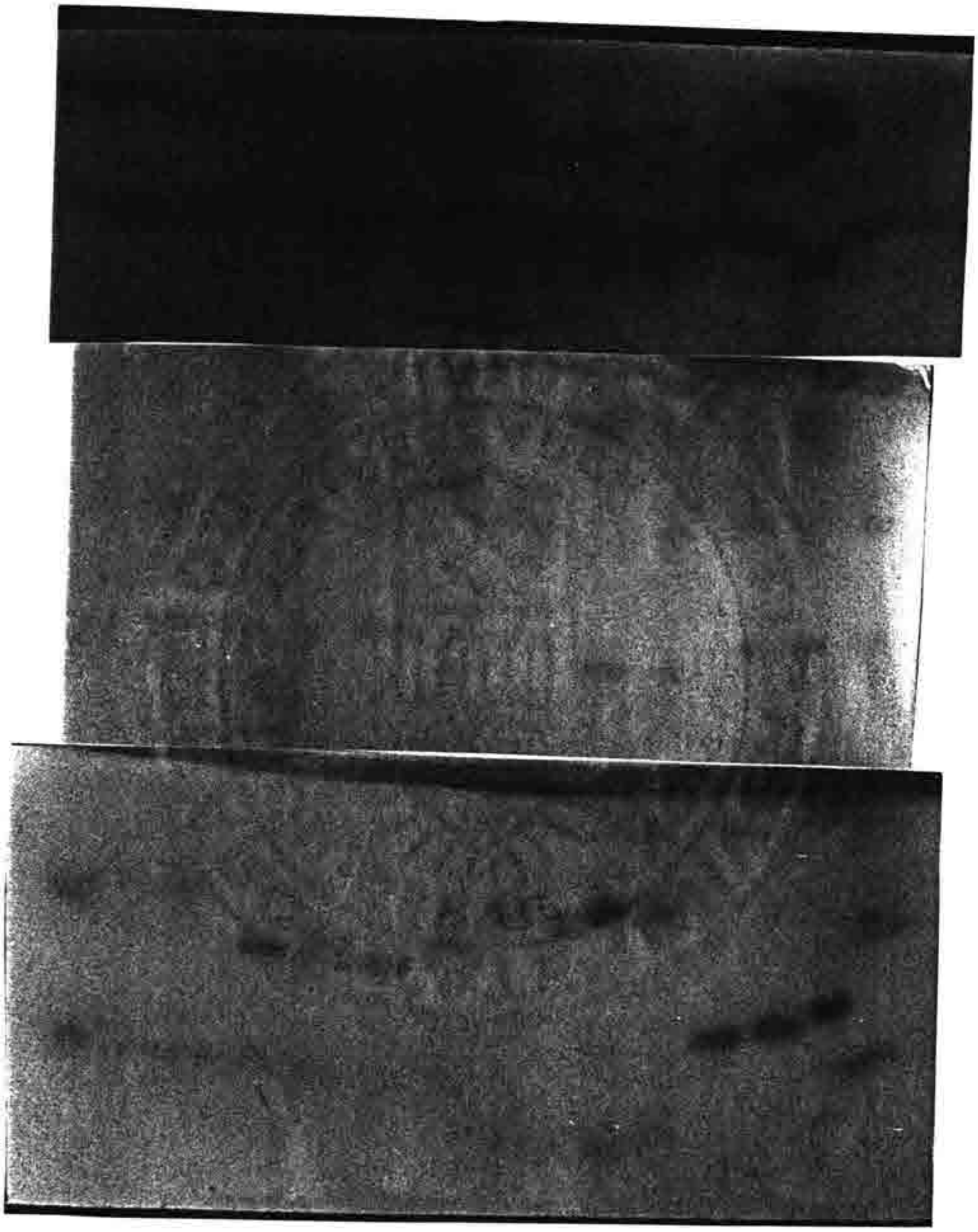
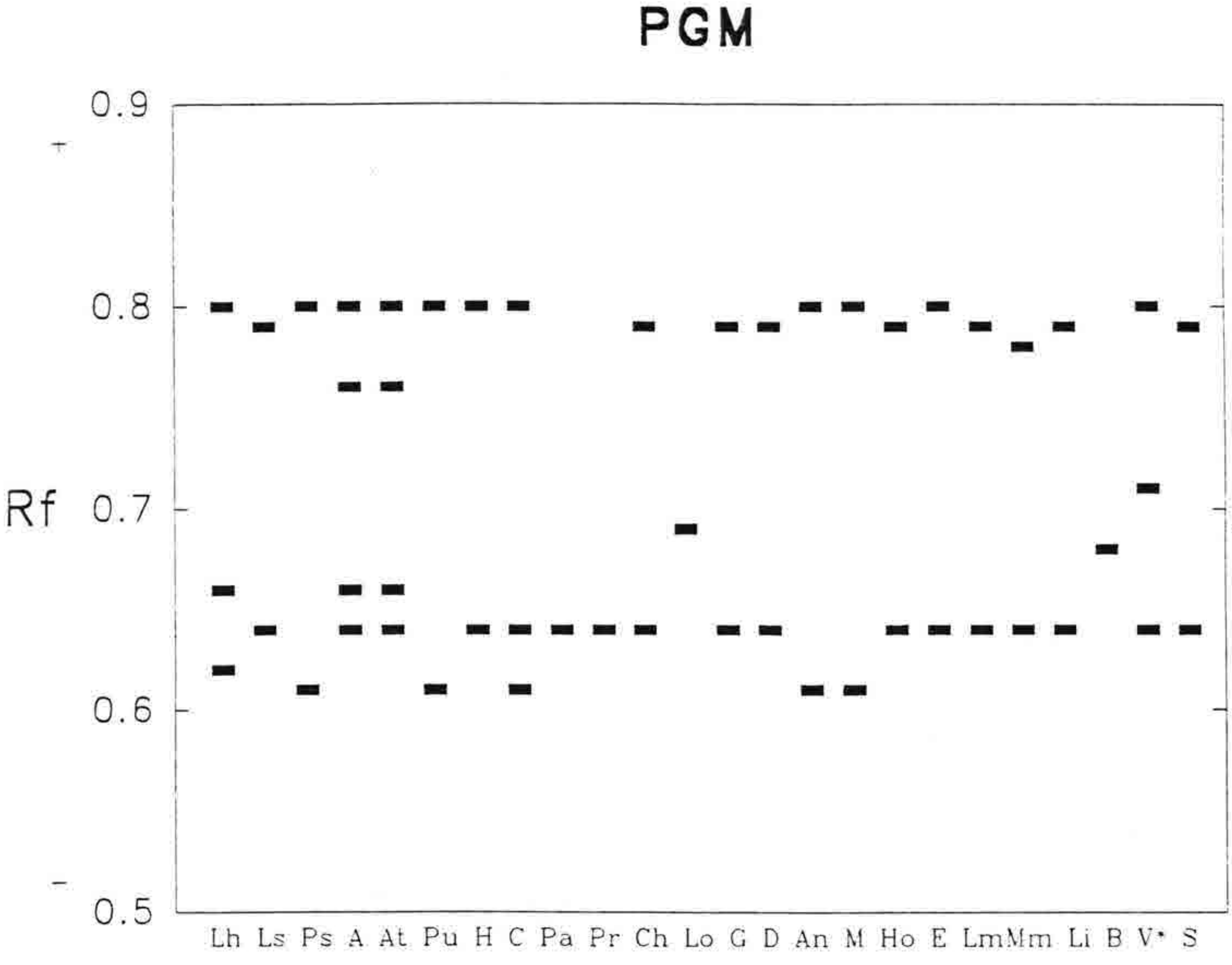


Fig. 4.2a. Separation of PGM isozymes



* Some variability was found in this species

Fig4.2b. Diagrammatic representation of PGM isozymes

Figure 4.3a. Phosphogluco-isomerase - species preparations

From left to right: P*, Ps, H, H, H, C, A, At, At, Ch, Lh, Lh, Lh, Ls, Ls, Ps, Pa, Pr, Lo

A, V, V, V, V, V, V, Pu, Pu, Pu, Ps, Lo, Lo, B, Ls

S, S, Pa, Pr, Pr, Li, Mm, Lm, E, Ps, Ho, M, An, D, G, Ls

*Abbreviations from Table 2.1.

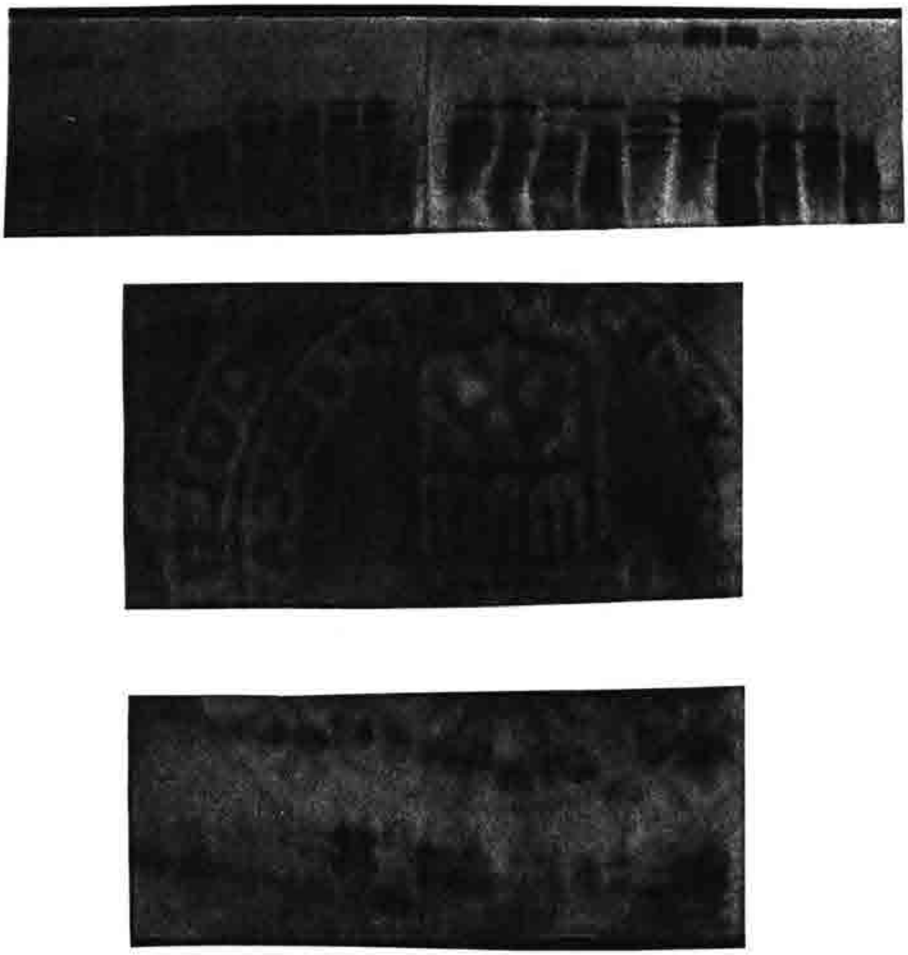
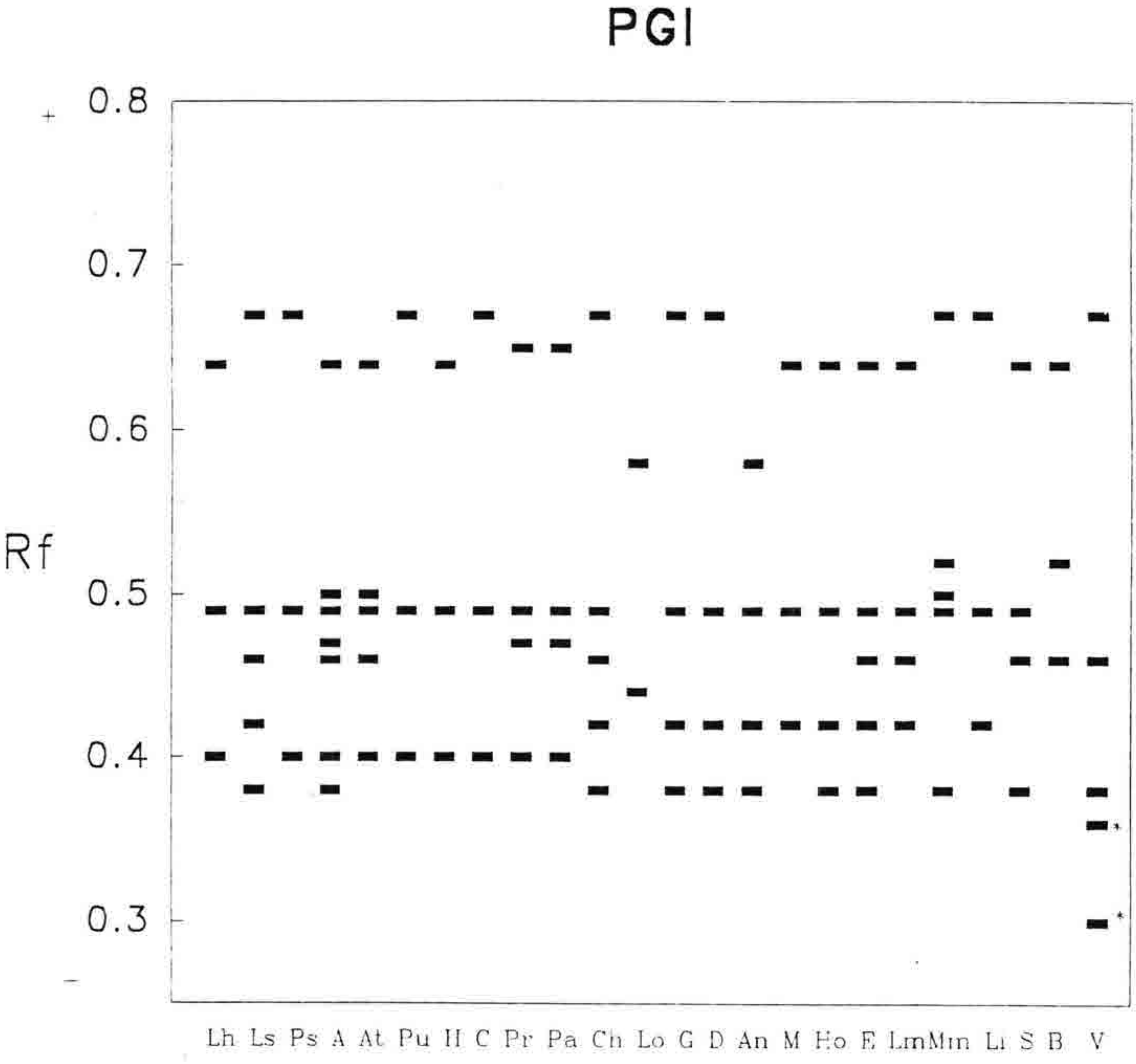


Fig.4.3a. Separation of PGI isozymes.



*Variable bands found only in some individuals

Fig 4.3b. Diagrammatic representation of PGI isozymes having from one to four alleles. *A. versicolor* showed some variability in the most cathodal of the allozymes, with bands being located at Rf 0.30 and 0.36 in some individuals, in addition to the more typical band at Rf 0.38. *Leontochir ovallei* had a unique type of isozyme banding pattern with only a single band in the cathodal zone at Rf 0.44 and a single band in the anodal zone at Rf 0.58. *Bomarea sp.* had only three bands altogether, at Rf 0.45, 0.52 and 0.64. *A. psittacina* and *A. pulchella* had the same isozyme banding pattern, as did the two color forms or *A. pelegrina*. *A. garaventae and* A. *diluta ssp diluta* also had the same pattern of four bands. The other 18 species and hybrids had unique banding patterns with this enzyme.

6-Phosphogluconate dehydrogenase (Fig.4.4)

There were two main areas of banding within the gel after staining for this enzyme. These areas were relatively close together at about Rf 0.50 and 0.47. *A. ligtu hyb, A. ligtu sp., A. pelegrina alba and rosea*, and *A. chilensis* all had the same pattern with two bands at Rf 0.50 and 0.47. *A. psittacina* and *A. pulchella* had the same patterns with bands at Rf 0.49, 0.52 and 0.54, but no bands in the region of Rf 0.47. The two color variants of *A. aurantiaca* had quite different patterns, with only four bands in the yellow-flowered variant, and five widely spaced bands in the orange-flowered variant. *A. haemantha hyb.* had three isozymes at Rf 0.50, 0.49 and 0.47. *A. caryophyllae hyb.* also had two bands at Rf 0.50 and 0.49 but had no band at Rf 0.47. *A. garaventae, A. angustifolia ssp angustifolia, A. exserens, A. ligtu* `Miyake spotless', and *A. ligtu ssp simsii* had the same pattern with two bands at Rf 0.46 and 0.50. *A. diluta ssp diluta* also had these same twobands and a further two bands at Rf 0.53 and 0.55. *A. hookeri ssp hookeri* had three bands at Rf 0.50, 0.48 and 0.46 while *A. magenta* also had bands at Rf 0.50 and 0.46 with two further bands in between at Rf 0.47 and 0.49. *A. magnifica ssp maxima* had three bands each in both the cathodal and the anodal region of the gel. This species thus had a very distinct pattern. One of the cathodal bands, Rf 0.44, and the common anodal band, Rf 0.50, was also found in *A. sierrae* which had one other band at Rf 0.47. *A. versicolor* had three bands at Rf 0.46, 0.48 and 0.50, the same pattern as seen for *A. hookeri ssp hookeri*. The related genus *Bomarea* had bands at Rf 0.45 and 0.53, as well as the common band at Rf 0.50. *Leontochir ovallei* had a pattern of four bands, three of which, Rf 0.48, 0.51 and 0.54 may be seen in some alstroemeria species. The fourth band was located at Rf 0.56. Ten species, hybrids or color

Figure 4.4a. Phosphogluconate dehdrogenase - species preparations

From left to right: Lh*, Lh, Ls, Ls, Ps, Pa, Pr, Lo

Ps, Ps, Pu, Pu, At, H, H, C, A, At, At, Ch

Pr, A, V, V, V, V, V, V, Pu, Pu, Pu, Ps, Lo, Lo, B, Pr

S, S, Pa, Pr, Pr, Li, Mm, Lm, E, A, Ho, M, An D, G, Ps

*Abbreviations from Table 2.1.

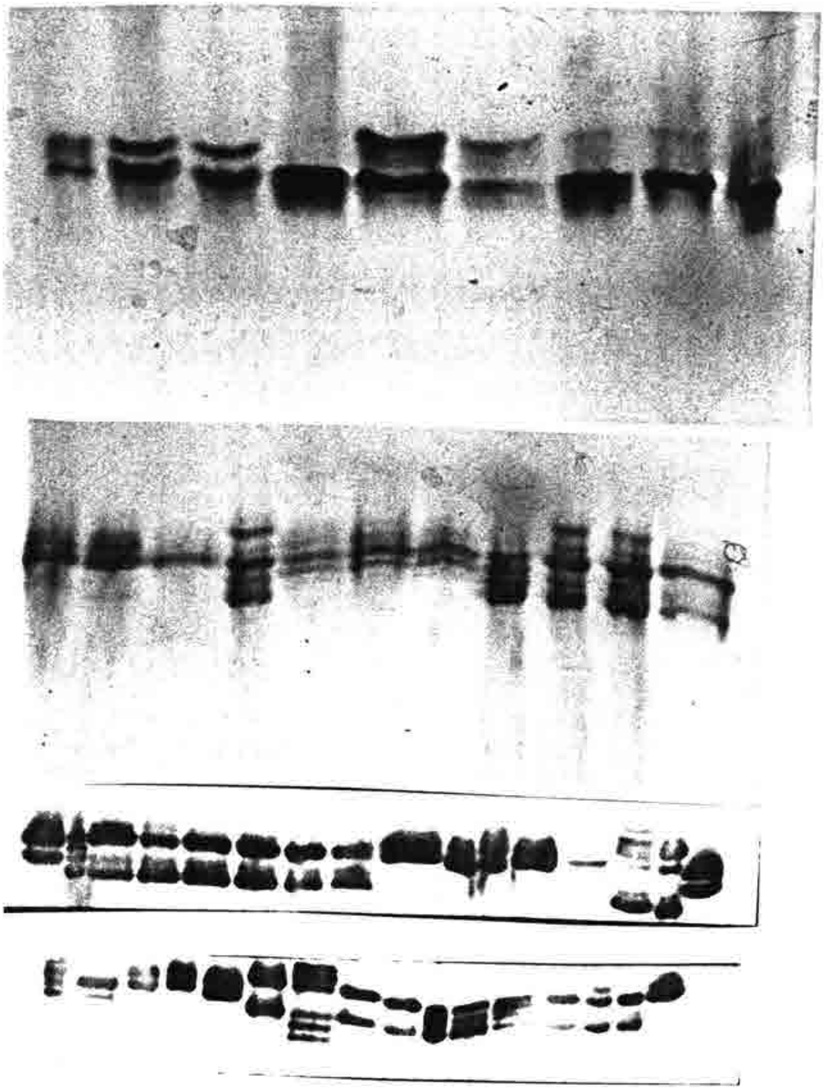


Fig.4.4a. Separation of 6-PGD isozymes.

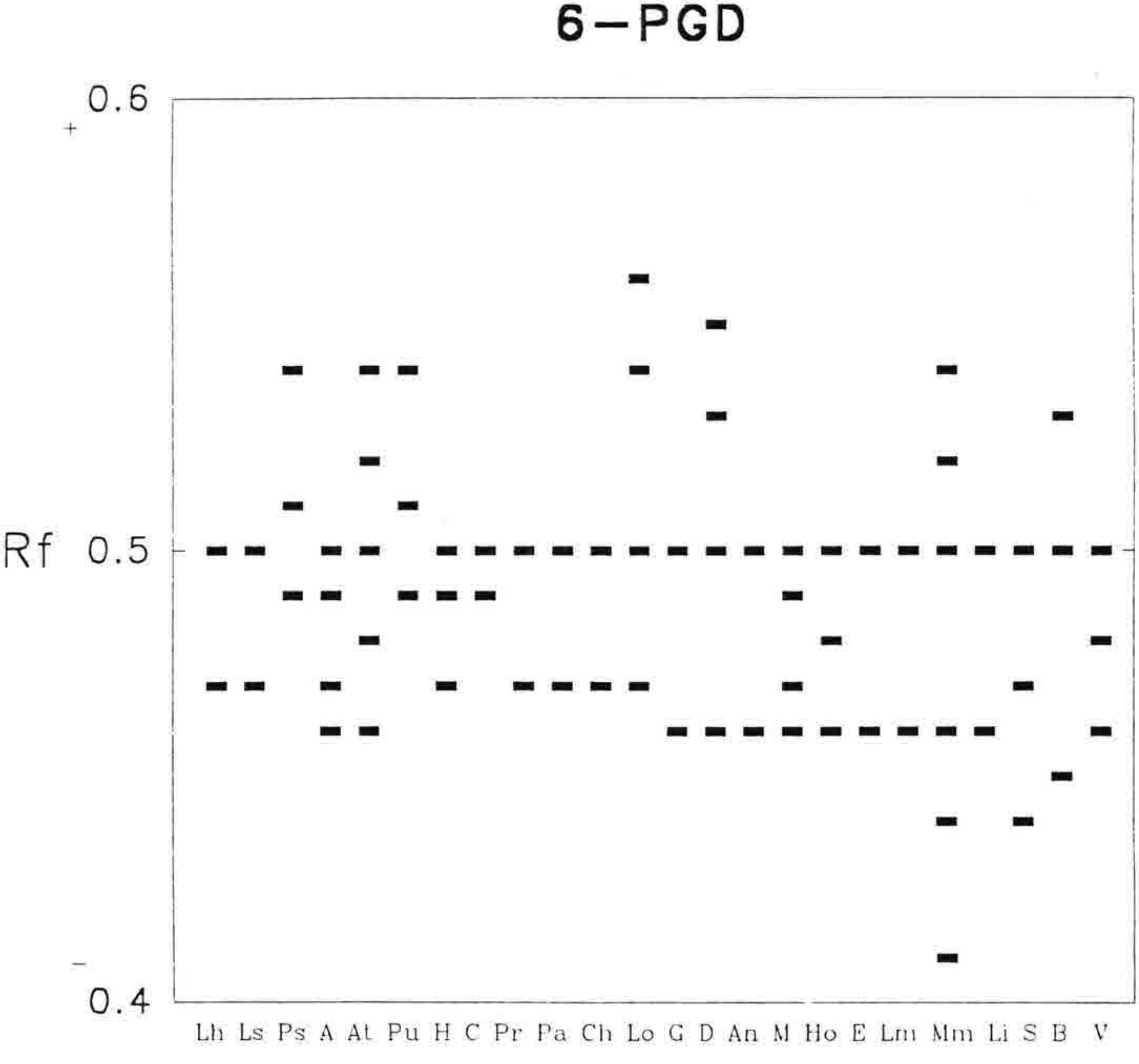


Fig. 4.4b. Diagrammatic representation of 6-PGD isozymes.

variants could be uniquely characterized using 6-PGD. *A. psittacina* and *A. pulchella* could also be separated from the other species based on their isozyme pattern for this enzyme system. Five species, hybrids and color variants shared a single pattern, two species shared another pattern, and the remaining five species shared another distinct pattern. A common band at Rf 0.50 was found in all species and hybrids except *A.psittacina* and *A. pulchella*.

Aspartate amino transferase (Fig.4.5)

The banding pattern for AAT includes up to seven allozymes per species. *A. chilensis* and *A. ligtu spp.* had the same pattern of four bands at Rf 0.47, 0.52, 0.55 and 0.57. *A. ligtu hyb.* had a different pattern from *A. ligtu sp.*, sharing only three bands at Rf 0.47, 0.52 and 0.55. The remaining three bands were located at Rf 0.45, 0.58 and 0.60, although the latter band was sometimes indistinct.

A. psittacina and *A. pulchella* had the same pattern of five bands, along with *A. caryophyllae hyb.* The two color variants of *A. aurantiaca* also had a similar pattern of six bands but in this instance the fifth band position was slightly different, the yellow form with a band at Rf 0.59 and the orange form with a band at Rf 0.57. Both color variants of *A. pelegrina* had the same pattern of four bands. *A. haemantha hyb* had bands at Rf 0.43, 0.52, 0.57, 0.59, and 0.61. Three common allozyme bands were found in *A. garaventae, A. hookeri ssp hookeri, A. exserens, A. ligtu* 'Miyake spotless', *A. magnifica ssp maxima* and *A. ligtu ssp simsii* at Rf 0.52, 0.55 and 0.58. *A. garaventae* also had four other bands, two cathodal and two anodal to the common three bands. The other five species and hybrids had between one and four other bands which were all cathodal to the common group of bands. *A. hookeri ssp hookeri* and *A. ligtu simsii* had the same banding pattern with a total of six bands. *A. magenta* had a total of only three bands at Rf 0.58, 0.52 and 0.49. *A. diluta ssp diluta* also had two of the most common bands at Rf 0.58 and 0.55, as well as bands at Rf 0.49, 0.43, 0.41 and 0.37, similar to the pattern seen for *A. exserens*, which also had a band at Rf 0.52. *A. versicolor* and *A. sierrae* also had bands at Rf 0.58 and 0.55, *A. versicolor* with one other band at Rf 0.46 and *A. sierrae* with two other bands at Rf 0.49 and 0.44. *A. angustifolia ssp angustifolia* had only one of the common bands at Rf 0.55 as well as two other bands at Rf 0.47 and 0.41. *Bomarea sp* and *Leontochir ovallei* were easily distinguished from the alstroemeria species and hybrids. *L. ovallei* had only three

Figure 4.5a. Aspartate amino transferase - species preparations

From left to right: H*, Lh, Hs, At, A, C, Ps, Lo, Pa

Pr, A, V, V, V, V, V, V, Pu, Pu, Pu, Ps, Lo, Lo, B, A

S, S, Pa, Pr, Pr, Li, Mm, Lm, E, Ls, Ho, M, An, D, G, A

*Abbreviations from Table 2.1.

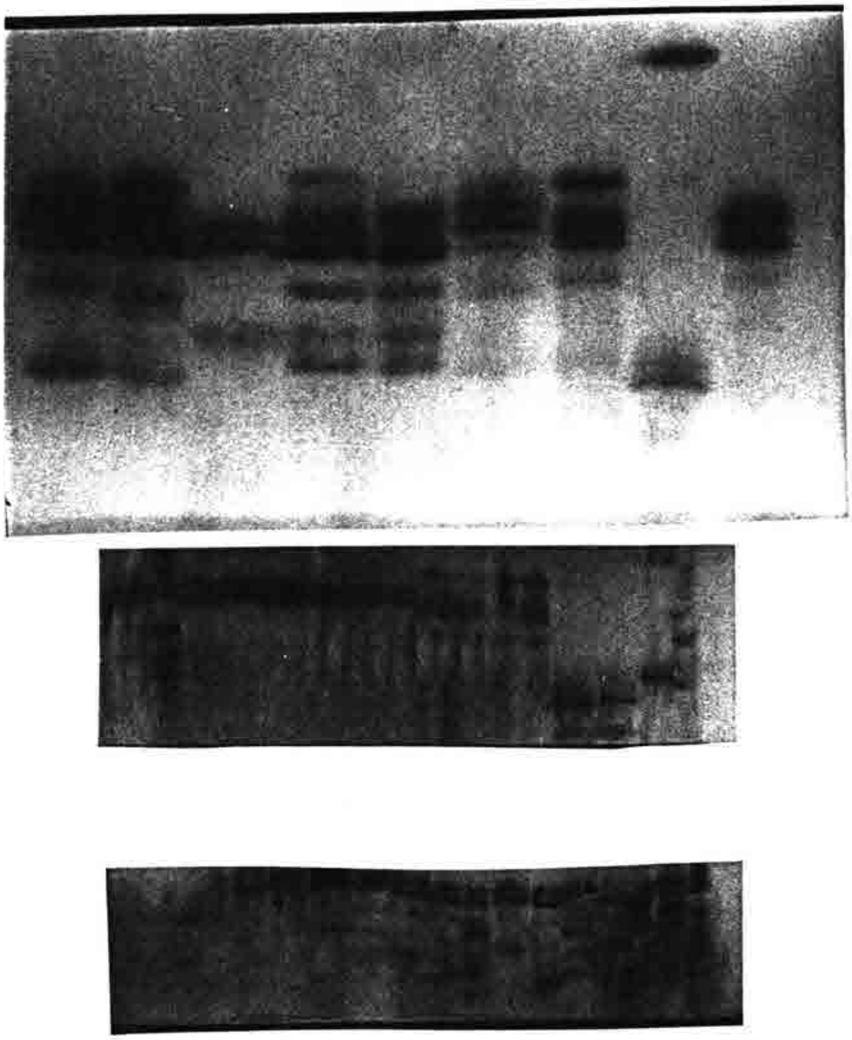


Fig.4.5a. Separation of AAT isozymes.

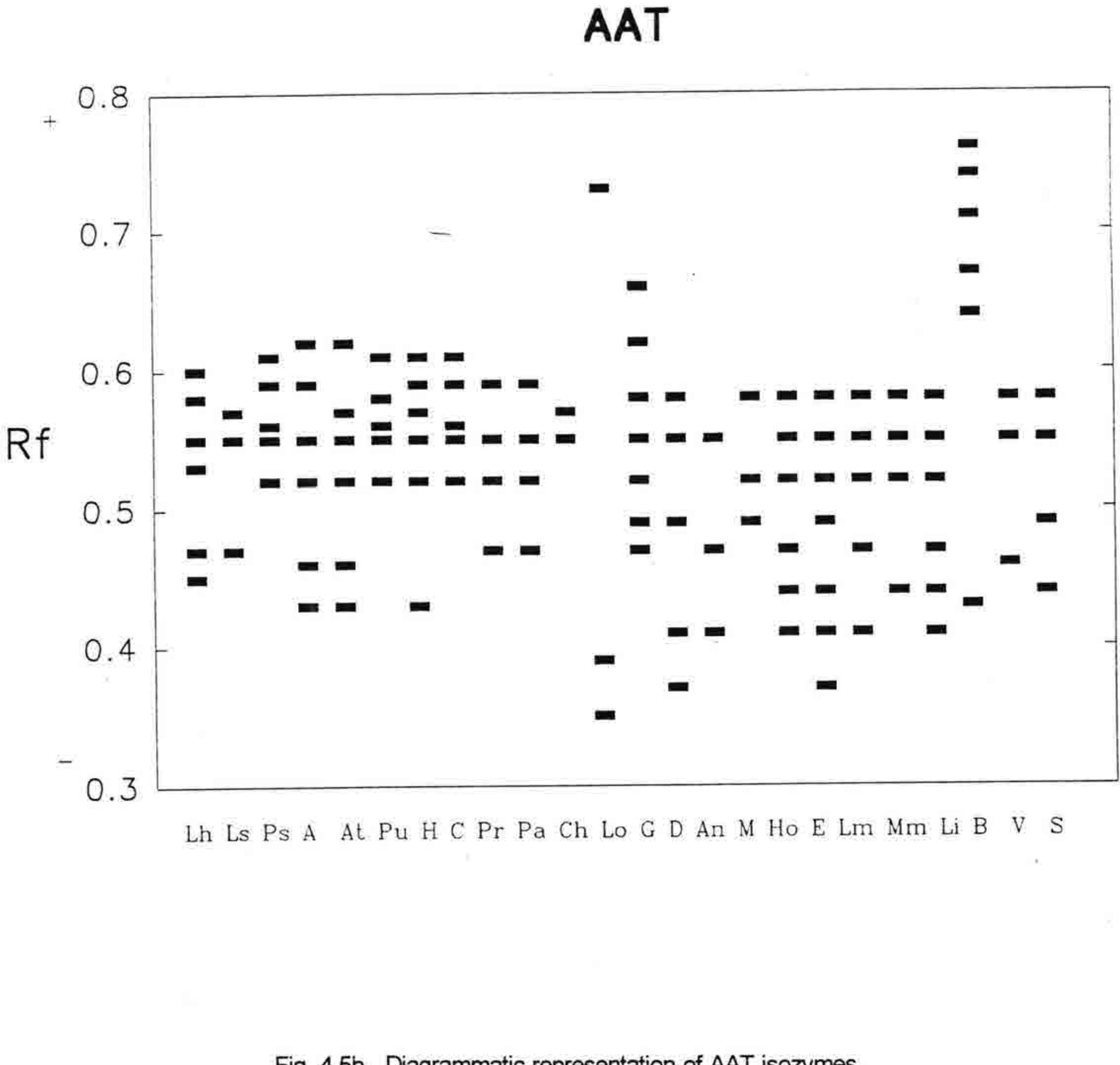


Fig. 4.5b. Diagrammatic representation of AAT isozymes.

bands, one anodal band at Rf 0.74 and two cathodal bands at Rf 0.40 and 0.43, the latter band sometimes very faint. *Bomarea sp* had a band at Rf 0.43 which was close to bands identified in alstroemeria species but the other five bands were located from Rf 0.64 to 0.76, outside the region of the gel in which most alstroemeria isozymes were located.

Fifteen species and hybrids including the two color forms of *A. aurantiaca* could be uniquely characterized using this enzyme system. The two color variants of *A. pelegrina* had the same isozyme banding pattern, as did *A. hookeri* and *A. ligtu ssp simsii*, *A. chilensis* and *A. ligtu spp*, and *A. psittacina*, *A. pulchella* and *A. caryophyllae hyb.*

Malic Enzyme (Fig.4.6)

There were two distinct areas of banding for this enzyme system. A cathodal region at about Rf 0.40 and an anodal region around Rf 0.52. The number of bands in each species varied from two to five. *A. ligtu spp*, *A. ligtu hyb*, the orange-flowered *A. aurantiaca*, *A. angustifolia ssp angustifolia*, *A. diluta ssp diluta* and *A. ligtu* `Miyake spotless' all had the same two bands, at Rf 0.42 and 0.52. The yellow-flowered variant of *A. aurantiaca* also had these two bands as well as three more cathodal bands at Rf 0.38, 0.40 and 0.41. *A. psittacina* and *A. pulchella* had the same pattern of two bands at Rf 0.42 and 0.50, while *A. pelegrina rosea*, *A. pelegrina alba* and *A. exserens* each had bands at Rf 0.36 and 0.54. *A. haemantha hyb* had a simple pattern of two bands at Rf 0.40 and 0.52 and *A. chilensis* had two bands at 0.42 and 0.54. *A. caryophyllae hyb* was more complex, with one band at Rf 0.38 and four bands in the anodal region of the gel from Rf 0.48 to 0.54. *A. caryophyllae* was the only specimen to have more than one band in the anodal portion of the gel. *A. magenta* had a band at Rf 0.52 with a second band at Rf 0.36. *A. ligtu ssp simsii* had *two cathodal bands at Rf 0.40 and 0.42 and a single anodal band at 0.54.* *A. garaventae* also had a total of three bands, a single anodal band at Rf 0.54 and two cathodal bands at Rf 0.39 and 0.40. *A. hookeri ssp hookeri* was interesting since it not only had bands at Rf 0.54 and 0.42, as found in many other species, but also had a median isozyme band at Rf 0.47. This band was also faintly visible in *A. diluta ssp diluta*, *A. angustifolia ssp angustifolia*, *A. ligtu* `Miyake spotless', and *A.* ligtu simsii but was not included in the diagrammatic representation due to its faint appearance.

Figure 4.6a. Malic enzyme - species preparations

From left to right: Pu*, Lh, C. AT, A, Ps, Pa, H, Ch, Ls

Pr, A, V, V, V, V, V, Pu, Pu, Ps, Lo, Lo, B, Pr

S, Pa, Pr, Pr, Li, Mm, Lm, E, LS, Ho, M, An, D, G, A

*Abbreviations from Table 2.1.

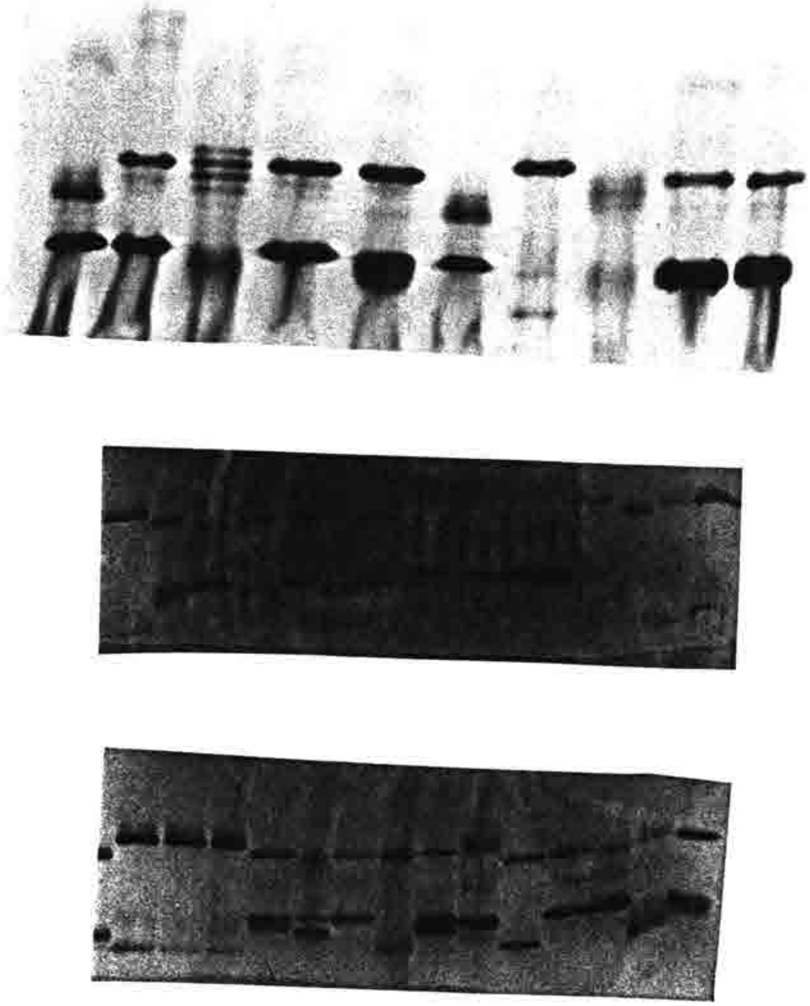
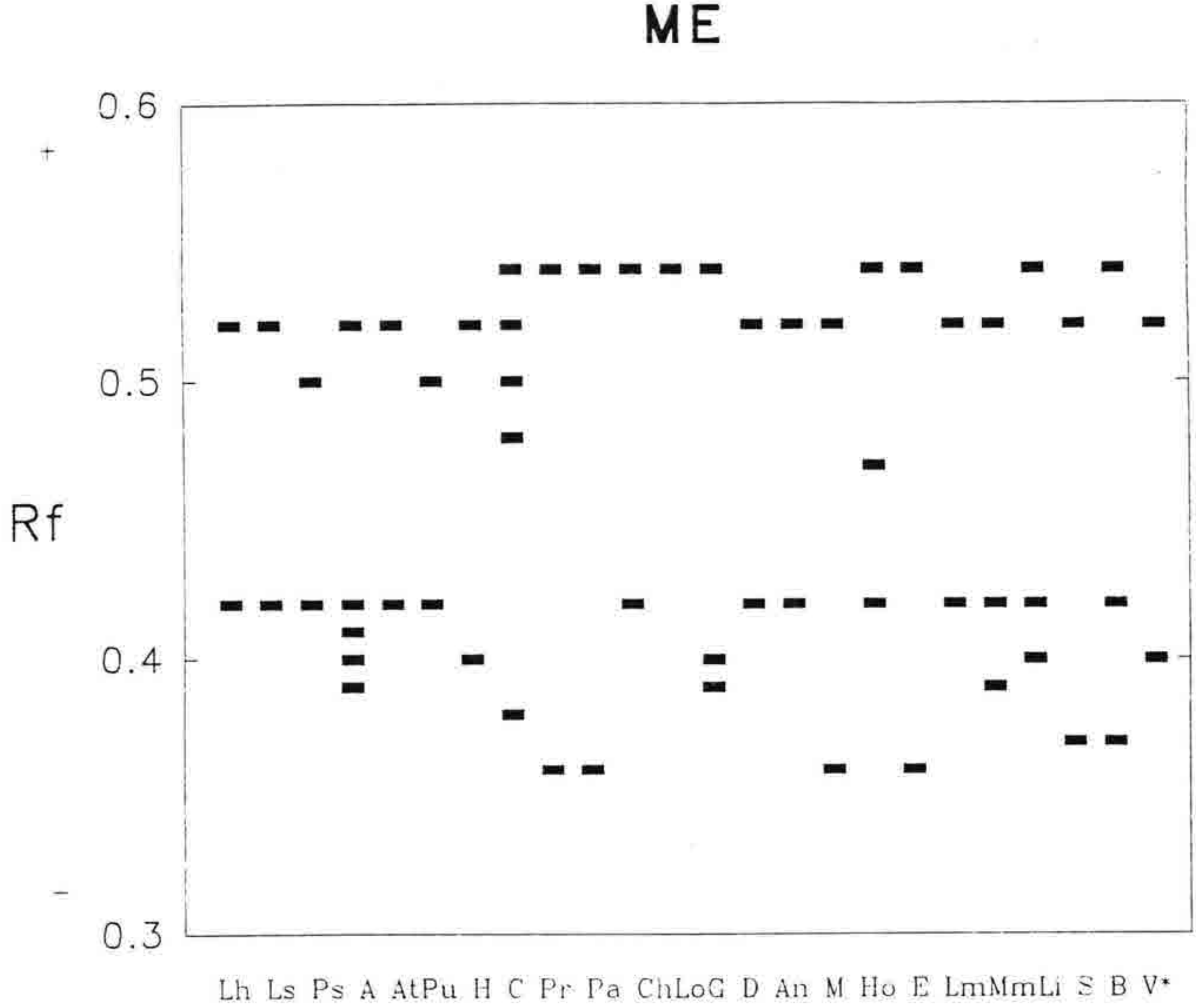


Fig.4.6a. Separation of ME isozymes.



*Some variability was found in this species

Fig. 4.6b. Diagrammatic representation of ME isozymes

A. magnifica ssp maxima had three bands, two cathodal at Rf 0.39 and 0.42 and one anodal at 0.52. *A. sierrae* had two bands at Rf 0.37 and 0.52. *A. versicolor* was variable for this enzyme system. It had only two bands present for each plant studied but these were at either Rf 0.40 or 0.41 and 0.51 or 0.52, with the most common combination being Rf 0.40 and 0.52 (four of the six plants studied). *Leontochir ovallei* and *Bomarea spp* each had a strong anodal band at 0.54. The position of the cathodal bands in *L. ovallei* was difficult to determine but those for *Bomarea sp* were at Rf 0.37 and 0.42. There were six species and hybrids which had the same isozyme banding pattern. *A. pelegrina rosea*, *A. pelegrina alba* and *A exserens* all had the same pattern which was different from all other species studied. The remaining examples each had a unique identification pattern.

Esterase (Fig.4.7)

Up to seven isozyme bands were found for each species or hybrid stained for esterase, with the minimum number of bands being three. *A. ligtu hyb* and *A. ligtu spp* differed only in the presence of an extra band at Rf 0.73 in *A. ligtu hyb*. The other five bands ranged from Rf 0.69 to 0.88. *A. psittacina* and *A. pulchella* had the same isozyme pattern, as did *A. pelegrina rosea* and *A. pelegrina alba*. A. *aurantiaca* had considerable differences between the yellow and the orange flowered variants. Both had three anodal bands at Rf 0.79, 0.84 and 0.87, however, the orange-flowered form also had three cathodal bands at Rf 0.69, 0.73 and 0.79. *A. haemantha hyb* had six bands between Rf 0.69 and 0.90. *A. caryophyllae hyb* had only four bands which were very distinctly separated into two groups, with two bands in the anodal region at Rf 0.84 and 0.87 and two bands in the cathodal region at Rf 0.69 and 0.74. *A. chilensis* had six isozyme bands between Rf 0.74 and 0.90, three of which were also found in *A. ligtu sp* and A. *ligtu hyb*. *A. garaventae* had a very similar pattern to *A. pelegrina* except that it had no band at Rf 0.88. *A. diluta ssp diluta* had only four isozyme bands in the central portion of the zone, at Rf 0.74, 0.77, 0.79 and 0.84. *A. angustifolia ssp angustifolia* also had two of these bands at Rf 0.77 and 0.79, as well as an anodal band at 0.90.

A. magenta had five bands from Rf 0.73 to 0.84 while the five bands of *A. hookeri* were found between Rf 0.77 and 0.90. *A. exserens* and *A. magnifica ssp maxima* had the greatest number of isozymes with seven bands each. Bands for both species were between Rf 0.68 and

Figure 4.7a. Esterase stain - species preparations

From left to right: Pu*, Lh, C, At, A, Ps, Pa. H, Ch, Ls

A, V, V, V, V, V, V, Pu, Pu, Ps, Lo, Lo, B, Ps, S, S, Pa, Pr
Pr, Li, Mm, E, A, Ho, M, An, D, G, Ls

*Abbreviations from Table 2.1.

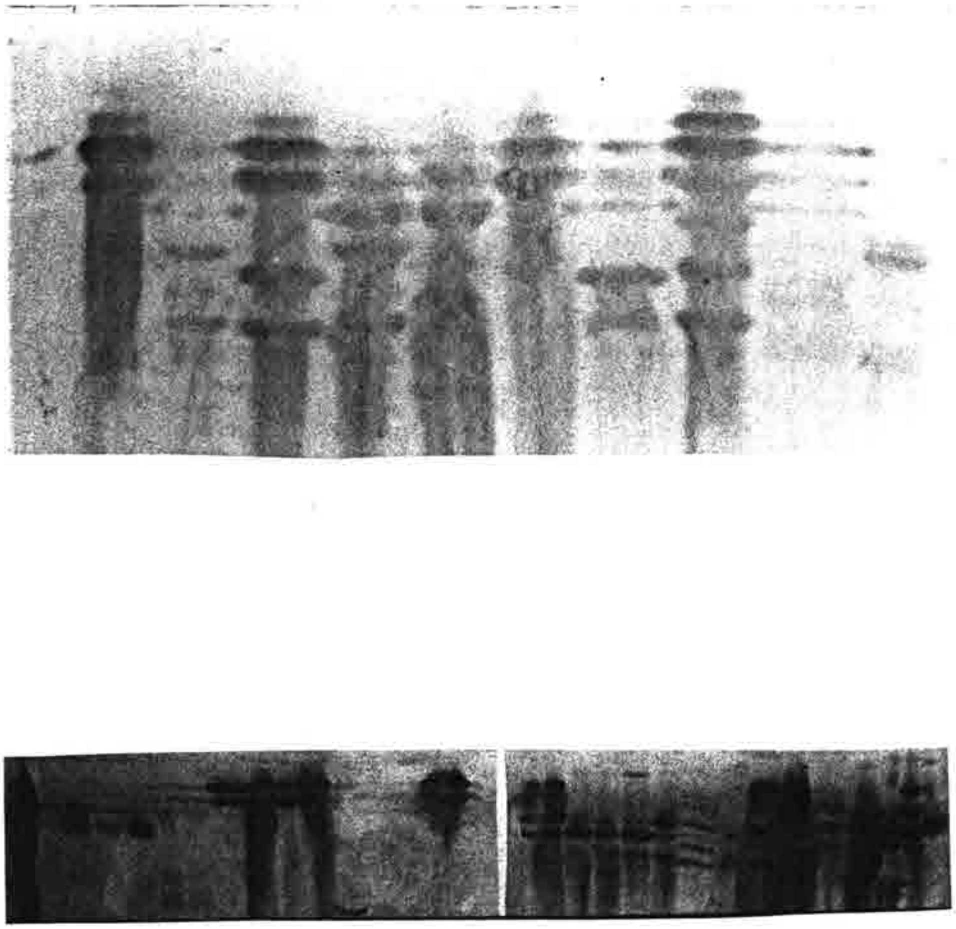


Fig.4.7a. Separation of EST isozymes.

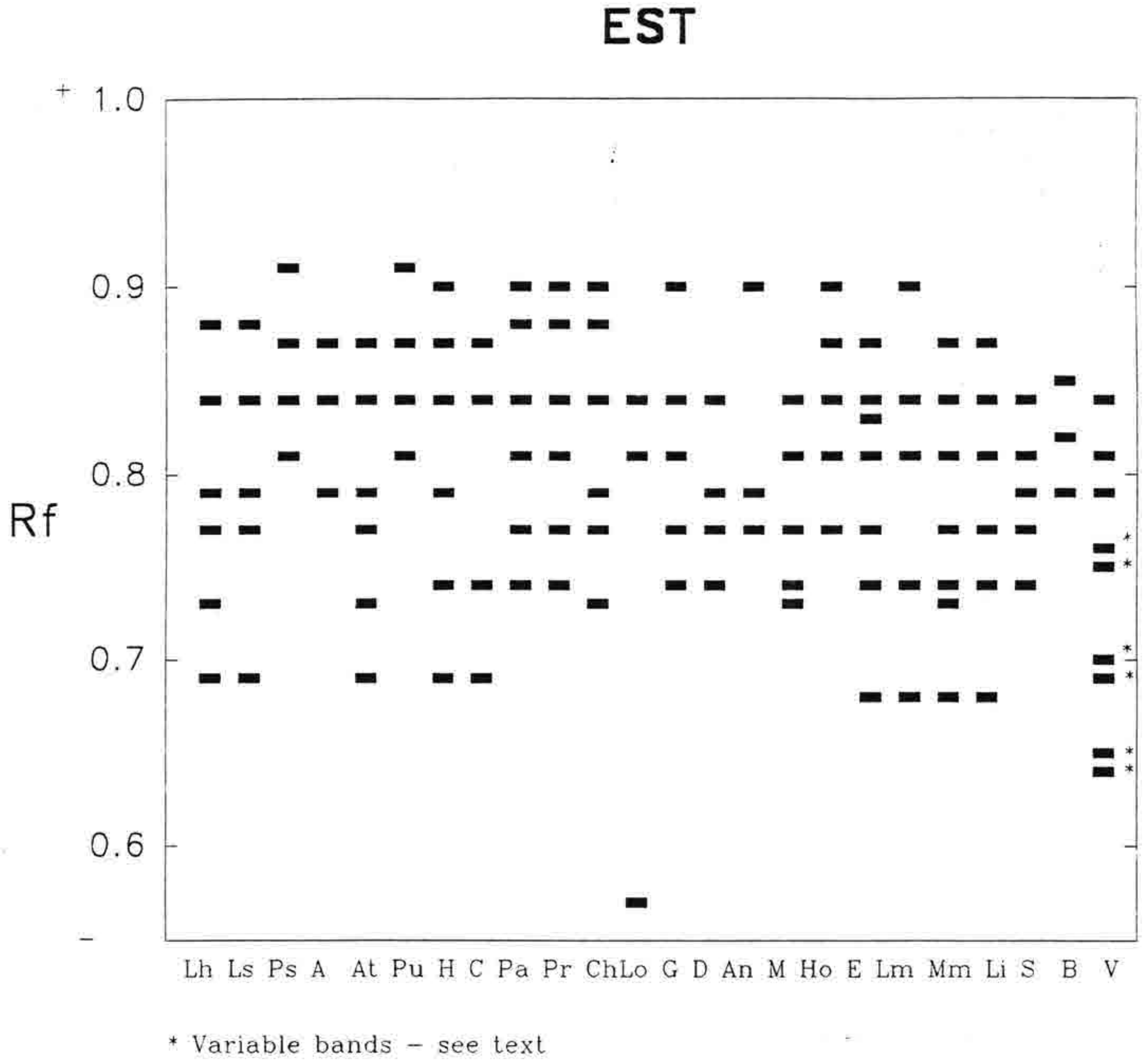


Fig. 4.7b. Diagrammatic representation of EST isozymes.

0.87. Six of these were common to both species, with *A. exserens* having a seventh band at Rf 0.83, while *A. magnifica ssp maxima* had a band at Rf 0.73. *A. ligtu simsii* also had the six bands from Rf 0.68 to 0.87 common to the former two species. *A. ligtu* `Miyake spotless' had a total of five bands at Rf 0.68, 0.74, 0.81, 0.84, and 0.90. *A. sierrae* had an isozyme pattern of four bands similar to *A. diluta ssp diluta*, at Rf 0.74, 0.77, 0.79, and 0.84, except that *A. sierrae* also had a fifth band at Rf 0.81. *A. versicolor* again showed great variability in the banding patterns of the six plants studied. One showed bands at only Rf 0.79, 0.81 and 0.84, three showed these three bands plus two other bands at Rf 0.75 and 0.76, and the other two plants had the three most anodal bands plus two groups of two bands each at Rf 0.66 and 0.67, and 0.60 and 0.62. *L. ovallei* had the most cathodal band of any species studied, with a band at Rf 0.57. The other two bands identified for *Leontochir* at Rf 0.81 and 0.84 were common to many other species examined. *Bomarea sp* had bands at Rf 0.79, 0.81 and 0.84, which were also common to many other species.

Esterase staining was more easily affected by environmental factors than the other enzyme systems used. Faint bands were sometimes seen in some species and at other times bands were missing altogether. Consequently, only the most consistent and strongest bands were included in the results. Streaking was also a problem more often with esterase, depending on the species being investigated and to some extent the time of year. Attempts were made to change the extraction procedure to try to eliminate these problems but this generally led to the loss of some of the bands. Streaking was also a major problem when using PGI but again no other extraction procedure was found that could eliminate this problem without also eliminating all of the enzyme activity.

Leucine amino peptidase (Fig.4.7)

The final enzyme system which was found to be useful in distinguishing the various species was LAP. Unfortunately, February was the only month during which well stained bands could be identified. *A. ligtu spp, A. ligtu hyb* and *A. chilensis* had the same two isozyme bands at 0.51 and 0.53. *A. psittacina* and *A. pulchella* each had the same isozyme pattern with bands at Rf 0.54 and 0.55. Four bands were common to both *A. haemantha hyb* and *A. caryophyllae hyb* at Rf 0.47, 0.49, 0.54 and 0.55. The two cathodal bands were also common to *A. pelegrina alba*. *A. pelegrina rosea* was not available for analysis with this enzyme. The two color variants of *A. aurantiaca* had very different isozyme patterns for LAP. The orange-flowered variant

Figure 4.8a. Leucine amino peptidase - species preparations

From left to right: Pu*, Lh, C, At, A, Ps, Pa, H, Ch, Ls

*Abbreviations from Table 2.1.

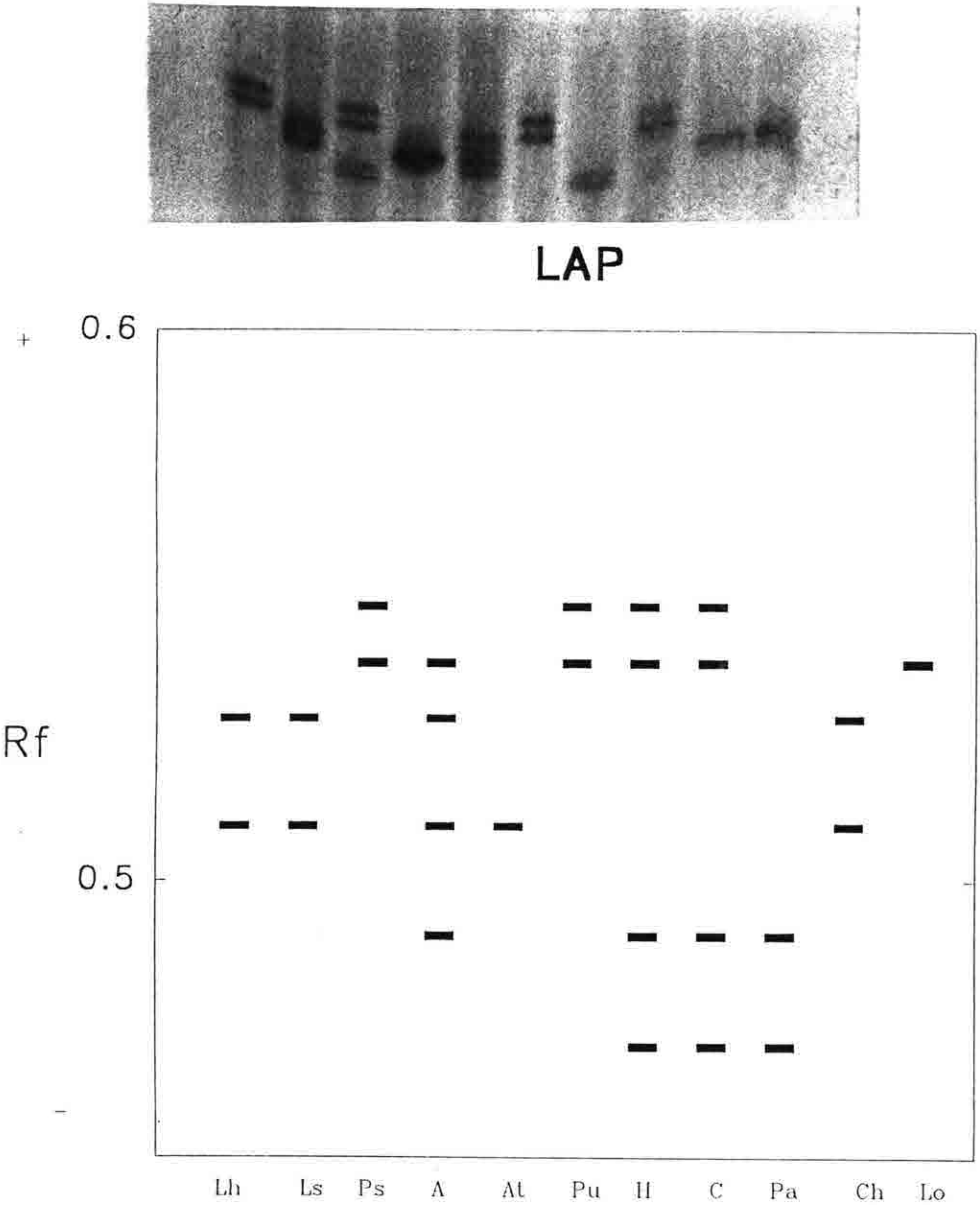


Fig.4.8a. Separation of LAP isozymes. b. diagrammatic representation

had only a single band at Rf 0.51, whereas the yellow- flowered variant had this band as well as three others at Rf 0.49, 0.53 and 0.54. *L. ovallei* had only a single band at Rf 0.54.

Not all species and hybrids were available for testing with this enzyme system at the critical time. They were examined as they became available but no usable results could be obtained.

CULTIVARS

From five to seven electrophoretic runs were made for all cultivars for each enzyme system being investigated. These experiments were run from March, 1993 until May, 1994.

Phospho-gluco mutase (Fig.4.9)

There are two distinct zones of activity for this enzyme at around Rf 0.64 and 0.80. Generally two bands are found in each zone for each cultivar but occasionally only a single band was found in one or both zones. Of the 23 cultivars examined 16 could be identified uniquely using this enzyme. The *Alstroemeria* cultivars 'Arizo', 'Eureka', 'Carmen', 'Mona Lisa', 'Luciana', 'Jubilee', and 'Marina' all had the same two cathodal bands at 0.59 and 0.64. 'Arizo', 'Marina', and 'Jubilee' also had identical anodal bands at Rf 0.80, while 'Carmen' and 'Mona Lisa' had the same two anodal bands at Rf 0.78 and 0.80. 'Eureka' had a single anodal band at Rf 0.79 while 'Luciana' had two anodal bands at Rf 0.77 and 0.80. 'Neva' and 'Yellow King' both had cathodal bands at Rf 0.60 and 0.64 but 'Neva' had two anodal bands at Rf 0.79 and 0.80, whereas 'Yellow King' had bands at Rf 0.76 and 0.80. 'Canaria' had one anodal band at 0.80 with two cathodal bands at Rf 0.62 and 0.66.

'Regina', 'Orchid', and 'Pink Triumph' also had bands at Rf 0.62 and 0.66. 'Orchid' had a single anodal band at Rf 0.78 and 'Pink Triumph' had two anodal bands at Rf 0.79 and 0.80. 'Regina' was the only cultivar in which an anodal isozyme band was not observed. 'Rosita' and 'Rosario' each had a single cathodal band at Rf 0.62, 'Rosita' also had two anodal bands at Rf 0.78 and 0.80 and 'Rosario' had only a single anodal band at Rf 0.80.

'Orange Beauty', 'Appelbloesem', 'Alnba', 'Jubilee', and 'Monika' were the other cultivars with a single anodal band at Rf 0.80 but they could all be distinguished by the positions of their cathodal bands. 'Orego' and 'Red Surprise' each had a single anodal band at Rf 0.78 while

Figure 4.9a. Phosphoglucomutase - cultivars

From left to right: Red surprise, Regina, Orego, Orchid, Rosario, Orange Beauty, Rosita, Pink Triumph, Neva, Mona Lisa, Monika, *A. ligtu* `Miyake Spotless', Carmen, Eureka, Arizo, Alnba, Appelbloesem, Luciana, Marina, King Cardinal, Texas, Yellow King, Canaria, Jubilee

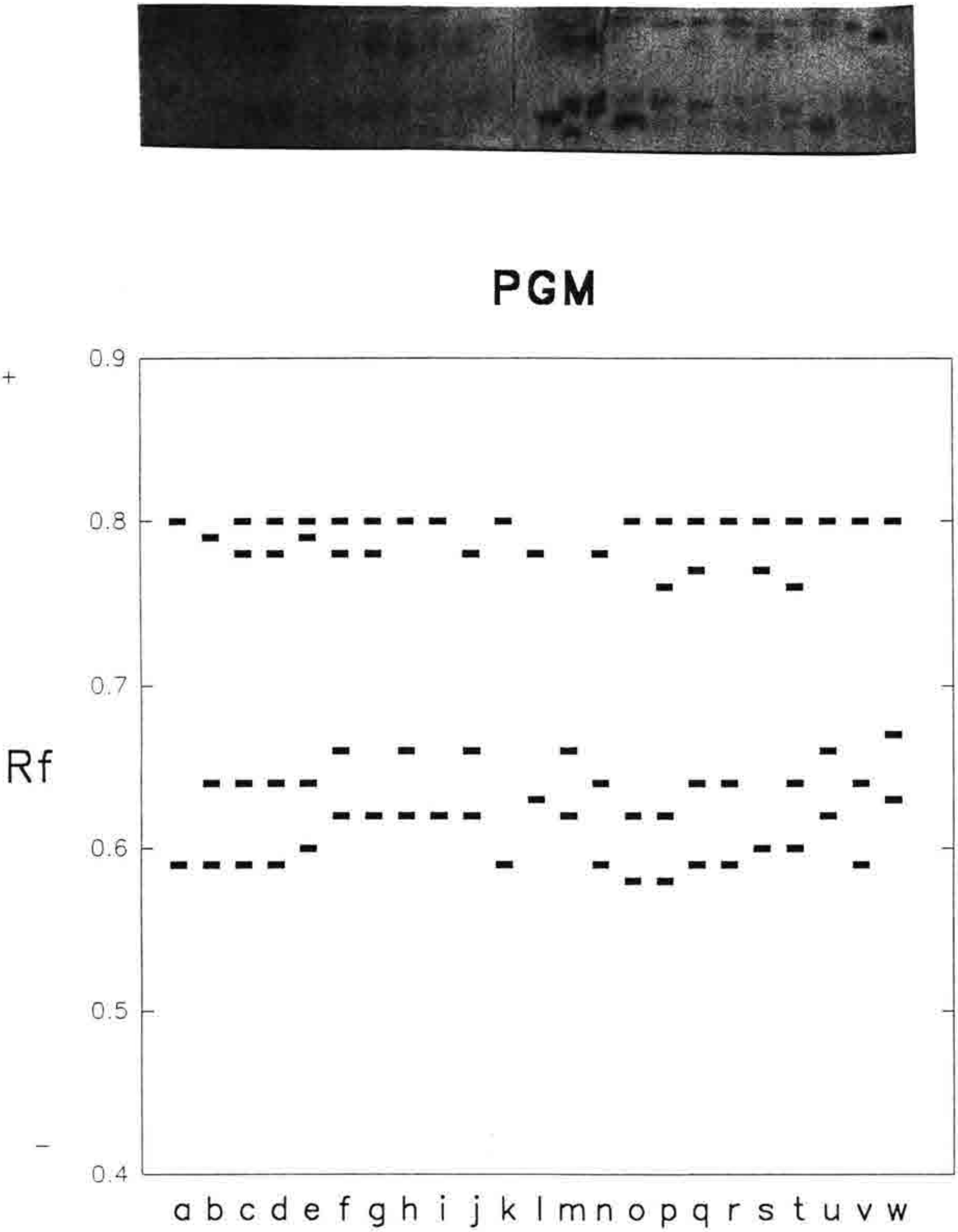


Fig.4.9a. Separation of PGM isozymes of cultivars. b. Diagrammatic representation

`Red Surprise' had two cathodal bands at Rf 0.59 and 0.62, and Orego had a single cathodal band at Rf 0.63. `Texas' had only a single cathodal band at 0.60 and two anodal bands at Rf 0.77 and 0.80 whereas `King Cardinal' had a symmetric series of four bands at Rf 0.58, 0.62, 0.76 and 0.80.

Eighteen cultivars could be uniquely identified using this enzyme system. Three cultivars had an identical pattern and two other cultivars also had the same pattern as one another.

Phospho-gluco isomerase (Fig.4.10)

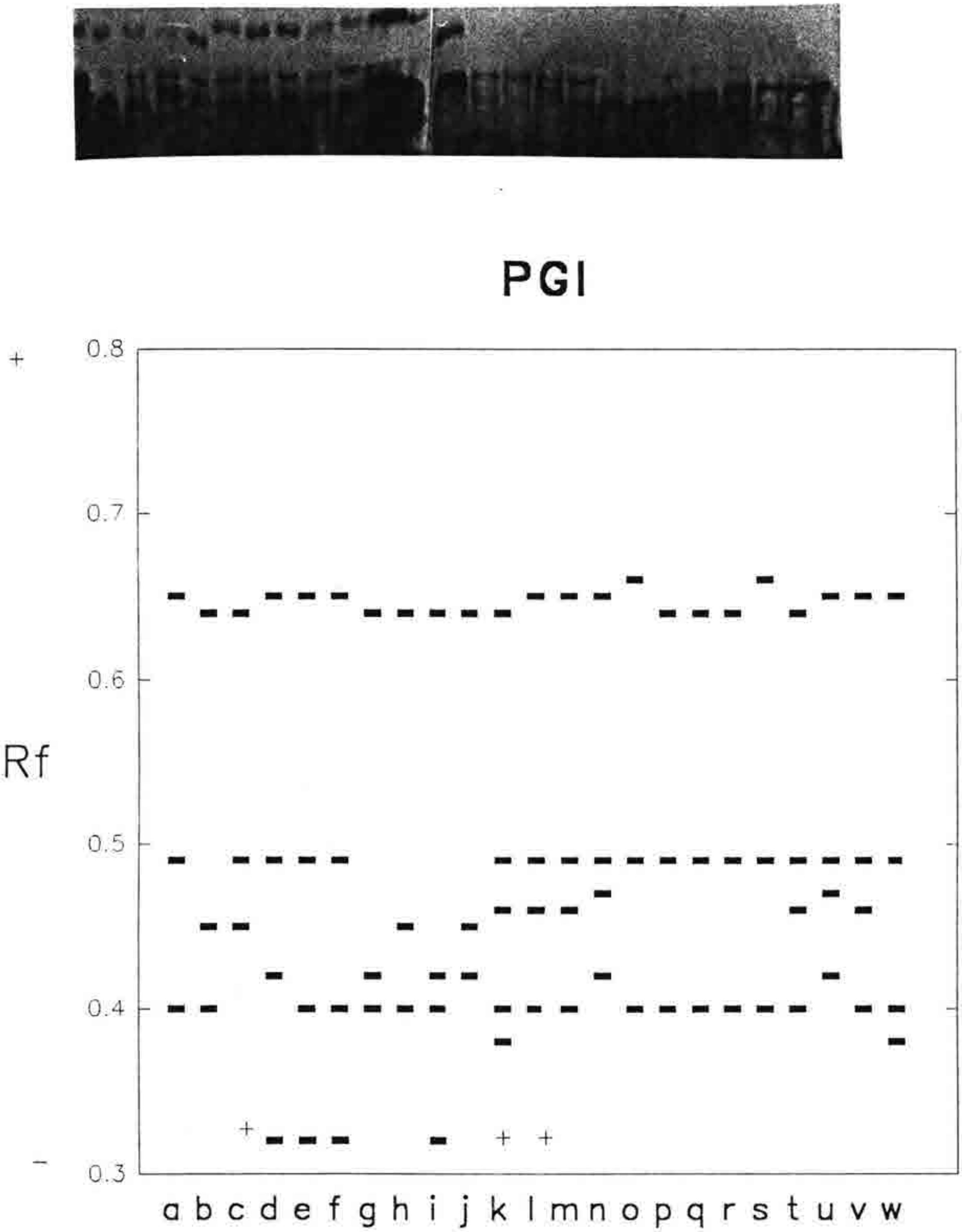
All of the anodal bands found for these 23 cultivars were at Rf 0.64, 0.65 or 0.66. Each cultivar had only a single anodal band. The arrangement of bands in the cathodal region was more complex, usually with two or three bands for each cultivar, although `Appelbloesem' had four isozyme bands in this region. Eighteen of the 23 cultivars had an isozyme band at Rf 0.49, the common band found in all the alstroemeria species except *A. versicolor*. Of these 18 cultivars, 14 also had a band at Rf 0.40. `Neva' and `Pink Triumph' had identical banding patterns with isozyme bands at Rf 0.32, 0.40, 0.49 and 0.65. `Arizo' had a very similar pattern but did not have a band at Rf 0.32. `Mona Lisa' also had a similar pattern with bands at Rf 0.32, 0.49 and 0.65. However, the fourth band was at Rf 0.42 and not at 0.40. `Alnba' and `Texas' also had identical patterns with bands at Rf 0.40, 0.49 and 0.66. `King Cardinal' , `Luciana', and `Jubilee' all had bands at Rf 0.40, 0.49 and 0.64.

`Orego', `Regina' and `Marina' had the same isozyme patterns with four bands at Rf 0.40, 0.46, 0.49 and 0.65. `Appelbloesem' and `Yellow King' had very similar patterns with bands at Rf 0.40. 0.46, 0.49 and 0.64, however, `Appelbloesem' also had a fifth band at Rf 0.38. ``Eureka' and `Carmen' both had bands at Rf 0.64 and 0.45 but the third band for `Eureka' was at Rf 0.40 whereas the third band for `Carmen' was at Rf 0.49.

`Rosita', `Orange Beauty', `Rosario', and `Orchid' all had a band at Rf 0.65 but were all missing the commonly observed band at Rf 0.49. `Rosita' and `Rosario' both had bands at Rf 0.40 and 0.42 and `Rosario' also had a fourth band at Rf 0.32. Orange Beauty' and `Orchid' both had a band at Rf 0.45 with the third band for `Orange Beauty' being at Rf 0.40 and that for `Orchid' at Rf 0.42.

Figure 4.10a. Phophoglucoisomerase - cultivars

From left to right: Monika, Marina, Canaria, Yellow King, Texas, Jubilee, Luciana, King Cardinal, Alnba, Red Surprise, Rosario, *A. chilensis*, Orego, Appelbloesem, Orchid, Rosario, Orange Beauty, Rosita, Pink Triumph, Neva, Mona Lisa, Carmen, Eureka, Arizo



+Faint bands are sometimes found near the cathodal end

Fig.4.10a. Separation of PGI isozymes of cultivars. b. diagrammatic representation

`Red Surprise' and `Canaria' had exactly the same isozyme banding pattern with bands at Rf 0.42, 0.48, 0.49 and 0.65. `Monika' also had bands at Rf 0.49 and 0.65 but the other two bands for this cultivar were at Rf 0.38 and 0.40.

Eleven of the cultivars had unique banding patterns for this enzyme system. There were five other patterns, two of which were common to three cultivars each, and three of which were common to two cultivars.

6-Phosphogluconate dehydrogenase (Fig.4.11)

All cultivars except `Eureka' had a clear band at Rf 0.50, the most common band observed in the species studied. There were from two to six bands for each cultivar, generally found between Rf 0.40 and 0.54. `Marina' and `Canaria' also had a band at Rf 0.39. Occasionally, faint bands were observed in the cathodal part of the stained zone of the gel, particularly for `Carmen' but these were not included in this analysis due to their inconsistent nature.

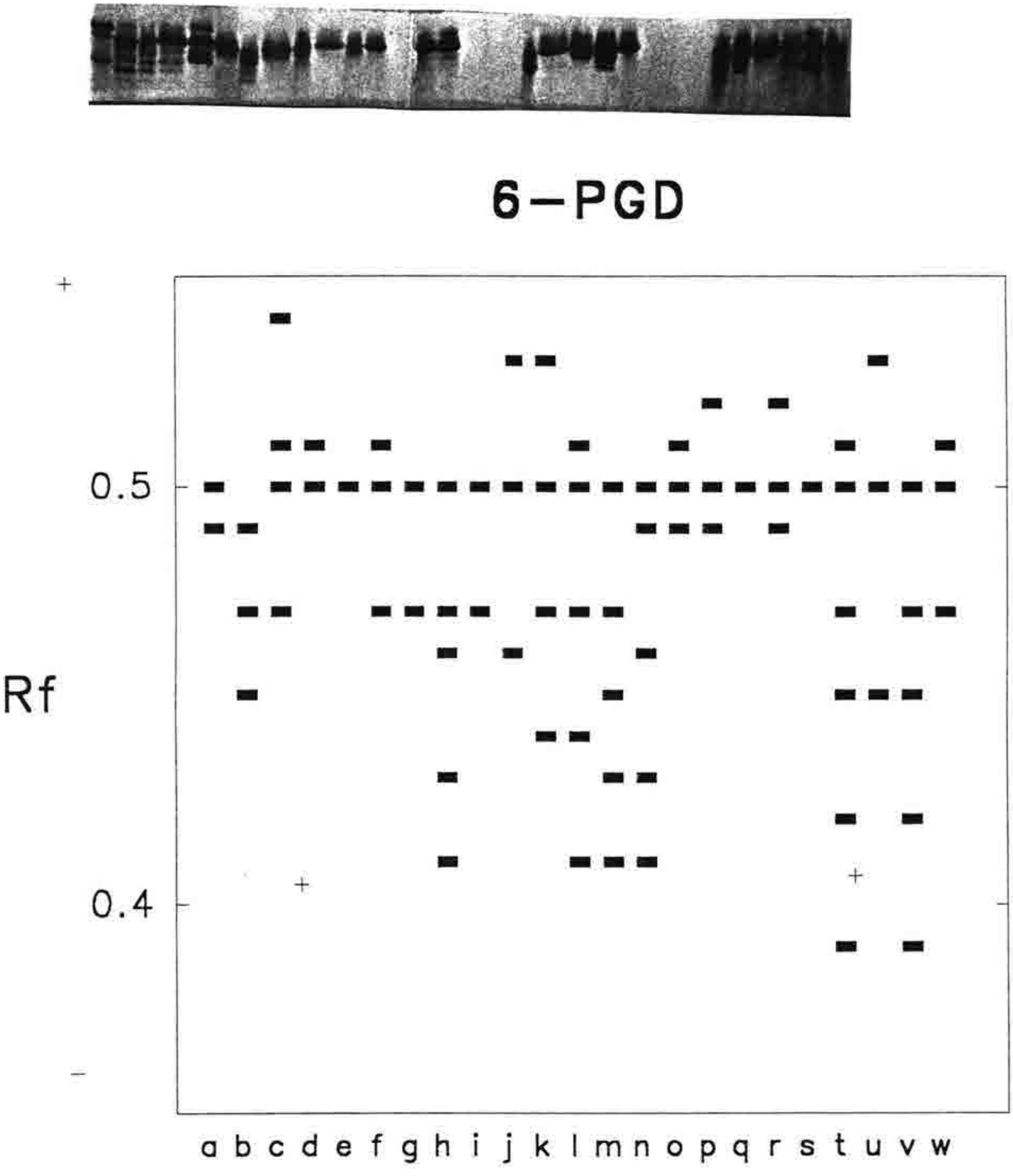
`Rosita' and `Rosario' had the same banding pattern at Rf 0.47 and 0.50. `Pink Triumph' and `Monika' also had these same two bands as well as a third band at Rf 0.51. `Carmen' had the same three bands as the latter two cultivars, as well as a band at Rf 0.54, the most anodal band observed in the cultivars examined. `Mona Lisa' also had isozyme bands at Rf 0.50 and 0.51 as did `Alnba', which also had a third band at Rf 0.49.

`Arizo' had only two bands at Rf 0.49 and 0.50. `King Cardinal' and `Jubilee' had identical bands at Rf 0.49, 0.50 and 0.52. Three cultivars, `Neva', `Luciana', and `Texas' had only a single band at Rf 0.50. `Regina' and `Orange Beauty' had a very similar pattern of five isozyme bands. Four of these bands, Rf 0.41, 0.43, 0.47, and Rf 0.50 were identical, however `Regina' had a fifth band at Rf 0.45 whereas `Orange Beauty' had its fifth band at Rf 0.46. `Red Surprise' had four bands in common with `Orange Beauty' at Rf 0.41, 0.43, 0.46 and 0.50. The fifth band for `Red Surprise' was at Rf 0.49.

`Eureka' had three bands at Rf 0.45, 0.47, and 0.49. `Orchid' also had three bands at Rf 0.46, 0.50, and 0.53. Two of these bands, Rf 0.50 and 0.53, were observed in `Appelbloesem' and `Canaria'. `Appelbloesem' also had two other bands at Rf 0.44 and 0.47 and `Canaria' had only one other band at Rf 0.45. `Orego' had three bands identical to `Appelbloesem' at Rf 0.44,

Figure 4.11a. 6-Phosphogluconate dehydrogenase - cultivars

From left to right: *A. pelegrina*, *Bomarea* Red Surprise, Regina, Orego, Orchid, Rosario, Orange Beauty, Rosita, Pink Triumph, Neva, Mona Lisa, Monika, *A. pelegrina*, Carmen, Eureka, Arizo, Alnba, Appelbloesem, Luciana, Marina, King Cardinal, Texas, Yellow King, Canaria, Jubilee



+ Faint bands are sometimes found at the cathodal end

Fig.4.11a. Separation of 6-PGD isozymes of cultivars. b. diagrammatic representation

0.47, and 0.50 but also had bands at Rf 0.41 and 0.51. `Marina' was the fourth cultivar which haa series of five isozyme bands. These were at Rf 0.39, 0.42, 0.45, 0.47, and 0.50. `Yellow King' had these five isozyme bands plus a sixth band at Rf 0.51.

There were sixteen cultivars that could be identified by their unique isozyme banding patterns for this enzyme. Three other patterns were common to either 2 or 3 of the cultivars.

Aspartate amino transferase (Fig.4.12)

A common band at Rf 0.55 was identified for all cultivars except `Neva'. A second band at Rf 0.57 was identified in 16 of the 23 cultivars. Identical banding patterns were observed in `Arizo' and `Eureka' with bands at Rf 0.55, 0.56, and 0.58. `Appelbloesem' and `Orego' had the same pattern of five bands at Rf 0.48, 0.52, 0.55, 0.57, and 0.59. `Carmen' and `Pink Triumph' also had the same pattern of three bands at Rf 0.55, 0.57, and 0.59. `Mona Lisa' had the same three bands along with a fourth isozyme band Rf 0.56. Isozyme bands at Rf 0.55 and 0.56 were identified in `Rosita', `Orange Beauty', and `Rosario'. `Orange Beauty' and `Rosario' also had a third band at Rf 0.51, while `Rosario' had a fourth band at Rf 0.59.

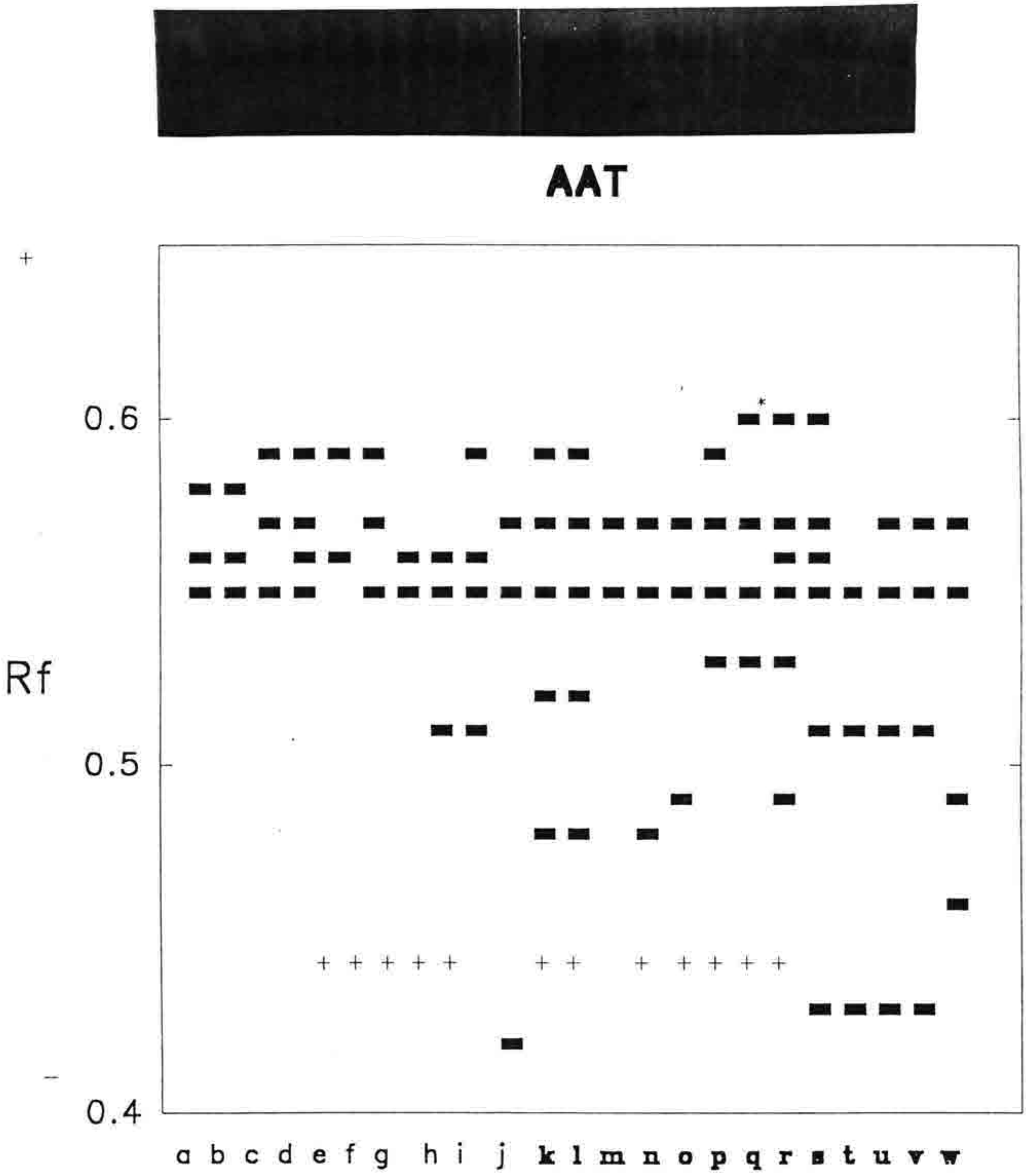
`Orchid' had the two common bands at Rf 0.55 and 0.57 as well as the most cathodal of all the isozyme bands identified at Rf 0.42. `Regina' had only the two most common bands, while `Red Surprise' and `Alnba' had these two bands plus a third band at Rf 0.48 and 0.49, respectively. `King Cardinal' had a total of four bands, the two common bands at Rf 0.55 and 0.57 as well as bands at Rf 0.53 and 0.59. `Luciana' also had four bands at Rf 0.53, 0.55, 0.57, and 0.60. The band at Rf 0.60 was not seen in all preparations.

`Jubilee' and `Texas' both had four identical bands at Rf 0.55, 0.56, 0.57, and 0.60. `Jubilee' had two other isozyme bands at Rf 0.49 and 0.53 while the other two bands for `Texas' were at Rf 0.43 and 0.51. The three bands of `Yellow King' were at Rf 0.43, 0.51 and 0.55. In two of seven experiments with this enzyme, a fourth band was also identified at Rf 0.57. The four bands of `Monika' were located at Rf 0.46, 0.49, 0.50, and 0.57. Four bands at Rf 0.43, 0.51, 0.55, and 0.57 were identified for both `Canaria' and `Marina'.

There were unique patterns identified for 15 of the cultivars. The remaining eight cultivars had a further four different patterns, each pattern being common to two cultivars. A series of faint cathodal bands were sometimes seen for many of the cultivars. These bands were difficult to discern precisely and so were not included in the analysis of cultivar differences.

Figure 4.12a. Aspartate amino transferase - cultivars

From left to right: *A. aurantiaca*, Monika, Marina, Carmen, Yellow King, Texas, Jubilee, Luciana, King Cardinal, Alnba, Red Surprise, Regina, *A. aurantiaca*, Orego, Appelbloesem, Orchid, Rosario, Orange Beauty, Rosita, Pink Triumph, Neva, Mona Lisa, Carmen, Eureka, Arizo



+ Series of faint bands sometimes found near the cathodal end
* Variable band not seen in all preparations

Fig.4.12a. Separation of AAT isozymes of cultivars. b. diagrammatic representation

Malic Enzyme (Fig.4.13)

There were two very distinct zones of activity for malic enzyme. The more anodal zone was between Rf 0.50 and 0.54, while the more cathodal zone was between Rf 0.37 and 0.43. Seventeen of the 23 cultivars had an isozyme band at Rf 0.54. `Eureka' and `Carmen' had identical banding patterns, with bands at Rf 0.42, 0.43 and 0.54. They were the only cultivars to have an isozyme band at Rf 0.43. `Yellow King' and `Monika' also had the same isozyme pattern of two bands at Rf 0.42 and 0.54. `Regina', `Rosita' and `King Cardinal' each had three bands at Rf 0.40, 0.42 and 0.54. `Pink Triumph' had these same three bands as well as a fourth band at Rf 0.39. `Red Surprise' and `Canaria' also had isozyme bands at Rf 0.40 and 0.42 but the anodal band for these cultivars was at Rf 0.52. `Orchid', `Appelbloesem' and `Alnba' had only two isozyme bands at Rf 0.40 and 0.54.

`Orego' and `Jubilee' had a cathodal band at Rf 0.40 and an anodal band at 0.52. `Luciana' and `Texas' each had two isozyme bands at Rf 0.38 and 0.54. `Orange Beauty' had two cathodal bands at Rf 0.41 and 0.42, and an anodal band at 0.54, the same pattern as `Mona Lisa'. `Marina' also had cathodal bands at Rf 0.41 and 0.42 but the anodal band for this cultivar was at Rf 0.52. `Arizo' had an anodal band at Rf 0.52 and a cathodal band at 0.41.

`Neva' and `Rosario' had the most cathodally located isozyme band at Rf 0.38. `Neva' had a second band at Rf 0.54 whereas `Rosario' had four more bands at Rf 0.40, 0.50, 0.52, and 0.54. `Rosario' had the most isozymes of any of the cultivars studied and was the only cultivar to have more than one isozyme band in the anodal portion of the gel.

There were only five cultivars which had unique banding patterns with this enzyme. Six other patterns were identified for each of two cultivars, while two patterns were each present in three cultivars. Thus there was a total of 13 different isozyme banding patterns for this enzyme.

Esterase (Fig.4.14)

All cultivars had isozyme bands at Rf 0.84 and 0.87. Ten cultivars also had an isozyme band at Rf 0.90 and seven had a band at Rf 0.91. The cultivars had from three to as many as eight bands for esterase enzyme. Three bands were identified for `Arizo' at Rf 0.84, 0.87 and 0.90. `Monika' and `Yellow King' also had three bands at Rf 0.80. 0.84 and 0.87. The latter two bands were also present for `Regina', with the third band at Rf 0.79.

Figure 4.13a. Malic Enzyme - cultivars

From left to right: *A. pelegrina, Bomarea*, Red Surprise, Regina, Orego, Orchid, Rosario, Orange Beauty, Rosita, Pink Triumph, Neva, Mona Lisa, Monika, *A. pelegrina*, Carmen, Eureka, Arizo, Alnba, Appelbloesem, Luciana, Marina, King Cardinal, Texas, Yellow King, Canaria, Jubilee

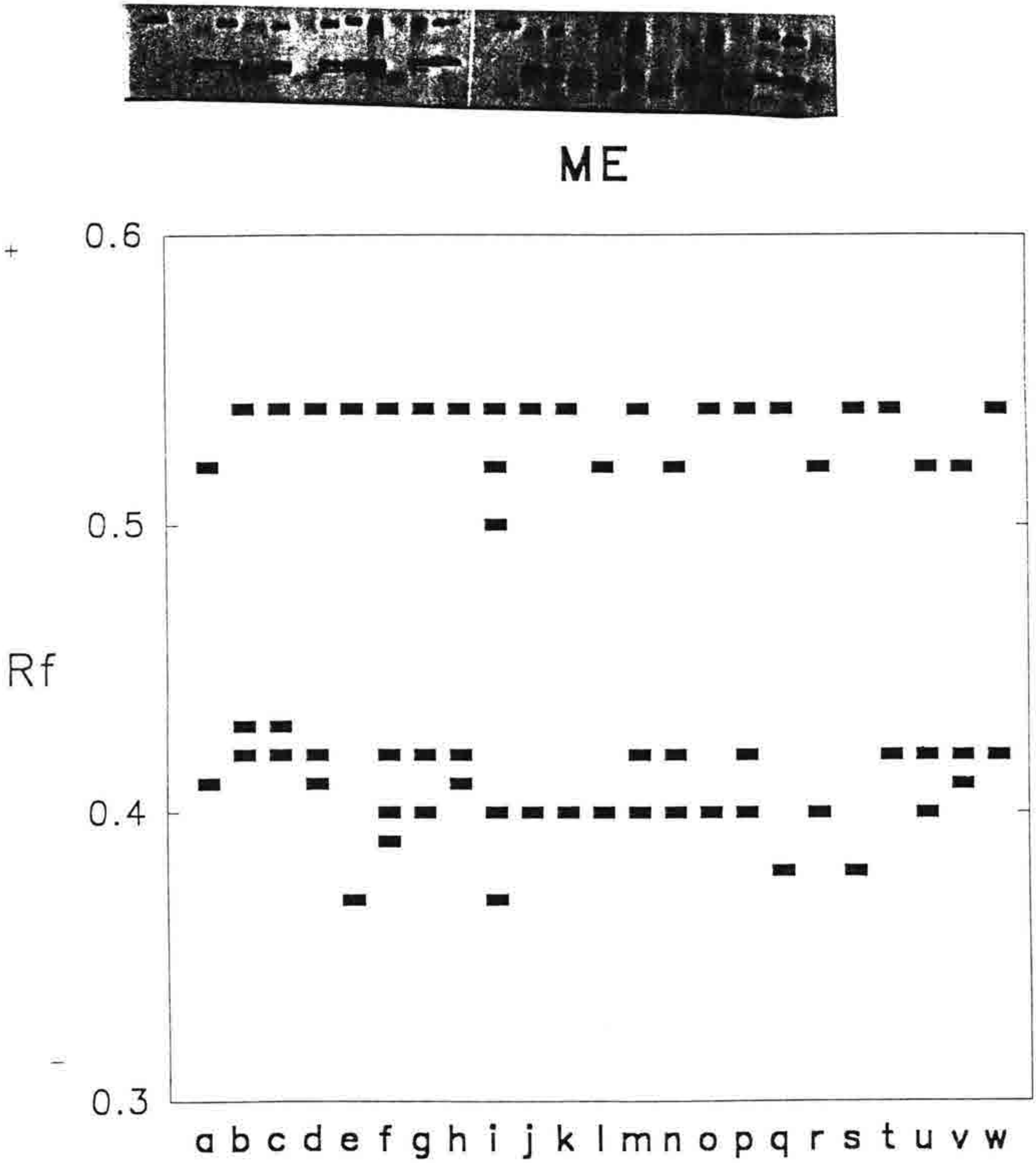
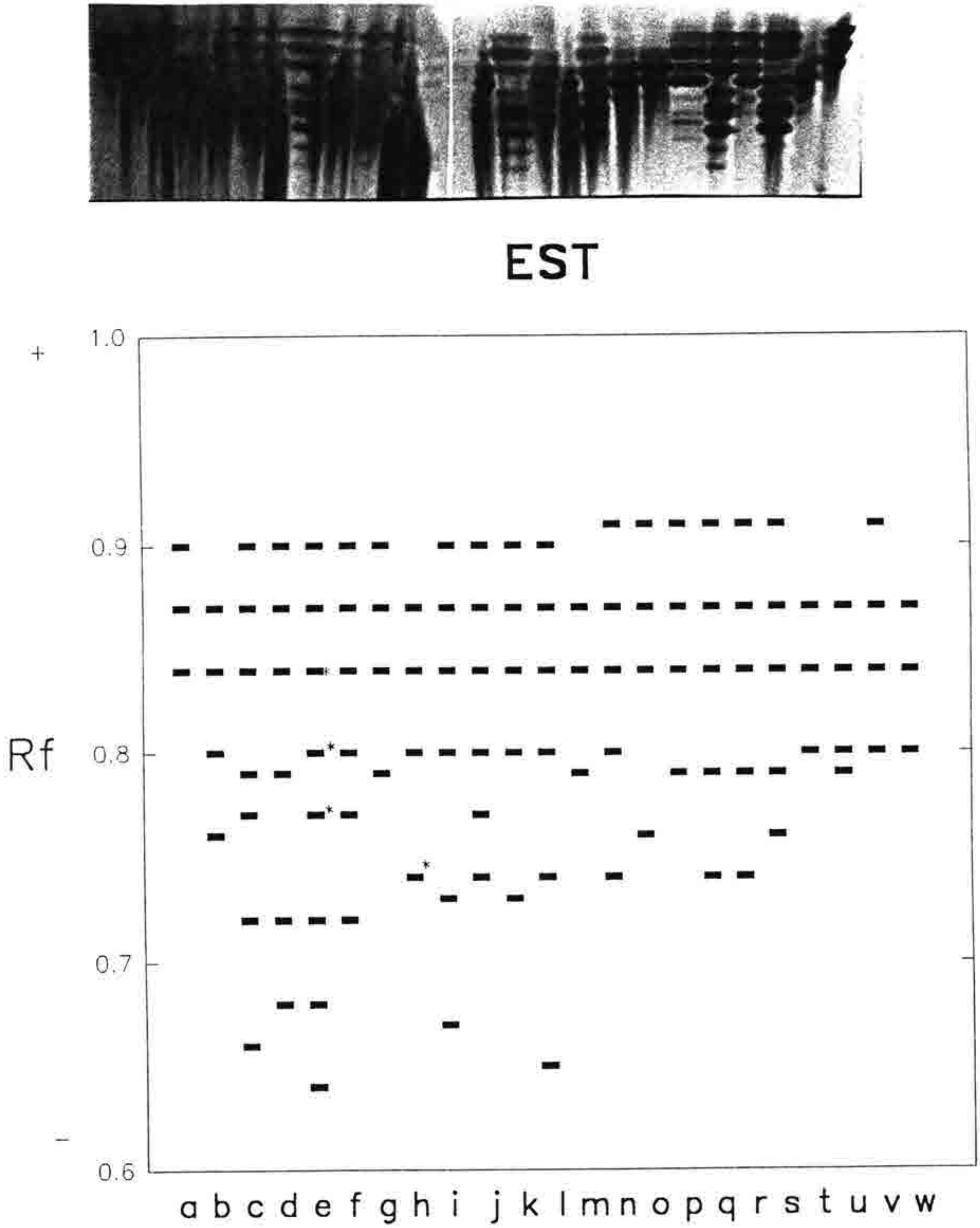


Fig.4.13a. Separation of ME isozymes of cultivars. b. diagrammatic representation

Figure 4.14a. Esterase - cultivars

From left to right: *A. aurantiaca*, Monika, Marina, Canaria, Yellow King, texas, Jubilee, Luciana, King Cardinal, Alnba, Red Surprise, Regina, *A. aurantiaca*, Orego, Appelbloesem, Orchid, Rosario, Orange Beauty, Rosita, Pink Triumph, Neva, Mona Lisa, Carmen, Eureka, Arizo



*Variable bands not all seen in all preparations

Fig.4.14a. Separation of EST isozymes of cultivars. b. diagrammatic representation

There were four identifiable bands for seven of the cultivars. `Eureka' had bands at Rf 0.76, 0.80, 0.84, and 0.87. `Rosita' had the two common isozyme bands at Rf 0.80 and 0.84 as well as bands at Rf 0.79 and 0.90. `Orange Beauty' had three bands at Rf 0.80, 0.84 and 0.87, as well as a fourth band at about 0.74. The position of this band varied slightly in different preparations at different times of the year and so only an average position has been indicated. `Alnba' also had four isozyme bands at Rf 0.76, 0.84, 0.87, and 0.91. It was one of only two cultivars which did not have a band near Rf 0.80. `King Cardinal' was very similar to `Alnba' with bands at Rf 0.84, 0.87 and 0.91 but the fourth band was at 0.79. `Canaria' and `Marina' had three bands in common at Rf 0, 0.84 and 0.87, however, the fourth band for `Canaria' was at Rf 0.79 while that for `Marina' was at Rf 0.91.

There were five cultivars that had five isozyme bands. `Appelbloesem' had bands at Rf 0.80, 0.84, 0.87, and 0.90 as well as a more cathodal band at 0.73. `Red Surprise' had the same four anodal bands as `Apelbloessem' but its fifth band was at Rf 0.74. `Luciana' and `Jubilee' had similar patterns of isozyme bands with bands at Rf 0.74, 0.79, 0.84, 0.87, and 0.91. `Texas' had the same four bands from Rf 0.79 to 0.91 but the fifth band was at 0.76.

Six isozyme bands were identified for five of the cultivars. All of these cultivars had three bands in common at Rf 0.84, 0.87 and 0.90. `Mona Lisa' also had three other bands at Rf 0.68, 0.72 and 0.79, while the other three bands of `Pink Triumph' were at Rf 0.72, 0.77 and 0.80. `Rosario' had bands at Rf 0.67, 0.73 and 0.80 as well as the three common bands, whereas `Orchid' had three other isozyme bands at Rf 0.74, 0.77 and 0.80. `Orego' had five bands in common with `Orchid' at Rf 0.74, 0.80, 0.84, 0.87, and 0.90 with a sixth band at 0.65. Seven bands were identified for `Carmen' at Rf 0.66, 0.72, 0.77, 0.79, 0.84, 0.87, and 0.90 and eight bands were found for `Neva' at Rf 0.64, 0.68, 0.72, 0.77, 0.80, 0.84, 0.87, and 0.90.

Despite problems with streaking of the gel for some or all of the cultivars in some of the electrophoretic runs, esterase bands were generally very intense and well defined and gave the most consistent results of all the enzyme systems used for cultivar identification. Only four of the cultivars could not be uniquely identified with this enzyme. Two groups of two cultivars each had the same isozyme pattern.

Chapter 5

DISCUSSION

In this study, seven different enzyme stains have been used to separate the twenty four species, hybrids and color variants of *Alstroemeria*. Due to the confused nomenclature and identification of some of the species, it has not always been possible to determine if different authors are in fact referring to the same species. Isozyme analysis may provide further evidence as to the true identities of some of these materials.

The majority of the species studied originated in Chile. *A. pulchella, A. psittacina* and *A. caryophyllae* were the exceptions, as they are native to Brazil.

Due to the limited amount of material available per species and to confusion in prior years with labelling of some greenhouse plants, there were insufficient numbers for genetic analysis of the enzyme systems. The difficulties in producing and germinating viable seed resulted in an inability to collect adequate data on the segregation of isozyme genes. Hypotheses on the possible structure of the active enzymes are thus made by reference to other published information and comparison of the banding patterns obtained. These hypotheses may be supported or refuted by future work.

Phosphogluco-mutase

The majority of species and hybrids had a single anodal and a single cathodal band when stained for PGM. *A. pelegrina,* however, had only a single cathodal band; *A. ligtu hyb* and both color variants of *A. aurantiaca* had two cathodal and two anodal bands; and *A. caryophyllae hyb* had two cathodal bands and a single anodal band. Since the cathodal and anodal bands were so widely separated, it seemed likely that they were each due to the action of separate genes. It has been shown that in many plant species PGM has both chloroplastic and cytosolic genes (Weeden and Gottlieb, 1980; Weeden et al., 1988). Chloroplastic enzymes are more highly conserved than cytosolic enzymes and often have fewer alleles which generally migrate closely together within the gel (Gottlieb, 1982).

It was difficult to determine which allozyme bands belonged to the proposed chloroplastic or cytosolic isozymes. Progeny of a cross between *A. versicolor* as the female parent and *A.*

aurantiaca as the male, had a single band in the anodal region of the gel, at Rf 0.80, as well as two cathodal bands at 0.64 and 0.66. Since chloroplastic genes are inherited only through the female parent, in this case *A. versicolor*, this suggested that the anodal isozymes were possibly due to the action of a chloroplastic gene. If this is correct, then the chloroplastic gene had four alleles, which produced allozymes that were closely spaced. The cytosolic gene would then have four alleles in the genus *Alstroemeria* and one other allele in *Leontochir ovallei* and *Bomarea spp.*

Since each species had only one or two alleles at each proposed locus, PGM was assumed to be monomeric. This agrees with previous work on peas, spinach (Weeden and Gottlieb, 1980), cotton (Suiter, 1988), salvia (Hashemi et al., 1993), citrus (Torres et al., 1978b), sour cherry (Beaver and Iezzoni, 1993), guayule (Hashemi and Estilai, 1992), raspberry (Cousineau and Donnelly, 1992), and apple (Weeden and Lamb, 1985).

Phosphogluco-isomerase

A single, clear anodal band was identified for each species studied. These bands were assumed to be encoded by a chloroplastic gene, since both chloroplastic and cytosolic gene loci have been identified for many plant species for this enzyme (Gottlieb, 1982; Weeden and Gottlieb, 1980; Bringhurst et al., 1981; Weeden et al., 1988). There were from one to six isozyme bands in the more cathodal region of the gel, for each species investigated. The total number of isozyme bands was eleven, including one band found only in *L. ovallei*. It was not possible to determine how many loci were involved in the formation of these isozyme bands and thus no determination of allelic bands can be made.

PGI has been shown to be a dimeric enzyme in many plant species, such as ryegrass (Nielsen, 1980), *Clarkia* (Gottlieb, 1982), cotton (Suiter,1988), salvia (Hashemi et al., 1993), apple (Weeden and Lamb, 1985), grape (Weeden et al., 1988) and potato (Oliver and Martinez-Zapater, 1985). The quaternary structure of PGI in alstroemeria and related species could not be determined from this study.

6-Phosphogluconate dehydrogenase

The clearest isozyme bands were obtained using 6-PGD, particularly for extractions made during the late spring and summer. The isozymes always migrated to a very narrow zone within the gel, with a common band in almost all species at Rf 0.50. The exceptions to this were *A. psittacina* and *A. pulchella* which had bands at Rf 0.51. These species were from Brazil, whereas all the others, except *A. caryophyllae hyb* were from Chile. A second band for all the Chilean species of alstroemeria was at 0.46 or 0.47.

It has been shown that 6-PGD has cytosolic and chloroplastic genes (Gottlieb, 1982; Ireland and Dennis, 1980; Suiter, 1988; Schnarrenberger et al., 1974) between which there is no intergenic dimerization. In maize, however, the isozymes of 6-PGD were found to form interlocus heterodimers (Gottlieb, 1982), suggesting that the subunits are both present in the same compartment. The quaternary structure of 6-PGD has been shown to be dimeric (Suiter, 1988; Oliver and Martinez-Zapater, 1985; Chyi and Weeden, 1984). In the present study, further investigation is needed before the compartment location and quaternary structure of 6-PGD isozymes in alstroemeria can be determined.

Ten species were readily identified with this enzyme stain, with the remaining species having common patterns.

<u>Aspartate amino transferase</u>

The isozyme banding pattern for AAT was more complex than the first three enzyme systems discussed. AAT has been variously described as both monomorphic and dimorphic (Torres et al., 1978a, b; Suiter, 1988; Arulsekar et al., 1985; Reyes and Collins, 1992). Some authors have also shown that in some species there are both plastid and cytoplasmic isozymes present (Weeden and Gottlieb, 1980; Weeden et al., 1988, Huang et al. 1976, Scandalios et al., 1975).

A common band at 0.55 was identified in all alstroemeria species and hybrids except for *A. hookeri ssp hookeri*. Neither *L. ovallei* nor *Bomarea spp.* had an isozyme at this position in the gel.

The distribution of the isozyme bands for this enzyme suggested that there were at least five *Aat* loci in alstroemeria. Based on this aussumption the most anodal loci, *Aat-1* had only two alleles found in *A. garaventae* and *A. aurantiaca* with another six alleles from *Bomarea spp* and *L. ovallei*. *Aat-2* is proposed to have five alleles with each species having one or two of these, as well as heterodimeric isozymes when two alleles are present. *Aat-3* had only one allele for most of the alstroemeria plants with the exception of *A. magenta*, which had no isozyme at this position. Another fixed isozyme was found at Rf 0.52 and may be the result of the action of a fourth proposed loci, *Aat-4*. No isozyme was found for *Aat-4* in *A. diluta ssp diluta, A. angustifolia ssp angustifolia, A. versicolor* or *A. sierrae*. The fifth proposed locus, *Aat-5*, had seven alleles in *Alstroemeria* and *Bomarea* with a further two alleles identified in *L. ovallei*. From one to four allozymes were present in each of the species. All of these loci had active products in tissue of leaf, flower and roots.

Gottlieb (1982) reports that three or four AAT isozymes is the minimum number reported in many conifers and flowering plants. Additional isozymes of the enzyme are often found, but none fewer. Torres et al. (1978a) suggested that the AAT enzyme system in avocado is controlled by four genes. The first two, *Aat-1* and *Aat-2* are dimeric enzymes. *Aat-3* had the same isozyme band in all varieties of avocado studied, and *Aat-4* is assumed to be a monomeric isozyme since individuals had either one or two isozymes.

Four *Aat* loci are also suggested for grape (Weeden et al., 1988). *Aat-1* and *Aat-2* are localized in the mitochondria or microbodies, *Aat-3* is a monomorphic plastid specific isozyme and *Aat-4* is located in the cytosol.

Goodman and Stuber (1983) report that all AAT isozymes in maize are dimers and are encoded by three loci. *Aat-1 (Got-3* in the original paper) migrates the fastest and is generally expressed in mitochondria. *Aat-2* (*Got-2)* codes for activities expressed in the plastid fraction and *Aat-3* (*Got-1)* is expressed in the glyoxysomes. No interlocus heterodimers are identified. Although six alleles have been identified at the *Aat-1* locus, the majority of lines surveyed showed only the same fixed allele. There are eight alleles at the *Aat-2* locus but again a single allele was fixed in a large proportion of nearly 700 racial collections. Nine alleles are identified at the *Aat-3* locus in maize and teosinte.

In wheat, Hart (1983) and Jaaska (1976, 1981) each found five sets of AAT genes. Jaaska (1983) describes three major and three minor AAT systems in rye species and triticale. *Aat-1* is a polymorphic locus with at least two alleles. The enzyme encoded at this locus is dimeric. *Aat-2* codes for an isozyme with three alleles, of which one allozyme is common to all rye species, as well as two other alleles, all of which may dimerize to form the active enzyme.

Of the three minor AAT systems discussed; one, between allozymes of AAT-2 and AAT-3, has a fixed, faint band common to all rye species, while the other two were observed in only a few species.

Rick (1983) identified four *Aat* loci in tomato. Five zones of AAT activity were reported on gels of peppers (McLeod et al., 1983) although later work suggests that there may be only four *Aat* loci, the other apparent loci being an allele of another loci. The most cathodal isozyme is monomeric in all taxa. In soybean, Kiang and Gorman (1983) identified three loci, two of which are responsible for cytosol-active bands and one which was located in the plastid.

The banding pattern for alleles from proposed locus *Aat-2*, for all alstroemeria species and hybrids, fits the model for a dimeric enzyme. There were either single allozyme bands indicating homozygous individuals or three bands for heterozygous individuals. No band was found in this region for *A. angustifolia ssp angustifolia* suggesting the presence of a null allele. Both the loci *Aat-3* and *Aat-4* had a single fixed allele for all species. It may be that one or both of these alleles were due to the action of chloroplastic loci. Further clarification is needed to establish the localization and quaternary form of isozymes from these proposed loci.

Allozymic bands from the proposed *Aat-5* loci did not fit a dimeric model. There were one, two, three, or four allozymes identified for each alstroemeria species or hybrid, except *A. caryophyllae hyb, A. pulchella* and *A. psittacina.* The latter three are all Brazilian species whereas the remaining species and hybrids originated in Chile.

The spacing of the multiple bands for some of the species suggests that the heterozymic isozyme formation normally found is a dimeric system. Other isozymes appeared to be functioning as monomers. This situation was also observed in citrus (Torres et al., 1978a) and avocado (Torres et al., 1978b). It has been suggested that possibly some subunits cannot dimerize with one another or that some isozymes are truly monomeric in some species and dimeric in others. Scandalios (1969) suggests that, in some instances, there is a strict affinity for identical polypeptide chains in the formation of dimers. Further study is needed to clarify this anomaly.

Malic enzyme

A tetrameric structure for ME was proposed by Goodman and Stuber (1983) for ME-1 in maize coleoptile tissue. A single zone of activity was observed for malic enzyme of cotton, with heterozygotes displaying a five band pattern, suggesting that the enzyme is tetrameric (Suiter, 1988). In citrus leaves, however, ME behaved as a monomer specified by two genes that produced isozymes in two well separated regions of the gel (Torres et al., 1982).

The ME isozymes of alstroemeria appeared to resemble those of citrus. There were two, well separated staining zones within the gel. In the anodal zone, all of the species and hybrids, except for *A. caryophyllae hyb.*, had a single isozyme band. *A. caryophyllae hyb.* had an evenly spaced series of four bands. This pattern suggests a trimeric structure for these isozymes, which would be very unusual. Alternatively, the isozymes may be monomers, with four alleles present in the tetraploid hybrid. There are four alleles for this locus in the materials studied.

The second proposed locus for ME had six alleles. Three of the alstroemeria species and the *Bomarea* spp. had two isozymes in this cathodal zone. The yellow flowered *A. aurantiaca* had an evenly spaced series of four bands, while the remaining 18 species and hybrids had only a single band. This pattern generally fits a monomeric model for isozyme structure. The inter-allelic bands seen in *A. aurantiaca* were possibly an artifact due to the extraction procedure, or may represent post-translational modification of the allozymes of this species.

The intermediate band seen clearly in *A. hookeri ssp hookeri* and faintly in four other species and hybrids, may have indicated the presence of another allele of one of the two loci. If, however, this band represents an intergenic (heterozygous) isozyme, the quaternary structure of at least one loci would have to be multimeric.

There were obvious differences in the intensity of each isozyme for the various species and hybrids. This ranged from very faint bands for *A. haemantha hyb* and *L. ovallei* to extremely thick, dark bands for *A. ligtu* species and hybrids and *A. psittacina*. However, since no attempt was made to quantify the amount of protein in each of the prepared samples it was not possible to determine whether or not this was due to dosage differences. Further investigation of this enzyme system is necessary to determine its quaternary structure and the number of alleles at each of its loci.

Esterase

A large number of esterase loci have been reported for many plant species. Esterases are a heterogeneous group of enzymes with low substrate specificity. *In vitro* they may hydrolyse a large number of different esters, although at different rates (Scandalios, 1969). The natural substrates and *in vivo* action of most of the esterases are not known. The artificial esterase substrate most commonly used is α-napthyl acetate.

Schwartz (1960) proposed that at the E-1 locus of maize, there are seven alleles and the functional maize esterases are dimers. Later work indicates that there are at least 10 loci for maize esterase isozymes (MacDonald and Brewbaker, 1975). Of the few loci that have been extensively studied, three of the maize esterases were found to be dimeric (Schwartz, 1964; Newton et al., 1982; Stuber and Goodman, 1982) and at least two others are monomeric (Harris, 1966; Brown and Allard, 1969). Mok (1981) also suggested that esterase systems of potato indicated that the active enzyme is a dimer. Desborough (1983) disagrees with this interpretation and suggests that potato isozymes may be tetramers.

For peppers, McLeod et al. (1983) decided that the three more anodal esterases are monomeric proteins, while the cathodal locus codes for a dimeric protein.
Rick (1983) also suggested that the seven loci of tomato are a mixture of monomers and dimers with three loci confirmed as coding for dimeric isozymes, three for monomeric and one being uncertain.

The banding pattern for most of the *Alstroemeria* species and hybrids was quite complex with from three to seven bands identified for each. A common band at Rf 0.84 was found in every species or hybrid except for *A. angustifolia ssp angustifolia*. *Bomarea spp.* had a slightly different allele at 0.85 while *A. exserens* had a second isozyme band at 0.83. This group of isozymes may be controlled by a single locus, probably specifying a monomeric enzyme.

The series of bands anodal to the common band, had either one or two bands for many species or hybrids, however, some species of *Alstroemeria*, as well as *L. ovallei* and *Bomarea spp* had no bands in this region. This set of bands may also be controlled by a single locus specifying monomeric isozymes.

The third proposed locus for EST had four alleles, with bands at Rf 0.77-0.82. Each species and hybrid had either one or two bands in this region indicating the probability of a monomeric isozyme. The exception was *A. sierrae* which had a series of three bands in this area. This may indicate that the isozymes for this loci are dimeric in *A. sierra* and possibly in some of the other species which have only a single band. Alternatively, the third band may be due to the activity of another locus.

The remaining cathodal bands in the gel may be due to the activity of one or two loci. Five of the *Alstroemeria* species as well as *Bomarea* species have no clear bands in this area. *A. versicolor* was interesting since all of the bands in this area appear as doublets. Doublet banding patterns are thought to be due to either post-translational modification or they could be artifacts of the extraction procedure (Suiter, 1988). This two-banded phenotype has been noted for LAP in citrus (Torres et al., 1982) and in cotton (Suiter, 1988).

Progeny testing is necessary to finally determine the number of loci and alleles involved in the presence of esterase isozymes. The quaternary structure of each of these isozymes also remains to be determined.

Leucine amino peptidase

There are a number of amino peptidases, some of which are more specific for certain N-terminal amino acids. Leucine amino peptidase is an enzyme that hydrolyzes the chromogenic substrate L-leucyl β-napthylamide. Its activity is accelerated by the presence of either Mg or Mn ions. Chemically, LAP is an excopeptidase which hydrolyses peptide linkages adjacent to the free α-amino groups of a peptide, resulting in a free amino acid and a smaller peptide. This process may then be repeated with the free amino group of the smaller peptide (Scandlios, 1969).

The activity of this enzyme was only high in alstroemeria during certain times of the year and thus only a few species were analyzed. Each species or hybrid had from one to four isozyme bands. These bands were clustered within a very small region of the gel, and the zymogram consisted of doublet isozymes. *A. ligtu spp, A. ligtu hyb* and *A. chilensis* all had the same doublet isozymes in the median portion of the region. *A. psittacina* and *A. pulchella* had the doublet isozymes in the same anodal or faster part of the region. *A.pelegrina alba* had a doublet isozyme pattern in the cathodal region of the gel. These species were all assumed to be homozygous for LAP. *A. haemantha hyb* and *A. caryophyllae hyb* had a series of four bands, comprised of two sets of doublet isozymes. The yellow-flowered *A. aurantiaca* also had two sets of doublet isozymes but in a more median part of the staining region. These three species and hybrids were assumed to be heterozygous for LAP. Single bands were identified for the orange-flowered *A. aurantiaca* and for *L. ovallei.*

The doublets occur in three different positions and were assumed to be coded for by three different alleles of a single locus. The patterns suggest that LAP is a monomeric isozyme. Further analysis is necessary to confirm or refute this assumption. Doublet bands for LAP have been noted in citrus (Torres et al., 1982), grape (Wolfe, 1976) and cotton (Suiter, 1988).

Two LAP loci have been identified in avocado (Torres et al., 1978a), cotton (Suiter, 1988), alfalfa (Quiros, 1983) and soybean (Kiang and Gorman, 1983). Where genetic testing was included, the LAP enzyme was shown to be monomeric. The two-banded doublet isozymes may be the result of post-translational modification, or they could be artifacts of the extraction procedure.

Isozyme characterization of species

For all enzyme systems except LAP, the zymograms for *A. pelegrina rosea* and *A.pelegrina alba* were exactly the same. No comparison was made for LAP. Seed for each of the plants used was from very different sources, suggesting that there is good conservation of isozymes throughout different populations of *A. pelegrina*. *A. pelegrina* is found along coastal areas of Chile, below 1000m, and between latitudes 32° and 33° S.

A. psittacina and *A. pulchella* are generally considered to be different names for the same species (Hannibal, 1941; Stinson, 1942; Robinson, 1963). Comparison of zymograms for individuals of these species show good agreement for all enzymes (LAP was not included).

The two color variants of *A. aurantiaca* have exactly the same zymogram only for PGM. For all of the other enzymes, the two variants have some isozyme bands in common as well as unique isozymes for each. The two color variants in our greenhouse could easily be distinguished based on their isozyme banding patterns. The enzyme system with the most obvious and consistent differences was ME. *A. aurantiaca* (*aurea*) species are found over a wide area of Chile from 36°- 42°S and spread throughout very different habitats. The results of the present study suggest that there may be wide differences among populations of *A. aurantiaca*. Several other plants, from a different source, are now being grown for isozyme analysis, to try to determine further population differences.

A. ligtu ssp. ligtu and *A. chilensis are also thought to be the same plant species* (Bayer, 1987). A comparison of the zymograms of *A. ligtu spp* and *A. chilensis* for the seven enzymes studied, showed that they are identical for four of the enzymes. Three enzyme systems, PGI, ME and EST showed some slight differences between plants of each separately identified species. For ME, a different anodal allele was present in each; for EST, four isozymes were common to both; and for PGI, four isozymes or allozymes were common. Some of these differences may simply be due to the presence of different alleles in the plants studied. *A. ligtu ssp ligtu* is distributed along coastal areas of Chile between 32° and 38°S, although it is also commonly found along river valleys further inland. It generally grows below 1000m.

It has been noted that *A.ligtu* hybrids are probably the result of a cross made in about 1927 between *A. ligtu spp* and *A. haemantha* (Gulmon and Mooney, 1980). The *A. haemantha* specimens in our collection are no longer true species but have hybridized with other materials in the greenhouse. However, a comparison of the banding patterns of *A. ligtu spp., A. haemantha* and *A. ligtu hyb* for six of the enzyme systems (not LAP) would seem to support this origin for the ligtu hybrids.

A. haemantha is thought to be another name for *A. ligtu ssp simsii* (Bailey, 1947; Baker, 1888; Uphof, 1952, Bayer, 1987). The latter has two color variations - orange/red and yellow.

A.haemantha has also been described as having either dark red, salmon rose or orange-yellow flowers (Robinson, 1963; Stinson, 1942). The leaves are generally described as ciliated and the petals are pointed and reflexed. A comparison of the banding patterns of the single individual of *A. ligtu ssp simsii* and the *A. haemantha* hybrids, showed that of the 22 isozyme loci in each of the two species, 10 were common to both. A wide range of individuals from each species needs to be studied before any conclusions can be drawn with regard to their identity to one another.

A.sierrae and *A. gayana* are different names for the same species, which is also known as *A. magnifica ssp magnifica (Bayer, 1987)*. *A. gayana* is thought to be an intermediate between *A. haemantha* and *A. pelegrina* (Baker, 1888). A comparison of the zymograms of *A. sierrae* with *A. pelegrina* and *A. haemantha* revealed that nine of the 18 isozymes of *A. pelegrina* and eight of 20 isozymes of *A. haemantha hyb* were the same as those found in *A. sierrae*. The best match of zymograms is found for EST in which every band in *A. sierrae* is also found in either *A.pelegrina* or *A. haemantha hyb.* A perfect match was also noted for the cytosolic PGM isozyme for all three species, and in PGD two of the three isozyme bands of *A. sierrae* are common to all three species.

L. ovallei and *Bomarea spp* were readily distinguishable from *Alstroemeria* species using isozyme analysis. *L. ovallei* was identified using the EST enzyme system; it had a cathodal band that was quite separate from the isozyme bands of all *Alstroemeria* and *Bomarea* which have been studied at this time. *Bomarea* and *L.ovallei* were also easy to identify using AAT. All *Alstroemeria* species and hybrids had isozyme bands in the center of the stained region of the gel whereas *Bomarea spp* and *L. ovallei* only had bands in the cathodal and anodal regions of the gel. The pattern for each is distinctive.

Identification of each of the other species can be achieved with only a few enzyme systems. *A. pelegrina rosea* and *A. pelegrina alba* will be discussed as a single species, *A. pelegrina*; while *A. pulchella* and *A. psittacina* will also be discussed as a single species under the latter designation. The few differences between *A. chilensis* and *A. ligtu spp* have already been discussed and comparisons at this time will be made only to *A. ligtu spp.*

There were eleven different isozyme patterns for *Alstroemeria* species and hybrids, for the isozymes at the cytosolic loci of PGI. Although there was some small variation in the position of the chloroplastic isozymes, these are generally not included in comparison of the zymograms, except in the case of *A. angustifolia ssp angustifolia.* In the latter species the migrational position of the chloroplastic isozyme was different enough from the other *Alstroemeria* species that it could be used to distinguish it.

The PGI pattern of cytosolic isozymes was unique for each color variant of *A. aurantiaca, A. pelegrina, A. magnifica ssp maxima, A. sierrae* and *A. versicolor. The patterns for A. chilensis* and *A. ligtu* 'Miyake spotless' were similar to that of *A. ligtu spp* except that the most cathodal band of *A. ligtu spp* was not always clearly seen in the first two species. These three species and hybrid, as well as *A. exserens* could not be clearly separated from each other based on their PGI zymograms (Table 5.1).

The other species fall into one of three other patterns. The second pattern was shown by *A. ligtu hyb, A. psittacina, A. haemantha hyb,* and *A. caryophyllae hyb.* The third pattern was shared by *A. garaventae, A diluta ssp diluta, A. hookeri ssp hookeri* and *A. angustifolia ssp angustifolia.* The latter species could then be distinguished from the first three by the unusual position of its chloroplastic isozyme. The fourth pattern was shared by only *A. magenta* and *A. ligtu ssp simsii.*

If the 6-PGD enzyme system was then utilized, the species with the second and fourth patterns of PGI could be separated. There were thirteen different zymograms for *Alstroemeria* species using 6-PGD. *A. ligtu hyb, A. psittacina, A. caryophyllae hyb,* and *A. haemantha hyb* all have different patterns which are easy to distinguish. Similarly, *A. ligtu ssp simsii* and *A. magenta* could be separated from one another using this enzyme. The group of four species which shared the first pattern for PGI, could be separated into two groups using 6-PGD. *A. ligtu* 'Miyake spotless' and *A. exserens* had the same pattern for 6-PGD, which separated them from *A. ligtu spp* and *A. chilensis.* Of the third group of PGI, *A. hookeri ssp hookeri* could be distinguished from *A. garaventae* and *A. diluta ssp diluta.*

A third enzyme system must then be used to separate *A. ligtu* 'Miyake spotless' and *A. exserens, and A. garaventae* and *A. diluta ssp diluta.* The AAT system revealed very different cathodal bands for *A. exserens* and *A. ligtu* 'Miyake spotless', with both the position and intensity of the banding being easily distinguishable. ME could just as easily have provided the information necessary to separate the two materials. Similarly, *A. garaventae* and *A. diluta ssp diluta* could be separated using either AAT or ME.

Therefore, using only a single enzyme, PGI, seven species or color variants could be clearly identified. The addition of the second enzyme, 6-PGD, provides the separation of a further eight species and hybrids, and the remaining four species and hybrid could be separated with a third enzyme system.

Table 5.1: Separation of species based on isozyme patterns

PGI-cytosolic	PGI-plastid	6-PGD	AAT Or ME
Separated		Separated	
A. aurantiaca - yellow		*A. ligtu ssp/* *A. chilensis*	*A. ligtu* `Miyake spotless'
A. aurantica - orange		*A. ligtu hyb*	*A. exserens*
A. pelegrina		*A. psittacina*	*A. garaventae*
A. magnifica ssp maxima		*A. haemantha hyb*	*A. diluta ssp diluta*
		A. caryophyllae hyb	
A. sierrae	*A. angustifolia* ssp. *angustifolia*	*A. hookeri ssp hookeri*	
A. versicolor		*A. magenta*	
		A. ligtu ssp simsii	
Not separated		Not separated	
A. ligtu spp/ *A. chilensis* *A. exserens* *A. ligtu* `Miyake spotless' *A. ligtu hyb.* *A. psittacina* *A. haemantha* *A. caryophyllae* *A. angustifolia ssp angustifolia* *A. hookeri ssp hookeri* *A. garaventae* *A. diluta ssp diluta* *A. magenta* *A. ligtu ssp simsii*		*A. ligtu* `Miyake spotless' *A. exserens* *A. garaventae* *A. diluta ssp diluta*	

Cultivar Identification

As expected from the hypothesized complicated pedigree of the *Alstroemeria* cultivars, great variation was found in all of the enzyme systems studied.

Using 6-PGD, nineteen different zymograms were identified for the 23 cultivars. Two cultivars, `Rosita' and `Rosario' each had isozyme bands at Rf 0.50 and 0.47; `King Cardinal' and `Jubilee' had the same zymogram of three bands, and `Neva', `Luciana' and `Texas' had the same single isozyme at 0.50.

If the zymograms for AAT is then considered `Neva', `Luciana' and `Texas' can be separated based on their unique banding patterns with this enzyme. Only the most consistent and clearest bands were used for this analysis. `Rosita' and `Rosario' could also be separated using the AAT zymogram, as could `King Cardinal' and `Jubilee'.

Using only two enzyme systems it was possible to uniquely identify all of the 23 cultivars included in this study.

The zymogram of the cultivars for ME revealed sixteen different isozyme patterns. Ten of the cultivars could be uniquely identified with this enzyme. The remaining cultivars could then be identified uniquely if the zymograms for AAT were then considered. Alternatively, fifteen cultivars could be uniquely identified if the zymograms for AAT were first considered. Using only the cytosolic isozyme patterns of PGI, the remaining cultivars could then be classified uniquely.

Very little is known about the parentage of most of the cultivars. Many of the commercially grown cultivars that have been studied are triploids or tetraploids (Tsuchiya et al, 1987; Hang and Tsuchiya, 1988) and are generally sterile.

A comparison of the banding patterns of the species and the cultivars was carried out. For every isozyme band in each cultivar the possible parental species which may have contributed this band were listed. This was carried out for each of the isozyme systems studied. These lists were then reviewed to determine which species contributed one or more bands to every enzyme system in the cultivar. With this process of elimination, species were identified which were likely to have been progenitors of the particular cultivar (Table 5.2). Since not all possible species have been included in this study, the results present only possible parents at this time. Further investigation is necessary to eliminate other species not yet studied or to give added evidence for the proposed parent species.

The triploid cultivar A. cv `Regina' is known to be the result of the cross A. pelegrina X A. aurantiaca (Heins and Wilkins, 1979), in which one of the parents probably contributed an

Table 5.2: Possible parents of cultivars studied

CULTIVAR	CHROMOSOME NUMBERS[a]	POSSIBLE PARENTS[b]
Arizo	2n=4x=32	A, H, C
Eureka	2n=2x=16	A, P
Carmen	2n=3x=24	A, At, H, P, Mm
Mona Lisa	2n=3x=24	Ls, A, At, P
Neva	2n=4x=32	P, H*(ME)
Pink Triumph	2n=3x=24	A, H
Rosita	2n=3x=24	A, At, LH
Orange Beauty	2n=3x+1=25	A, Lh
Rosario	2n=4x+1=33	At, H, C, Lh
Orchid	2n=2x=16	A
Apelbloessem	2n=3x=24	A, H
Orego	2n=4x=32	P, H, Ls
Regina	2n=3x=24	A, At, P
Red Surprise	2n=3x=24	An, Mm, Li
Alnba	2n=4x=32	H, At, Ps*(ME)
King Cardinal	2n=3x=24	A, At, H
Luciana	2n=4x-1=31	C, A
Jubilee	2n=4x=32	A, At, H, C
Texas	2n=4x=32	A, At, H, C
Yellow King	2n=3x=24	A, At, Ps
Canaria	2n=2x=16	A
Marina	2n=3x=24	A, At, H, Mm, Ps
Monika	2n=3x=24	A, At, Ch, Ps

[a] Tsuchiya et al., 1987; Hang and Tsuchiya, 1988
[b] For abbreviations see Table 3.1
*No isozyme bands for this species were found for the listed enzyme

unreduced gamete.

Comparison of the isozyme bands in the cultivar `Regina' with those of the species studied, showed that the probable parents of this triploid cultivar were A. pelegrina and *A. aurantiaca*. This agrees with the information on the parentage of `Regina' published by Heins and Wilkins (1979) after consultation with M.C. Van Staavaren.

A. cv `Walter Fleming' is reported to be the result of a cross between *A. violaceae* and either *A. haemantha* (Robinson, 1963) or *A. aurantiaca* (Heins and Wilkins, 1979; Healy and Wilkins, 1989). The cultivar A. cv `Orchid' is known to be derived from A. cv `Walter Fleming' through the process of mutagenesis using X-rays (Broerjtes and Verboom, 1974). A. cv `Canaria' was then derived from `Orchid' by the same procedure (Micke et al., 1985).

Analysis of the isozyme bands of A. cv `Orchid' and A. cv `Canaria' indicated that *A. aurantiaca* was a possible progenitor of both of these cultivars. All of the other species included in this study were eliminated since there were not bands common to the species for every enzyme, in the cultivar. Since *A. violaceae* was not included in this study its isozyme band positions are unknown, however, it would appear likely that based on the cultivar isozyme bands, *A. aurantiaca* and not *A. haemantha* was the other likely parent.

A. aurantiaca is also named as one of the parents of a three way cross, which resulted in the `Parigo' hybrids (Goemans, 1962). *A. ligtu* species are also mentioned as not being involved with the production of these hybrids. The Parigo hybrids included in this study were `Carmen' and `Marina'. Examination of the isozyme bands indicated that four of the species studied could have been involved in the parentage of each these cultivars. For A. cv `Carmen' these were *A. aurantiaca* (either color), *A. haemantha, A. magnifica ssp maxima* and *A. pelegrina*. For A. cv `Marina' they were *A. aurantiaca* (either color), *A. haemantha, A. magnifica ssp maxima* and *A. psittacina*. Further investigation of these and other species is necessary before any firm conclusions can be made.

A. cv `Appelbloesem' is a cultivar developed through X-ray mutation of A. cv `King Cardinal' (Micke et al., 1985). Comparison of their isozyme bands with those of the species studied showed that both A. *aurantiaca* and *A. haemantha* were probable parents for both cultivars. Examination of the karyotype of both cultivars revealed that they both contain two different genomes, with two homologous genomes of one parent and one genome from the other parent contributing to the triploid karyotype (Hang and Tsuchiya, 1988).

The other diploid cultivar `Eureka' is comprised of two non-homologous genomes (Hang and Tsuchiya, 1988). Examination of the isozyme bands suggested that *A. aurantiaca* and *A. pelegrina* may have been the parental species for this cultivar.

Until all of the most common *Alstroemeria* species have been examined, the parentage of the cultivars cannot be established definitively. However, comparison of the isozyme bands in both the species and the cultivars may be a relatively easy and accurate method with which to narrow the number of probable parents and thus facilitate a more rapid breeding program.

It should be noted that isozyme bands were identified in some cultivars that did not appear in any of the species studied. There may be a number of reasons for this. First, mutagenic treatment of some of the cultivars may have caused slight differences in their enzyme structure, resulting in a shifting of the band position within the gel. Secondly, inaccuracy in measurement of band position may result in slight differences in band position and hence identification of a separate band which may not in fact be different. Thirdly, there are many other species that still need to be examined and the unknown bands may belong to one of these.

In order to clarify the possible parentage of these cultivars, further isozyme analysis needs to be carried out using other species and other enzyme systems. Complimentary to this investigation, analysis of the karyotype and Giemsa banding pattern of the chromosomes of the species and cultivars may assist in the definite identification of parents of these cultivars.

Chapter 6

INTRODUCTION

Although some limited research has been undertaken in the last 100 years there has been no extensive genetic analysis of the genus *Alstroemeria* or its related genera *Leontochir* and *Bomarea*. The earliest studies of these genera were in the late 1800's (Strasburger, 1882, Guignard, 1884) and chromosome numbers were reported by Sato (1938), Whyte (1929) and Taylor (1926). There have been limited studies since that time with only about ten species studied (Darlington and Wylie, 1955; Tsuchiya et al., 1987, 1989; Hang and Tsuchiya, 1988).

Despite the large number of cultivars that have been developed in the last 30 years there is very little published information about their origins or even the chromosome numbers. Some cultivars were reported to be triploid based on their radiosensitivity (Broertjes and Verboom, 1974) but until the 1980's very little new information was available on the karyotypes of the cultivars.

Chromosome studies of *Alstroemeria* species and cultivars by Tsuchiya et al. (1987, 1989), Tsuchiya and Hang (1987), Hang and Tsuchiya (1988), Rustanius et al. (1991) and Stephens et al. (1993) added greatly to our understanding of this genus. They reported that the chromosome number of all the species studied was $2n=2x=16$ in agreement with earlier work. The chromosome number of the cultivars varies from diploid to triploid to tetraploid. This is somewhat surprising given the diploid nature of all the species studied to date. Most of the commercially important cultivars are the result of hybridization and/or mutagen treatment (Broertjes and Verboom, 1974).

Many of the most important cultivars used for cut-flower production are polyploid. They are prized especially for their long vase life as well as for the variety of their colors. The cultivars currently in production in the United States were developed primarily in Europe and their parentage is unknown. Occasionally the breeders have identified one of the parents used in their programs but since these cultivars are often the result of three way crosses it is impossible to try to recreate the original crosses. This problem is further compounded by the use of mutagenic treatments which were widely used to create new cultivars.

It is important that cultivars are developed in the United States for use by local growers. This would provide a much cheaper plant to the grower and, in turn, to the consumer. Since there are more than 60 species of *Alstroemeria* it is difficult to develop an effective breeding program. Many of these species are incompatible when hybridized and special techniques such as embryo rescue are needed to maintain the development of the young plant. It is therefore important that the number of species involved in a breeding program be minimized and that the ones chosen should have the highest probability of providing useful offspring. Extensive analysis of *Alstroemeria* species and cultivars is needed in order to identify the species that were most likely used to create the original hybrids so that these species can then be utilized in a new breeding program.

The present study was developed to continue work already begun by Tsuchiya and his research group. Brief analysis of many cultivars and species were carried out from 1987 to 1991 by this group. More detailed analysis of eight species and one hybrid of *Alstroemeria* as well as one species each of *Bomarea* and *Leontochir ovallei* were undertaken in the current reseach. Similar analysis of 22 cultivars was also included.

Karyotype analysis of each of the species was used to determine the general chromosome complement with respect to size and shape of chromosomes and the presence or absence of satellites. Detailed analysis of the related genera *Bomarea* and *Leontochir* was also included to allow a comparison to be made between the genomes of each of the three genera. Detailed Giemsa banding studies were used to try to determine whether there was any homology between chromosomes of different species which were of similar size and shape. Comparisons were then made between the chromosomes in the species and those in the cultivars to help decide if certain chromosomes within the cultivars may have arisen from particular species.

Using this approach it was possible to determine which species may have been probable parents for some of the cultivars. Since only a limited number of species have been studied at this time many more have to be included before any conclusions can be made as to the exact parentage of the cultivars examined.

Part one of this research involved characterizing the species and cultivars by the use of isozyme banding patterns. The effectiveness of this technique, combined with the cytogenetic study described in part two, for the identification and characterization of species and cultivars is discussed in the conclusion.

Chapter 7

LITERATURE REVIEW

A. MORPHOLOGY

Alstroemeria, which are native to South America, were first introduced to Europe by Father Fueillée in 1714. He gave seeds of *Alstroemeria pelegrina* to Alstromer who passed them on to his friend Carolus Linnaeus. The genus was named in honor of Alstromer and this first species was described and illustrated by Linnaeus (Linneaus, 1762). The Spanish botanist, Fueillée, indicated that *A. pelegrina* was a favorite flower of the Incas and was often found in the gardens of the Inca rulers. It is sometimes referred to as the Peruvian or Inca Lily. *A. pelegrina* was introduced to Kew gardens in 1753 (Robinson, 1963).

There are over 60 species of *Alstroemeria* which are native to climates that range from dry coastal sand dunes, deserts, river valleys, semi-woodlands and up to the snowline of the Andes (Hughes et al, 1986).

The taxonomic placement of *Alstroemeria* has been somewhat confused, as it was sometimes placed in the family *Amaryllidaceae* or the family *Lilliaceae*. By 1985, however, Dahlgren et al. (1985) had further investigated the phylogeny of the genus and concluded that it should be classified as:

CLASS: Monocotyledons
SUPERORDER: Liliiflorae
ORDER: Liliales
FAMILY: Alstroemeriaceae
GENUS: Alstroemeria

The genus *Alstroemeria* is composed of herbaceous perennials that have a white, fleshy rhizome from which arise the aerial shoots as well as a fibrous root system. These roots are modified to store nutrients and water and often contain a considerable amount of starch. The majority of the aerial stem length has no axillary buds, with one axillary bud at just the bottom two nodes of the shoot. The first bud allows for the continuation of the main rhizome and second bud may grow out to form a lateral rhizome. Each new aerial shoot arises from the first node of the previous shoot, and may be vegetative or reproductive depending on environmental conditions (Hughes et al., 1986). The stems may be up to 4m long or so small that they are barely visible above the surface *(A. pygmaea*). The leaves are narrow, and as they unfold they rotate 180°, so that they are upside down or resupinate. The leaf base is more or less sessile with the leaves being lanceolate or linear in shape. Stomates are present on the side facing the ground.

The branches end in umbel-like inflorescences which may have from one (*A. pygmeae*) to 10-30 flowers (*A. aurantiaca*). The flowers are often subtended by green, leaf-like bracts which vary from very minute to quite large. The brightly colored flowers are often spotted and are trimerous, epigynous, bisexual and actinomorphic or lightly 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 with each cell containing 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 pollen has generally matured and been released before the stigma is receptive (protandry), thus promoting cross-pollination of flowers. The fruit is a dehiscent, locilucidally 3-valved capsule and the seeds are globose (Dahlgren et al., 1985; Uphof, 1952).

B. KARYOTYPE ANALYSIS

Chromosome studies of *Alstroemeria* were first published by Strasberger in 1882, who recorded a chromosome number of n=8 in microspores of *A. chilensis*. Guignard (1884) studied *A. pelegrina* and *A. psittacina* which both showed n=8 chromosomes. These results were included in a paper by Taylor (1926) along with his own study of *A. braziliensis* which also showed 2n=16 chromosomes. Whyte (1929) published a study of the chromosomes of *Alstroemeria* and *Bomarea*. The chromosome numbers for *A. aurantiaca, A. pulchella* and *A. haemantha* were all 2n=16, in agreement with Taylor's earlier work. *Bomarea caldasiana* was reported to have n=9 chromosomes.

Sato (1938) studied the karyotypes of several families within *Amaryllidaceae* including *A. chilensis* and *A. pulchella* and also confirmed that both have 2n=16 chromosomes. In "Chromosome Atlas of Flowering Plants" (Darlington and Wylie, 1955) La Cour is credited with reporting the chromosome numbers for *A. ligtu, A. rosea* and *A. campaniflora* to be 2n=16.

Tsuchiya and Hang (1987, unpublished data) studied 11 species in the genus *Alstroemeria*. They found that all 11 species, *A. aurantiaca, A. psittacina, A. pulchella, A. pelegrina, A. versicolor, A. haemantha, A. chilensis, A. caryophyllae, A. ligtu, A. sierrae* and *A. hookeri*, all had 2n=16 chromosomes, in agreement with the earlier work. Rustanius et al. (1991) studied *A. ligtu* hybrids and also found the chromosome number to be 2n=16.

Taylor (1926) grouped the chromosomes of *A. braziliensis* into six classes. In the first class is the largest chromosome pair which "has the fiber constriction near it's center". The second pair were long chromosomes with well developed short arms about 2-3 times as long as broad. There were four pairs of acrocentric chromosomes, one pair of which has satellites. The seventh pair was a much shorter version of the second, while the eighth pair was much smaller than the first pair and had a submedian fiber attachment. This is similar to Whyte's (1929) description, although Whyte noted the additional observation of the large distal satellite on the large submetacentric pair of chromosomes.

Sato (1938) found some differences between *A. pulchella* and *A. chilensis*. *A. pulchella* was observed to have somewhat shorter chromosomes than *A. chilensis*. These included one pair of long chromosomes with submedian constriction and one pair of medium chromosomes with subterminal constrictions. He observed six pairs of short chromosomes, four with almost terminal constrictions, one pair with subterminal constrictions and the remaining pair with median constrictions. One pair of the acrocentrics had a satellite at its proximal end. The median chromosomes also seemed to exhibit a secondary constriction in the distal arms and thus were SAT-chromosomes. This description of *A. pulchella* karyotype is slightly different from the one given by Whyte. Whyte described two large metacentric chromosomes while Sato observed two medium subtelocentrics.

For *A. chilensis*, Sato found two pairs of long chromosomes with submedian constrictions, four short pairs of chromosomes with subterminal constrictions and two pairs of short chromosomes with submedian constrictions. Satellites were noted on the short arm of one pair of long chromosomes, as well as on two pairs of the short chromosomes.

Tsuchiya and Hang (1989) reported that of the 11 species they studied most have a common constitution of two groups of four chromosomes in their genomes. The first group has four pairs of metacentric, submetacentric or subtelocentric chromosomes of different sizes, most without satellites. Four pairs of acrocentric chromosomes with or without satellites comprise the second group. *A. ligtu* and *A. ligtu* hybrids showed some variation from this general karyotype (Rustanius et al., 1991; Tsuchiya et al., 1987; Tsuchiya and Hang, 1989). The first group consists of five chromosome pairs including one pair of large submetacentric and one pair of small metacentric chromosomes both with satellites. The second group consists of only three pairs of acrocentric chromosomes with two pairs showing satellites.

There were different degrees of variation among species, with only minor differences between some, but greater differences for others. Also, karyotypic polymorphism was found in different plants within species such as *A. versicolor* (Tsuchiya and Hang, 1989) and *A. pelegrina* (Stephens et al., 1993). In *A. pelegrina* the smallest submetacentric pair of chromosomes were observed to have satellites on one or both members of the chromosome pair. It was also noted that some species had a heterozygous chromosome complement, with one or more non-homologous pairs in their somatic cells (Tsuchiya and Hang, 1987). Meiosis, however, was fairly normal with most species showing eight bivalents, although occasionally seven bivalents and two univalents were observed in a few sporocytes of *A. ligtu, A. pelegrina* and *A. pulchra* (Tsuchiya and Hang, 1989).

Information on the karyotypes of the commercial cultivars is very scarce. Generally it is limited to an indication of chromosome number only. The late Professor T. Tsuchiya and various members of his research group have conducted brief karyotype analyses of 25 cultivars to determine their chromosome constitution and/or number.

Of the 25 cultivars, 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 the last one was $2n=4x-1=31$. Meiotic behavior showed irregularities at various stages caused by abnormal pairing behavior. Consequently, most of the cultivars, including diploid, showed no or extremely low, pollen fertility. Good pollen fertility was noted in many of the tetraploid cultivars, and interestingly in the hypertriploid `Orange Beauty' (Tsuchiya and Hang, 1987).

Twenty one cultivars were studied in more detail by Hang and Tsuchiya (1988) and Tsuchiya et al. (1987). Preliminary results of karyotype analysis of the diploid cultivars 'Orchid`, 'Canaria`, 'Eureka`, and 'Zebra` indicate their hybrid origin. The genomes 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 SATS. In 'Eureka` all four pairs of acrocentrics were noted to carry satellites whereas in 'Zebra` only seven acrocentrics had satellites (Hang and Tsuchiya, 1988). Seven acrocentrics were also identified in 'Orchid` (which was mutated to form 'Zebra`), while 'Canaria` showed only two pairs of SAT-chromosomes (Tsuchiya et al., 1987). 'Orchid` and 'Canaria` each have four unmatched chromosomes in their genomes; three metacentrics of different sizes and one small submetacentric.

The twelve triploid cultivars and the hypertriploid 'Orange Beauty` all showed the three largest metacentric or submetacentric chromosomes. The number and size of the other chromosomes differed in each cultivar. Three non-homologous genomes were postulated for the chromosome complements of 'Yellow King` and 'Mona Lisa`, with two homologous and one different genome in the cultivars 'King Cardinal`, 'Appelblosem` and 'Pink Triumph`. Analysis of the chromosome complements illustrated by Tsuchiya, et al. (1987) suggests that 'Campfire`, 'Red Surprise', `Regina', 'Marina` and 'Pink Perfection` also have two homologous and one non-homologous genome, while 'Carmen` and possibly 'Rosita` have three non-homologous genomes. The additional notable feature of 'Rosita` is the presence of a small subtelocentric or submetacentric chromosome with a distal satellite. 'Orange Beauty` has 24 chromosomes that are from two homologous and one different genome plus an extra telocentric (or acrocentric) chromosome. The third genome also includes a small subtelocentric chromosome with a tiny satellite on the short arm, which appears identical to the one seen in 'Rosita`.

The tetraploid cultivar 'Jubilee` has a chromosome complement which can be divided into two groups of two genomes each. Chromosome size and shape indicate homology or close homology of two pairs of chromosomes, but there is one unique medium-sized submetacentric chromosome with a tiny satellite. This is thought to be different from the medium-sized sub-metacentric SAT-chromosome found in the *A. ligtu* hybrids. *A.* 'Luciana` (2n=4x-1=31) is thought to contain at least two, but possible three different genomes (Hang and Tsuchiya, 1988). The tetraploid 'Rosario` (2n=4x+1=33) also appears from the illustration (Tsuchiya et al., 1987) to consist of two different genomes, the extra chromosome being acrocentric. There are also satellites visible on one of the two small submetacentric chromosomes, similar to those found in 'Rosita` and 'Orange Beauty`.

Since mutation breeding, using X-rays, has been a commonly used procedure in the formation of alstroemeria cultivars, not all of the chromosomes may be traceable to a specific parent.

Many of the chromosome pairs identified in the cultivars and possibly in some of the species may appear to be homologous when in fact there may be some differences between them. In order to determine a more exact homology of the chromosomes in both species and cultivars Tsuchiya and Hang (1989) and Hang and Tsuchiya (1987) used Giemsa-banding techniques. Although only limited details have been published, they indicate that useful information on the nature of chromosome polymorphism between presumed homologous chromosomes and between plants within a species can be determined as well as information on the evolutionary and phylogenetic relationships among species.

C. GIEMSA BANDING

Although chromosomes may show little or no structural differentiation along their length, banding patterns may be produced through differential binding of dyes or fluorescent chemicals. In many instances a single dye may result in uniform coloration of the chromosomes unless a specific pretreatment regime is first used. "As well as being of immense practical value in identifying chromosomes and parts of chromosomes, banding and the study of its mechanisms provides an insight into the highest level of chromosome organization below that of the whole chromosome" (Sumner, 1990).

The concept that chromatin was composed of both euchromatin and heterochromatin was introduced by Heitz in 1928. Following this discovery a large number of techniques for the specific staining of heterochromatin were developed. Since 1968, however, modern techniques have been developed that have widespread, practical application.

Caspersson et al. (1968, 1969a, 1969b) first used fluorochrome (quinacrine mustard) to show variation in the intensity of fluorescence along chromosomes and these bands are now known as Q-bands. Since then other base-specific fluorochromes have been used, singly or in combination. The invention of *in situ* hybridization led to the development of C-banding and G-banding techniques. C-banding demonstrates constitutive heterochromatin and was first observed in mouse chromosomes by Pardue and Gall (1970). They noted that when using *in situ* hybridization to locate satellite DNA the centromeres were more strongly stained with Giemsa dye. Through a series of less drastic pretreatments, e.g. proteolytic enzymes such as trypsin, G-bands are revealed with Giemsa staining of higher vertebrate chromosomes, especially of man. R-bands, which are the reverse pattern of that found with G-banding, are used in France, but are not widely used in the rest of the world (Sumner, 1990).

The classical definition of heterochromatin is that it is a region or regions along the length of the chromosome that remain condensed at interphase. However, it has not yet been shown that all heterochromatic bands do actually remain condensed at interphase, or that all areas that remain condensed at interphase are stained by any of the banding techniques in use (John and King, 1977; Camacho et al. 1984).

It was initially believed that the preferential binding of the Giemsa dye to heterochromatin was due to differences in renaturation times of the DNA. The chromosomal DNA was thought to denature during alkaline treatments, and then the most highly repetitive DNA, in the C-bands, renatured most rapidly and so stained most strongly. However, it has been demonstrated that C-bands do not always contain highly repetitive DNA (see Sumner, 1990, Table 4.1) and that denaturation of chromosomal DNA by alkaline treatment is largely irreversible (Holmquist, 1979).

C-banding has been shown to involve the preferential removal of DNA from non-C-banded regions of chromosomes, with the C-banded regions thus having a greater amount of DNA (Comings et al., 1973; Pathak and Arrighi, 1973). This loss of DNA during C-banding is due to the pre-treatment process prior to staining with Giemsa dye. Preliminary treatment with 0.2N HCl depurinates the DNA. Alkaline treatment then results in β-elimination and breakage at the depurinated sites. The depurinated DNA is then extracted during incubation in warm saline. The overall result is smaller fragments of DNA which are more easily lost from the chromosomes (Holmquist, 1979).

In order for this mechanism to explain C-banding it is still necessary to understand why DNA is extracted preferentially from non-C-banded regions of the chromosomes. There is some evidence that there are proteins in chromatin that bind more strongly to highly repetitive DNAs (Musich et al., 1977; Hsieh and Brutlag, 1979) in some organisms, and so the C-band DNA is less able to be extracted.

Identification of chromosomes using C-banding has proved very valuable, especially in insects and plants which do not exhibit G- or R-bands. Chromosomes can be characterized by the size and position of the C-bands, i.e. pericentromeric, interstitial or terminal. C-bands may appear as a solid block of material across the whole width of the chromosome or may be narrow bands, or even dots on each chromatid. Chromosomes which lack C-bands entirely are rare, and when observed are probably due to inappropriate treatment for the species or to loss of small bands through excessive treatments (Sumner, 1990).

Specific procedures do not always define all types of heterochromatin (Stack et al. 1974) and a lack of centromeric heterochromatin, such as observed by Filion (1974) in *Tulipa,* does not necessarily preclude its existence. Similarly, the limited amount of heterochromatin revealed in the cultivar *T.* "Turkestanica" as compared to that seen in *T.* "Spring Song" and *T.* "Queen of Night" may be a result of the inability of the technique to demonstrate its presence. On the other hand, Schweizen (1973) noted that *Fritillaria meleagris* chromosomes are essentially free of bands but a tiny terminal or subterminal band in the short arm of some sub-telocentric chromosomes indicated that the Giemsa method has in fact worked.

Vosa (1973) determined the C-banded variants for the six pairs of chromosomes of *Scilla* *sabirica*, with five of these six having an unbanded variant. In all, 42 banding patterns were identified for the chromosomes of the 20 plants examined. On the other hand, the amount and location of heterochromatin varied little between the various species and cultivars of barley (*Hordeum spp.*) (Vosa, 1976). It was observed that the similarity of banding patterns found both within and between species did not allow their use as outright specific markers. Later work by Linde-Laursen (1978a,b), Noda and Kasha (1978), and Singh and Tsuchiya (1981) indicated that each chromosome of barley possesses a different degree of constitutive heterochromatin, which allows identification of chromosomes 1 through 4, previously almost indistinguishable. A different technique, N-banding was used by Islam (1980) and then by Singh and Tsuchiya (1982) in combination with C-banding patterns to further delineate the barley karyotype.

In rye (*Secale cereale* L.), Giemsa C-banding has been used to help identify individual rye chromosomes (Sarma and Natarajan, 1973; Weimark, 1975; Fujigaka and Tsuchiya, 1985, 1990) as well as to identify rye chromosomes in Triticale. Rye chromosomes have blocks of telomeric heterochromatin whereas wheat chromosomes show only some centromeric heterochromatin and /or small intercalary bands (Merker, 1973; Sarma and Natarajan, 1973).

Weimark (1975), however, found that C-banding revealed not only terminal bands but also intercalary and centromeric banding patterns in rye. The intercalary and centromeric bands were generally small and faintly stained with the centromeric bands appearing as two small dots, one on each of the two chromatids, at or near the centromere. A high level of heteromorphism of the heterochromatic regions of the rye chromosomes has also been noted at the level of group, plant and individual chromosomes (Merker, 1973; Sarma and Natajaran, 1973; Weimark, 1975).

Fujigaka and Tsuchiya (1985) used a sequential acetocarmine Giemsa staining technique to identify individual chromosomes in a haploid plant of an inbred line of common rye. Each chromosome could be precisely identified, even in acetocarmine stained cells; and the combined acetocarmine-Giemsa technique allowed precise chromosome measurements to be made as well as localizing heterochromatic bands to the correct chromosomes. This technique was suggested to help eliminate problems associated with the polymorphic nature between and within species of rye.

Acetocarmine Giemsa C-banding of inbred rye was also used in the identification of seven primary trisomics (Fujigaka and Tsuchiya, 1990). A complete set of primary trisomics was thus able to be unequivocally established in an inbred line of rye.

Heterogeneity of the heterochromatin of wheat was revealed by Endo and Gill (1984) through an improved N-banding technique. Schlegel and Gill (1984) used the same approach to try to identify additional heterochromatic regions along the rye chromosomes. Three of the seven chromosomes of rye were identified individually by their N-banding pattern. However, a comparison among chromosomes stained by N-banding and C-banding techniques (Singh and Tsuchiya, 1982) showed no major difference between the quality and quantity of banding patterns obtained from either method at mid-metaphase.

Heteromorphy for banding was also shown to occur within species of *Anemone* as well as between species (Marks and Schweizer, 1974). Heteromorphism of heterochromatin and Nucloelar Organizer Regions is probably universal throughout the animal and plant kingdoms and is certainly widespread in groups such as higher plants, insects and vertebrates, which have been studied intensively by cytogeneticists (Sumner, 1990). The heteromorphism shown in plants may provide favorable material for studying the relationship between banding heteromorphism and phenotypic effects. There have been a number of examples of correlation between total DNA per nucleus and various phenotypic and environmental effects for a number of plants (Bennett, 1985). For example, *Zea mays* has varying amounts of heterochromatin between different strains, which can be correlated with total nuclear DNA content and geographical location. Northern varieties have lower DNA C-values and less heterochromatin than southern varieties, which would result in a shorter cell cycle, and the production of more cells. This then results in a larger, earlier maturing plant. However, heteromorphism of C-bands can also be found within inbred lines of maize (Rayburn et al., 1985). These observations suggest that heteromorphism of heterochromatin may be involved in the variations in total DNA which lead to these phenotypic effects (Sumner, 1990).

Although Giemsa banding has a relatively long history only a few genera have been studied to date, generally cereal crops. At this time no information has been located relating to Giemsa banding of *Alstroemeria* chromosomes. It is important to understand the development of the existing hybrids of *Alstroemeria* in order to develop new hybrids in a short space of time. The characterization of species with regard to Giemsa band locations may provide a method for identification of individual chromosomes. These chromosomes may then be identified in each cultivar and probable parentage determined.

Chapter 8

MATERIALS AND METHODS

A total of 31 *Alstroemeria* species, hybrids and cultivars, *Leontochir ovallei* and *one Bomarea spp* (Table 9.1) were studied to determine karyotypes and characteristic Giemsa-banding patterns of each chromosome complement. The plants were all container-grown in a greenhouse under natural daylength with temperatures ranging from 10°C in winter to 28°C in summer. Shading with 30% shadecloth was provided.

Root tips were collected between 3 and 5p.m. The plants were gently removed from the pots and white, actively growing root tips were cut and placed in 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 of less than 18 hours resulted in nicely constricted chromosomes but they were difficult to separate. The longer pre-treatment resulted in well separated chromosomes with clearly visible satellites.

After pre-treatment 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 root tips were then transferred to a solution of 0.7% carmine in 45% acetic acid (acetocarmine). Preparations were made using Tsuchiya's modified acetocarmine squash (Tsuchiya, 1971). The root tips were left in the acetocarmine stain for a minimum of one week before squashes were made, and were usable for up to a month.

For each plant a minimum of ten cells was examined to determine or confirm the chromosome number. A number of cells from each plant were selected and photographed with a Carl Zeiss Photomicroscope II. Chromosomes in these cells were well separated and any secondary constrictions were visible. Five cells were chosen and were photographed. Measurements were made of lengths of long arm, short arm, total length and length of satellite. An average of these measurements was used to describe the general karyotype of the species or cultivar.

The chromosomes were arranged into two groups. The first group contained all metacentric, submetacentric and subtelocentric chromosomes. The second group contained the acrocentric chromosomes. The length of all the chromosomes, excluding the satellites, was measured and chromosomes in each of the two groups were arranged in order of descending size and number from one to either 16, 24 or 32/33. Apparently homologous pairs or groups were determined based on centromeric position, length of chromosomes and the presence or absence of satellites. These chromosomes could be arranged to form a karyotype.

For the species, averages of the paired chromosomes were determined to provide total length, relative length, index and ratio. Actual chromosome lengths cannot be used as a means of comparison between plants or species, since these lengths will vary due to the degree of chromosome contraction at various mitotic stages, variations in chemical agents, the duration of treatment or the squash treatment 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 is calculated as the ratio of the long arm to the 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 (Table 8.1). However, the more common alternate terms, metacentric, submetacentric, subtelocentric or acrocentric were utilized for descriptive purposes since they are more widely used and understood.

In order to compare the chromosomes of each species to one another, idiograms were developed. The long and short arm measurements of each chromosome pair in the genome were used to construct the idiogram. These were made relative to one another by using a conversion factor to multiply the actual long and short arm measurements. The respective conversions factors were calculated by dividing the relative total genome length, which was set at 100 units for each species, by the actual genome length. An idiogram could then be drawn for each species using these adjusted measurements (Schlarbaum and Tsuchiya, 1984).

After photographing cells from at least three different squashes, for a minimum total of 10 cells, the slide was frozen at -70°C for 30 minutes and coverslip was remo ved. Immediately, the slides were placed in 98% alcohol for 5 minutes and then transferred to 100% alcohol for a further five minutes. The slides were dried in a desiccator, over silica gel, for at least two weeks.

The dry slides were first placed in 0.2M HCl at 52°C for two minutes, to soften cell walls and allow better penetration of the Giemsa dye. A Giemsa C-banding technique developed by A. Hang (personal communication) was then followed. After a brief wash in dH_2O the slides were

Table 8.1. Centromere position and chromosome description (from Levan *et al.*, 1964)

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

incubated in a 7 to 8% solution of $Ba(OH)_2$ at 30°C for 7 to 10 minutes. They were washed three times in warm distilled water (45°C) for a total washing time of 10 minutes or more. This is to ensure that the Barium is removed from the slide. The slides were incubated in three changes of 2XSSC (saline sodium citrate) at pH7. The first incubation was at room temperature for 15 minutes, the second at 30°C for 20 minutes, and the final change at 52°C for 60 to 70 minutes. After washing three times in distilled water, the slides were air dried for 10-15 minutes and stained in 1 to 2% Giemsa dye, diluted with equal volumes of phosphate buffers A and B (A= 14.2g/l Na_2PO_4, B=13.6g/lKPO_4) for ½ to 2 hours. Each slide was individually checked every few minutes to obtain optimum staining. The slide was air dried and mounted with a drop of permount.

The same cells photographed following acetocarmine staining were rephotographed at the same magnification with Techpan Black and White 135 film with a Zeiss Photomicroscope II.

Chapter 9

RESULTS

At least twenty cells of each *Alstroemeria* species, hybrid and cultivar and for a *Leontochir ovallei* and *Bomarea* species were examined under the microscope (Table 9.1). The chromosome number for all *Alstroemeria* species was 2n=2x=16 and 2n=2x=18 for the other two genera. A minimum of five cells were selected and photographed for further analysis of the general karyotype for each species and cultivar. Measurements were made of chromosomes in all five cells and these were averaged to give measurements of total chromosome lengths, lengths of long arm, short arm and satellite. Satellite lengths were not included in total chromosome length measurements as they were often very tiny and difficult to measure accurately. They were not observed in all preparationa and therefore could not be measured in all cases. A relative length for each of these measurements was then calculated in order to compare chromosomes among the different species.

The karyotypes of all ten species and one hybrid showed some similarities. The *Alstroemeria* chromosomes could be divided into two groups. The first group contained four or five pairs of metacentric, submetacentric or subtelocentric chromosomes of different sizes, some of which had satellites. The second group contained three or four pairs of acrocentric chromosomes, some with satellites. *L. ovallei* had four pairs of chromosomes in the first group and five pairs of acrocentric chromosomes, with three pairs carrying satellites. The *Bomarea* sp. had five pairs of chromosomes in the first group and four pairs of acrocentric chromosomes. All of the acrocentrics had satellites.

The Giemsa banding patterns for each chromosome pair within the species and cultivar is also presented in order to aid in distinguishing among apparently homologous chromosomes of the various species. Some species and cultivars did not show any heterochromatic bands using the present Giemsa banding technique (Table 9.1).

Table 9.1. **Plants used for karyotype analysis and Giemsa banding**

Species or Cultivar	Acquis-ition number	Karyotype analysis	Giemsa banding
A. pelegrina	91H-1-1	Yes	yes
A. pulchella	#1	yes	yes
A. aurantiaca	86H-44-1	yes	yes
A. psittacina		yes	no
A. ligtu spp.	86H-35-5	yes	yes
A. ligtu hyb	86H-21-3	yes	yes
A. chilensis	86H-40-1	yes	yes
A. sierrae	87H-24-3	yes	yes
A. versicolor	92H-10-1	yes	yes
L. ovallei	89H-9-1	yes	yes
Bomarea spp	89H-10-1	yes	yes
\`Arizo'		yes	no
\`Eureka'		yes	yes
\`Carmen'		yes	yes
\`Mona Lisa'		yes	yes
\`Neva'		yes	no
\`Pink Triumph		yes	yes
\`Rosita'		yes	yes
\`Orange Beauty'		yes	yes
\`Rosario'		yes	no
\`Orchid'		yes	yes
\`Appelbloesem'		yes	yes
\`Orego'		yes	no
\`Red Surprise		yes	yes
\`Alnba'		yes	no
\`King Cardinal'		yes	yes
\`Luciana'		yes	no
\`Jubilee'		yes	no
\`Texas'		yes	no
\`Yellow King'		yes	yes
\`Canaria'		yes	yes
\`Marina'		yes	yes
\`Monika'		yes	yes

SPECIES

A. pelegrina

The average measurements of five cells of a single plant were calculated (Table 9.2) and these were then used to construct Table 9.3. These average measurements for each pair of chromosomes were then used to describe the karyotype of this species. A representative cell is shown in Fig. 9.1a. This species exhibited the most common karyotype. It had four pairs of metacentric or submetacentric chromosomes and four pairs of acrocentric chromosomes. The largest pair of chromosomes in the first group was more than twice the length of the next largest chromosomes of the complement. The acrocentric chromosomes in the second group had very tiny short arms. Pairs number 5 and 7 had satellites which were of similar size to the short arms. Chromosome pair number 6 had the largest short arms of all the acrocentric chromosomes.

Only limited Giemsa banding patterns were identified for this species using the present technique. Chromosome pair number 2 had two small black spots on either side of the centromere for both chromosomes of the pair (Fig. 9.1b.)

A. pulchella

The average measurements of five cells from a single plant were calculated (Table 9.4) and the average for each chromosome pair was then determined (Table 9.5). A representative cell is shown in Fig. 9.2a and the Giemsa banding pattern is presented in Fig 9.2b.

This species had the common karyotype for *Alstroemeria* with four pairs of metacentric, submetacentric or subtelocentric chromosomes in the first group of the complement and four pairs of acrocentric chromosomes in the second group. Satellites were found on one or both chromosomes of three of the acrocentric chromosome pairs.

All chromosome pairs, except number 2, had heterochromatic bands. These varied from small spots on the distal end of the chromosome arms to more defined bands across both chromatids. There was some heteromorphy with respect to band location between chromosomes of some of the pairs. For example, for chromosome pair 4 one chromosome had a heterochromatic band on the long arm close to the centromere while the other chromosome of the pair had a band which was halfway along the length of the long arm. In chromosome pair 6 only one chromosome of the pair showed heterochromatic regions.

Table 9.2. Average measurements of chromosomes of *A. pelegrina*

Chromosome	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	13.9	13.5		27.4	14.8	1.03	m
2	13.3	12.1		25.4	13.7	1.10	m
3	7.5	6.1		13.6	7.2	1.23	m
4	7.0	5.1		12.1	6.5	1.38	m
5	4.2	3.4		7.6	4.1	1.21	m
6	3.8	3.1		6.9	3.7	1.20	m
7	4.7	1.5		6.2	3.3	3.00	st
8	4.4	1.5		5.9	3.2	2.81	sm
9	11.3	0.4	0.2	11.7	6.3	25.00	t
10	10.9	0.6	0.2	11.5	6.2	19.54	t
11	10.2	0.5		10.7	5.8	18.47	t
12	9.7	0.6		10.3	5.6	15.88	t
13	9.8	0.3	0.2	10.1	5.5	31.74	t
14	9.3	0.3	0.4	9.6	5.2	33.11	t
15	7.8	0.4		8.3	4.5	17.75	t
16	7.5	0.3		7.8	4.2	30.00	t

Table 9.3. Average measurements of chromosome pairs of *A. pelegrina*.

Chromosome pair	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	13.6	12.8		26.4	28.5	1.06	m
2	7.3	5.6		12.7	13.7	1.30	m
3	4.0	3.3		7.3	7.8	1.21	m
4	4.5	1.6		6.1	6.6	2.81	sm
5	11.1	0.5	0.2	11.6	12.6	22.20	t
6	9.9	0.5		10.4	11.3	19.80	t
7	9.6	0.3	0.3	9.9	10.7	32.00	t
8	7.7	0.3		8.0	8.7	25.67	t

*RL=Relative Length
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

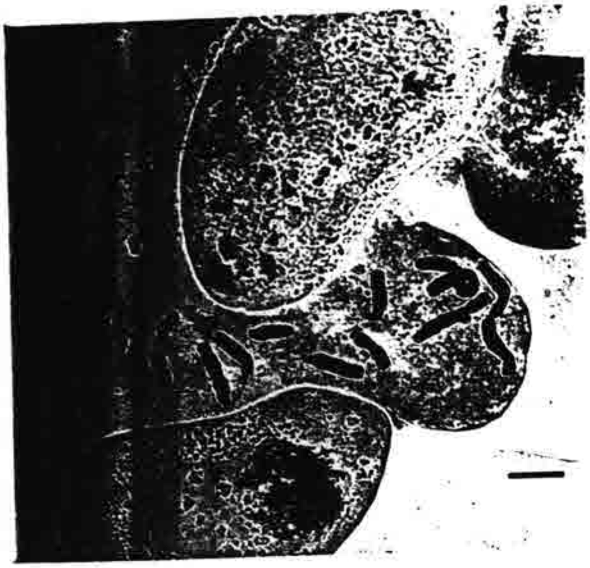
Table 9.4. Average measurements of chromosomes of *A. pulchella*.

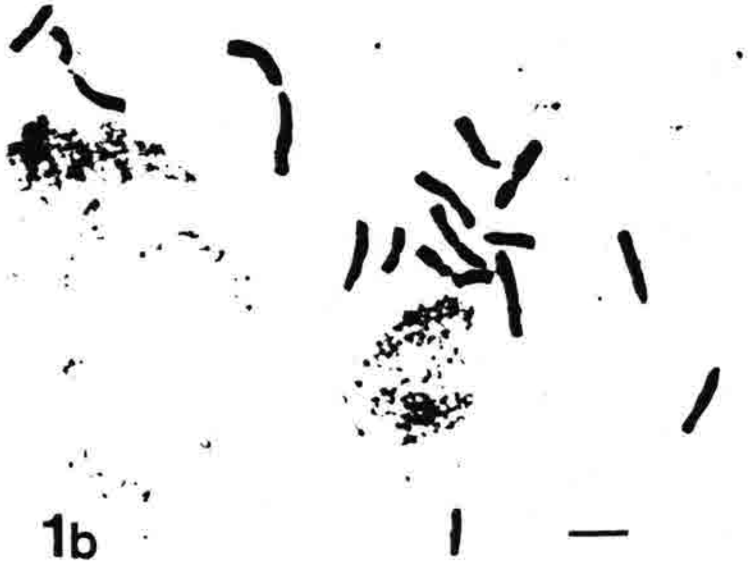
Chromosome	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	11.3	11.1		22.4	13.9	1.02	m
2	10.7	10.6		21.2	13.2	1.01	m
3	10.2	3.0		13.2	8.2	3.44	st
4	9.8	2.9		12.7	7.9	3.37	st
5	3.8	3.1		6.9	4.3	1.23	m
6	3.5	3.2		6.7	4.1	1.09	m
7	4.4	1.7		6.1	3.8	2.53	sm
8	4.2	1.7		6.0	3.7	2.44	sm
9	10.4	0.2		10.6	6.6	45.13	t
10	9.8	0.1		9.9	6.2	65.13	t
11	8.6	0.4		9.0	5.6	22.05	t
12	7.8	0.1	0.7	7.9	4.9	65.08	t
13	7.5	0.3	0.4	7.8	4.7	27.63	t
14	7.2	0.4	0.2	7.7	4.8	16.02	t
15	6.5	0.3	0.2	6.9	4.3	20.38	t
16	6.0	0.4		6.4	4.0	14.26	t

Table 9.5. Average measurements of chromosomes pairs of *A. pulchella*.

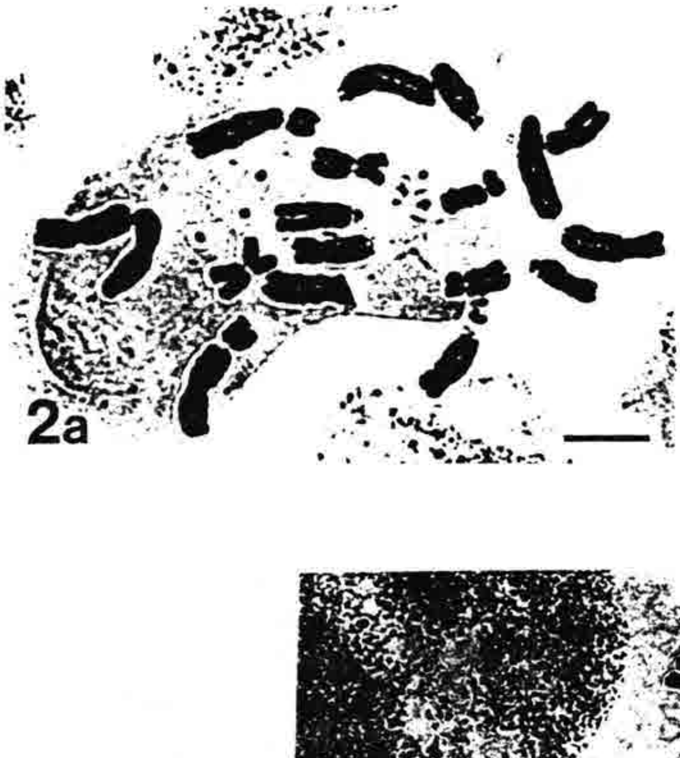
Chromosome pair	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	11.0	10.8		21.8	27.0	1.01	m
2	10.0	2.9		12.9	16.0	3.41	st
3	3.6	3.2		6.8	8.4	1.16	m
4	4.3	1.7		6.0	7.5	2.48	sm
5	10.1	0.2		10.3	12.7	53.05	t
6	8.2	0.3	0.7	8.5	10.5	31.54	t
7	7.3	0.4	0.3	7.7	9.5	20.39	t
8	6.3	0.4	0.2	6.7	8.3	16.95	t

*RL=Relative Length
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

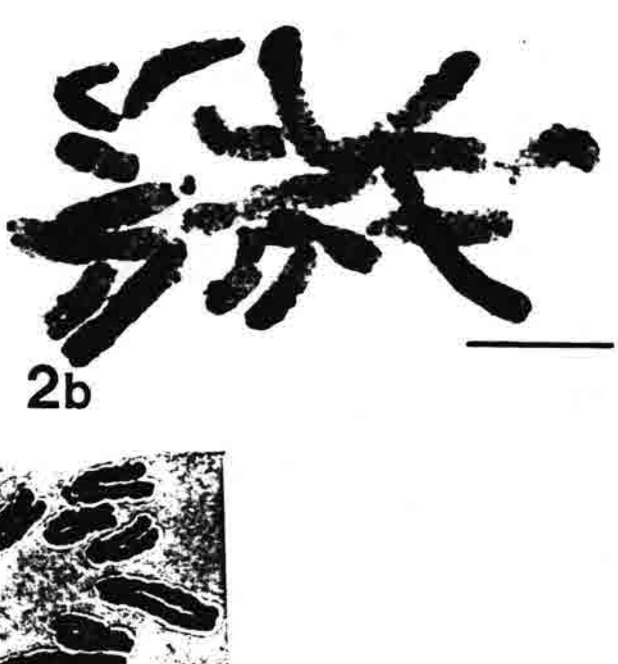




1b



2a



2b

3

A. psittacina

Five cells were photographed and used for measurements. The average measurements of these five cells were then calculated (Table 9.6) and used to determine the average measurements for each chromosome pair of the species (Table 9.7). These measurements were then used to describe the karyotype of this species. A representative cell is depicted in Fig. 9.3. No Giemsa bands were found for this species.

A. psittacina had the same basic karyotype as the first two species. There were four pairs of metacentric, submetacentric or subtelocentric chromosomes in one group of the complement and four pairs of acrocentric chromosomes in the second group. Three pairs of acrocentric chromosomes had satellites on one chromosome of the pair.

A. aurantiaca

Observation of cells of each of the color variants of *A. aurantiaca* revealed no morphological differences between them. Consequently, only the results for the yellow-flowered variant are presented. Five cells were photographed and measured and the average measurements are presented in Table 9.8. An average of these results for each chromosome was then calculated (Table 9.9) and used to describe the karyotype of this species. A representative cell is depicted in Fig. 9.4a and the Giemsa banding pattern is shown in Fig 9.4b.

This species also had the same basic karyotype as the previous ones. There were four pairs of metacentric, submetacentric or subtelocentric chromosomes in one group of the chromosome complement and four pairs of acrocentric chromosomes in the second group. Two of the acrocentric chromosome pairs had small satellites.

Giemsa banding revealed unique patterns for the chromosomes of this species. All chromosomes, except pair number 1, had heterochromatic regions. Chromosome pair 2 had a heterochromatic band at the distal end of the short arm as well as an occasional band at the distal end of the long arm. Chromosome pair 3 very distinctive with a wide heterochromatic band at the distal end of both arms of the chromosomes. Subtelocentric pair number 4 had a broad band at the distal end of the short arm with a narrower band often observed at the distal end of the long arm. A broad band was in the center of the long arm. The acrocentric chromosomes varied from a single narrow heterochromatic band on the long

Table 9.6. Average measurements of chromosomes of A. *psittacina*.

Chromosome	L* µ	S* µ	SAT* µ	Total* µ	RL*	Ratio	Centromere position
1	12.8	12.1		24.9	13.9	1.05	m
2	11.8	11.2		23.0	12.9	1.05	m
3	12.1	3.1		15.2	8.5	3.90	st
4	11.3	3.2		14.5	8.1	3.48	st
5	4.4	3.8		8.2	4.6	1.16	m
6	4.0	3.2		7.2	4.0	1.23	m
7	5.2	1.9		7.1	4.0	2.80	sm
8	4.8	1.8		6.6	3.7	2.74	sm
9	11.6	0.3		11.9	6.6	46.40	t
10	11.0	0.1		11.1	6.2	109.80	t
11	8.9	0.6		9.5	5.3	14.64	t
12	8.6	0.3	0.3	8.9	5.0	27.00	t
13	8.3	0.4	0.3	8.7	4.9	21.31	t
14	8.0	0.4		8.4	4.7	21.05	t
15	7.2	0.4		7.6	4.3	20.66	t
16	6.5	0.2	0.1	6.7	3.8	28.22	t

Table 9.7. Average measurements of chromosomes pairs of *A. psittacina*.

Chromosome pair	L* µ	S* µ	SAT* µ	Total* µ	RL*	Ratio	Centromere position
1	12.3	11.7		24.0	26.8	1.05	m
2	11.7	3.2		14.9	16.6	3.66	st
3	4.2	3.5		7.7	8.6	1.20	m
4	5.0	1.8		6.8	7.6	2.78	sm
5	11.3	0.2		11.5	12.8	56.50	t
6	8.8	0.5	0.3	9.3	10.4	17.60	t
7	8.2	0.4	0.3	8.6	9.6	20.50	t
8	6.9	0.3	0.1	7.2	8.0	23.00	t

*RL=Relative Length
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

Table 9.8. Average measurements of chromosomes of *A. aurantiaca.*

Chromosome	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	11.1	9.8		20.9	11.3	1.13	m
2	10.0	9.8		19.8	10.7	1.02	m
3	7.8	5.6		13.4	7.2	1.38	m
4	6.6	4.6		11.0	6.0	1.44	m
5	5.1	4.8		9.9	5.3	1.05	m
6	4.9	4.7		9.6	5.2	1.04	m
7	7.6	2.3	0.2	9.9	5.3	3.30	st
8	6.6	2.3		8.9	4.8	2.90	sm
9	10.8	1.5	0.1	12.3	6.6	7.20	t
10	10.4	0.4	0.1	10.8	5.8	23.57	t
11	10.0	0.6		10.6	5.7	17.86	t
12	9.5	0.7		10.2	5.5	12.84	t
13	9.6	0.5		10.1	5.5	20.06	t
14	9.2	0.2		9.4	5.1	48.37	t
15	8.5	0.6	0.3	9.1	4.9	15.18	t
16	8.3	0.3	0.2	8.6	4.6	33.00	t

Table 9.9. Average measurements of chromosome pairs of *A. aurantiaca.*

Chromosome pairs	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	10.5	9.8		20.3	21.9	1.07	m
2	7.2	5.1		12.3	13.2	1.40	m
3	5.0	4.8		9.8	10.5	1.04	m
4	7.1	2.3	0.2	9.4	10.1	3.09	st
5	10.6	1.0	0.1	11.6	11.5	10.89	t
6	9.8	0.7		10.6	11.2	15.00	t
7	9.4	0.3		9.7	10.5	27.70	t
8	8.4	0.4	0.2	8.8	9.5	20.40	t

*RL=Relative Length
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

arm of chromosome pair 6, the combination of a broad and a narrow band on the long arm, chromosome pairs 5 and 7, to a very broad band on the long arm as well as bands on the short arm and the satellites on chromosome pair 8.

A. chilensis

A total of five cells of a single plant of *A. chilensis* were photographed and measured. The average measurements for each chromosome were calculated (Table 9.10) and used to obtain average values for each chromosome pair (Table 9.11). A representative cell is shown in Fig. 9.5a and the Giemsa banding pattern in Fig. 9.5b.

The chromosomes of *A. chilensis* can be divided into two groups, as for the previous species. However, for this species there were five pairs of chromosomes in group one and only three pairs in group two. Group one chromosomes were metacentric, submetacentric or subtelocentric, whereas group two chromosomes were acrocentric. Satellites were on one chromosome each of pairs 3 and 5 and on both chromosomes of pairs number 6, 7 and 8.

All of the chromosomes of this species revealed heterochromatic bands. Thin bands were on the proximal end of one arm of a chromosome of pair number 1 and both chromosomes of pair number 2. Chromosome pair number 3 had heterochromatic bands near the centromere of one arm and at the distal end of the other arm. Faint bands were at the distal end and near the centromere of one arm of chromosomes of pair 4 with a wide band in the middle of the other arm.

The subtelocentric chromosome pair number 5 had the same relative length as the subtelocentric chromosome pair number 4 of *A. aurantiaca*. However, one chromosome of this pair in *A. chilensis* had a small satellite on the short arm. The distal half of the short arm of both chromosomes was heterochromatic with only faint bands in the middle of the long arm. This pattern of banding therefore distinguishes this chromosome pair from the similar shaped pair found in *A. aurantiaca*.

Two heterochromatic bands were present on the long arms of the chromosomes of pairs number 6 and 7. The satelites of chromosome pair 8 were heterochromatic and two faint heterochromatic spots were in the proximal quarter of the long arm.

Table 9.10: Average measurements for *A. chilensis*.

Chromo-some	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	13.5	12.8		26.3	11.1	1.02	m
2	13.0	10.9		23.9	10.0	1.19	m
3	11.8	9.5	0.3	21.3	8.9	1.24	m
4	10.7	8.5		19.2	8.1	1.26	m
5	8.1	6.7		14.8	6.2	1.21	m
6	7.8	7.0		14.8	6.2	1.11	m
7	7.7	6.4		14.1	5.9	1.20	m
8	6.3	5.8		12.1	5.1	1.09	m
9	10.0	2.5	0.2	12.5	5.3	4.00	st
10	8.3	3.1		11.4	4.8	2.68	sm
11	14.6	0.8	0.3	15.4	6.5	18.25	t
12	12.3	0.3	0.9	12.6	5.3	41.00	t
13	10.9	1.3	0.2	12.2	5.1	8.38	t
14	10.0	0.6	0.5	10.6	4.5	16.67	t
15	9.8	0.6	0.9	10.4	4.4	16.33	t
16	6.3	0.4	0.9	6.7	2.8	15.75	t

Table 9.11. Average measurements for chromosome pairs of *A. chilensis*.

Chromo-some pair	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	13.3	11.9		25.2	21.1	1.12	m
2	11.3	9.0	0.3	29.3	17.0	1.36	m
3	8.0	6.7		14.7	12.4	1.19	m
4	7.0	6.1		13.1	11.0	1.15	m
5	9.2	2.8	0.2	12.0	10.1	3.29	st
6	13.5	0.6	0.6	14.1	11.8	22.50	t
7	10.5	1.0	0.3	11.5	9.6	10.50	t
8	8.1	0.5	0.9	8.6	7.2	11.40	t

*RL=Relative Length
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

Figure 9.4a. *A. aurantiaca* - acetocarmine stain b. *A. aurantiaca* - Giemsa stain
one subtelocentric covers the
end of one acrocentric.

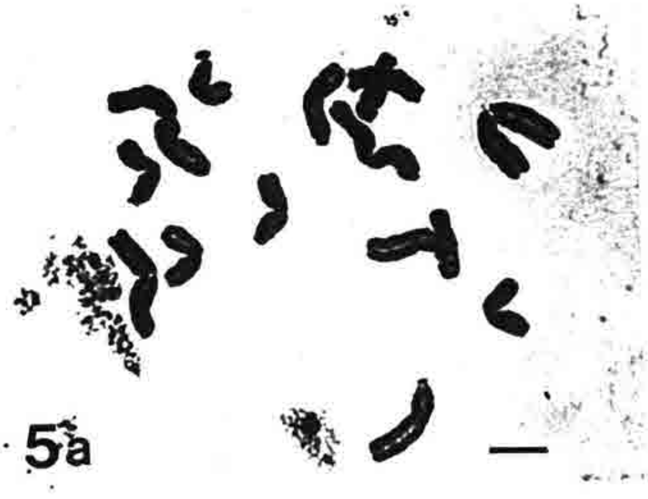
Figure 9.5a *A. chilensis* - acetocarmine stain b. *A. chilensis* - Giemsa stain
one acrocentric is missing

Figure 9.6a. *A. ligtu* - acetocarmine stain b. *A. ligtu* - Giemsa stain

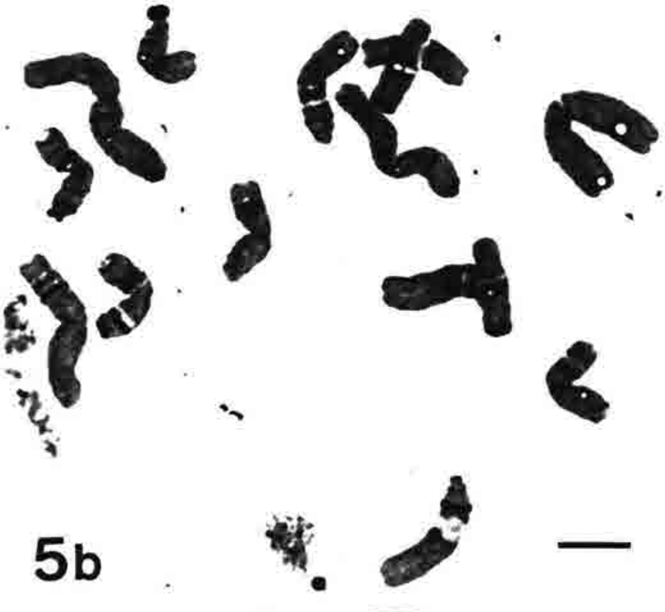
Bar = 10μ

4a

4b

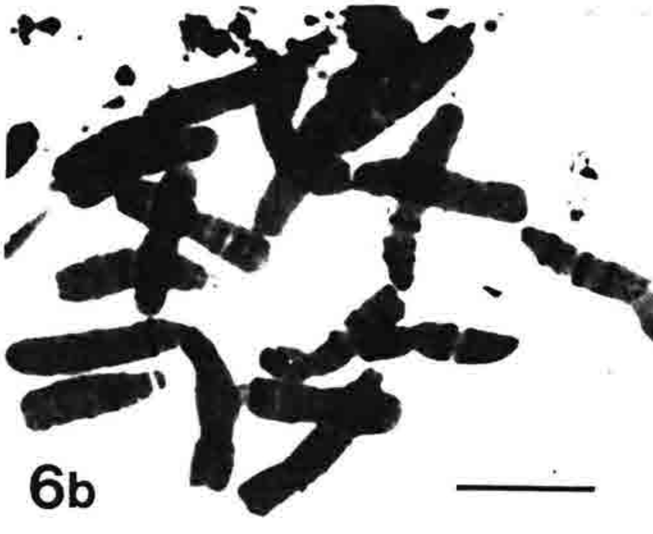


5a



5b

6a



6b

A. ligtu

Five cells of a single plant of *A. ligtu* species were photographed and measured. The averages of these measurements were calculated (Table 9.12) and used to construct Table 9.13. These average chromosome values were then used to describe the karyotype of this species. A representative cell is shown in Fig. 9.6a and the Giemsa banding pattern is illustrated in Fig. 9.6b.

The general karyotype of this plant was similar to that for *A. chilensis*. There were two groups of chromosomes in the genome. The first consisted of five pairs of metacentric and submetacentric chromosomes and the second group had three pairs of acrocentric chromosomes. Satellites were found on one chromosome of each of pairs number 3 and 4, and both chromosomes of pairs number 6 and 8.

The arrangement of the karyotype was slightly different between *A. ligtu* and *A. chilensis.* The smallest chromosome pair in *A. chilensis* was a subteocentric pair whereas in *A. ligtu* a submetacentric pair was the second smallest pair of group one. The relative lengths of each of these pairs in the two species was almost the same and the ratio of each was close to 3.0.

Pair number 1 was about the same relative length as pair number 1 of *A. chilensis* and also had a thin heterochromatic band at the proximal end of one arm of one chromosome of the pair. Chromosome pair number 2 had a wide heterochromatic band in the middle of the shorter arm of the chromosome, with two narrow bands near the centromere of the longer arm. There were heterochromatic bands at the centromere of chromosome pair number 3 and a wider band near the distal end of the shorter arm.

The satellite on one chromosome of pair number 4 was heterochromatic. A wide heterochromatic band was at the distal end of the short arm and a narrower band was at the cnetromere. A third band was faintly visible at the distal end of the long arm. Chromosome pair number 5 had five heterochromatic bands; near the centromere and the distal end of the one arm, two bands close together in the middle of the other arm, and a wider band at the distal end of this arm.

Three heterochromatic bands were in the proximal half of the long arm of acrocentric chromosome pair number 6. Chromosome pairs number 7 and 8 had wide heterochromatic bands on the long arm and narrower bands at the centromeres.

Table 9.12. Average measurements of chromosomes of *A. ligtu* species.

Chromosome	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	13.0	11.7		24.7	11.1	1.11	m
2	12.2	11.1		23.3	10.4	1.10	m
3	9.3	8.8		18.1	8.1	1.06	m
4	9.5	7.5		17.0	7.7	1.27	m
5	6.6	5.0		11.6	5.2	1.32	m
6	6.3	5.0	0.5	11.3	5.1	1.27	m
7	8.1	3.0	0.7	11.1	5.0	2.27	sm
8	7.9	3.1		11.0	4.9	2.57	sm
9	5.6	5.2		10.8	4.8	1.06	m
10	5.7	5.1		10.8	4.8	1.10	m
11	13.6	0.7	0.2	14.3	6.4	19.69	t
12	12.7	0.9	0.2	13.6	6.1	14.22	t
13	11.4	0.6		12.0	5.4	17.88	t
14	11.4	0.3		11.7	5.3	42.40	t
15	9.5	0.3	0.4	9.8	4.4	10.79	t
16	9.4	0.2	0.4	9.6	4.3	46.90	t

Table 9.13. Average measurements of chromosome pairs of A. *ligtu* species.

Chromosome pair	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	12.6	11.4		24.0	21.5	1.11	m
2	9.4	8.1		17.5	15.8	1.16	m
3	6.4	5.0	0.5	11.4	10.3	1.29	m
4	8.0	3.0	0.7	11.0	9.9	2.66	sm
5	5.6	5.2		10.8	9.6	1.08	m
6	13.1	0.8	0.2	13.9	12.5	16.60	t
7	11.4	0.5		11.9	10.7	24.87	t
8	9.5	0.2	0.4	9.7	8.7	37.88	t

*RL=Relative Length
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

A *ligtu* hybrid

The average measurements of five cells from a single *A. ligtu* hybrid plant were taken (Table 9.14) and were used to calculate the average measurements for each chromosome pair in this plant (Table 9.15). These average measurements for the chromosome pairs were used to describe the karyotype. A representative cell is depicted in Fig. 9.7a and the Giemsa banding pattern is shown in Fig. 9.7b.

This hybrid plant had the same chromosome arrangement of its genome as *A. ligtu* species. There were five pairs of metacentric or submetacentric chromosomes in one group and three pairs of acrocentric chromosomes in the second group. Both chromosomes of pairs number 3 and 4 had satellites but oniy one chromosome of pair number 5 had a satellite. In the second group both chromosomes of pair number 6 and one chromosome of pair number 7 had satellites.

Chromosomes number 1, 3, 6, 9, 11, 12, 14 and 16 had almost the same relative lengths as similarly shaped chromosomes of *A. ligtu*. A narrow heterochromatic band was at the proximal end of the longer arm of chromsome 1 of *A. ligtu* hybrid, the same as that for chromosome 1 of *A. ligtu*. The banding pattern for chromosome number 3 of *A. ligtu* was similar as that found for chromosome 3 of *A. ligtu*. Chromosome number 6 of *A. ligtu* hybrid had a very similar banding pattern to the chromosomes of pair number 3 of *A. ligtu* with heterochromatic bands at the centromere, the distal end of one arm and the center of the other arm.

Both chromosomes of pair number 4 of *A. ligtu* hybrid were of the same relative length and both carried small satellites. These chromosomes were similar to chromosome 8 of *A. ligtu* and had a similar pattern of heterochromatic bands, with a wide band close to the distal end of the short arm, a narrow band closer to the centromere and one or two bands near the distal end on the long arm.

Chromosome number 9 of *A. ligtu* hybrid was almost the same as the chromosomes of pair number 5 of *A. ligtu*, however, a satellite was observed on the chromosome in the hybrid but not on those in the species. The banding pattern of the chromosome of *A. ligtu* hybrid was slightly different from that of the chromosomes in the species, with only a centromeric band and a wider band in the middle of the longer arm.

Chromosomes number 11 and 12 of both species and hybrid were of the same relative length and similar shape, however, the heterochromatic bands were different. The

Table 9.14. Average measurements of chromosomes of *A. ligtu* hybrid.

Chromosome	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	12.5	11.6		23.1	10.9	1.07	m
2	10.8	10.1		20.9	9.8	1.06	m
3	9.9	7.3		17.2	8.1	1.35	m
4	8.7	6.6		15.3	7.2	1.32	m
5	6.5	5.8	0.4	12.3	5.8	1.12	m
6	6.1	5.2	0.4	11.3	5.3	1.17	m
7	7.6	2.5	0.4	10.1	4.7	3.04	st
8	6.8	3.3	0.3	10.1	4.7	2.10	sm
9	5.4	5.0	0.3	10.4	4.9	1.10	m
10	5.0	4.3	0.6	9.3	4.4	1.17	m
11	13.1	0.6	0.5	13.7	6.4	22.60	t
12	12.5	0.4	0.5	12.9	6.1	32.05	t
13	11.8	0.6		12.4	5.8	18.70	t
14	11.3	0.3	0.3	11.6	5.4	43.50	t
15	10.3	0.6		10.9	5.1	16.27	t
16	8.8	0.7		9.5	4.5	11.95	t

Table 9.15. Average measurements of chromosome pairs of *A. ligtu* hybrid.

Chromosome pair	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	11.1	10.9		22.0	20.7	1.07	m
2	9.3	7.0		16.3	15.3	1.34	m
3	6.3	5.5	0.4	11.8	11.1	1.14	m
4	7.2	2.9	0.4	10.1	9.4	2.49	sm
5	5.2	4.6	0.4	9.8	9.3	1.13	m
6	12.8	0.4	0.5	13.2	12.5	26.15	t
7	11.5	0.4	0.3	11.9	11.4	25.66	t
8	9.6	0.6		10.2	9.6	13.83	t

*RL=Relative Length
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

Figure 9.7a. *A. ligtu* hybrid - acetocarmine stain b. *A. ligtu* hybrid - Giemsa stain

Figure 9.8. *A. sierrae* - acetocarmine stain

Figure 9.9. *A. versicolor* - Giemsa stain

Bar = 10µ


7a


7b


8


9

banding pattern for *A. ligtu* hybrid revealed only a single band in the proximal one third of the long arm. Chromosome 14 of the hybrid was also the same relative length as chromosome 13 of the species and the heterochromatic banding pattern was similar, with a band near the centromere and a wider band just distal to the first. The smallest acrocentric chromosome of *A. ligtu* hybrid was close in size to chromosome 15 of the species and had a similar pattern of two bands in the proximal portion of the long arm of the chromosome.

The other chromosomes of *A. ligtu* hybrid could not be matched to those of the species and are presumed to be derived from the other parent of the hybrid. It is interesting to note the high level of similarity between the banding patterns of the chromosomes in each pair suggesting that the chromosomes of the other parent are very similar to those of *A. ligtu* species.

A. sierrae

Measurements were made on five cells of a single plant of *A. sierrae*. The average of these measurements for each chromosome in the complement (Table 9.16) were then used to calculate the average measurements for each chromosome pair (Table 9.17). These measurements were then used to describe the karyotype. A representative cell is depicted in Fig. 9.8. No Giemsa banding patterns were observed using the present technique.

Karyotype analysis indicated that this species had four pairs of metacentric, submetacentric and subtelocentric chromosomes in the first group of the complement. There were four pairs of acrocentric chromosomes in the second group, all four of which had satellites on both chromosomes of the pair.

The interesting difference between this species and the other *Alstroemeria* is the presence of two submetacentric or subtelocentric pairs of chromosomes in group one. These were of a different relative length than any similar chromosomes and did not have any heterochromatic bands, unlike those of the other species.

A. sierrae was the only species studied in which every acrocentric chromosome had a satellite. These satellites were quite large and were often larger than the short arm of the chromosome.

Table 9.16. Average measurements of chromosomes of *A. sierrae*.

Chromosome	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	12.3	11.6		23.9	14.1	1.06	m
2	12.2	11.6		23.8	14.1	1.06	m
3	8.4	2.0		10.4	6.2	4.16	st
4	8.4	2.1		10.5	6.2	4.07	st
5	3.2	3.1		6.4	3.7	1.02	m
6	2.9	2.6		5.5	3.3	1.12	m
7	4.0	1.6		5.6	3.3	2.24	sm
8	3.7	1.6		5.3	3.1	2.27	sm
9	11.0	0.9	1.6	11.9	7.1	11.74	t
10	10.5	0.9	0.6	11.4	6.8	11.17	t
11	9.8	1.0	0.8	10.8	6.4	9.89	t
12	9.1	0.5	1.1	9.6	5.7	16.47	t
13	8.8	0.7	0.6	9.5	5.6	13.06	t
14	8.5	0.7	0.8	9.2	5.4	12.38	t
15	7.2	0.6	0.6	7.8	4.5	11.41	t
16	6.8	0.6	0.9	7.4	4.4	10.75	t

Table 9.17. Average measurements of chromosome pairs of *A. sierrae*.

Chromosome pair	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	12.3	11.6		23.9	28.2	1.06	m
2	8.4	2.0		10.4	12.3	4.10	st
3	3.0	2.9		5.9	7.0	1.06	m
4	3.8	1.6		5.4	6.4	2.36	sm
5	10.8	0.9	1.1	11.7	13.8	11.46	t
6	9.4	0.8	1.0	10.2	12.0	12.24	t
7	8.6	0.7	0.8	9.3	11.0	12.71	t
8	7.0	0.6	0.7	7.6	8.9	10.75	t

*RL=Relative Length
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

A. versicolor

An average of measurements of each chromosome was made on five cells from a single plant (Table 9.18). The average measurements for each chromosome pair was then calculated (Table 9.19). These measurements were then used to describe the karyotype of this species. A representative cell and the Giemsa banding pattern is shown in Fig. 9.9.

This species had the typical *Alstroemeria* karyotype. There were two groups of chromosomes in the complement. The first group had four pairs of metacentric and submetacentric chromosomes and the second group had four pairs of acrocentric chromosomes. Satellites were observed on chromosomes of both groups.

Neither chromosome in pair number 1 had heterochromatic bands. A narrow band was near the middle of the longer arms of chromosomes in pair number 2 with a distal band on the shorter arm. Pair number 3 had a wide band in the middle of each arm. The submetacentric chromosomes of pair number 4 had a heterochromatic band near the middle of the short arm and a broader band in the middle of the long arm. Only faint scattered spots of heterochromatin were noted on the chromosomes of acrocentric pair number 5, while two distinct bands were in the proximal half of the chromosomes of pair 7. A single heterochromatic band was in the proximal quarter of the long arm of the chromosomes of pair 8.

Leontochir ovallei

The karyotype of the genus *Leontochir* was quite similar to that for *Alstroemeria*. There were 2n=2x=18 chromosomes in *L. ovallei*. They could be divided into two groups. The first group consisted of four pairs of metacentric or subtelocentric chromosomes. The second group consisted of five pairs of acrocentric chromosomes some of which carried satellites. The extra two chromosomes not found in the alstroemeria were in this second group.

Measurements were made of chromosomes from five different cells of a single plant. The measurements were averaged for each chromosome (Table 9.20). An average measurement for each chromosome pair was then calculated (Table 9.21) and was used to describe the karyotype. A representative cell is shown in Fig. 10a and the Giemsa banding pattern is illustrated in Fig. 10b.

Table 9.18. Average measurements of chromosomes of *A. versicolor.*

Chromosome	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	9.4	8.6		18.1	11.2	1.08	m
2	8.4	6.8		15.2	9.4	1.26	m
3	6.6	4.9		11.5	7.1	1.35	m
4	5.4	5.0		10.4	6.5	1.08	m
5	4.9	4.3		9.2	5.7	1.14	m
6	4.7	4.0		8.7	5.4	1.18	m
7	5.5	2.8		8.3	5.1	1.96	sm
8	4.8	2.2	0.1	7.0	4.3	2.18	sm
9	10.2	0.4		10.6	6.6	25.50	t
10	10.0	0.2		10.2	6.3	50.00	t
11	9.5	0.5		10.0	6.2	19.00	t
12	9.2	0.1		9.3	5.8	131.43	t
13	8.5	0.1	0.2	8.6	5.3	85.00	t
14	8.4	0.01		8.4	5.2	840.00	t
15	7.9	0.6	0.3	8.3	5.1	13.17	t
16	6.5	0.2	0.3	6.7	4.1	32.50	t

Table 9.19. Average measurements of chromosome pairs of *A. versicolor.*

Chromosome pair	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	8.9	7.8		16.7	20.6	1.14	m
2	6.0	5.0		11.0	13.6	1.20	m
3	4.8	4.2		9.0	11.1	1.14	m
4	4.8	2.2	0.1	7.7	9.4	2.08	sm
5	10.1	0.3		10.1	12.9	33.67	t
6	9.3	0.5		9.8	12.0	18.60	t
7	8.5	0.1	0.2	8.6	10.5	141.67	t
8	7.2	0.4	0.3	7.6	9.2	18.00	t

*RL=Relative Length
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

Table 9.20. Average measurements of chromosomes of *Leontochir ovallei*.

Chromosome	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	6.7	5.9		12.6	7.0	1.14	m
2	5.9	5.5		11.4	6.4	1.08	m
3	5.0	4.0		9.0	5.0	1.25	m
4	4.8	4.0		8.8	4.9	1.21	m
5	7.5	1.2		8.7	4.9	6.29	st
6	7.1	1.4		8.5	4.8	5.06	st
7	6.8	1.5		8.3	4.6	4.57	st
8	6.0	1.7		7.7	4.3	3.62	st
9	11.8	0.6		12.4	6.9	20.26	t
10	11.4	0.3		11.7	6.6	34.49	t
11	10.7	0.6		11.3	6.3	18.43	t
12	10.6	0.5	0.3	11.1	6.3	20.06	t
13	10.0	0.3	0.6	10.3	5.8	30.12	t
14	9.4	0.5		9.9	5.6	19.26	t
15	9.1	1.2		10.3	5.8	7.61	t
16	8.7	1.2		9.9	5.6	7.01	t
17	8.4	0.7	0.6	9.1	5.1	11.32	t
18	8.1	0.8	0.6	8.9	5.0	10.04	t

Table 9.21. Average measurements of chromosome pairs of *Leontochir ovallei*.

Chromosome pair	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	6.3	5.7		12.0	13.5	1.11	m
2	4.9	4.0		8.9	10.0	1.23	m
3	7.3	1.3		8.6	9.6	5.6	st
4	6.4	1.6		8.0	8.9	4.06	st
5	11.6	0.5		12.1	13.5	25.15	t
6	10.7	0.6	0.3	11.3	12.6	19.03	t
7	9.7	0.4	0.6	10.1	11.3	23.63	t
8	8.9	1.2		10.1	11.3	7.28	t
9	8.3	0.8	0.6	9.1	10.1	10.59	t

*RL=Relative Length
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite.

Heterochromatic bands were on each chromosome of the complement of *Leontochir ovallei*. The first two metacentric pairs had a small band at the distal end of each arm of the chromosomes while the subtelocentric chromosome pairs number 3 and 4 had a band at the distal end of the long arm only.

The four pairs of acrocentric chromosomes had similar heterochromatic banding patterns with a wide band at the middle of the long arm of both of the chromosomes of each pair. The entire short arm of chromosomes of pair number 8 were heterochromatic.

The largest pair of metacentric chromosomes in *L. ovallei* were only about half the size of many of the largest *Alstroemeria* metacentrics.

Bomarea species

The chromosome number for this genus was 2n=2x=18. The karyotype was similar to that for the *Alstroemeria* species. The chromosomes could be divided into two groups. There were five pairs of metacentric, submetacentric and subtelocentric chromosomes in the first group. The second group of chromosomes had four pairs of acrocentric chromosomes all of which had a satellite on one or both chromosomes of the pair.

Measurements were made of chromosomes in five cells and average measurements for each chromosome were calculated (Table 9.22). Average measurements for each pair of chromosomes were then calculated (Table 9.23) and were used to describe the karyotype of this species. A representative cell is depicted in Fig. 9.11a and the Giemsa banding pattern is shown in Fig. 9.11b.

No heterochromatic bands were on chromosomes of pairs number 1, 4, 6 or 9. Thin heterochromatic bands were noted on the remaining chromosome pairs. Chromosome pair number 2 had a band at the proximal end of the long arm while the metacentric chromosomes of pair number 3 had a band in the middle of the long arm. The smallest submetacentric chromosomes also had a faint band at the proximal end of the long arm. The acrocentric chromosome pair 7 had two bands in the distal portion of the long arm of the chromosomes while chromosome pair number 8 had a thin band at the proximal end of the long arm.

The largest metacentric chromosome pair was also quite small compared to the *Alstroemeria* species, although it was larger than the same pair in *Bomarea*.

Table 9.22. Average measurements of chromosomes of *Bomarea spp.*

Chromo-some	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	5.9	4.7		10.6	8.7	1.25	m
2	4.9	4.8		9.7	7.9	1.02	m
3	6.1	1.7		7.8	6.4	3.59	st
4	5.2	1.6		6.8	5.6	3.25	st
5	3.7	3.5		7.2	5.9	1.06	m
6	2.9	2.6		5.5	4.5	1.11	m
7	5.2	1.3		6.5	5.3	3.47	st
8	4.5	1.3		5.8	4.8	3.46	st
9	3.2	1.4		4.6	3.8	2.29	sm
10	2.7	1.1		3.8	3.1	2.45	sm
11	9.4	0.4		9.8	8.0	23.50	t
12	7.8	0.4	0.1	8.2	6.7	19.50	t
13	7.0	0.3	0.1	7.3	6.0	23.33	t
14	6.2	0.6	0.2	6.8	5.6	10.33	t
15	5.4	0.5	0.2	5.9	4.8	10.80	t
16	4.9	0.3	0.2	5.2	4.3	16.73	t
17	4.6	0.7	0.3	5.3	4.3	6.57	st
18	4.6	0.6		5.2	4.3	7.67	t

Table 9.23. Average measurements of chromosome pairs of *Bomarea spp.*

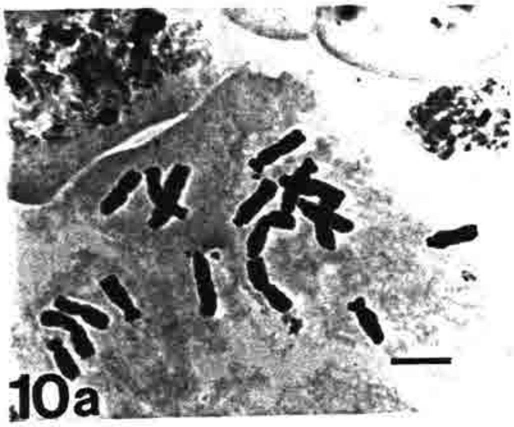
Chromo-some pair	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	5.4	4.7		10.1	16.6	1.15	m
2	5.7	1.7		7.4	12.0	3.35	st
3	3.3	3.1		6.4	10.4	1.06	m
4	4.9	1.3		6.2	10.1	3.73	st
5	3.0	1.3		4.3	6.9	2.30	sm
6	8.6	0.4	0.1	9.0	14.7	21.50	t
7	6.6	0.5	0.1	7.1	11.6	13.20	t
8	5.2	0.4	0.2	5.6	9.1	13.00	t
9	4.6	0.6	0.3	5.2	8.6	7.67	t

*RL=Relative Length
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

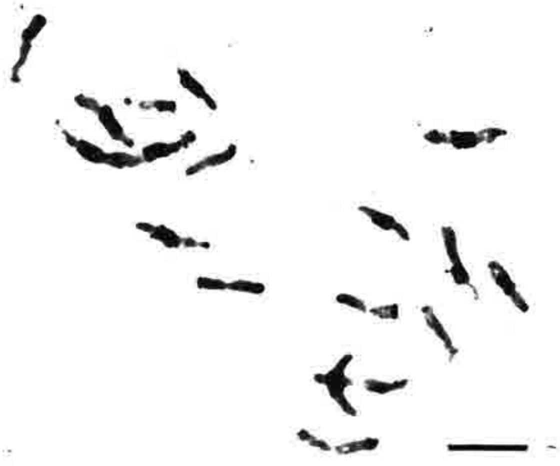
Figure 9.10a. *Leontochir ovallei* - acetocarmine stain b. *L. ovallei* - Giemsa stain

Figure 9.11a. *Bomarea* species - acetocarmine stain b. *Bomarea* species - Giemsa stain

Bar = 10µ

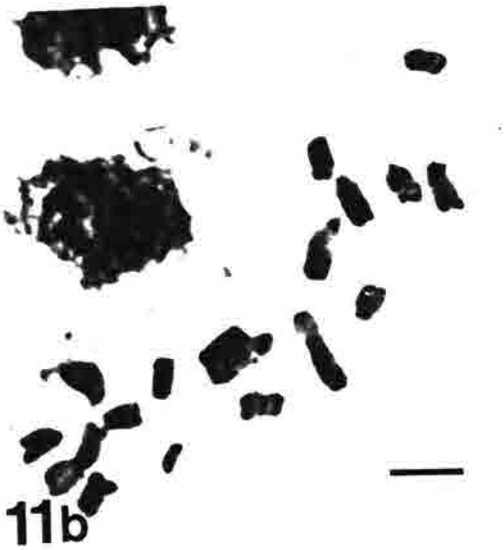


10a



10b

11a



11b

CULTIVARS

The karyotypes and chromosome numbers of the cultivars varied widely. Each chromosome is discussed separately since individual chromosomes within each cultivar may have different origins. Where it can be shown that more than one copy of a single genome is present within a cultivar the average values for the chromosome pairs of that genome were considered. The chromosomes were arranged into two groups. The first group contained all the metacentric, submetacentric or subtelocentric chromosomes and the second group contained all the acrocentric chromosomes. Chromosomes within each group were arranged in order of descending length.

The Giemsa banding pattern of the chromosomes of the cultivars was used to help determine which of the possible species was the probable contributor to the each cultivar. In some cases, however, no Giemsa banding pattern could be determined using the present staining techniques (Table 9.1).

Diploid cultivars

A. \`Canaria'

The chromosome number of this cultivar was 2n=2x=16. The karyotype had nine metacentric or submetacentric chromosomes and seven acrocentric chromosomes. Five of the acrocentric chromosomes had small satellites on the short arms (Fig. 9.12a). Chromosome number 7 was very close to having a ratio which would classify it as acrocentric rather than subtelocentric.

Average measurement of five cells of a single plant (Table 9.24) were used to describe each chromosome of the genome. A representative cell is shown in Fig. 9.12a and Fig. 9.12b shows the Giemsa banding pattern.

Chromosome 1: This metacentric chromosome had an average total length of 19.4µ and a relative length of 11.7%. Small heterochromatic bands were at the distal end of each chromatid on both arms of the chromosome.

Chromosome 2: The second largest chromosome was also a metacentric with an average total length of 18.9µ and a relative length of 11.4%. A small heterochromatic band was at the distal end of each chromatid of the shorter arm of this chromosome.

Chromosome 3: The third metacentric chromosome had an average total length of 16.2µ and a relative length of 9.8%. A heterochromatic band was observed at the distal end of both of the chromosome arms.

Table 9.24. Average measurements of chromosomes of *A.* `Canaria'.

Chromo-some	L* µ	S* µ	SAT* µ	Total* µ	RL*	Ratio	Centromere position
1	10.6	8.8		19.4	11.4	1.21	m
2	10.0	8.9		18.9	11.4	1.13	m
3	9.1	7.1		16.2	9.8	1.28	m
4	5.2	4.9		10.1	6.0	1.06	m
5	7.6	1.8		9.4	5.7	4.22	st
6	7.0	1.8		8.8	5.3	3.89	st
7	5.5	0.8		6.3	3.8	6.90	st
8	2.8	2.6		5.4	3.2	1.10	m
9	3.2	1.3		4.5	2.7	2.46	sm
10	10.9	0.6	0.3	11.5	6.9	19.89	t
11	10.8	0.2	0.1	11.0	6.6	54.00	t
12	10.3	0.4	0.2	10.3	6.4	25.75	t
13	9.7	0.3	0.2	10.0	6.0	32.33	t
14	8.7	0.3		9.0	5.4	29.00	t
15	7.9	0.1	0.2	8.0	4.8	79.00	t
16	7.1	0.3		7.4	4.4	23.67	t

*RL=Relative Length
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

Chromosome 4: The metacentric chromosome 4 was much smaller than the first three metacentrics. It had an average total length of 10.1μ and a relative length of 9.8%. There was a wide heterochromatic band at the distal end of each chromosome arm which was about ¼ the length of each arm.

Chromosome 5: The fifth chromosome of this cultivar was a subtelocentric chromosome with an average total length of 9.4μ. The relative length was 5.7%. There was a heterochromatic band at the distal end of each chromosome arm and a wide heterochromatic band in the middle of the long arm. This wide band covered about one third of the length of the chromosome arm.

Chromosome 6: The second subtelocentric chromosome had an average total length of 8.8μ and a relative length of 5.3%. A faint heterochromatic band was visible on the distal end of the long arm of this chromosome.

Chromosome 7: The smallest subtelocentric chromosome was much smaller than the first two. It had an average total length of 6.3μ and a relative length of 3.8%. There was no heterochromatic staining on this chromosome.

Chromosome 8: The smallest metacentric chromosome had an average total length of 5.4% and a relative length of 3.2%. No heterochromatic bands were visible on this chromosome.

Chromosome 9: The last chromosome in the first group of the genome was a small submetacentric. It had an average total length of 4.5μ and a relative length of 2.7%. There was a small heterochromatic region at the distal end of each chromatid of the long arm of the chromosome. A second band was at the proximal end of the long arm near the centromere.

Chromosome 10: The largest acrocentric chromosome had an average total length of 11.5μ and a relative length of 6.9%. This chromosome had a satellite which was about half the length of the short arm. There was a wide heterochromatic band beginning at the middle of the long arm of the chromosome and covering about one third of the length of the arm. A narrower band was just proximal to this band.

Chromosome 11: The second acrocentric chromosome had a long arm which was almost the same size as that of chromosome 10. It had an average total length of 11.0μ and a relative length of 6.6%. There was a narrow heterochromatic band at the distal end of the long arm, a wide band covering the middle third of the arm and a narrow band proximal to this. The small satellite was not heterochromatic.

Chromosome 12: This acrocentric chromosome had a satellite which was about half the size of the short arm. Any heterochromatic areas on this chromosome were indistinct in this preparation.

The average total length of this chromosome was 10.3µ and the relative length was 6.4%.

Chromosome 13: This chromosome had an average total length of 10.0µ and a relative length of 6.4%. The satellite was about the same size as the short arm. A broad heterochromatic band began at the middle of the long arm of the chromosome and covered about ⅓of the length of the arm. A narrow band was proximal to this.

Chromosome 14: The fifth acrocentric chromosome of this genome had no satellite. It had an average relative length of 9.0µ and a relative length of 6.0%. There was a thin heterochromatic band just proximal to the middle of the long arm of this chromosome.

Chromosome 15: This chromosome had a satellite which was about twice the length of the short arm. The average total length was 8.0µ and the relative length was 4.8%. No heterochromatic bands were apparent on this chromosome.

Chromosome 16: The smallest acrocentric chromosome had an average total length of 7.4µ and a relative length of 4.4%. There was no satellite on this chromosome and no heterochromatic bands were visible.

A. `Eureka'

The chromosome number of this cultivar was 2n=2x=16. The average measurements of five cells of a single plant were used to describe the karyotype of this cultivar (Table 9.25). The karyotype consisted of eight metacentric, submetacentric or subtelocentric chromosomes and eight acrocentric chromosomes (Fig. 9.13a). The Giemsa banding pattern is shown in Fig. 9.13b.

Chromosome 1: The largest metacentric chromosome had an average total length of 24.7µ and a relative length of 13.3%. There was no heterochromatic banding on this chromosome.

Chromosome 2: The second metacentric chromosome had an average total length of 22.1µ and a relative length of 11.9%. No heterochromatic bands were visible on this chromosome.

Chromosome 3: The average total length of this metacentric chromosome was 15.4µ and the relative length was 8.5%. There was a narrow heterochromatic band at the distal end of the shorter arm.

Chromosome 4: The fourth metacentric chromosome of the genome had an average total length of 10.7µ and a relative length of 5.7%. There was a heterochromatic band at the distal end of each arm of the chromosome.

Table 9.25. Average measurements of chromosomes of *A.* `Eureka'.

Chromo-some	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	12.9	11.8		24.7	13.3	1.09	m
2	11.3	10.8		22.1	11.9	1.05	m
3	8.9	6.5		15.4	8.3	1.37	m
4	5.4	5.3		10.7	5.7	1.01	m
5	8.6	2.2		10.8	5.8	3.87	st
6	7.4	2.2		9.6	5.2	3.37	st
7	3.4	2.8		6.2	3.3	1.22	m
8	3.9	1.4		5.3	2.9	2.69	sm
9	12.2	0.6		12.8	6.9	20.33	t
10	11.1	0.5	0.2	11.6	6.3	22.20	t
11	10.5	0.3	0.4	10.8	5.8	35.00	t
12	9.9	0.4	0.3	10.3	5.6	24.75	t
13	9.4	0.4	0.2	9.8	5.3	23.50	t
14	9.0	0.2	0.4	9.2	5.0	45.00	t
15	7.8	0.4		8.2	4.4	19.50	t
16	6.4	0.5		6.8	3.7	12.80	t

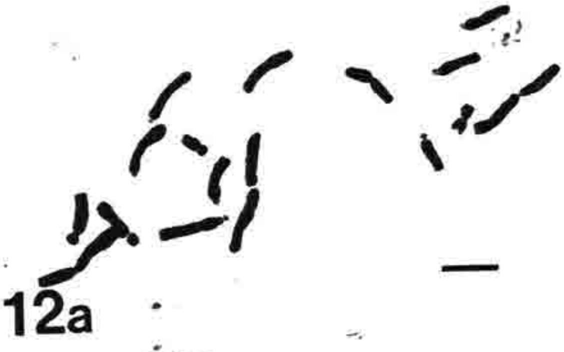
*RL=Relative Length
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

Figure 9.12a. A `Canaria' - acetocarmine stain b. A `Canaria' - Giemsa stain

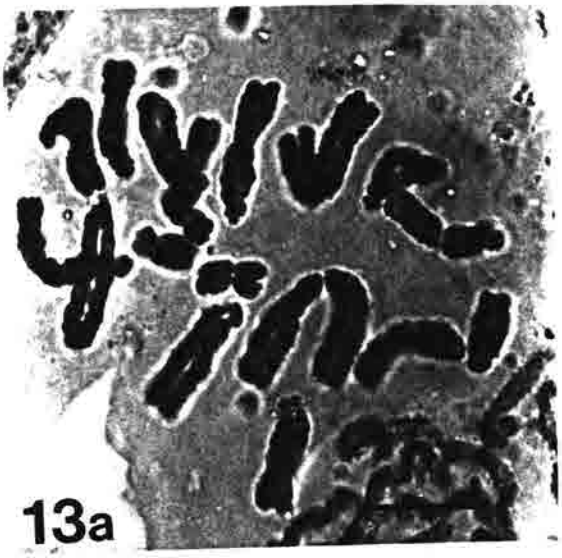
Figure 9.13a. A `Eureka' - acetocarmine stain b. A `Eureka - Giemsa stain

Figure 9.14a. A `Orchid' - acetocarmine stain b. A `Orchid' - Giemsa stain
Both large metacentric chromosomes are broken.

Bar = 10µ


12a


12b


13a


13b


14a


14b

Chromosome 5: The largest subtelocentric chromosome had an average total length of 10.8µ and a relative length of 5.8%. Heterochromatic bands were at the distal end of both arms of the chromosome. The long arm also had a broad heterochromatic band covering the middle third of the length of the chromosome.

Chromosome 6: The other subtelocentric chromosome of this genome had an average total length of 9.6µ and a relative length of 5.2%. There was a narrow heterochromatic band at the proximal end of the short arm near the centromere.

Chromosome 7: This metacentric chromosome had an average total length of 6.2µ and a relative length of 3.3%. There were no heterochromatic bands on this chromosome.

Chromosome 8: The smallest chromosome in group one was the smallest of all the chromosomes in the genome with an average total length of 5.3µ. The relative length was 2.9%. This submetacentric chromosome had a heterochromatic band at the centromere.

Chromosome 9: The largest acrocentric chromosome had an average total length of 12.8% and a relative length of 6.9%. This chromosome had the largest short arm of all the acrocentric chromosomes of the genome and did not have a satellite. A broad heterochromatic band began at the middle of the long arm and covered about ¼ of the length of the arm. A narrow heterochromatic band was proximal to this.

Chromosome 10: This acrocentric chromosome had a satellite which was less than half the length of the short arm. The average total length of the chromosome was 11.6µ and the relative length was 6.3%. There was a broad heterochromatic band from the middle of the long arm of the chromosome covering about ¼ of it's length and a narrow band proximal to this.

Chromosome 11: The third chromosome of the second group was an acrocentric with an average total length of 10.8µ and a relative length of 5.8%. The satellite on this chromosome was about the same size as the short arm. A broad heterochromatic band was at the middle of the long arm of the chromosome and covered about ⅓ of the length of the arm.

Chromosome 12: This chromosome had an average total length of 10.3µ and a relative length of 5.6%. The satellite was about the same size as the short arm. A narrow heterochromatic band was at the proximal end of the long arm of the chromosome but near the middle of the arm.

Chromosome 13: This was an acrocentric chromosome with an average total length of 9.8µ and a relative length of 5.3%. The satellite was about half the size of the short arm. There were no heterochromatic bands on this chromosome.

Chromosome 14: The fifth acrocentric chromosome had an average total length of 9.2µ and a relative length of 5.0%. The satellite was about twice the length of the short arm. There was a thin heterochromatic band at the proximal end of the short arm of the chromosome.

Chromosome 15: This small acrocentric chromosome had an average total length of 8.2µ and a relative length of 4.4%. There was no satellite on this chromosome. No heterochromatic bands were visible on this chromosome.

Chromosome 16: The smallest acrocentric chromosome had an average total length of 6.8µ and a relative length of 3.7%. No satellite was on this chromosome and there were no heterochromatic regions.

A. `Orchid'

This cultivar had a chromosome number of 2n=2x=16. Average measurements were calculated from measurements of five cells from a single plant (Table 9.26). These measurements were used to describe the karyotype of this plant. This cultivar had eight chromosomes in the first group of metacentric, submetacentric or subtelocentric chromosomes. There were eight acrocentric chromosomes in the second group. This was a similar karyotype to the other two diploid cultivars. Four of the acrocentric chromosomes had satellites (Fig. 9.14a). Heterochromatic bands are illustrated in Fig. 14b.

Chromosome 1: The largest metacentric chromosome of this cultivar had an average total length of 19.6µ and a relative length of 12.5%. There were no heterochromatic bands on this chromosome.

Chromosome 2: This metacentric chromosome was almost the size of the largest chromosome of the genome. It had an average total length of 17.0µ and a relative length of 10.9%. There were no heterochromatic bands on this chromosome.

Chromosome 3: This chromosome was much smaller than the first two. The average total length of this chromosome was 13.3µ and the relative length was 8.5%. This was a metacentric chromosome which had two heterochromatic bands at the distal end of the shorter arm. One band was located right at the end of the arm and the second band was narrower and located just proximal to the first one.

Chromosome 4: This metacentric chromosome had one arm much shorter than the other. The average total length was 11.7µ and the relative length was 8.5%. There was a broad

Table 9.26. Average measurements of chromosomes of *A.* `Orchid'.

Chromo-some	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	10.2	9.4		19.6	12.5	1.09	m
2	9.7	7.3		17.0	10.9	1.33	m
3	7.8	5.5		13.3	8.5	1.42	m
4	7.2	4.5		11.7	7.5	1.61	m
5	7.0	1.7		8.7	5.6	4.12	st
6	6.4	1.6		8.0	5.1	4.00	st
7	2.8	2.5		5.3	3.4	1.12	m
8	3.3	1.2		4.5	2.9	2.75	sm
9	10.1	0.2	0.1	10.3	6.6	50.50	t
10	9.6	0.3	0.4	9.9	6.3	32.00	t
11	9.4	0.5	0.2	9.9	6.3	18.80	t
12	9.4	0.5	0.6	9.9	6.3	18.80	t
13	7.8	0.4		8.2	5.2	19.50	t
14	7.3	0.5		7.8	5.0	14.60	t
15	6.5	0.4		6.9	4.4	16.25	t
16	5.4	0.2		5.6	3.6	27.00	t

*RL=Relative Length
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

heterochromatic band at the distal end of both arms of the chromosome and small heterochromatic region on each chromatid at the centromere.

Chromosome 5: The largest of the two subtelocentric chromosomes had an average total length of 8.7µ and a relative length of 5.6%. There was a broad heterochromatic band at the distal end of the short arm covering about ⅓ of the arm. A thin band was at the distal end of the long arm as well as a broad band from the middle of the arm, covering about ⅓ of the arm length. There was a heterochromatic region on each chromatid at the centromere.

Chromosome 6: The second subtelocentric chromosome had an average total length of 8.0µ and a relative length of 5.1%. The short arm was entirely heterochromatic. There was a narrow heterochromatic band at the distal end of the long arm of the chromosome.

Chromosome 7: This was the smallest metacentric chromosome in the genome. It had an average total length of 5.3µ and a relative length of 3.4%. There was a small heterochromatic region on both chromatids of the chromosome at the centromere.

Chromosome 8: The tiny submetacentric chromosome was the smallest chromosome in the genome. The average total length was 4.5µ and the relative length was 2.9%. A small heterochromatic region was on both chromatids at the centromere.

Chromosome 9: The largest acrocentric chromosome in the genome had an average total length of 10.3µ and a relative length of 6.6%. The satellite was about half the size of the short arm and appeared to be heterochromatic. There were two heterochromatic bands on the long arm. A broad band started at the middle of the chromosome and covered about ¼ the length of the arm. A narrower band was proximal to the broad band.

Chromosome 10: This acrocentric chromosome had an average total length of 9.9µ and a relative length of 6.3%. The satellite was about the same size as the short arm. There was a narrow heterochromatic band at the proximal end of the long arm of the chromosome as well as a broad band in the middle of the arm. This band covered about ⅓ of the length of the chromosome arm.

Chromosome 11: There was a small satellite on this acrocentric chromosome that was about half the size of the short arm. The average total length of this chromosome was 9.9µ and the relative length was 6.3%. The short arm of this chromosome was heterochromatic. There was a heterochromatic band at the proximal end of the long arm of the chromosome and another band just proximal to the mid-point of the chromosome arm.

Chromosome 12: The fourth acrocentric chromosome had a satellite about the size of the short arm. The average length of this chromosome was 9.9µ and the relative length was 6.3%. This chromosome had the same shape and size as chromosome 11. There was a much larger satellite on this chromosome, however, and the heterochromatic bands were different. The satellite was heterochromatic and there was a thin heterochromatic band at the proximal end of the long arm. There was a broad band starting at the middle of the arm and covering about ¼ of the length of the arm. Another thin heterochromatic band was proximal to this broad band.

Chromosome 13: This acrocentric chromosome had an average total length of 8.2µ and a relative length of 5.2%. There were no satellites on this chromosome and no heterochromatic bands were visible.

Chromosome 14: The sixth acrocentric chromosome was similar in size to chromosome 13. The average total length of this chromosome was 7.8µ and the relative length was 5.0%. No satellites were on this chromosome. There was a thin heterochromatic band at the proximal end of the long arm of the chromosome.

Chromosome 15: This acrocentric chromosome had an average total length of 6.9µ and a relative length of 4.4%. There was a thin heterochromatic band at the proximal end of the long arm of the chromosome.

Chromosome 16: The smallest acrocentric chromosome had an average total length of 5.6µ and a relative length of 3.6%. There was a thin heterochromatic band at the proximal end of the long arm of the chromosome.

Triploid Cultivars

The relative lengths of these chromosomes was determined by multiplying the average lengths by a conversion factor. This factor was determined by dividing 150 by the total length of the whole genome since the cells of the triploid cultivars contained 1½ times the chromosome number of the diploid species and cultivars. Measurements were made of five cells of a single plant of each cultivar and these were then averaged to determine the average lengths of each chromosome, and long arms, short arms and satellites.

A. `King Cardinal'

The average measurements of the chromosomes of this cultivar (Table 9.27) were used to describe the karyotype. This cultivar had twelve metacentric, submetacentric or subtelocentric chromosomes in one group and twelve acrocentric chromosomes in the second group (Fig. 9.15a). The Giemsa banding pattern is shown in Fig. 9.15b.

Chromosome 1: This large metacentric chromosome had an average total length of 27.3µ and a relative length of 14.4. No heterochromatic bands were on this chromosome .

Chromosome 2 : This metacentric chromosome had an average total length of 23.0µ and a relative length of 12.2. The primary constriction was in the median region very close to the median point. No heterochromatic bands were visible on this chromosome.

Chromosome 3: The third metacentric chromosome had a total length of 21.5µ and a relative length of 11.4. There were no heterochromatic bands on this chromosome.

Chromosome 4: This medium-sized metacentric chromosome had an average total length of 16.4µ and a relative length of 8.7. The shorter arm of this chromosome was much shorter than the longer arm. There was a narrow heterochromatic band on the shorter arm close to the centromere.

Chromosome 5: The first of the two subtelocentric chromosomes of this genome had an average total length of 15.2µ and a relative length of 8.0. There was a heterochromatic band at about the middle of the long arm.

Chromosome 6: The largest of the submetacentric chromosomes had an average total length of 12.1µ and a relative length of 6.4. The longer arm of this chromosome was shorter than the arms of the largest three metacentrics. The short arm of this chromosome had a heterochromatic band which covered half of it's width at the distal end. There was a narrow heterochromatic band at the distal end of the long arm of the chromosome as well as a broad band. This broad band covered about half of the length of the arm from just before the middle of the arm towards the distal end.

Chromosome 7: The fifth pair of metacentric chromosomes had an average total length very close to that of chromosome 6. The average total length was 11.6µ and the relative length was 6.1. Broad heterochromatic bands were at the distal end of both arms of the chromosome.

Chromosome 8: This metacentric chromosome had an average total length of 10.9µ and a relative length of 5.8. No clear heterochromatic bands were identified on this chromosome.

Chromosome 9: This chromosome was metacentric with an average total length of 8.2µ and a relative length of 4.3. There were no heterochromatic bands on this chromosome.

Chromosome 10: The second subtelocentric chromosome had an average total length of 7.0µ and a relative length of 3.7. A heterochromatic band was at the centromere.

Table 9.27. Average measurements of chromosomes of *A.* \`King Cardinal'.

Chromo-some	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	14.8	12.5		27.3	14.4	1.18	m
2	11.6	11.4		23.0	12.2	1.01	m
3	11.1	10.4		21.5	11.4	1.06	m
4	10.0	6.4		16.4	8.7	1.56	m
5	11.8	3.4		15.2	8.0	3.51	st
6	8.9	3.2		12.1	6.4	2.80	sm
7	7.0	4.6		11.6	6.1	1.52	m
8	5.8	5.2		10.9	5.8	1.11	m
9	4.4	3.8		8.2	4.3	1.16	m
10	5.6	1.4		7.0	3.7	4.00	st
11	4.3	1.7		6.0	3.2	2.53	sm
12	3.1	2.7		5.8	3.1	1.15	m
13	12.4	0.5		12.9	6.8	24.80	t
14	11.6	0.5		12.1	6.4	23.20	t
15	10.9	0.6		11.5	6.1	18.12	t
16	10.4	0.4		10.8	5.7	18.22	t
17	10.2	0.1		10.3	5.4	102.00	t
18	9.9	0.3		10.2	5.4	33.00	t
19	9.1	0.7	0.3	9.8	5.2	13.00	t
20	8.9	0.5		9.4	5.0	17.80	t
21	8.6	0.4		9.0	4.8	21.50	t
22	8.0	0.3		8.3	4.4	26.67	t
23	7.4	0.3		7.7	4.1	24.67	t
24	7.1	0.3		7.4	3.9	23.67	t

*RL=Relative Length with a total genome length of 150%.
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

Chromosome 11: This small submetacentric chromosome one of the two smallest chromosomes in the genome. It had an average total length of 6.0µ and a relative length of 3.2. There was a heterochromatic band in the middle of the short arm of the chromosome.

Chromosome 12: The smallest chromosome of group one was the smallest chromosome of the whole genome. This metacentric chromosome had an average total length of 5.8µ, almost the same size as the submetacentric chromosome 11. The relative length of this chromosome was 3.1. No heterochromatic bands were located on this chromosome.

Chromosome 13: The largest acrocentric chromosome was less than half the size of the largest metacentric in the genome. It had an average total length of 12.9µ and a relative length of 6.8. A broad heterochromatic band was at the middle of the long arm of the chromosome that covered about ⅓ of the length of the chromosome. Another heterochromatic region was located at the proximal end of the long arm near the centromere.

Chromosome 14: This acrocentric chromosome had an average total length of 12.1µ and a relative length of 6.4. There was a narrow heterochromatic band at the distal end of the long arm of this chromosome as well as a faint band at the proximal end near the centromere. There was a broad band in the distal half of the arm covering about half the arm's length. There was a narrow band proximal to this broad band.

Chromosome 15: The average total length of this acrocentric chromosome was 11.5µ and the relative length was 6.1. No heterochromatic bands were identified on this chromosome.

Chromosome 16: This acrocentric chromosome had an average total length of 10.8µ and a relative length of 5.7. There was a heterochromatic band about ⅓ the distance along the length of the long arm at the proximal end.

Chromosome 17: The fifth acrocentric chromosome had a tiny short arm. This chromosome had an average total length of 10.3µ and a relative length of 5.4. No heterochromatic bands were identified on this chromosome.

Chromosome 18: This acrocentric chromosome was almost the same average total length as chromosome 17. It had a much larger short arm however. There was a heterochromatic band in the middle of the long arm of the chromosome and another band at the distal end of the short arm.

Chromosome 19: This was the only acrocentric which had a satellite. The satellite was about half the size of the short arm. The average total length was 9.8µ and the relative length was 5.2. There appeared to be a heterochromatic band at the distal end of the short arm.

Chromosome 20: The average total length of this acrocentric chromosome was 9.4µ and the relative length was 5.0. No heterochromatic bands were revealed on this chromosome.

Chromosome 21: This acrocentric chromosome had an average total length of 9.0µwiht a relative length of 4.8. There was a heterochromatic band at the distal end of the long arm and the short arm appeared to be heterochromatic.

Chromosome 22: This chromosome was an acrocentric with an average total length of 8.3µ and a relative length of 4.4. There was a wide heterochromatic band near the middle of the long arm of the chromosome and a narrow band at the distal end.

Chromosome 23: The average relative length of this acrocentric chromosome was 7.7µ. It's relative length was 4.1. There was a heterochromatic band at the distal end of the long arm of the chromosome and a faint band at the proximal end. The short arm was only faintly heterochromatic.

Chromosome 24: The smallest acrocentric chromosome had an average total length of 7.4µ and a relative length of 3.9. The short arm of the chromosome was heterochromatic.

A. \`Marina'

The chromosome number for *A.* \`Marina' was 2n=3x=24. The average measurements for five cells of a single plant (Table 9.28) were used to describe the karyotype of this cultivar. The karyotype had twelve metacentric, submetacentric and subtelocentric chromosomes in one group of the genome and twelve acrocentric chromosomes in group two (Fig. 9.16a). The Giemsa banding pattern is shown in Fig. 9.16b. Six of the acrocentric chromosomes carried satellites.

Chromosome 1: This metacentric chromosome had an average total length of 20.4µ and a relative length of 13.5. The primary constriction was in the median region close to the median point. There were no heterochromatic bands on this chromosome.

Chromosome 2: The second metacentric chromosome was slightly smaller than the first. It had an average total length of 18.8µ and a relative length of 12.4. The primary constriction was in the median region. No heterochromatic regions were evident on this chromosome.

Table 9.28. Average measurements of chromosomes of A. ‘Marina'.

Chromosome	L μ	S μ	SAT μ	Total μ	RL*	Ratio	Centromere position
1	10.3	10.1		20.4	13.5	1.02	m
2	9.8	9.0		18.8	12.4	1.10	m
3	9.3	8.7		18.0	11.9	1.07	m
4	10.2	2.8		13.0	8.6	3.64	st
5	6.8	4.7		11.5	7.6	1.45	sm
6	6.7	4.0		10.7	7.0	1.68	m
7	7.4	2.7		10.0	6.6	2.74	sm
8	4.5	4.1		8.6	5.7	1.10	m
9	3.6	2.7		6.2	4.1	1.33	m
10	4.4	1.2		5.6	3.7	3.67	st
11	3.4	1.1		4.5	3.0	1.55	sm
12	2.4	2.1		4.5	3.0	1.14	m
13	9.9	0.6		10.5	6.9	16.50	t
14	9.4	0.2		9.6	6.3	47.00	t
15	9.1	0.5	0.8	9.6	6.3	18.20	t
16	8.7	0.3	0.2	9.0	6.0	29.00	t
17	8.2	0.4		8.7	5.7	20.50	t
18	8.0	0.6		8.6	5.7	13.33	t
19	7.5	0.2		7.7	5.1	37.50	t
20	7.0	0.2		7.2	4.7	35.00	t
21	6.5	0.3	0.6	6.8	4.5	21.67	t
22	6.1	0.1	0.5	6.2	4.1	61.00	t
23	5.3	0.2	0.2	5.5	3.6	26.50	t
24	4.9	0.3	0.2	5.2	3.4	16.33	t

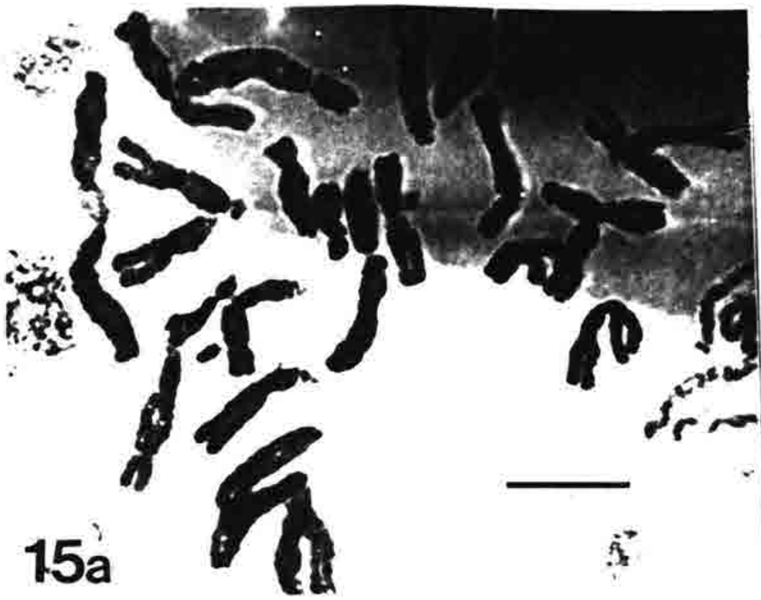
*Relative Length with a total genome length of 150%.
L = Long arm
S = Short arm
Total excluding satellite

Figure 9.15a. A `King Cardinal' - acetocarmine stain b. A `King Cardinal' - Giemsa stain several chromosomes have been washed away.

Figure 9.16a. A `Marina' - acetocarmine stain b. A `Marina' - Giemsa stain Medium metacentric is broken

Figure 9.17a. A `Yellow King' - acetocarmine stain b. A `Yellow King - Giemsa stain

Bar = 10µ



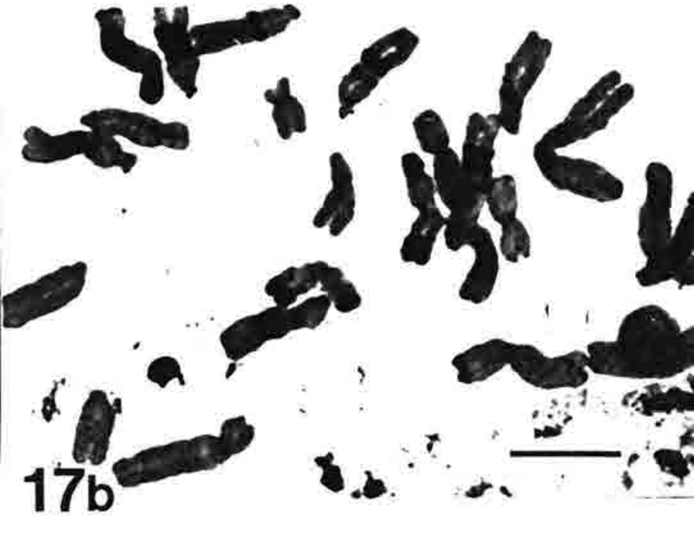
15a

15b

16a

16b

17a



17b

Chromosome 3: This chromosome was also metacentric and was close in size to chromosome number 2. The average total length was 18.0µ and the relative length was 11.9. There were no heterochromatic bands present on this chromosome.

Chromosome 4: The fourth chromosome in the genome was a large subtelocentric with an average total length of 13.0µ. The relative length of this chromosome was 8.6. No heterochromatic bands or regions were revealed with Giemsa banding.

Chromosome 5: The largest of the three submetacentric chromosomes of this genome had an average total length of 11.5µ and a relative length of 7.6. There were small heterochromatic dots on the proximal end of the long arm next to the centromere.

Chromosome 6: This metacentric chromosome had an average total length of 10.7µ and a relative length of 7.0. The shorter arm had a heterochromatic band at the distal end and a heterochromatic dot on each chromatid proximal to the first band at about one third of the length of the arm.

Chromosome 7: The next largest chromosome in the genome was the second submetacentric. It had an average total length of 10.0µ and a relative length of 6.6. This chromosome had two broad heterochromatic bands and one narrow one. The narrow band was at the distal end of the long arm. The middle ⅓ of the chromosome was also heterochromatic as was the distal half of the short arm.

Chromosome 8: The fifth metacentric chromosome was less than half the size of the first three metacentrics. It had an average total length of 8.6µ and a relative length of 5.7. There were heterochromatic bands at the distal end of both chromosome arms.

Chromosome 9: The next metacentric chromosome had an average total length of 6.2µ and a relative length of 4.2. No heterochromatic bands were visible on this chromosome.

Chromosome 10: The second subtelocentric chromosome was less than half the size of the first one. It had an average total length of 5.6µ and a relative length of 3.7. No heterochromatic bands were visible on this chromosome.

Chromosome 11: The last submetacentric chromosome was one of the smallest chromosomes in the genome. It had an average total length of 4.5µ and a relative length of 3.0. There were no heterochromatic regions along the length of this chromosome.

Chromosome 12: The smallest metacentric chromosome was the same size as the smallest submetacentric. It had an average total length of 4.5µ and a relative length of 2.9. No heterochromatic regions could be discerned on this chromosome.

Chromosome 13: The largest acrocentric chromosome had an average total length of 10.5µ, about half the size of the largest chromosome in the genome. It had one of the largest short arms of any of the chromosomes of this group. A heterochromatic band was located just proximal to the middle of the long arm of the chromosome.

Chromosome 14: The second acrocentric chromosome had an average total length of 9.6µ and a relative length of 6.3. It had a very short short arm and no satellites were present. There was a heterochromatic band proximal to the middle of the long arm of the chromosome.

Chromosome 15: This acrocentric chromosome carried a very large satellite. The chromosome had the same average total length as chromosome 14 at 9.6µ, but had a shorter long arm and a longer short arm than the latter. The relative length of this chromosome was 6.3. There was a broad heterochromatic band starting at the middle of the long arm of the chromosome and covering about half of the distal half of the arm. Another, narrower, band was proximal to the broad band.

Chromosome 16: The satellite on this acrocentric chromosome was much smaller than on chromosome number 15. The chromosome had an average total length of 9.0µ and a relative length of 6.0. There was a broad heterochromatic band from the middle of the long arm of the chromosome and covering about ½ of the distal half of the arm. A second narrow band was proximal to this. The satellite had heterochromatic dots.

Chromosome 17: This acrocentric chromosome had an average relative length of 8.7µ and a relative length of 5.7. There was a broad heterochromatic band covering the middle one ⅓ of the long arm of the chromosome and a narrow band just proximal to this. The short arm also appeared to be heterochromatic.

Chromosome 18: The sixth acrocentric chromosome had about the same average total length as chromosome number 17. It's length was 8.6µ and the relative length was 5.7. Faint heterochromatic regions appeared to be near the middle of the long arm and on the short arm but these were indistinct. The short arm of this chromosome was the largest of any of the acrocentric chromosomes.

Chromosome 19: The next acrocentric chromosome was much smaller than the previous one with an average total length of 7.7µ and a relative length of 5.1. No heterochromatic bands were identified on this chromosome.

Chromosome 20: This acrocentric chromosome had an average total length of 7.2µ and a relative length of 4.7. It also did not show any heterochromatic regions.

Chromosome 21: There was a large satellite on the short arm of this acrocentric chromosome which was about twice the size of the short arm. The average total length of this chromosome was 6.8µ and the relative length was 4.5. There were no heterochromatic regions on this chromosome.

Chromosome 22: This acrocentric chromosome also had a large satellite. The short arm was very tiny. The average total length was 6.2µ and the relative length was 4.1. There was a small heterochromatic band at the middle of the long arm of the chromosome.

Chromosome 23: This small acrocentric chromosome had a satellite which was the same size as the short arm. The average total length was 5.5µ and the relative length was 3.6. An indistinct heterochromatic region appeared to be near the middle of the chromosome.

Chromosome 24: The small satellite on this acrocentric chromosome was smaller than the short arm of the chromosome. This chromosome had an average total length of 5.2µ and a relative length of 3.4. The short arm had faint heterochromatic dots.

A. `Yellow King'

The chromosome number was 2n=3x=24. The average measurements of five cells of a single plant of this cultivar (Table 9.29) were used to describe the karyotype of this cultivar. There were twelve metacentric, submetacentric and subtelocentric chromosomes in the first group of the genome and twelve acrocentric chromosomes in the second group (Fig. 9.17a). This was the same as for the other cultivars discussed. Four of the acrocentric chromosomes carried satellites. The Giemsa banding pattern of this cultivar is depicted in Fig. 9.17b.

Chromosome 1: This large metacentric chromosome had the primary constriction in the median region. It had an average total length of 21.1µ and a relative length of 12.5. There was a

Table 9.29. Average measurements of chromosomes of *A.* `Yellow King'.

Chromosome	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	11.0	10.1		21.1	12.5	1.09	m
2	10.3	9.5		19.8	11.9	1.08	m
3	9.4	8.7		18.1	10.6	1.08	m
4	10.4	2.9		13.3	7.8	3.57	st
5	6.8	6.3		13.1	7.7	1.08	m
6	7.7	3.2		10.9	6.5	2.39	sm
7	7.1	3.0		10.1	5.9	2.37	sm
8	5.0	4.4		9.4	5.5	1.14	m
9	4.7	3.7		8.4	5.0	1.27	m
10	6.3	1.7		8.0	4.7	3.68	st
11	3.9	3.7		7.6	4.5	1.05	m
12	3.1	1.3		4.4	2.6	2.38	sm
13	11.7	0.8		12.5	7.4	14.63	t
14	10.9	0.4		11.3	6.7	27.25	t
15	9.8	0.6		10.4	6.1	16.33	t
16	9.5	0.4		9.9	5.8	23.75	t
17	9.4	0.5		9.9	5.8	18.80	t
18	9.1	0.5	0.2	9.6	5.6	18.20	t
19	8.7	0.2	0.3	8.9	5.3	43.50	t
20	8.5	0.3		8.8	5.2	28.33	t
21	8.2	0.3	0.4	8.5	5.0	27.33	t
22	8.1	0.2	0.4	8.3	4.9	40.50	t
23	6.8	0.5		7.3	4.3	13.60	t
24	6.0	0.6		6.6	3.9	10.00	t

*RL=Relative Length with a total genome length of 150%.
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

heterochromatic band at the distal end of the longer arm and some heterochromatic spots near the centromere .

Chromosome 2: The average total length of this metacentric chromosome was 19.8µ and the relative length was 11.9. There were no heterochromatic bands on this chromosome.

Chromosome 3: The third metacentric chromosome had an average total length of 18.1µ and a relative length of 10.6. There were no heterochromatic bands on this chromosome.

Chromosome 4: The fourth chromosome of this cultivar was subtelocentric with an average total length of 13.3µ and a relative length of 7.8. This chromosome had no heterochromatic bands.

Chromosome 5: The fourth metacentric chromosome was very close in size to the subtelocentric chromosome 4. It had an average total length of 13.1µ and a relative length of 7.7. There were wide heterochromatic bands covering the distal third of both the chromosome arms with a narrower band just proximal to this on the shorter arm.

Chromosome 6: The largest submetacentric chromosome had an average total length of 10.9µ and a relative length of 6.5. The distal ⅔ of the short arm was heterochromatic. There was a narrow heterochromatic band at the distal end of the long arm and a broad band covering the middle third of this arm.

Chromosome 7: The second submetacentric chromosome had an average total length of 10.1µ and a relative length of 5.9. There were heterochromatic dots at the distal end of the short arms. The long arm had a broad band covering the middle ⅓ of the arm as well as a narrower band proximal to this.

Chromosome 8: This metacentric chromosome was about half the size of the first two metacentrics in the genome. It had an average total length of 9.4µ and a relative length of 5.5. There were no heterochromatic bands on this chromosome.

Chromosome 9: The sixth metacentric chromosome had an average total length of 8.4µ and a relative length of 5.0. There were broad heterochromatic bands at the distal ends of both chromosome arms and heterochromatic dots proximal to the bands on the longer arm.

Chromosome 10: The tenth chromosome in the genome was the second subtelocentric. It had an average total length of 8.0µ and a relative length of 4.7. The distal half of the short arm was heterochromatic. There was a heterochromatic band at the distal end of the long arm and a broad band covering ⅔ of the distal half of the arm.

Chromosome 11: This was a small metacentric chromosome with an average total length of 7.6µ and a relative length of 4.5 The primary constriction was in the median region near the centromere. There were small heterochromatic dots near the distal end of each arm as well as near the centromere.

Chromosome 12: The smallest chromosome in group one was the smallest of all chromosomes in the genome. It had an average total length of 4.4µ and a relative length of 2.6. This submetacentric chromosome did not have any heterochromatic regions.

Chromosome 13: The largest acrocentric chromosome had an average total length of 12.5µ and a relative length of 7.4. There was a broad heterochromatic band from the middle of the long arm which covered ⅔ of the proximal half of the arm. A narrower band was distal to this and a very narrow band was near the distal end of the arm.

Chromosome 14: The average total length of this acrocentric chromosome was 11.3µ. and the relative length was 6.7. A heterochromatic band was near the middle of the long chromosome arm and a broad band was distal to this and covered about ¼ of the length of the arm.

Chromosome 15: This acrocentric chromosome was very similar in appearance to chromosome 14. The distribution of heterochromatin was the same. The average total length of the chromosome was 10.4µ and the relative length was 6.1. The short arm of this chromosome was proportionally much larger than that on chromosome 14.

Chromosome 16: The next acrocentric chromosome had an average total length of 9.9µ and a relative length of 5.8. There were heterochromatic dots distributed in the distal half of the long arm of the chromosome and small heterochromatic dots near the centromere.

Chromosome 17: This chromosome was the same size as chromosome 16. It's long arm was slightly shorter and it's short arm slightly longer than the latter chromosome. There was no heterochromatin visible in this chromosome.

Chromosome 18: This acrocentric chromosome carried a small satellite about half the size of it's short arm. It had an average total length of 9.6µ and a relative length of 5.6. The short arm and the satellite were both heterochromatic. The long arm had a heterochromatic band about a third of the distance from the centromere to the distal end and another broader band distal to this.

Chromosome 19: The satellite on this acrocentric chromosome was slightly larger than the short arm. The chromosome had an average total length of 8.9µ and a relative length of 5.3. The satellite was heterochromatic as was the short arm of the chromosome. The long arm had a narrow band about ¼ of the distance from the centromere to the distal end and a broad band just distal to this covered the arm to the middle.

Chromosome 20: This acrocentric chromosome did not have a satellite. It had an average total length of 8.8µ and a relative length of 5.2. There was a single heterochromatic band about ⅓ of the distance from the centromere to the distal end of the chromosome.

Chromosome 21: This chromosome carried a satellite slightly larger than the short arm which was heterochromatic. There was also a heterochromatic band on the long arm of the chromosome at about ⅓ of its length. The average total length of this chromosome was 8.5µ and the relative length was 5.0.

Chromosome 22: The satellite on this acrocentric chromosome was about twice the size of the short arm which was quite small. The average total length of this chromosome was 8.3µ and the relative length was 4.9.

Chromosome 23: This small acrocentric chromosome did not carry a satellite. It had an average total length of 7.3µ and a relative length of 4.3. There were scattered spots of heterochromatin along the distal half of the long arm of the chromosome. A small spot of heterochromatin was also located at the proximal end of the small arm.

Chromosome 24: The smallest chromosome of group two was also one of the two smallest chromosomes in the whole genome. The average total length was 6.6µ and the relative length was 3.9. This acrocentric chromosome had one of the largest short arms of all the chromosome of group two. There were small areas of heterochromatin throughout the distal end of the long arm of the chromosome as well as a small spot of heterochromatin at the proximal end of the arm, next to the centromere.

A. \`Appelbloesem'

This cultivar had a chromosome number of 2n=3x=24. The karyotype was described by the average measurements of chromosomes in five cells of a single plant (Table 9.30). This karyotype was generally the same as that for the other triploid cultivars. There were twelve metacentric, submetacentric and subtelocentric chromosomes in group one of the genome and twelve acrocentric chromosomes in group two. One chromosome in group one carried a satellite and six chromosomes in group two carried satellites (Fig. 9.18a). Some of these chromosomes had heterochromatic bands when stained with Giemsa dye (Fig. 9.18b).

Table 9.30. Average measurements of chromosomes of A. `Appelbloesem'.

Chromo-some	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	12.4	12.0		24.4	13.4	1.04	m
2	11.7	10.7		22.4	12.3	1.09	m
3	10.7	9.6		20.3	11.1	1.11	m
4	8.6	6.1		14.7	8.1	1.11	m
5	11.9	2.9		14.8	8.1	4.10	st
6	7.9	3.4		11.3	6.2	2.32	sm
7	5.4	5.0		10.4	5.7	1.28	m
8	5.4	4.2		9.6	5.3	1.30	m
9	4.8	3.9		8.7	4.8	1.25	m
10	3.5	3.0		6.5	3.6	1.15	m
11	4.5	1.5	0.2	6.0	3.3	2.99	sm
12	4.4	1.5		5.9	3.2	2.93	sm
13	12.3	0.5		12.8	7.0	24.60	t
14	11.7	0.3		12.0	6.6	39.00	t
15	10.9	0.4		11.3	6.2	27.25	t
16	10.6	0.4	0.4	11.0	6.0	26.50	t
17	10.2	0.4	0.3	10.6	5.8	25.50	t
18	9.6	0.5		10.1	5.5	19.20	t
19	9.4	0.2	0.3	9.6	5.3	47.00	t
20	9.1	0.2	0.2	9.3	5.1	45.50	t
21	8.4	0.5		8.9	4.9	16.80	t
22	7.9	0.3	0.6	8.2	4.5	26.33	t
23	7.4	0.4	0.3	7.8	4.3	18.50	t
24	6.6	0.2		6.7	3.7	33.00	t

*RL=Relative Length with a total genome length of 150%.
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

This karyotype was generally the same as that for the other triploid cultivars. There were twelve metacentric, submetacentric and subtelocentric chromosomes in group one of the genome and twelve acrocentric chromosomes in group two. One chromosome in group one carried a satellite and six chromosomes in group two carried satellites (Fig. 9.18a). Some of these chromosomes had heterochromatic bands when stained with Giemsa dye (Fig. 9.18b).

Chromosome 1: This was a very large metacentric chromosome which did not have any heterochromatic bands. It had an average total length of 24.4µ and a relative length of 13.4. The primary constriction was in the median region close to the median point.

Chromosome 2: The second metacentric chromosome in the genome had an average total length of 22.4µ and a relative length of 12.3. There were no heterochromatic bands on this chromosome.

Chromosome 3: The metacentric chromosome number 3 had an average total length of 20.3µ and a relative length of 11.1. There were no heterochromatic bands visible on this chromosome.

Chromosome 4: This metacentric chromosome had an average total length of 14.7µ and a relative length of 8.1. It was much smaller than the first three metacentric chromosomes. There was a heterochromatic band at the distal end of the shorter arm of the chromosome.

Chromosome 5: The only subtelocentric chromosome in the genome of this cultivar had an average total length of 14.8µ and a relative length of 8.1. There were no heterochromatic bands on this chromosome. The long arm of this chromosome was about the same size as the arms of the two largest metacentrics.

Chromosome 6: The largest submetacentric chromosome in the genome had an average total length of 11.3µ and a relative length of 6.2. This chromosome had a heterochromatic band at the distal end of the long arm. There was a broad heterochromatic band covering the middle ⅓ of the long arm of the chromosome and the distal half of the short arm was also heterochromatic.

Chromosome 7: The next metacentric chromosome had a heterochromatic band at the distal end of the shorter chromosome arm. This chromosome had an average total length of 10.4µ and a relative length of 5.7.

Chromosome 8: Metacentric chromosome 8 had an average total length of 9.6µ and a relative length of 5.3. There were heterochromatic bands at the distal ends of both arms of the chromosome.

Chromosome 9: This was a metacentric chromosome with an average total length of 8.7µ and relative length of 4.8. There were no heterochromatic bands on this chromosome.

Chromosome 10: The smallest metacentric chromosome did not have any heterochromatic regions. It had an average total length of 6.5µ and a relative length of 3.6.

Chromosome 11: This submetacentric chromosome had a small satellite on the short arm. There were no heterochromatic regions on either the satellite or the body of the chromosome. The average total length of the chromosome was 6.0µ and the relative length was 3.2.

Chromosome 12: The second submetacentric chromosome was almost the same size as the first but was not satellited. It also had no heterochromatic regions. the average total length of this chromosome was 5.9µ and the relative length was 3.2.

Chromosome 13: The largest acrocentric chromosome had an average total length of 12.8µ and a relative length of 7.0. This chromosome had one of the largest short arms in this genome. There was a heterochromatic band just proximal to the middle of the long arm of the chromosome and another heterochromatic band at the proximal end of the long arm.

Chromosome 14: This acrocentric chromosome had small heterochromatic dots just distal to the proximal end of the long arm of the chromosome. There was another heterochromatic spot on one of the chromatids of the long arm at about the middle of the arm.

Chromosome 15: The third acrocentric chromosome did not have any heterochromatic areas. It had an average total length of 11.3µ and a relative length of 6.2.

Chromosome 16: This acrocentric chromosome had a satellite which was about the same size as the short arm. The average total length was 11.0µ and the relative length was 6.0. The satellite was heterochromatic and there were two heterochromatic bands along the long arm of the chromosome. There was a narrow band at about ⅓ of the distance along the arm from the centromere and a wider band covering about ¼ of the length of the chromosome starting at the middle of the arm.

Chromosome 17: The second satellited acrocentric chromosome had a satellite that was slightly smaller than the short arm. This chromosome had an average total length of 10.6µ and a relative length of 5.8. There was a broad heterochromatic band from the middle of the long arm covering about ⅓ of the length of the arm.

Chromosome 18: This acrocentric chromosome was unsatellited. It had an average total length of 10.1µ and a relative length of 5.5. There were two heterochromatic bands on the long arm of the chromosome. One was proximal to the middle of the arm and a slightly wider band was distal to the middle.

Chromosome 19: The satellite on this acrocentric chromosome was slightly larger than the short arm. The short arm was quite short. There were no heterochromatic bands on this chromosome. The average total length of this chromosome was 9.6µ and the relative length was 5.3.

Chromosome 20: This acrocentric chromosome had a satellite which was the same size as the small short arm. The average total length was 9.3µ and the relative length was 5.1. No heterochromatic bands were detected on this chromosome.

Chromosome 21: The average total length of this chromosome was 8.9µ and the relative length was 4.9. There was no satellite present and the short arm was one of the largest for the acrocentric chromosomes. There was no heterochromatic staining of this chromosome.

Chromosome 22: This acrocentric chromosome had the largest satellite in the genome. It was twice the size of the short arm of this chromosome. The average total length of this chromosome was 8.2µ and the relative length was 4.5. No heterochromatic bands were present on this chromosome.

Chromosome 23: The satellite on this acrocentric chromosome was about the same size at the short arm. The average total length of the chromosome was 7.8µ and the relative length was 4.3. There was no heterochromatic staining of this chromosome.

Chromosome 24: The smallest acrocentric chromosome was not satellited. The short arm was quite small. No heterochromatic bands were identified on this chromosome. The average total length of the chromosome was 6.7µ and the relative length was 3.7.

A. `Pink Triumph'

The chromosome number of this cultivar was 2n=3x=16. The average measurements of five cells from a single plant (Table 9.31) were used to describe the karyotype of this cultivar. The karyotype was the same as for the other triploid cultivars with twelve metacentric, submetacentric and subtelocentric chromosomes in one group of the genome and twelve acrocentric chromosomes in the second group. Six of the acrocentric chromosomes had satellites (Fig. 9.19a). Only seven of these chromosomes had heterochromatic bands (Fig. 9.19b).

Chromosome 1: This large metacentric chromosome had the primary constriction in the median region close to the median point. There were no heterochromatic bands on this chromosome. The average total length of the chromosome was 26.6µ and the relative length was 14.1.

Chromosome 2: The second metacentric chromosome had an average total length of 23.7µ and a relative length of 12.6. The primary constriction was in the median region close to the median point. There were no heterochromatic bands on this chromosome.

Table 9.31. Average measurements of chromosomes of A. `Pink Triumph'.

Chromo-some	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	13.9	12.7		26.6	14.1	1.09	m
2	12.3	11.4		23.7	12.6	1.07	m
3	9.9	8.9		18.8	10.0	1.11	m
4	8.6	6.4		15.0	7.9	1.34	m
5	11.5	3.2		14.7	7.8	3.59	st
6	9.0	3.8		12.8	6.8	2.37	sm
7	6.3	5.0		11.3	6.0	1.26	m
8	5.6	4.5		10.1	5.3	1.24	m
9	4.6	3.5		8.1	4.3	1.32	m
10	5.3	1.9		7.2	3.8	3.31	st
11	3.6	3.2		6.8	3.6	1.13	m
12	4.4	1.6		6.0	3.2	2.75	sm
13	12.3	0.7	0.9	12.0	6.8	17.57	t
14	11.8	0.3	1.2	12.1	6.4	39.33	t
15	10.7	1.2		11.9	6.3	8.92	t
16	10.4	0.4	0.6	10.8	5.7	26.00	t
17	10.3	0.2	0.3	10.5	5.5	51.50	t
18	9.8	0.6	1.5	10.4	5.5	16.33	t
19	9.5	0.5		10.0	5.3	19.00	t
20	9.3	0.3		9.6	5.0	31.00	t
21	8.1	0.5		8.7	4.6	16.20	t
22	7.3	0.6		7.9	4.2	12.17	t
23	7.0	0.5		7.5	4.0	14.00	t
24	6.5	0.5	0.1	7.0	3.7	13.00	t

*RL=Relative Length with a total genome length of 150%.
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

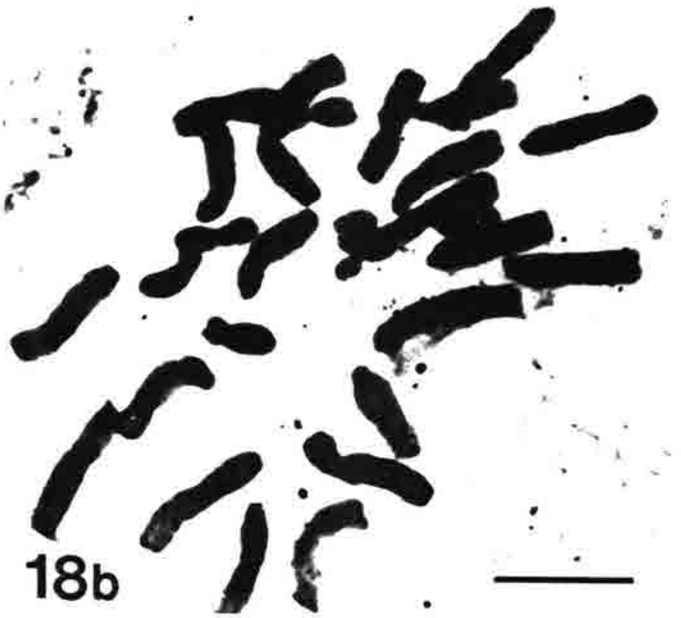
Figure 9.18a. A. `Appelbloesem' - acetocarmine stain One large metacentric chromosome is broken. b. A. `Appelbloesem - Giemsa stain

Figure 9.19a. A. `Pink Triumph' - acetocarmine stain b. A. `Pink Triumph' - Giemsa stain

Figure 9.20a. A. `Mona Lisa' - acetocarmine stain b. A. `Mona Lisa' - Giemsa stain

Bar = 10µ

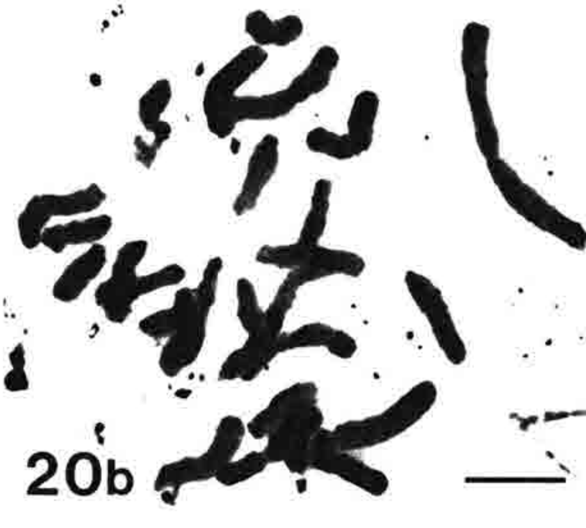

18a


18b


19a


19b


20a


20b

Chromosome 3: This metacentric chromosome was considerably smaller than the previous two with an average total length of 18.8µ and a relative length of 10.0. Treatment with Giemsa dye did not reveal any heterochromatic bands on this chromosome.

Chromosome 4: Metacentric chromosome 4 had a heterochromatic band at the distal end of the shorter arm. The average total length of this chromosome was 15.0µ and the relative length was 7.9.

Chromosome 5: The largest subtelocentric chromosome of the genome had an average total length of 14.7µ and a relative length of 7.8. This chromosome was not much smaller than chromosome number 4. No heterochromatic bands were visible on this chromosome.

Chromosome 6: This submetacentric chromosome had an average total length of 12.8µ and a relative length of 6.8. There were two heterochromatic bands on this chromosome. The broadest band was towards the distal end of the long arm and covered about ⅓ of the length of the arm. The second band was at the distal end of the short arm and was half the length of the short arm.

Chromosome 7: The next chromosome in the genome was metacentric in shape. The average total length was 11.3µ and the relative length was 6.0. There was a heterochromatic band at the distal end of both arms of the chromosome with the band on the longer arm being slightly wider than the other.

Chromosome 8: This metacentric chromosome had an average total length of 10.1µ and a relative length of 5.3. There were no heterochromatic bands on this chromosome.

Chromosome 9; Metacentric chromosome 9 had an average total length of 8.1µ and a relative length of 4.3. No heterochromatic bands were on this chromosome.

Chromosome 10: The second subtelocentric chromosome had a broad heterochromatic band in the middle of the long arm of the chromosome. The average total length of this chromosome was 7.2µ and the relative length was 3.8.

Chromosome 11: The smallest metacentric chromosome had an average total length of 6.8µ and a relative length of 3.6. There were no heterochromatic bands on this chromosome. This chromosome was about ¼ the size of the largest metacentric.

Chromosome 12: The smallest chromosome in group one was a small submetacentric one with an average total length of 6.0µ and a relative length of 3.2. There were no heterochromatic bands on this chromosome.

Chromosome 13: There was a large satellite on the short arm of this acrocentric chromosome. The short arm was relatively large. The average total length of the chromosome was 12.0µ and the relative length was 6.8. Giemsa staining did not reveal any heterochromatic regions on this chromosome.

Chromosome 14: This acrocentric chromosome carried a large satellite that was three times the size of the short arm. The average total length of the chromosome was 12.1µ and the relative length was 6.4. There were two heterochromatic bands on the long arm of the chromosome. A narrow band was in the proximal portion of the long arm about ⅓ of the distance along the arm, the other, broader band was at the middle of the arm.

Chromosome 15: No satellite was on this acrocentric chromosome which had an average total length of 11.9µ . The relative length was 6.3. The short arm of this chromosome was very large. No heterochromatic bands were located on this chromosome.

Chromosome 16: The satellite on this acrocentric chromosome was slightly larger than the short arm of the chromosome. The average relative length was 10.8µ and the relative length was 5.7. The heterochromatic bands on the long arm of this chromosome were near the middle of the arm. There was a narrow band just proximal to the middle of the arm and a broader band at the middle of the arm.

Chromosome 17: This acrocentric chromosome had a small satellite slightly larger than the small short arm and only half the size of the satellite on chromosome 16. This chromosome was 10.5µ in average total length with a relative length of 5.5. There was a single heterochromatic band on the long arm of the chromosome about ⅓ of the distance from the centromere to the distal end.

Chromosome 18: There was a very large satellite on this acrocentric chromosome. The short arm of the chromosome was also relatively large. The average total length of the chromosome was 10.4µ and the relative length was 5.5. There were no heterochromatic bands present.

Chromosome 19: No satellite was present on this chromosome. It had an average total length of 10.0µ and a relative length of 5.3. No heterochromatic bands were revealed on this chromosome.

Chromosome 20: The average total length of this acrocentric chromosome wa 9.6µ and the relative length was 5.0. There were no heterochromatic bands present.

Chromosome 21: This acrocentric chromosome had an average total length of 8.7µ and a relative length of 4.6. No heterochromatic bands were present on this chromosome.

Chromosome 22: There were no heterochromatic bands on this small acrocentric chromosome with an average total length of 7.9µ. The relative length of the chromosome was 4.2.

Chromosome 23: This acrocentric chromosome had an average total length of 7.5µ and a relative length of 4.0. There were no heterochromatic bands on this chromosome.

Chromosome 24: The smallest acrocentric chromosome had a tiny satellite on it's short arm. The average total length was 7.0µ and the relative length was 3.7.

A. `Mona Lisa'

The chromosome number of this cultivar was 2n=3x=24. Measurements were made of chromosomes in five cells of a single plant (Table 9.32). These measurements were used to describe the karyotype of this cultivar. Giemsa staining revealed only two chromosomes with clear heterochromatic bands for this cultivar. This may be due to the procedure used or may reflect a lack of heterochromatin in many chromosomes. Since most of the chromosomes in this cultivar revealed no heterochromatin further modification of the staining procedure will need to be made to determine if heterochromatic bands can be defined.

Individual measurements of chromosome total length, long arm length, short arm length and length of any satellites are provided in Table 9.32. The relative total length of the chromosomes was also included to facilitate the comparison of the chromosomes in this cultivar with those of the species.

The general karyotype of `Mona Lisa' is the same as that for the other cultivars. There were twelve chromosomes in the first group of the genome which were metacentric, submetacentric or subtelocentric, one of which was satellited. The second group consisted of twelve acrocentric chromosomes of which four carried satellites (Fig. 9.20).

There were seven metacentric chromosomes in the first group. These ranged in size from an average total length of 25.9µ to only 5.9µ. Their relative lengths were from 14.3 to 3.3. Five submetacentric chromosomes were also in the first group. These ranged form 14.6µ to 6.5µ in average total length and 8.0 to 3.6 in relative length. Chromosome number 11 had broad heterochromatic bands at the distal ends of each of its arms. The final chromosome in group one was a small subtelocentric which carried a satellite about half the size of the short arm. This subtelocentric was the same size as the smallest submetacentric and is one of the smallest chromosomes in the genome. It had a broad heterochromatic band at the distal end of the short arm and another broad band in the middle of the long arm.

The second group of twelve acrocentric chromosomes ranged in size from 13.0µ to 7.3µ. This was a relative length range of 7.2 to 4.0. Chromosome numbers 15, 16, 17 and 22 carried

Table 9.32. Average measurements of chromosomes of A. `Mona Lisa'.

Chromo- some	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	13.3	12.6		25.9	14.3	1.05	m
2	12.2	11.3		23.5	12.8	1.08	m
3	10.4	10.4		20.8	11.4	1.00	M
4	10.6	4.0		14.6	8.0	2.65	sm
5	7.4	6.5		13.9	7.6	1.14	m
6	6.5	5.3		11.8	6.5	1.23	m
7	8.1	3.1		11.2	6.2	2.61	sm
8	7.1	2.8		9.9	5.4	2.54	sm
9	4.1	3.4		7.5	4.1	1.20	m
10	5.4	1.8		7.2	4.0	3.00	sm
11	4.8	1.7		6.5	3.6	2.82	sm
12	5.6	0.9	0.5	6.5	3.6	6.22	st
13	3.1	2.8		5.9	3.3	1.11	m
14	12.9	0.2		13.0	7.2	61.33	t
15	11.9	0.6	0.4	12.5	6.8	19.83	t
16	11.5	0.7	0.1	12.2	6.7	16.43	t
17	10.0	0.6	1.0	10.6	5.8	16.67	t
18	9.6	0.3		9.9	5.4	32.00	t
19	9.0	0.1		9.1	5.0	90.00	t
20	8.4	0.7		9.1	5.0	12.00	t
21	7.3	1.0	0.1	8.3	4.6	7.30	t
22	7.8	0.3		8.1	4.4	26.00	t
23	6.9	0.4		7.3	4.0	17.25	t
24	6.7	0.6		7.3	4.0	11.17	t

*RL=Relative Length with a total genome length of 150%.
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

satellites. The satellite on chromosome 15 was slightly smaller than the short arm and the satellites on chromosomes 16 and 22 were much smaller than the short arm of the chromosomes. The satellite on chromosome 17 was larger than the short arm of the chromosome.

A. 'Monika'

The chromosome number of this cultivar was 2n=3x=24. The measurements of five cells of a single plant were used to describe the karyotype of the cultivar (Table 9.33). The karyotype consisted of twelve metacentric, submetacentric and subtelocentric chromosomes in group one of the genome of which one carried a small satellite. The second group of the genome had twelve acrocentric chromosomes of which four carried satellites (Fig. 9.21a). Giemsa staining resulted in heterochromatic bands on many of these chromosomes (Fig. 9.21b).

Chromosome 1: This large metacentric chromosome had an average total length of 25.6µ and a relative length of 13.1. There were no heterochromatic bands visible on this chromosome.

Chromosome 2: The second metacentric chromosome in the genome had an average total length of 23.3µ and a relative length of 11.9. There did not appear to be any heterochromatic bands on this chromosome.

Chromosome 3: There were no heterochromatic bands identified for this metacentric chromosome. It had an average total length of 22.0µ and a relative length of 11.2.

Chromosome 4: Heterochromatic bands were at the distal ends of both chromosome arms. This metacentric chromosome had an average total length of 16.0µ and so was much smaller than the first three metacentrics. It had a relative length of 8.2.

Chromosome 5: The largest submetacentric chromosome of the genome had an average total length of 15.9µ and a relative length of 8.1. There were heterochromatic bands on the distal half of the short arm as well as a narrower band at the distal end of the long arm. A broad band covered about ¼ of the length of the long arm from the middle towards the distal end.

Chromosome 6: This metacentric chromosome had an average total length of 12.6µ and a relative length of 6.4. There were faint heterochromatic spots at the centromere and at the distal end of the shorter arm.

Chromosome 7: The second submetacentric chromosome had an average total length of 11.3µ and a relative length of 5.7. There was a heterochromatic band at the distal end of the short arm and another heterochromatic region near the centromere.

Table 9.33. Average measurements of chromosomes of *A.* 'Monika'.

Chromosome	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	13.3	12.3		25.6	13.1	1.07	m
2	11.8	11.5		23.3	11.9	1.02	m
3	11.4	10.6		22.0	11.2	1.08	m
4	8.8	7.2		16.0	8.2	1.22	m
5	11.4	4.5		15.9	8.1	2.53	sm
6	6.9	5.7		12.6	6.4	1.21	m
7	8.4	2.9		11.3	5.7	2.90	sm
8	5.6	5.3		10.9	5.6	1.06	m
9	5.0	4.3		9.3	4.7	1.16	m
10	5.8	2.2		8.0	4.1	2.64	sm
11	5.6	1.6	0.3	7.3	3.7	3.50	st
12	3.6	3.2		6.8	3.5	1.13	m
13	13.9	1.0		14.9	7.6	13.90	t
14	11.9	0.2		12.1	6.2	59.50	t
15	11.8	0.3		12.1	6.2	39.33	t
16	11.2	0.5	0.6	11.7	6.0	22.40	t
17	11.0	0.2	0.3	11.3	5.7	55.00	t
18	9.8	0.2		10.0	5.1	49.00	t
19	9.7	0.2	0.3	9.9	5.0	48.50	t
20	9.3	0.4		9.7	4.9	23.25	t
21	8.4	0.6	0.3	9.0	4.6	14.00	t
22	7.5	0.4		7.9	4.0	18.75	t
23	6.9	0.6		7.4	3.8	11.50	t
24	6.1	0.6		6.7	3.4	10.17	t

*RL=Relative Length with a total genome length of 150%.
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

Chromosome 8: This was a metacentric chromosome with an average total length of 10.9µ and a relative length of 5.6. There was a heterochromatic band at the distal end of the longer arm and faint heterochromatic spots near the centromere.

Chromosome 9: No heterochromatic bands were present on this chromosome. The average total length was 9.3µ and the relative length was 4.7.

Chromosome 10: This was the third submetacentric chromosome in the genome and had an average total length of 8.0µ. The short arm had a broad heterochromatic band at the distal end covering most of the arm. A second narrower band was at the distal end of the long arm.

Chromosome 11: This was the only chromosome in the first group of the genome to carry a satellite. The satellite was much smaller than the short arm. The average total length of this subtelocentric chromosome was 7.3µ and the relative length was 3.7. The entire short arm appeared to be heterochromatic.

Chromosome 12: The smallest chromosome in group one was a metacentric chromosome with an average total length of 6.8µ and a relative length of 3.5. There were heterochromatic bands at the distal ends of both chromosome arms.

Chromosome 13: The largest acrocentric chromosome had a relatively large short arm. It had an average total length of 14.9µ and a relative length of 7.6. There appeared to be a heterochromatic band on the proximal end of the short arm of the chromosome.

Chromosome 14: This acrocentric chromosome had a small short arm and an average total length of 12.1µ. The relative length was 6.2. There was a narrow heterochromatic band at the proximal end of the chromosome about one third of the distance along the chromosome.

Chromosome 15: This chromosome was about the same size as chromosome 14. It was an acrocentric with an average total length of 12.1µ and a relative length of 6.2. No heterochromatic areas were identified for this chromosome.

Chromosome 16: This acrocentric chromosome carried the largest satellite in the whole genome. The satellite was about the same size as the short arm of this chromosome. The average total length of the chromosome was 11.7µ and the relative length was 6.0. There was a broad heterochromatic band in the middle of the long arm of the chromosome and a narrow band at the proximal end next to the centromere. The satellite had small heterochromatic dots.

Chromosome 17: The satellite on this acrocentric chromosome was slightly larger than the small short arm. It had an average total length of 11.3µ and a relative length of 5.7. There was a broad heterochromatic band at the proximal end of the chromosome long arm covering about one third of its length.

Chromosome 18: This acrocentric chromosome did not have a satellite. The average total length was 10.0µ and the relative length was 5.1. There was a narrow heterochromatic band at the distal end of the long arm as well as a broad band from the middle of the arm towards the distal end.

Chromosome 19: The average total length of this acrocentric chromosome was 9.9µ and it's relative length was 5.0. It had a small satellite just larger than the short arm. There was a broad heterochromatic band at the distal end of the long arm of the chromosome starting at the middle and a narrower band proximal to this.

Chromosome 20: This acrocentric chromosome did not carry a satellite. It had an average total length of 9.7µ and a relative length of 4.9. There was a heterochromatic band at the distal end of the long arm of this chromosome.

Chromosome 21: A narrow heterochromatic band was near the distal end of the long arm of this chromosome and another fainter band was near the middle of the arm.The chromosome was acrocentric and had an average total length of 9.0µ and a relative length of 4.6. The satellite was about one half the size of the short arm.

Chromosomes 22-24: These were all acrocentric chromosomes with no satellites. They varied from 7.9 - 6.7µ in average total length and 4.0-3.4 in relative length. There were no heterochromatic bands visible on any of these chromosomes.

A. `Rosita'

The chromosome number of this cultivar was 2n=3x=24. Measurements were made of the chromosomes in five cells of a single plant. These were then averaged to provide average values for chromosome length, arm lengths and satellite size (Table 9.34). These average measurements were used to describe the karyotype of the plant. There were twelve metacentric, submetacentric and subtelocentric chromosomes in the first group of the genome and twelve acrocentric chromosomes in the second group. Four of the acrocentric chromosomes carried a satellite (Fig. 9.22a). Some of the chromosomes of this cultivar had bands which stained with Giemsa dye (Fig. 9.22b).

Table 9.34. Average measurements of chromosomes of A. `Rosita'.

Chromo-some	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	12.3	11.9		24.2	13.5	1.04	m
2	11.2	10.3		21.5	12.0	1.09	m
3	8.6	6.6		15.4	8.6	1.30	m
4	11.8	3.4		15.2	8.5	3.47	st
5	8.8	3.5		12.3	6.9	2.51	sm
6	6.6	5.0		11.6	6.5	1.32	m
7	5.4	5.0		10.4	5.8	1.08	m
8	7.0	2.6		9.7	5.4	2.69	sm
9	4.8	3.7		8.5	4.7	1.30	m
10	6.1	2.2		8.3	4.6	2.77	sm
11	3.3	3.2		6.5	3.6	1.03	m
12	4.5	1.4	0.6	5.9	3.3	3.21	st
13	11.2	0.4	0.7	11.6	6.5	28.00	t
14	10.8	0.4		11.2	6.2	27.00	t
15	11.0	0.1	0.3	11.1	6.2	110.00	t
16	9.8	0.4	0.2	10.3	5.8	24.50	t
17	9.8	0.3		10.1	5.7	32.67	t
18	9.7	0.3		10.0	5.6	32.33	t
19	9.0	0.7		9.7	5.4	12.86	t
20	9.4	0.2		9.6	5.4	47.00	t
21	8.8	0.4	0.4	9.2	5.1	22.00	t
22	7.7	0.7		8.4	4.6	11.00	t
23	7.2	0.5		7.7	4.3	14.40	t
24	6.6	0.4		7.0	3.9	16.50	t

*RL=Relative Length with a total genome length of 150%.
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

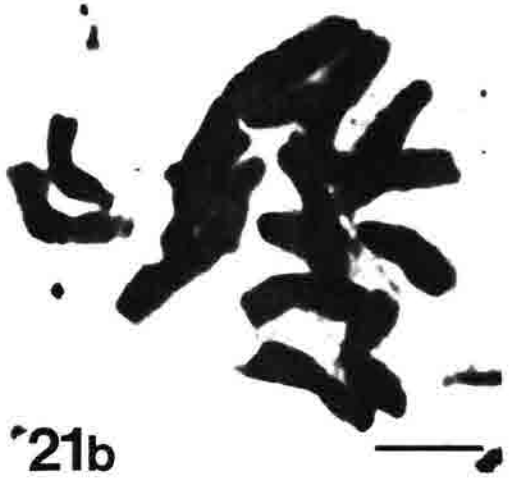
Figure 9.21a. A `Monika' - acetocarmine stain b. A `Monika' - Giemsa stain
Smallest subtelocentric chromo- Banding pattern for 15
some is missing. chromosomes only.

Figure 9.22a. A `Rosita - acetocarmine stain b. A `Rosita' - Giemsa stain

Figure 9.23a. A `Carmen' - aacetocarmine stain b. A` Carmen' - Giemsa stain

Bar = 10µ

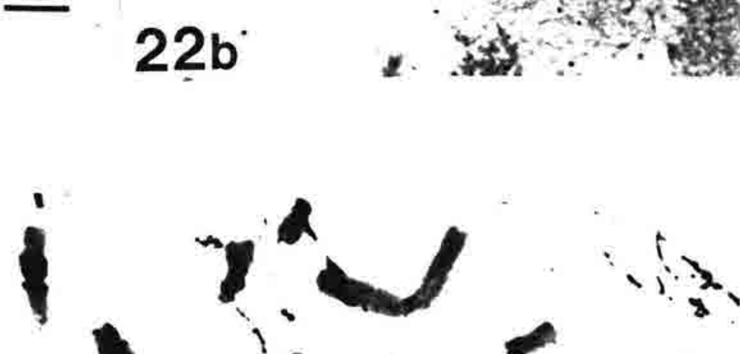

21a


21b


22a


22b


23a


23b

Chromosome 1: This was a metacentric chromosome with an average total length of 24.1µ and a relative length of 13.5. The primary constriction was in the median region close to the median point. There were no heterochromatic bands on this chromosome.

Chromosome 2: This metacentric chromosome was only slightly smaller than the first chromosome with an average total length of 21.5µ. It's relative length was 12.0. No heterochromatic bands were located on this chromosome.

Chromosome 3: This was a much smaller metacentric chromosome less than ⅔ the size of the first chromosome. It had an average total length of 15.4µ and a relative length of 8.6. There were no heterochromatic bands revealed on this chromosome.

Chromosome 4: The largest subtelocentric chromosome in the genome had an average total length of 15.2µ, similar in size to chromosome 3. The relative length was 8.5. This chromosome had a heterochromatic band in the middle of the long arm.

Chromosome 5: The first submetacentric chromosome appeared to have no heterochromatic bands. It had an average total length of 12.3µ and a relative length of 6.9.

Chromosome 6: The next metacentric chromosome had an average total length of 11.6µ and a relative length of 6.5. There were no heterochromatic bands on this chromosome.

Chromosome 7: This metacentric chromosome had it's primary constriction very close to the median point. It had an average total length of 10.4µ and a relative length of 5.8. There were heterochromatic bands at the distal ends of both arms of the chromosome.

Chromosome 8: The second submetacentric chromosome had an average total length of 9.7µ and a relative length of 5.4. The distal half of the short arm was entirely heterochromatic. There was a narrow heterochromatic band at the distal end of the long arm of the chromosome and a broad band covering about a third of the long arm from the middle towards the distal end.

Chromosome 9: This metacentric chromosome also had no heterochromatic bands. It had an average total length of 8.5µ and a relative length of 4.7.

Chromosome 10: The smallest submetacentric chromosome in the genome had an average total length of 8.3µ and a relative length of 4.6. No heterochromatic bands were visible on this chromosome.

Chromosome 11: This small metacentric chromosome did not have any heterochromatic bands. It had an average total length of 6.5µ, about ¼ the size of the largest metacentric. The relative length was 3.6.

Chromosome 12: The smallest chromosome in group one was a subtelocentric chromosome with a large satellite. The satellite was heterochromatic. The average total length of this chromosome was 5.9µ and the relative length was 3.3.

Chromosome 13: This was an acrocentric chromosome with two heterochromatic bands. A narrow band was just proximal to the middle of the long arm of the chromosome and a broad band was just distal to it. The average total length of this chromosome was 11.6µ and the relative length was 6.5. There was a large satellite which was almost twice the size of the short arm.

Chromosome 14: The average total length of this acrocentric chromosome was 11.2µ and the relative length was 6.2. There was no satellite on this chromosome. There was a single narrow heterochromatic band about a third of the distance along the chromosome long arm from the centromere.

Chromosome 15: The second acrocentric chromosome with a satellite had an average total length of 11.1µ and so was quite similar in size to chromosome 14. It had a satellite which was about three times the size of the short arm of the chromosome. There was a broad heterochromatic band in the middle of the long arm of the chromosome covering about a quarter of the arms length. The relative length of this chromosome was 6.2.

Chromosome 16: The satellite on this acrocentric chromosome was about half the size of the short arm. The average total length of the chromosome was 10.3µ and the relative length was 5.8. There was a narrow heterochromatic band just proximal to the middle of the chromosome and a broad band distal to the middle.

Chromosomes 17-24: None of these acrocentric chromosomes had any heterochromatic bands using this technique. They ranged in size from 10.1-7.0µ. The relative lengths were from 5.7-3.9. Chromosome number 21 had a small satellite the same size as it's short arm. Chromosomes number 19, 22 and 23 had relatively large short arms.

A. `Carmen'

The chromosome number of this cultivar was 2n=3x=24. It had the same basic karyotype as the other cultivars studied. The averages of measurements made of the chromosomes in five cells of a single plant (Table 9.35) were used to describe the karyotype. There were twelve metacentric, submetacentric and subtelocentric chromosomes in the first group of the genome and twelve acrocentric chromosomes, one of which carried a satellite, in the second group (Fig.

23a). Many of these chromosomes had areas which stained with Giemsa dye to reveal the location of heterochromatin (Fig. 23b).

Chromosome 1: This was a large metacentric with an average total length of 25.5µ and a relative length of 13.0. There were no heterochromatic bands on this chromosome.

Chromosome 2: The second metacentric chromosome was similar in size to the first at an average total length of 24.0µ and a relative length of 12.2. It also had no heterochromatic bands.

Chromosome 3: This was very close in size to the other two metacentrics. It had an average total length of 22.9µ and a relative length of 11.7. There were no distinct heterochromatic bands on this chromosome.

Chromosome 4: The heterochromatic bands on this chromosome were very distinctive. They were at the distal end of each arm of the chromosome. The chromosome is metacentric with an average total length of 16.9µ and a relative length of 8.6.

Chromosome 5: This subtelocentric chromosome had an average total length of 15.0µ and a relative length of 7.7. The distal half of the short arm was heterochromatic as was a small band at the distal end of the long arm of the chromosome. There was a broad band from the middle of the long arm towards the distal end of the arm.

Chromosome 6: The largest submetacentric chromosome in the genome had an average total length of 13.6µ and a relative length of 6.9. There were narrow heterochromatic bands at the proximal ends of both arms of the chromosome next to the centromere.

Chromosome 7: This metacentric chromosome had an average total length of 11.8µ and a relative length of 6.0. Heterochromatic banding was not determined for this chromosome.

Chromosome 8: The next submetacentric chromosome in the genome had an average total length of 9.8µ and a relative length of 5.0. There were faint heterochromatic bands on the proximal end of the short arm of the chromosome and possibly on the proximal end of the long arm.

Chromosome 9: Metacentric chromosome 9 had a heterochromatic band at the distal end of the long arm of the chromosome. The average total length of this chromosome was 8.8µ and the relative length was 4.5.

Chromosome 10: The smallest metacentric chromosome was also the smallest chromosome in the genome. It had an average total length of 6.5µ and a relative length of 3.3. There were no heterochromatic bands on this chromosome.

Table 9.35. Average measurements of chromosomes of *A.* `Carmen'.

Chromosome	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	13.2	12.3		25.5	13.0	1.07	m
2	12.4	11.6		24.0	12.2	1.07	m
3	12.2	10.7		22.9	11.7	1.14	m
4	9.1	7.8		16.9	8.6	1.16	m
5	11.5	3.5		15.0	7.7	3.29	st
6	9.4	4.2		13.6	6.9	2.23	sm
7	6.4	5.4		11.8	6.0	1.18	m
8	6.3	3.5		9.8	5.0	1.80	sm
9	4.8	4.0		8.8	4.5	1.19	m
10	3.3	3.2		6.5	3.3	1.05	m
11	4.1	1.9		6.0	3.0	2.16	sm
12	4.2	1.3		5.5	2.8	3.23	st
13	14.1	0.7		14.8	7.5	20.14	t
14	12.8	0.2	0.5	13.0	6.7	64.00	t
15	12.3	0.5	0.5	12.8	6.5	24.60	t
16	11.5	0.5	0.4	12.0	6.1	23.00	t
17	11.0	0.7		11.7	6.0	15.71	t
18	10.8	0.6		11.4	5.8	18.00	t
19	10.1	0.6	0.5	10.7	5.5	16.83	t
20	9.4	0.2		9.6	4.9	47.00	t
21	8.4	0.6		9.0	4.6	14.00	t
22	7.5	0.5		8.0	4.0	15.00	t
23	7.1	0.3	0.3	7.4	3.7	23.67	t
24	6.3	0.7		7.0	3.6	9.00	t

*RL=Relative Length with a total genome length of 150%.
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

Chromosome 11: The third submetacentric chromosome did not appear to have any heterochromatic bands. The average total length of the chromosome was 6.0µ and the relative length was 3.0.

Chromosome 12: This small metacentric chromosome had an average total length of 5.5µ, similar in size to chromosome 11. It's relative length was 2.8. There were heterochromatic dots at the centromere, probably on the proximal end of the short arm.

Chromosome 13: The largest acrocentric chromosome had an average total length of 14.8µ and a relative length of 7.5. No heterochromatic bands were identified for this chromosome.

Chromosome 14: This acrocentric chromosome had a satellite which was about twice as large as the short arm. It had an average total length of 13.0µ and a relative length of 6.7. There was a narrow heterochromatic band near the middle of the long arm of the chromosome and a broad band distal to this. Heterochromatic dots were on the satellite.

Chromosome 15: There was very little size difference between this chromosome and chromosome 14. This acrocentric chromosome also carried a satellite on its short arm which was about the same size as the arm. The average total length of this chromosome was 12.8µ and the relative length was 6.5. There were two heterochromatic dots at the middle of the long arm of the chromosome and a fainter pair of dots just distal to this.

Chromosome 16: The satellite on this acrocentric chromosome was a little smaller than the short arm. The chromosome had an average total length of 12.0µ and a relative length of 6.1. The satellite was heterochromatic and there was a narrow heterochromatic band proximal to the middle of the long arm with a broad band covering the middle third of the arm.

Chromosome 17: There was no satellite on this acrocentric chromosome. It had an average total length of 11.7µ and a relative length of 6.0. This chromosome had a large short arm. There were heterochromatic dots at the distal end of the short arm. A narrow heterochromatic band was at about the middle of the long arm with a broad band distal to this and covering about ¼ of the length of the arm.

Chromosome 18: Chromosome 18 also had a relatively large short arm. It had an average total length of 11.4µ and a relative length of 5.8. Faint heterochromatic spots were near the middle of the long arm of the chromosome but no organized bands were observed.

Chromosome 19: The satellite on this chromosome was a little smaller than the large short arm. This acrocentric chromosome had an average total length of 10.7µ and a relative length of 5.5. There were heterochromatic dots at the distal end of the short arm of the chromosome as well as at the proximal end of the long arm next to the centromere. A band of heterochromatin was just proximal to the middle of the long arm.

Chromosomes 20-22: None of these three acrocentric chromosomes appeared to have any heterochromatic regions. They varied from 9.6-8.0µ in average total length and from 4.9-4.0 in relative length.

Chromosome 23: This acrocentric chromosome had a satellite the same size as the short arm. It had an average total length of 7.4µ and a relative length of 3.7. There was a narrow heterochromatic band at the middle of the long arm of this chromosome.

Chromosome 24: The smallest acrocentric chromosome had an average total length of 7.0µ and a relative length of 3.6. The short arm of this chromosome was relatively large. It was entirely heterochromatic. There was a narrow heterochromatic band near the proximal end of the long arm of the chromosome and another faint band towards the distal end.

A. `Red Surprise'

The number of chromosomes in this cultivar was 2n=3x=24. The average measurements of the chromosomes of five cells of a single plant (Table 9.36) were used to describe the karyotype of this plant. This cultivar had twelve metacentric, submetacentric and subtelocentric chromosomes in group one and twelve acrocentric chromosomes in group two. Eight of the acrocentric chromosomes had satellites (Fig. 9.24a). Many of these chromosomes had heterochromatic bands (Fig. 9.24b).

Chromosome 1: The largest metacentric chromosome had an average total length of 20.7µ and a relative length of 13.6. There was a heterochromatic band near the middle of the longer arm of the chromosome with a broader band distal to this. A faint heterochromatic band was also near the centromere.

Chromosome 2: This metacentric chromosome was almost the same size as chromosome 1. It had an average total length of 19.7µ and a relative length of 12.6. There were no heterochromatic bands visible on this chromosome.

Chromosome 3: There was a heterochromatic band near the end of the shorter arm of this metacentric chromosome. The average total length of this chromosome was 15.8µ and the relative length was 10.4.

Chromosome 4: This metacentric chromosome was almost the same size as chromosome 3. It had an average total length of 15.7µ and a relative length of 10.4. There was a faint

Table 9.36. Average measurements of chromosomes of *A.* `Red Surprise'.

Chromosome	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	11.4	9.3		20.7	13.6	1.22	m
2	10.8	8.9		19.7	12.6	1.22	m
3	9.1	6.7		15.8	10.4	1.03	m
4	8.9	6.8		15.7	10.4	1.31	m
5	6.3	5.7		11.9	7.9	1.10	m
6	6.9	4.5		11.4	7.5	1.51	m
7	4.5	3.7		8.2	5.4	1.20	m
8	5.2	2.6		7.8	5.2	2.00	sm
9	3.3	2.8		6.1	4.0	1.17	m
10	4.4	1.4		5.8	3.8	3.14	st
11	3.1	2.3		5.4	3.6	1.35	m
12	3.4	1.1		4.5	2.9	3.10	st
13	9.5	0.6	0.5	10.1	6.7	15.83	t
14	8.8	0.6	0.7	9.4	6.2	14.80	t
15	8.4	0.4	0.3	8.8	5.8	21.00	t
16	8.1	0.2	0.2	8.3	5.5	40.50	t
17	7.9	0.4	0.4	8.3	5.5	19.75	t
18	7.7	0.2		7.9	5.2	38.50	t
19	7.3	0.4		7.7	5.1	18.25	t
20	7.0	0.2	0.1	7.1	4.7	35.00	t
21	6.6	0.4		7.0	4.7	16.50	t
22	6.4	0.3		6.7	4.4	21.33	t
23	5.8	0.4	0.4	6.2	4.1	14.50	t
24	5.0	0.5	0.3	5.5	3.6	10.00	t

*RL=Relative Length with a total genome length of 150%.
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

heterochromatic band near the centromere of this chromosome but no other bands were present.

Chromosome 5: The next metacentric chromosome had an average total length of 11.9µ and a relative length of 7.9. There was a heterochromatic band at the distal end of the shorter arm of this chromosome.

Chromosome 6: This was a metacentric chromosome with an average total length of 6.9µ and a relative length of 7.5. There was a narrow heterochromatic band near the distal end of the longer arm.

Chromosome 7: The next metacentric chromosome had an average total length of 8.2µ and a relative length of 5.4. There was a heterochromatic band at the distal ends of both arms of the chromosome.

Chromosome 8: This submetacentric chromosome had three heterochromatic regions. There was a band at the distal end of the long arm of the chromosome and a broad band covering about half the length of the arm. Almost all of the short arm was heterochromatic.

Chromosome 9: This metacentric chromosome had an average total length of 6.1µ and a relative length of 4.0. There were no heterochromatic bands on this chromosome.

Chromosome 10: Subtelocentric chromosome 10 had an average total length of 5.8µ and a relative length of 3.8. There were two thin bands at the distal end of the long arm of this chromosome.

Chromosome 11: The smallest metacentric chromosome had an average total length of 5.4µ and a relative length of 3.6. There were no heterochromatic bands visible on this chromosome.

Chromosome 12: The smallest chromosome in the first group had a thin heterochromatic band at the centromere. It had an average total length of 4.5µ and a relative length of 2.9.

Chromosome 13: The largest acrocentric chromosome had a satellite that was about the same size as the short arm. The average total length of this chromosome was 10.1µ and the relative length was 6.7. There was a broad heterochromatic band from the middle of the long arm of the chromosome which covered about ⅓ of the length of the arm.

Chromosome 14: This acrocentric chromosome had a large satellite, about the same size as the short arm. The average total length of this chromosome was 9.4µ and the relative length was 6.2. The satellite was entirely heterochromatic and there was another heterochromatic band about ⅓ of the distance along the length of the chromosome towards the distal end.

Chromosome 15: The third acrocentric chromosome had a satellite about the size as the short arm. There were no heterochromatic bands on this chromosome. The average total length of this chromosome was 8.8µ and the relative length was 5.8.

Chromosome 16: The satellite on this acrocentric chromosome was the same size as the small short arm. No heterochromatic bands were revealed on this chromosome. It had an average total length of 8.3µ and a relative length of 5.5.

Chromosome 17: This acrocentric chromosome also had a satellite the same size as the short arm. It had an average total length of 8.3µ, the same size as chromosome 16. The relative length of this chromosome was 5.5. There was a thin heterochromatic band just proximal to the middle of the long arm of the chromosome and a broader band distal to this which covered about ⅓ of the length of the chromosome.

Chromosome 18: There was no satellite on this acrocentric chromosome. It had an average total length of 7.9µ and a relative length of 5.2. There was no heterochromatic banding on this chromosome.

Chromosome 19: The average total length of this acrocentric chromosome was 7.7µ and the relative length was 5.1. There were two narrow heterochromatic bands close together near the middle of the long arm of the chromosome.

Chromosome 20: There was a tiny satellite about half the size of the short arm on this acrocentric chromosome. The average total length was 7.1µ and the relative length was 4.7. No heterochromatic bands were visible on this chromosome.

Chromosome 21: This acrocentric had no satellite and was about the same size as chromosome 20 with an average total length of 7.0µ. The relative length of this chromosome was 4.7. There were no heterochromatic bands present on this chromosome.

Chromosome 22-24: The last three acrocentric chromosomes in group two did not appear to have any heterochromatic bands. These chromosomes ranged in size from 6.7-5.5µ, with relative lengths of 4.4 -3.6. Chromosome 23 had a satellite the same size as the short arm of the chromosome and chromosome 24 had a satellite slightly smaller than it's short arm.

A. `Orange Beauty'

This cultivar was a hypertriploid with a chromosome number of 2n=3x+1 =25. Measurements of the chromosomes of five cells of a single plant (Table 9.37) were used to

Table 9.37. Average measurements of chromosomes of A. `Orange Beauty'.

Chromosome	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	14.5	13.3		27.8	12.8	1.09	m
2	13.6	12.7		26.3	12.1	1.07	m
3	12.3	11.4		23.7	10.9	1.08	m
4	11.6	6.9		18.5	8.5	1.68	m
5	13.3	3.8		17.1	7.9	3.50	st
6	7.3	5.8	0.6	13.1	6.0	1.26	m
7	9.9	2.6		12.5	5.8	3.80	st
8	6.3	5.4		11.7	5.4	1.09	m
9	5.6	4.8		10.4	4.8	1.17	m
10	7.9	2.5		10.4	4.8	3.20	st
11	5.0	1.7	0.3	6.7	3.1	2.90	sm
12	3.3	3.2		6.5	3.0	1.03	m
13	15.2	0.2	0.1	15.4	7.1	76.00	t
14	13.8	0.2	0.4	14.0	6.4	69.00	t
15	13.1	0.1	0.3	13.2	6.1	131.00	t
16	12.5	0.4		12.9	5.9	31.25	t
17	12.1	0.3	0.5	12.4	5.7	40.33	t
18	11.7	0.3	0.3	12.0	5.5	39.00	t
19	11.6	0.2	0.4	11.8	5.4	58.00	t
20	10.6	0.3	0.1	10.9	5.0	35.33	t
21	10.6	0.2		10.8	5.0	53.00	t
22	10.0	0.2	0.3	10.2	4.7	50.00	t
23	9.4	0.4		9.8	4.5	23.50	t
24	8.1	0.4		8.5	3.9	20.25	t
25	7.4	0.2	0.1	7.6	3.5	37.00	t

*RL=Relative Length
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

Figure 9.24a. A. `Red Surprise' - acetocarmine stain

b. A. `Red Surprise' - Giemsa stain

Figure 9.24a. A. `Orange Beauty' - acetocarmine stain

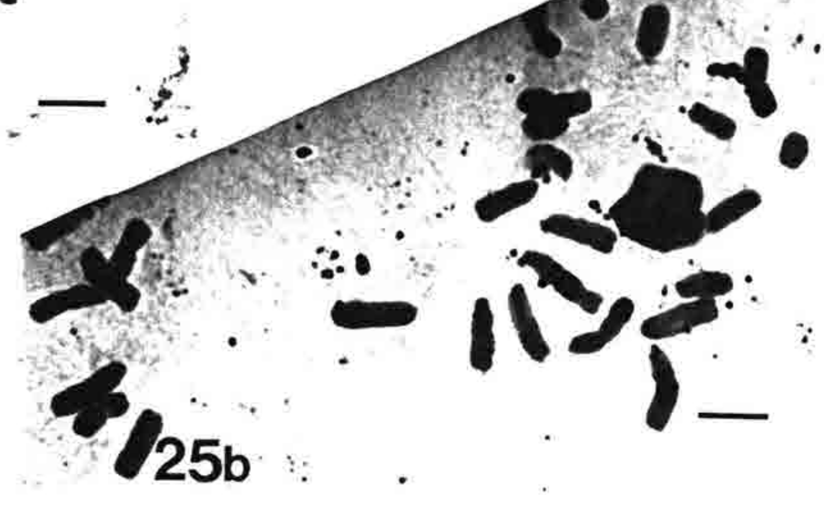
b. A. `Orange Beauty' - Giemsa stain

Bar = 10µ


24a


24 b


25a


25b

describe the karyotype of this plant. The karyotype consisted of twelve chromosomes in group one which were metacentric, submetacentric and subtelocentric in shape. The second group contained thirteen acrocentric chromosomes of which nine carried satellites (Fig. 9.25a). Some of these chromosomes revealed heterochromatic bands when stained with Giemsa dye (Fig. 9.25b). The general karyotype of this cultivar was essentially the same as for the other triploid cultivars with the exception that there was an extra chromosome in the second group of the genome.

Chromosome 1: This was a large metacentric chromosome with an average total length of 27.8μ and a relative length of 12.8. There were no heterochromatic bands on this chromosome.

Chromosome 2: The second metacentric chromosome was almost the same size as the first with an average total length of 26.3μ. The relative length of this chromosome was 12.1. The primary constriction was in the median region close to the median point. There were no heterochromatic bands on this chromosome.

Chromosome 3: This chromosome also did not appear to have any heterochromatic bands present. It had an average total length of 23.7μ and a relative length of 10.9.

Chromosome 4: This chromosome had a ratio which was very close to being submetacentric. The average total length was 18.5μ and the relative length was 8.5. There was a narrow heterochromatic band at the distal end of the shorter arm of the chromosome.

Chromosome 5: This was a subtelocentric chromosome with an average total length of 17.1μ and a relative length of 7.9. A narrow heterochromatic band was at the middle of the long arm of the chromosome with a broader band distal to this covering about ¼ of the length of the arm.

Chromosome 6: The next metacentric chromosome had a satellite at the end of the short arm. It had an average total length of 13.1μ and a relative length of 6.0. There was a heterochromatic band at the centromere and another band at the distal end of the longer arm. A faint band was sometimes visible at the distal end of the shorter arm.

Chromosome 7: This subtelocentric chromosome had an average total length of 12.5μ and a relative length of 5.8. The short arm was almost entirely heterochromatic.

Chromosome 8: Metacentric chromosome 8 had wide heterochromatic bands at the distal ends of both arms of the chromosome. It had an average total length of 11.7μ and a relative length of 5.4.

Chromosome 9: This was also a metacentric chromosome with an average total length of 10.4μ and a relative length of 4.8. It had a broad heterochromatic band covering the distal half of the shorter arm and a narrow band at the distal end of the longer arm.

Chromosome 10: The smallest subtelocentric chromosome had an average total length of 10.4µ. the same size as chromosome 9. It had a relative length of 4.8. There was a broad heterochromatic band at the distal end of the short arm of the chromosome and a narrow band at the distal end of the long arm. A broad band was at the middle of the long arm and covered about half the length of the arm.

Chromosome 11: There was a small satellite on the short arm of submetacentric chromosome 11. The satellite appeared to be heterochromatic and there was a narrow heterochromatic band at the centromere. The average total length was 6.7µ and the relative length was 3.1.

Chromosome 12: This tiny metacentric chromosome had an average total length of 6.5µ and a relative length of 3.2, close to that of chromosome 11. There was a heterochromatic band near the centromere of this chromosome.

Chromosome 13: The largest acrocentric chromosome had an average total length of 15.4µ. This chromosome carried a small satellite about half the size of the short arm and had a relative length of 7.1. There was a narrow heterochromatic band just proximal to the middle of the long arm and a broad band distal to this.

Chromosome 14: This acrocentric chromosome had a satellite about twice the size of the short arm. It had an average total length of 14.0µ and a relative length of 6.4. The heterochromatic bands were similar to those of chromosome 13 with a narrow band just proximal to the middle of the chromosome and a broad band distal to the first.

Chromosome 15: The satellite on this chromosome was also much larger than the short arm of the chromosome. The average total length was 13.2µ and the relative length was 6.1. There was a narrow heterochromatic band proximal to the middle of the long arm of the chromosome and a broad band distal to this.

Chromosome 16: This was one of only four acrocentric chromosomes in this genome that did not carry a satellite. It had an average total length of 12.9µ and a relative length of 5.9. There was a narrow heterochromatic band about a third of the distance along the long arm of the chromosome from the centromere to the distal end.

Chromosome 17: There was a relatively large satellite on this chromosome which was larger than the short arm. The average total length of the chromosome was 12.4µ and the relative length was 5.7. It was not much different in size to chromosome 16. The heterochromatic band on this chromosome was also in a similar location to that of chromosome 16. It was a narrow band located about one third of the distance along the long arm from the centromere to the distal end.

Chromosome 18: This acrocentric chromosome carried a satellite the same size as the short arm. It had an average total length of 12.0μ and a relative length of 5.5. There was a narrow heterochromatic band about ⅓ of the distance along the long arm from the centromere and a broad band distal to this.

Chromosome 19-24: The last six acrocentric chromosomes did not appear to have any heterochromatic regions. They ranged in average total length from 11.8- 7.6μ with a range in relative length of 5.4-3.4. Chromosomes 19 and 22 carried satellites which were larger than their short arms while chromosomes 20 and 25 had satellites which were smaller than their short arms.

Tetraploid cultivars

None of the eight tetraploid or near tetraploid cultivars revealed any heterochromatic areas when stained with Giemsa dye. Although a number of different attempts were made only an occasional heterochromatic dot or series of dots could be discerned. Since there was no definitive and consistent heterochromatic staining of these cultivars only a general description of the chromosome size and shape will be presented.

Measurements were made of chromosomes in five cells of a single plant for each cultivar. The average total length, long arm length, short arm length and length of any satellites was determined. These average measurements were used to describe the karyotype of each of the cultivars.

The relative length of the chromosomes for these cultivars was determined by multiplying the measured average length by a conversion factor. This conversion factor was determined by adding the lengths of all chromosomes in the genome and dividing this into 200, since there were essentially two genomes in each cell as compared to the diploid cultivars and species.

A. `Jubilee'

This cultivar had a chromosome number of 2n=4x=32. Average measurements (Table 9.38) revealed that there were sixteen chromosomes in group one of the genome. This group consisted of ten metacentric, four subtelocentric and two submetacentric chromosomes. Group two of the genome consisted of sixteen acrocentric chromosomes of which two carried satellites (Fig. 26). A representative cell is depicted in Fig. 9.26.

Table 9.38. Average measurements of chromosomes of *A.* `Jubilee'.

Chromo-some	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	13.0	9.9		22.9	15.1	1.32	m
2	11.4	10.8		22.2	14.7	1.05	m
3	10.7	10.3		21.0	13.9	1.04	m
4	10.4	9.0		19.4	12.8	1.15	m
5	10.4	2.7		13.1	8.7	3.85	st
6	8.9	2.7		11.6	7.6	3.30	st
7	6.5	4.6		11.1	7.4	1.41	m
8	5.1	3.9		9.0	6.0	1.31	m
9	4.0	3.3		7.3	4.8	1.21	m
10	3.2	2.8		6.0	4.0	1.14	m
11	4.8	1.1		5.9	3.9	4.36	st
12	2.9	2.6		5.5	3.6	1.11	m
13	3.8	1.6		5.4	3.6	2.38	sm
14	4.1	1.3		5.4	3.6	3.15	st
15	2.3	2.2		4.5	2.9	1.04	m
16	3.0	1.0		4.0	2.6	3.00	sm
17	11.3	0.2		11.5	7.6	56.50	t
18	9.9	0.4		10.3	6.9	24.75	t
19	9.3	0.3		9.6	6.4	31.00	t
20	8.8	0.2	0.3	9.0	5.9	44.00	t
21	8.6	0.4		9.0	5.9	21.50	t
22	8.4	0.1	0.3	8.5	5.6	84.00	t
23	8.4	0.1		8.5	5.6	84.00	t
24	8.0	0.4		8.4	5.6	20.00	t
25	7.8	0.4		8.2	5.4	19.50	t
26	7.2	0.3		7.5	5.0	24.00	t
27	6.9	0.6		7.5	5.0	11.50	t
28	6.9	0.3		7.2	4.8	23.00	t
29	6.7	0.4		7.1	4.7	16.75	t
30	6.3	0.3		6.6	4.4	21.00	t
31	5.5	0.2		5.7	3.8	27.50	t
32	5.0	0.3		5.3	3.5	16.67	t

*RL=Relative Length with a total genome length of 200%.
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

The largest metacentric chromosomes ranged in average length from 22.9 to 19.4µ (relative lengths 15.1 to 12.8). The next two chromosomes were subtelocentric and were 13.1µ and 11.6µ in average total length. The next four chromosomes were metacentric in shape and were from 11.1µ to 6.0µ in average total length. The third subtelocentric chromosome was 5.9µ in length, very similar to the metacentric before it. The following three chromosomes were all about the same size, 5.5µ or 5.4µ, although they had different shapes. The last two chromosomes in group one were tiny with one being a metacentric with an average total length of 4.5µ and the other being a submetacentric with an average total length of 4.0µ.

The acrocentric chromosomes in this genome ranged from 11.5-5.3µ in average total length. This was a range in relative length of 7.6-3.5. Some of these chromosomes had very tiny short arms. Chromosomes 20 and 22 carried satellites which were larger than their short arms.

A. `Luciana'

The chromosome number for this cultivar was 2n=4x-1=31. Average measurements for this cultivar (Table 9.39) revealed a karyotype that was very similar to that for *A.* `Jubilee'. There were sixteen metacentric, submetacentric and subtelocentric chromosomes in group one of the genome but only fifteen acrocentric chromosomes in group two (Fig. 9.27). Three of the acrocentric chromosomes had satellites. A representative cell is shown in Fig. 9.27.

There were ten metacentric chromosomes, four subtelocentric chromosomes and two submetacentric chromosomes in this genome. The first five largest chromosomes were all metacentric and ranged in average total length from 26.5-16.8µ. This was a range in relative length of 15.4-9.7. These five were followed by two subtelocentric chromosomes with average total lengths of 15.2µ and 12.8µ (relative lengths 8.8 and 7.4 respectively). Three metacentric chromosomes with average total lengths of 13.7-7.2µ and relative lengths of 7.9-4.2 were next in order of size. A subtelocentic (6.9µ) and a submetacentric (6.7µ) of similar size were next in size followed by two metacentrics. These had an average total length of 3.4µ and 3.2µ each. The fourth subtelocentric had the same size as the smallest metacentric. The smallest chromosome in group one was a small submetacentric with an average total length of 5.1µ and a relative length of 2.9. This chromosome carried a tiny satellite on its short arm.

The fifteen acrocentric chromosomes ranged in average total length from 16.2 to 5.7µ. This was a range in relative length of 9.3 to 3.3. Chromosome 21 had a tiny satellite and chromosome 30 had a satellite the same size as its short arm. Chromosome 26 had a relatively large short arm as did chromosome 31.

Table 9.39. Average measurements of chromosomes of A. 'Luciana'.

Chromosome	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	13.7	12.8		26.5	15.4	1.07	m
2	12.9	12.1		25.0	14.5	1.07	m
3	11.9	11.4		23.3	13.6	1.04	m
4	10.5	8.4		18.9	11.0	1.25	m
5	9.8	7.0		16.8	9.7	1.38	m
6	12.2	3.0		15.2	8.8	4.07	st
7	10.0	2.8		12.8	7.4	3.57	st
8	8.2	5.5		13.7	7.9	1.49	m
9	4.9	3.9		8.8	5.1	1.26	m
10	4.0	3.2		7.2	4.2	1.25	m
11	5.2	1.7		6.9	4.0	3.06	st
12	5.0	1.7		6.7	3.9	2.94	sm
13	3.1	2.8		5.9	3.4	1.11	m
14	2.9	2.7		5.6	3.2	1.07	m
15	4.3	1.3		5.6	3.2	3.31	st
16	3.8	1.3	0.1	5.1	2.9	2.92	sm
17	15.8	0.4		16.2	9.3	39.50	t
18	11.2	0.4		11.6	6.7	28.00	t
19	11.2	0.2		11.4	6.6	56.00	t
20	10.5	0.2		10.7	6.2	52.50	t
21	9.6	0.3	0.04	9.9	5.8	32.00	t
22	9.4	0.3		9.7	5.6	31.33	t
23	9.0	0.1		9.1	5.3	90.00	t
24	8.6	0.4		9.0	5.2	21.5	t
25	8.6	0.2		8.8	5.1	43.00	t
26	8.3	0.8		9.1	5.3	10.38	t
27	8.2	0.4		8.6	5.0	20.50	t
28	7.6	0.4		8.0	4.6	19.00	t
29	6.9	0.3		7.2	4.2	23.00	t
30	6.7	0.2	0.2	6.8	4.0	33.50	t
31	5.1	0.6		5.7	3.3	8.50	t

*RL=Relative Length with a total genome length of 200%.
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

Figure 9.26. A. `Jubilee' - acetocarmine stain

Figure 9.27. A. `Luciana' - acetocarmine stain
Two large metacentric chromosomes are broken.

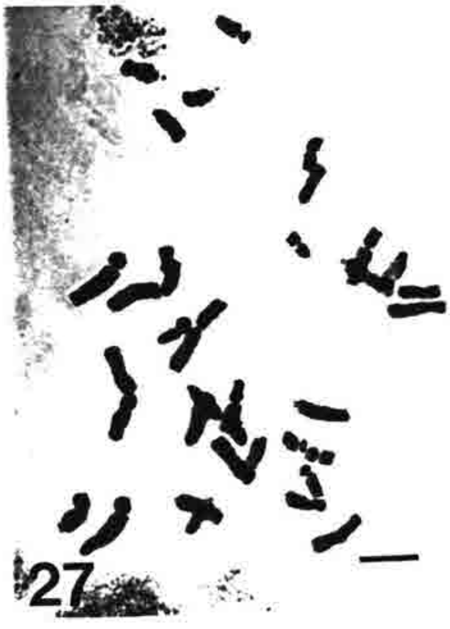
Figure 9.28. A. `Rosario' - acetocarmine stain

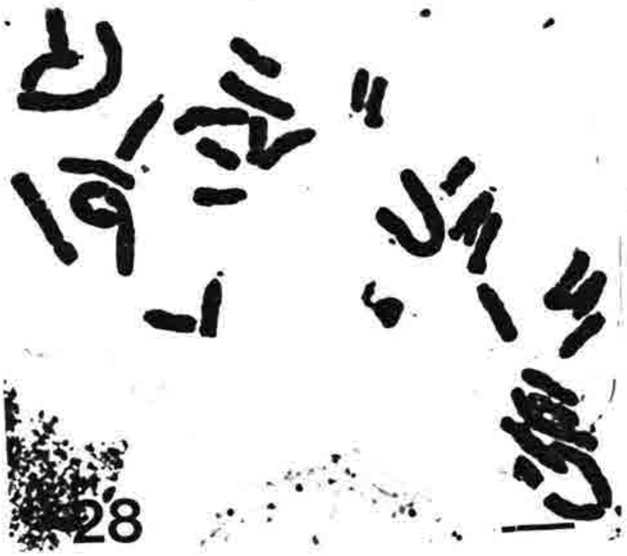
Figure 9.29. A. `Orego' - acetocarmine stain

Figure 9.30. A. `Arizo' - acetocarmine staine

Bar = 10µ


26


27


28


29


30

A. `Rosario'

The chromosome number for this cultivar was 2n=4x+1=33. The average measurements of five cells of a single plant (Table 9.40) were used to describe the karyotype of this cultivar. There were sixteen chromosomes in group one of the genome and seventeen chromosomes in group two. The extra chromosome is thus found in group two of the genome. A representative cell is depicted in Fig. 9.28.

Group one of the genome had eleven metacentric, two subtelocentric and three submetacentric chromosomes while group two had seventeen acrocentric chromosomes (Fig. 28). Two of the acrocentric chromosomes carried satellites which were larger than their short arms.

The first four chromosomes in group one were metacentrics which ranged in average total length from 27.4 to 21.8µ and in relative length from 15.4 to 12.2. These were followed by the two subtelocentrics which were 16.7µ and 15.4µ in average total length (9.4 and 8.6 in relative length respectively). Five metacentric chromosomes were next in size with an average total length of from 10.4µ to 7.9µ and a relative length range of 6.4-4.4. The last five chromosomes in group one consisted of three submetacentric and two metacentric chromosomes which ranged from 6.6µ to 5.1µ in average total length.

The fifteen acrocentric chromosomes ranged from an average total length of 12.8µ to 6.2µ. The short arms were generally similar in size, however, chromosomes 27 and 30 carried large satellites on their short arms.

A. `Orego'

This cultivar had a chromosome number of 2n=4x=32. Average measurements of the chromosomes in five cells (Table 9.41) were used to describe the karyotype. There were sixteen chromosomes in group one of the genome. These were metacentric, submetacentric and subtelocentric in shape. Sixteen acrocentric chromosomes were in group two (Fig. 9.29). A representative cell is shown in Fig. 9.29.

There were nine metacentric chromosomes, three subtelocentric chromosomes and four submetacentric chromosomes in this genome. The largest four metacentrics ranged in average total length from 28.5-22.8µ and a relative length range of 15.1-12.1. A subtelocentric and two submetacentrics were next in size and were from 15.5-12.4µ in average total size and 8.2-6.6 in relative size. Of the three metacentrics which followed, two were very similar in size at 12.2µ and 12.1µ and the third one was slightly smaller at 10.5µ in average total length. The relative lengths were 6.5-5.6. The second subtelocentric chromosome had the same average total length as the

Table 9.40. Average measurements of chromosomes of *A.* `Rosario'.

Chromo- some	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	14.5	12.9		27.4	15.4	1.13	m
2	12.9	12.1		25.0	14.0	1.07	m
3	12.6	10.8		23.4	13.1	1.17	m
4	11.3	10.5		21.8	12.2	1.07	m
5	13.4	3.3		16.7	9.4	4.06	st
6	12.0	3.4		15.4	8.6	3.53	st
7	6.6	4.8		11.4	6.4	1.38	m
8	6.2	4.2		10.4	5.8	1.47	m
9	5.6	4.0		9.6	5.3	1.39	m
10	5.6	3.4		9.0	5.1	1.65	m
11	4.1	3.8		7.9	4.4	1.08	m
12	4.8	1.8		6.6	3.7	2.67	sm
13	3.3	3.1		6.4	3.6	1.05	m
14	4.4	1.5		5.9	3.3	2.93	sm
15	3.0	2.6		5.6	3.1	1.16	m
16	3.8	1.3		5.1	2.9	2.92	sm
17	12.5	0.3		12.8	7.2	41.67	t
18	11.5	0.2		11.7	6.6	57.50	t
19	10.7	0.4		11.0	6.2	26.75	t
20	10.3	0.4		10.7	6.0	25.75	t
21	9.7	0.3		10.0	5.6	32.33	t
22	9.3	0.3		9.6	5.3	31.00	t
23	8.9	0.4		9.3	5.2	22.25	t
24	8.9	0.1		9.0	5.0	89.00	t
25	8.6	0.4		9.0	5.0	21.50	t
26	8.3	0.4		8.7	4.9	20.75	t
27	7.9	0.3	0.7	8.2	4.6	26.33	t
28	7.9	0.4		8.3	4.6	19.75	t
29	7.8	0.3		8.1	4.5	26.00	t
30	7.3	0.4	0.6	7.7	4.3	18.25	t
31	7.1	0.2		7.3	4.0	35.5	t
32	6.7	0.2		6.9	3.9	33.50	t
33	5.7	0.5		6.2	3.5	11.40	t

*RL=Relative Length with a total genome length of 200%.
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

Table 9.41. Average measurements of chromosomes of *A.* \`Orego'.

Chromo-some	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	15.1	13.4		28.5	15.1	1.13	m
2	14.1	12.7		26.8	14.2	1.11	m
3	12.9	11.2		24.1	12.8	1.16	m
4	12.5	10.3		22.8	12.1	1.21	m
5	11.8	3.7		15.5	8.2	3.19	st
6	10.0	3.3		13.3	7.0	3.00	sm
7	9.3	3.1		12.4	6.6	3.00	sm
8	7.4	4.8		12.2	6.5	1.54	m
9	7.3	4.8		12.1	6.4	1.52	m
10	6.8	3.7		10.5	5.6	1.78	sm
11	8.3	2.2		10.5	5.6	3.77	st
12	6.1	2.2		8.3	4.4	2.77	sm
13	3.6	3.4		7.0	3.7	1.06	m
14	4.9	1.8		6.7	3.5	2.72	sm
15	4.6	1.5		6.1	3.2	3.07	st
16	3.0	2.7		5.7	3.0	1.11	m
17	13.4	1.0		14.4	7.6	13.40	t
18	11.6	0.3		11.9	6.3	38.67	t
19	11.0	0.3	0.4	11.3	6.0	36.67	t
20	11.0	0.1	0.3	11.1	5.9	110.00	t
21	10.5	0.1		10.6	5.6	105.00	t
22	10.0	0.3		10.3	5.5	33.33	t
23	9.6	0.5		10.1	5.4	19.20	t
24	9.6	0.2	0.3	9.8	5.2	48.00	t
25	9.3	0.4		9.7	5.1	23.25	t
26	9.0	0.3		9.3	4.9	30.00	t
27	8.5	0.3	0.4	8.8	4.7	28.33	t
28	7.7	0.6		8.3	4.4	12.83	t
29	7.9	0.2		8.1	4.3	39.50	t
30	6.7	0.6	0.1	7.3	3.9	11.17	t
31	5.9	0.4		6.3	3.4	14.75	t
32	4.8	0.4		5.2	2.8	12.00	t

*RL=Relative Length with a total genome length of 200%.
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

last metacentric at 10.5µ. The five smallest chromosomes in group one contained two submetacentrics, two metacentrics and a subtelocentric chromosome. These ranged from 8.3-5.7µ in average total length and 4.4 to 3.0 in relative length.

The sixteen acrocentric chromosomes in this cultivar were from 14.4µ to 5.2µ in average total length and from 7.6 to 2.8 in relative length. There were satellites in chromosomes 19, 20, 24 and 27 which were a little larger than the short arms of the chromosomes and a satellite on chromosome 30 which was much smaller than the short arm of the chromosome.

A. `Arizo'

The chromosome number for this cultivar was 2n=4x=32. The average measurements of chromosomes in five cells of a single plant (Table 9.42) were used to describe the karyotype of the cultivar. This cultivar differed slightly from the others discussed since it had seventeen chromosomes in group one and only fifteen chromosomes in group two.Two of the submetacentric chromosomes of group one and five of the acrocentric chromosomes of group two carried small satellites (Fig. 9.30).

There were eleven metacentric, two subtelocentric and four submetacentric chromosomes in the first group of the genome. The four largest metacentrics had an average total length of from 20.1µ to 17.5µ. This was a relative length range from 14.7 to 12.8. The largest subtelocentric had an average total length of 13.1µ and it was followed by another metacentric which had an average total length of 10.9µ and a relative length of 7.9. The second subtelocentric had an average total length of 10.1µ and a relative length of 7.4. This was followed by the largest submetacentric chromosome which had an average total length of 8.5µ and a relative length of 6.2. This chromosome had a ratio which was close to the dividing line between metacentric and submetacentric chromosomes. There were then three more metacentrics from 7.9µ to 5.9µ in average total length (5.8 to 4.3 relative length). The second submetacentric chromosome had an average total length the same as the preceding metacentric. This was followed by another submetacentric which carried a tiny satellite on it's short arm. The average total length of this chromosome was 5.2µ and the relative length was 3.8. The next metacentric chromosome had the same average total length as the satellited submetacentric and it was followed by another satellited submetacentric of similar size (5.1µ). This chromosome also carried a tiny satellite on its short arm. The two smallest chromosomes in group one were a submetacentric and a metacentric with average total lengths of 4.6µ and 4.4µ respectively.

Table 9.42. Average measurements of chromosomes of *A.* \`Arizo'.

Chromosome	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	10.3	9.8		20.1	14.7	1.04	m
2	10.1	9.6		19.7	14.4	1.05	m
3	9.4	8.9		18.3	13.4	1.05	m
4	9.2	8.3		17.5	12.8	1.11	m
5	10.5	2.6		13.1	9.5	4.04	st
6	6.3	4.6		10.9	7.9	1.37	m
7	7.9	2.2		10.1	7.4	3.59	st
8	5.4	3.1		8.5	6.2	1.73	sm
9	4.4	3.5		7.9	5.8	1.24	m
10	4.2	3.6		7.8	5.7	1.18	m
11	3.1	2.8		5.9	4.3	1.11	m
12	4.3	1.6		5.9	4.3	2.69	sm
13	3.8	1.4	0.1	5.2	3.8	2.71	sm
14	2.7	2.5		5.2	3.8	1.07	m
15	3.6	1.5	0.1	5.1	3.7	2.40	sm
16	3.1	1.5		4.6	3.3	2.07	sm
17	2.4	2.0		4.4	3.2	1.20	m
18	9.2	0.3	0.1	9.5	6.9	30.67	t
19	8.6	0.2		8.8	6.4	43.00	t
20	8.3	0.2		8.5	6.2	41.50	t
21	7.7	0.5	0.2	8.1	5.9	15.40	t
22	7.4	0.6		7.9	5.8	12.50	t
23	7.0	0.2		7.2	5.2	35.00	t
24	6.6	0.4	0.3	7.0	5.1	16.50	t
25	6.5	0.3		6.8	4.9	21.67	t
26	6.5	0.4		6.9	5.0	16.25	t
27	6.4	0.3	0.2	6.7	4.9	21.33	t
28	5.6	0.5	0.1	6.1	4.5	11.20	t
29	5.3	0.3		5.6	4.1	17.67	t
30	5.2	0.4		5.6	4.1	13.00	t
31	4.6	0.5		5.1	3.7	9.20	t
32	4.1	0.4		4.5	3.3	10.25	t

*RL=Relative Length with a total genome length of 200%.
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

The fifteen acrocentric chromosomes ranged in average total length from 9.5µ to 4.5µ. This was a relative length range of 6.9 to 3.3. Chromosomes 18, 21, 24, 27 and 28 carried small to tiny satellites on their short arms that were all smaller than the short arms.

A. `Alnba'

The chromosome number for this cultivar was 2n=4x=32. The average measurements for it's chromosomes (Table 9.43) were used to describe the karyotype. There were only fourteen chromosomes in group one of the genome, two of which carried satellites. The second group had eighteen acrocentric chromosomes of which seven carried satellites (Fig. 9.31).

There were five metacentric chromosomes which ranged in average total length from 30.7µ to 15.1µ with a relative length range of 15.7 to 7.7. The largest subtelocentric chromosome had an average total length of 13.7µ and a relative length of 7.1. The next three metacentric chromosomes had an average total length of from 12.8µ to 9.1µ and a relative length ranging from 6.5 to 4.6. There was a small satellite on the short arm of chromosome 7. The smallest five chromosomes in group one consisted of three subtelocentric and two metacentric chromosomes. These were from 7.9µ to 5.9µ in average total length and 4.0 to 3.0 in relative length. The very smallest subtelocentric chromosome had a satellite that was about half the size of the short arm.

The eighteen acrocentric chromosomes were from 14.6µ to 5.3µ in average total length. They were from 7.5 to 2.7 in relative length. Chromosomes 15, 21 and 23 had satellites the same size or slightly smaller than the short arms while chromosomes 19, 20, 25 and 26 had satellites which were larger than the short arms.

A. `Texas'

This cultivar had a chromosome number of 2n=4x=32. The karyotype was determined from the average measurements of chromosomes from five cells of a single plant (Table 9.44). There were sixteen metacentric, submetacentric and subtelocentric chromosomes in group one and sixteen acrocentric chromosomes in group two (FIg. 9.32). Four of the acrocentric chromosomes carried small satellites on their short arms.

There were eleven metacentric, two subtelocentric and three submetacentric chromosomes in the first group of chromosomes in the genome. The first five metacentrics were from 28.9µ to 16.6µ in average total length and 16.7 to 9.6 in relative length. The largest

Table 9.43. Average measurements of chromosomes of *A.* `Alnba'.

Chromo-some	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	15.9	14.8		30.7	15.67	1.08	m
2	15.1	14.6		29.7	15.2	1.03	m
3	14.8	13.9		28.6	14.6	1.06	m
4	13.3	12.0		25.3	12.9	1.11	m
5	8.8	6.3		15.1	7.7	1.40	m
6	10.5	3.2		13.7	7.1	3.28	st
7	7.6	5.2	0.2	12.8	6.5	1.46	m
8	5.3	4.6		9.9	5.1	1.15	m
9	4.9	4.2		9.1	4.6	1.17	m
10	6.1	1.8		7.9	4.0	3.39	st
11	4.0	3.5		7.5	3.8	1.14	m
12	5.6	1.5		7.1	3.6	3.73	st
13	3.5	3.1		6.6	3.4	1.13	m
14	4.6	1.3	0.7	5.9	3.0	3.54	st
15	14.2	0.4	0.1	14.6	7.5	35.50	t
16	12.6	0.3		12.9	6.6	42.00	t
17	12.1	0.2		12.3	6.3	60.50	t
18	11.7	0.1		11.8	6.0	117.00	t
19	11.1	0.3	0.5	11.4	5.8	37.00	t
20	10.6	0.3	0.6	10.9	5.5	35.33	t
21	10.6	0.2	0.1	10.8	5.5	53.00	t
22	9.4	1.3		10.7	5.4	7.23	t
23	10.0	0.2	0.2	10.2	5.2	50.00	t
24	9.3	0.6		9.9	5.0	15.50	t
25	9.7	0.2	0.3	9.9	5.0	48.50	t
26	8.6	0.2	0.4	8.8	4.5	43.00	t
27	8.5	0.3		8.8	4.5	28.30	t
28	8.2	0.4		8.6	8.4	20.50	t
29	7.7	0.2		7.9	4.0	38.50	t
30	7.0	0.5		7.5	3.8	14.00	t
31	6.6	0.5		7.1	3.6	13.20	t
32	5.3	0.01		5.3	2.7	530.00	t

*RL=Relative Length with a total genome length of 200%.

L = Long arm

S = Short arm

SAT=Satellite

Total= Total length excluding satellite

Table 9.44. Average measurements of chromosomes of *A.* `Texas'.

Chromosome	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	16.2	12.7		28.9	16.7	1.27	m
2	15.1	13.4		28.5	16.5	1.13	m
3	12.1	10.5		22.6	13.1	1.16	m
4	9.9	9.3		19.2	11.1	1.06	m
5	8.7	7.8		16.6	9.6	1.11	m
6	10.0	3.2		13.2	7.7	3.12	st
7	7.6	5.6		13.2	7.7	1.36	m
8	5.8	4.7		10.5	6.1	1.35	m
9	4.7	3.9		8.6	5.0	1.20	m
10	5.6	2.4		8.0	4.6	2.34	sm
11	5.7	2.3		8.0	4.6	2.48	sm
12	4.0	3.3		7.3	4.3	1.20	m
13	4.5	2.0		6.5	3.8	2.25	sm
14	3.0	2.6		5.6	3.3	1.13	m
15	4.0	1.3		5.3	3.1	3.08	st
16	2.8	2.5		5.3	3.1	1.11	m
17	13.8	0.7		14.5	8.4	19.71	t
18	11.0	0.1		11.1	6.4	110.00	t
19	10.4	0.3		10.7	6.2	34.67	t
20	9.8	0.6	0.2	10.4	6.0	16.33	t
21	9.8	0.5		10.3	6.0	19.60	t
22	9.6	0.1		9.7	5.6	96.00	t
23	9.4	0.1	0.3	9.5	5.5	94.00	t
24	8.3	1.0		9.3	5.4	8.3	t
25	7.8	0.5		8.3	4.8	15.6	t
26	7.4	0.7	0.3	8.1	4.7	10.57	t
27	7.4	0.3	0.2	7.7	4.4	24.67	t
28	7.2	0.4		7.6	4.4	18.00	t
29	6.7	0.2		6.9	4.0	33.50	t
30	6.2	0.2		6.4	3.7	31.00	t
31	5.0	0.1		5.1	3.0	50.00	t
32	4.4	0.3		4.8	2.8	14.67	t

*RL=Relative Length with a total genome length of 200%.
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

Figure 9.31. A. `Alnba' - acetocarmine stain

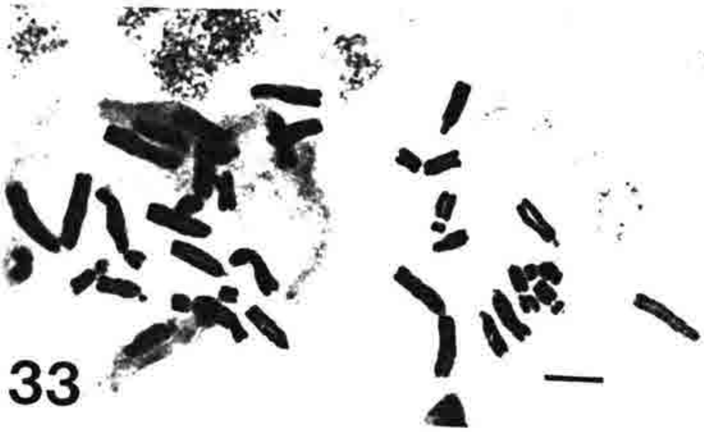
Figure 9.32. A. `Texas' - acetocarmine stain

Figure 9.33. A. `Neva' - acetocarmine stain

Bar = 10μ

31

32



33

subtelocentric chromosome had an average total length of 13.2µ and a relative length of 7.7. The next metacentric chromosome was the same size as the preceding subtelocentric and it was followed by two other metacentrics of 10.5µ and 8.6µ average total length. The following two submetacentrics were the same size at 8.0µ, a relative length of 4.6. The last five chromosomes in group one consisted of three metacentrics, a submetacentric and a subtelocentric chromosome and were from 7.3µ to 5.3µ in average total length. Their relative lengths were from 4.3 to 3.1.

The sixteen acrocentric chromosomes were from 14.5µ to only 4.8µ in average total length. This was a range in relative length of 8.4 to 2.8. Chromosomes 20, 26 and 27 had satellites on the short arms which were smaller than the short arms. The short arms for chromosomes 20, 24 and 26 were relatively large. The satellite on chromosome 23 was larger than the short arm.

A. Neva'

The chromosome number for this cultivar was 2n=4x=32. The average measurements of five cells (Table 9.45) were used to describe the karyotype. The karyotype consisted of only fourteen chromosomes in group one and eighteen chromosomes in group two of the genome (Fig. 9.33). There were nine metacentric, four submetacentric and one subtelocentric chromosome in the first group of the genome and eighteen acrocentric chromosomes in the second group.

The largest two metacentric chromosomes in the genome had average total lengths of 28.1µ and 25.1µ respectively and these were followed by two large submetacentrics which had average total lengths of 18.1µ and 15.6µ. This was the only tetraploid which did not have four or five of the largest chromosomes in it's genome. It had only two large metacentrics. The large submetacentric chromosomes were followed by six metacentrics with average total lengths from 14.3µ to 8.7µ. The relative lengths of these chromosomes was from 7.7 to 4.7. The following submetacentric chromosome had a tiny satellite on the short arm. It had an average total length of 7.5µ and a relative length of 4.1. The smallest three chromosomes in the first group were metacentric, subtelocentric and submetacentric in shape and were from 6.9µ to 6.0µ in average total length. Their relative lengths were from 3.7 to 3.2. The smallest submetacentric chromosome had a small satellite on the short arm.

The average total length of the eighteen acrocentric chromosomes was from 14.4µ to 6.1µ and 7.8 to 3.3 in relative length. There were large satellites on chromosomes 24, 25 and 28 which were up to three times larger than the short arms and a small satellite on chromosome 27, about the same size as the short arm.

Table 9.45. Average measurements of chromosomes of A. `Neva'.

Chromo-some	L* μ	S* μ	SAT* μ	Total* μ	RL*	Ratio	Centromere position
1	14.4	13.7		28.1	15.2	1.05	m
2	13.1	12.0		25.1	13.6	1.08	m
3	12.7	5.4		18.1	9.8	2.35	sm
4	11.8	3.8		15.6	8.4	3.10	sm
5	8.2	6.1		14.3	7.7	1.34	m
6	6.7	5.8		12.5	6.8	1.16	m
7	6.6	5.8		12.4	6.7	1.14	m
8	5.5	3.8		9.3	5.0	1.43	m
9	5.3	3.8		9.1	4.9	1.39	m
10	4.8	3.9		8.7	4.7	1.22	m
11	5.6	1.9	0.1	7.5	4.1	2.95	sm
12	3.8	3.1		6.9	3.7	1.18	m
13	4.7	1.4		6.1	3.3	3.36	st
14	4.5	1.5	0.2	6.0	3.2	3.00	sm
15	14.1	0.3		14.4	7.8	47.00	t
16	13.0	0.3		13.3	7.2	43.33	t
17	12.3	0.7		13.0	7.0	17.57	t
18	12.0	0.3		12.3	6.7	40.00	t
19	11.6	0.3		11.9	6.4	38.67	t
20	10.9	0.8		11.7	6.3	13.63	t
21	10.7	0.8		11.5	6.2	13.38	t
22	11.3	0.1		11.4	6.2	113.00	t
23	10.3	0.5		10.8	5.8	20.60	t
24	9.8	0.6	0.7	10.4	5.6	16.33	t
25	9.5	0.4	0.7	9.9	5.3	23.75	t
26	8.9	0.7		9.6	5.2	12.71	t
27	9.1	0.2	0.3	9.3	5.0	45.50	t
28	8.9	0.4		9.3	5.0	22.25	t
29	8.3	0.2	0.6	8.5	4.6	41.50	t
30	7.6	0.6		8.2	4.4	12.67	t
31	6.8	0.6		7.4	4.0	11.33	t
32	5.8	0.3		6.1	3.3	19.33	t

*RL=Relative Length with a total genome length of 200%.
L = Long arm
S = Short arm
SAT=Satellite
Total= Total length excluding satellite

Chapter 10

DISCUSSION

Using both karyotype analysis and Giemsa banding it was possible to identify individual chromosomes within each species. The identification of a particular species could then be described in terms of the characteristics of each chromosome pair in it's genome. Characterization and identification of cultivars was also possible using these techniques. Each cultivar studied had a unique complement of chromosomes which completely identified the cultivar. This procedure may thus provide another method of identification for the patenting of the cultivars and prevent confusion among those that are morphologically similar.

Comparisons with paired chromosomes of species revealed that although the heterochromatic regions of the paired chromosomes were almost always the same there were differences in their relative lengths. There were also some instances in which only one chromosome of the pair carried a satellite. Since only one plant for each species was extensively analyzed, future studies are needed to determine whether this heterozygosity is found in every plant within the species or whether the these chromosomes are polymorphic with regard to the presence of satelites. Polymorphism with respect to presence or absence of satellites was reported in *A. pelegrina* (Stephens et al. 1993).

Analysis of the karyotypes of eight *Alstroemeria* species and one hybrid revealed that they all had a similar karyotype. All of these species were diploids (2n=2x=16). The genome of each species could be divided into two groups. The first group had four or five pairs of metacentric, submetacentric or subtelocentric chromosomes some of which carried satellites. The second group had three or four pairs of acrocentric chromosomes with from one to four pairs carrying satellites. This agrees with earlier work by Taylor (1926), Whyte (1929), Sato (1938), Darlington and Wylie (1955), Tsuchiya and Hang (1989), Rustanius et al. (1991) and Stephens et al.(1993).

The largest pair of chromosomes in every species was a metacentric pair. The primary constriction for each of these pairs was very close to the median point. The relative lengths of these chromosome pairs varied among the species. The largest pair was found in *A. pelegrina* with a relative length of 28.5%, closely followed by *A. sierrae* at 28.2%. The largest chromosomes of the other species were from 27.0% to 20.6% in the order of *A. pulchella, A. psittacina, A. aurantiaca, A. chilensis, A. ligtu* species, *A. ligtu* hybrid and *A. versicolor.* The largest chromosome pairs of *A. pulchella* and *A. psittacina* were nearly the same relative length. Similarly, *A. ligtu spp.* and *A. chilensis* had large metacentric chromosome pairs of similar size while *A. ligtu* hybrid had a chromosome pair that was slightly shorter than the former two species.

The second chromosome pair was also metacentric for six of the species. For the other three, *A. sierrae, A. pulchella* and *A. psittacina,* the second chromosome pair was subtelocentric in shape. *A. chilensis* had the largest metacentric chromosome pair 2 at a relative length of 17.0%. *A. ligtu* species and *A. ligtu* hybrid had chromosome pairs number 2 which were just over 15.0% in relative length while *A. pelegrina, A. versicolor* and *A. aurantiaca* had pairs which were 13.7, 13.6 and 13.2% relative length respectively. Sato (1938) noted a satellite on one pair of long chromosomes of this species. However, he classified the chromosomes observed as having submedian constrictions rather than median. Since visual analysis of the chromosomes, without measurements, may suggest that they are in fact submetacentric in shape it is presumed that the long submetacentric chromosomes of Sato are the same as the long metacentrics described in this study.

The second pair of chromosomes in the other three species were subtelocentrics which were 16.6% in relative length in *A. psittacina,* 16.0% in *A. pulchella* and 12.3% in *A. sierrae.* The presence of a pair of medium sized subtelocentric chromosomes in *A. pulchella* was noted by Sato (1938). He also indicated that the chromosomes of *A. chilensis* were larger than those of *A. pulchella* but comparison of the relative lengths given in this study would indicate that, in fact, the opposite is true.

The third pair of chromosomes in all the species studied was a medium-small metacentric pair. These varied from 12.4% relative length in *A. chilensis* to only 7.0% in *A. sierrae.* A satellite was carried on the shorter arm of one or both chromosomes of the pair in *A. ligtu* species and hybrid. *A. chilensis* also had a small satellite on one chromosome of this pair. Rustanius et al. (1991) and Tsuchiya et al. (1987) also describe medium submetacentric chromosomes in *A. ligtu* hybrids which carry satellites on their shorter arms. However, measurement of these chromosomes in the published figures for this hybrid give a ratio of long-short arm lengths of around 1.1 or 1.2 or an index of 0.8-0.9. This agrees with the published index of Rustanius et al. of about 0.88 and indicates that these satellited chromosomes are in fact metacentric.

For all species except *A. chilensis* the fourth pair of chromosomes was submetacentric or subtelocentric. *A. aurantiaca* had one chromosome of pair 4 that was subtelocentric and one that was submetacentric. The averages and ratio for the two chromosomes (3.09) indicated that although this pair was classed as subtelocentric it was very close to the dividing ratio for submetacentric chromosomes. *A. chilensis* had a metacentric chromosome as the fourth largest pair of its genome. The submetacentric chromosome pairs ranged in relative length from 10.1% in *A. aurantiaca* to 6.4% in *A. sierrae*. The metacentric chromosome pair of *A. chilensis* had a relative length of 11.0%. The relative lengths of *A. pulchella* and *A. psittacina* were almost exactly the same for this pair of chromosomes. Satellites were present on at least one chromosome of this pair in *A. ligtu* hybrid and *A. versicolor*. No satellites were reported for this chromosome pair in *A. ligtu* hybrid in previous research (Rustanius et al., 1991; Tsuchiya et al., 1987). This chromosome pair is known to be polymorphic with respect to satellite presence or absence in *A. pelegrina* (Stephens et al., 1993), however, for the present plant no satellites were observed.

Chromosome pair 5 constituted the fifth pair of chromosomes of group one for *A. chilensis, A. ligtu* species and *A. ligtu* hybrid. It was a metacentric chromosome pair in both ligtu species and hybrid with a relative length of around 9.5%. In *A. chilensis* this pair was a submetacentric pair with a relative length of 10.1%. There were satellites on this chromosome pair in both *A. chilensis* and *A. ligtu* hybrid. This observation agrees with earlier work with *A. chilensis* (Sato, 1938) and *A. ligtu* hybrid (Rustanius et al., 1991; Tsuchiya and Hang, 1987).

For the remaining species the fifth chromosome pair was acrocentric in shape. The relative lengths of these chromosomes varied from 13.8% for *A. sierrae* to 11.5% for *A. aurantiaca*. *A. psittacina* and *A. pulchella* had chromosome pairs that were of approximately the same size. Satellites were present on both chromosomes of this pair for *A. sierrae, A. pelegrina* and *A. aurantiaca.*

The sixth pair of chromosomes in all species was acrocentric. These varied in relative length from 12.5% in both *A ligtu* species and hybrid to 10.5% and 10.4% in *A. pulchella* and *A. psittacina* respectively. Satellites were present on one or both chromosomes of the pair for *A. ligtu* species, *A. ligtu* hybrid, *A. sierrae, A. chilensis, A. psittacina* and *A. pulchella.* Sato (1938) also noted satellites on one pair of acrocentrics of *A. pulchella* and Rustanius et al. (1991) and Tsuchiya and Hang (1987) described two pairs of satellited acrocentric chromosomes for *A. ligtu* hybrid.

The next chromosome pair was also an acrocentric pair in all of the species studied. The relative length varied from 11.4% for *A. ligtu* hybrid to 9.6% and 9.5% for *A. psittacina* and *A. pulchella* respectively. Satellites were present on one or both chromosomes of the pair for *A. ligtu* hybrid, *A. sierrae, A. pelegrina, A. versicolor, A. chilensis, A. psittacina* and *A. pulchella.*

The smallest pair of acrocentric chromosomes in all the species varied from 9.5% relative length in *A. aurantiaca* to 8.0% in *A. psittacina.* All species except *A. ligtu* hybrid and *A. pelegrina* carried small satellites on this pair of chromosomes. Both of these latter species were previously reported to have satellites (Rustanius et al., 1991; Stephens et al., 1993). This may indicate that there is some polymorphism of these chromosomes with respect to the presence or absence of satellites or may be an artifact of the preparation technique.

Only one pair of acrocentric chromosomes for *A. pulchella* and two pairs for *A. chilensis* were identified by Sato (1938) as carrying satellites as compared with three and four pairs, respectively, identified in this study. This may reflect the discriminating ability of the different preparation techniques used or may be an example of polymorphism in these species. Polymorphism with respect to satellite presence or absence has been reported in *A. pelegrina* (Stephens et al., 1993) and polymorphism within the genome was observed by Tsuchiya and Hang (1989) in *A. versicolor.*

There were several chromosomes which were common to more than one species. *A. auarantiaca* and *Leontochir ovallei* both had a medium-sized metacentric chromosome with a relative length of about 10%, which had broad heterochromatic bands at the distal end of both of the arms. A medium-sized subtelocentric or submetacentric chromosome was also noted in *A. ligtu, A. chilensis, A. aurantiaca* and *A. versicolor* which had a relative length of between 9.4% and 10.1%. This chromosome had a broad heterochromatic band at or near the distal end of the short arm in all of these species. *A. aurantiaca* and *A. versicolor* also had broad bands in the middle of the long arm of this chromosome.

Both *Leontochir ovallei* and *Bomarea* species had similar karyotypes to the *Alstroemeria* species although they each had an extra pair of chromosomes. Whyte (1929) reported that this extra pair of chromosomes for *Bomarea* species should be placed in group two as an extra acrocentric pair. He also noted that this brought the total number of acrocentric chromosome pairs to four. The present study also found four pairs of acrocentric chromosomes but since this was the same for many of the *Alstroemeria* species it may not represent an extra pair in this group. The extra pair of chromosomes for *Bomarea* could be placed in group one of the genome if comparison is made to the majority of species studied. However, *A. ligtu* species and hybrid and *A. chilensis* also had five pairs of chromosomes in this group and only three pairs in the second group and so it was difficult to determine in which group the extra chromosome pair should be included.

The largest pair of acrocentric chromosomes in *Bomarea* were the largest of any of the species studied. The other three pairs were of similar sizes to those of the other species. The largest metacentric chromosomes, however, were much smaller than the largest ones in the other species. In fact, this chromosome was similar in total relative total length to just the longest arm of many of the large metacentrics of the other species. Similarly, the second pair of chromosomes in *Bomarea* was of a similar total relative length to the shorter arm of the largest metacentric of the other species, although it was subtelocentric in shape.

The second metacentric pair of chromosomes in *Bomarea* had a relative length that was very close to the length of the third chromosome pair of many of the other species. The second subtelocentric pair of chromosomes was exactly the same relative length as the subtelocentric pair of *A. aurantiaca* and the submetacentric pair of *A. chilensis.*

The final pair of chromosomes in group one of *Bomarea* was a small submetacentric pair that had a very similar relative length to the submetacentric pairs of *A. sierrae, A. pelegrina, A. pulchella* and *A. psittacina.*

The largest pair of acrocentric chromosomes in *Bomarea* was larger than those found in the other species although the difference was not great, 14.4% in *Bomarea* as compared to 13.8% in *A. sierrae.* The other three chromosome pairs of this group had similar sizes to other acrocentric pairs in the *Alstroemeria* species. All four pairs of acrocentric chromosomes in *Bomarea* carried small satellites as did *A. chilensis* and *A. sierrae.*

These observations suggest that the extra pair of chromosomes in *Bomarea* are part of the first group of chromosomes. It seems likely that either the chromosomes in the largest metacentric pair in the *Alstroemeria* species became disjoined and formed two smaller pairs or that the chromosomes of the two smaller pairs of *Bomarea* fused together to form the larger chromosomes found in the *Alstroemeria.* Unfortunately no Giemsa bands were identified for any of the chromosomes involved and so definite conclusions cannot be drawn at this time. Modification of the Giemsa banding technique may enable this point to be clarified at a later date.

Leontochir ovallei also had an extra pair of chromosomes as compared to the *Alstroemeria* species. Since this was the only species to have five pairs of acrocentric chromosomes in group two of the genome it is assumed that the extra pair were in this group. The two metacentric chromosomes of *L. ovallei* were very small when compared to the two largest group one chromosomes of the other species studied.

The range in relative lengths of the acrocentric chromosome of *L. ovallei* was very similar to those of the *Alstroemeria* species, especially *A. sierrae*. The extra chromosome pair may be due to non-disjunction of chromosomes of pair number 7 since the relative lengths of these chromosomes was the same as those for chromosomes of pair 8. Giemsa banding patterns for the long arms of both chromosome pairs 7 and 8 appeared to be exactly the same. The larger short arm of chromosome pair 8 was of a similar size to the combined lengths of the short arm and the satellite of chromosome pair 7.

Idiograms for each species were constructed from the relative lengths of the long arms, short arms and satellites of each chromosome pair (Fig. 10.1). These allow direct comparisons to be made among the species with respect to chromosome lengths and shapes.

A further means of comparison is provided by the diagrammatic representation of each genome (Fig. 10.2). These were constructed with the relative length of the chromosome pair as the x-axis and the index of the pair as the y-axis. The index is the reciprocal of the ratio or the short arm length divided by the long arm length.

It has been proposed that *A. chilensis* and *A. ligtu* species are in fact different names for the same species (Bayer, 1987). Comparison of the karyotype for each of these species does not support this proposal. The largest metacentric chromosomes, while being of similar size, had some differences. One chromosome of this pair in *A. chilensis* had a thin heterochromatic band on the proximal end of the shorter arm of one chromosome of the pair while *A. ligtu* had a heterochromatic band on both chromosomes of the pair. The other chromosome of *A. chilensis* did not have any heterochromatic regions which stained with Giemsa dye.

The second pair of chromosomes for each species was also of similar size and shape, however, the Giemsa banding pattern for each was different. For *A. chilensis* there was a thin heterochromatic band in the middle of the shorter arm of the chromosome. One chromosome also carried a small satellite. For *A. ligtu* there was a wide heterochromatic band in the middle

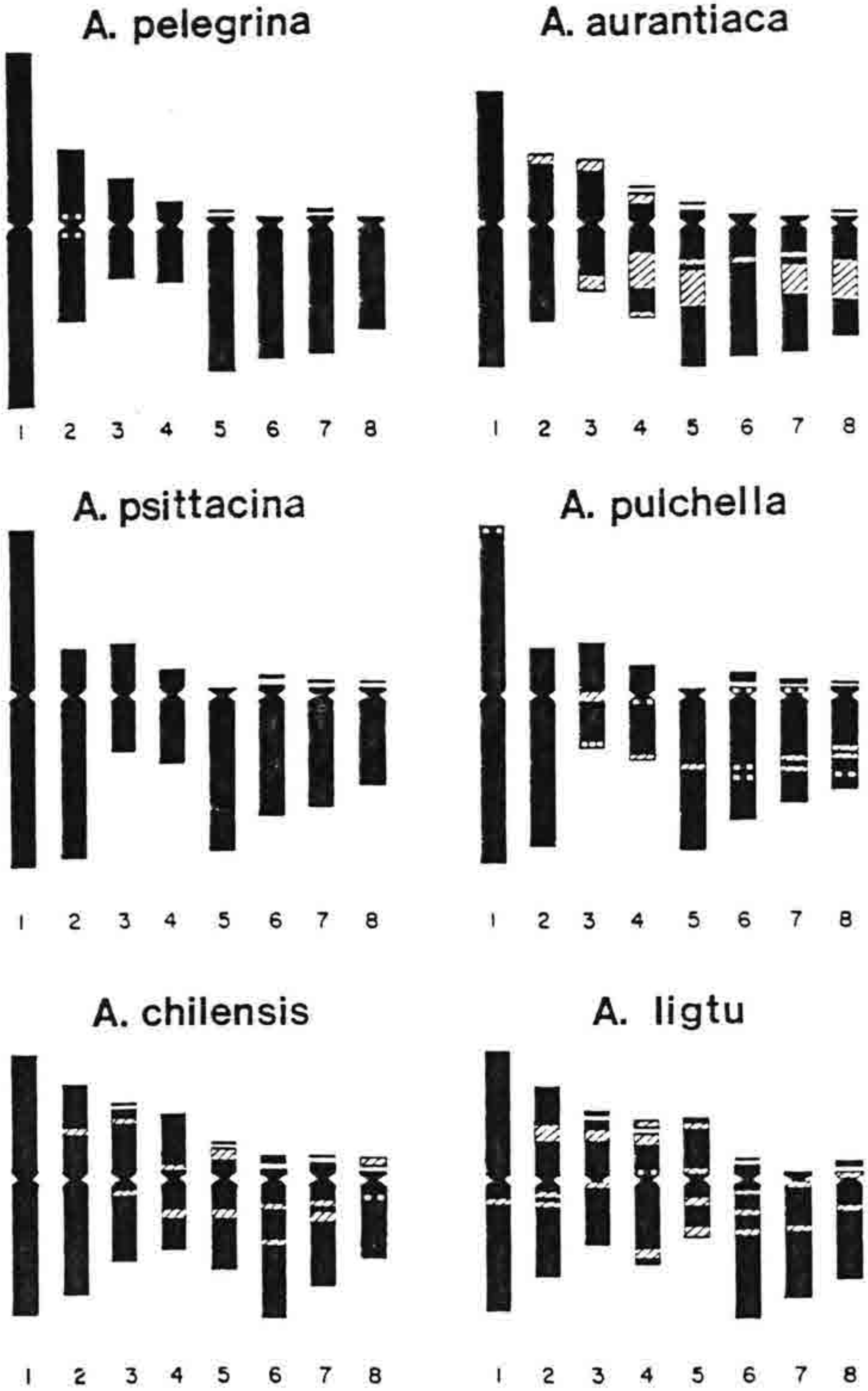


Fig. 10.1: Idiograms of Alstroemeria, Leontochir and Bomarea species.

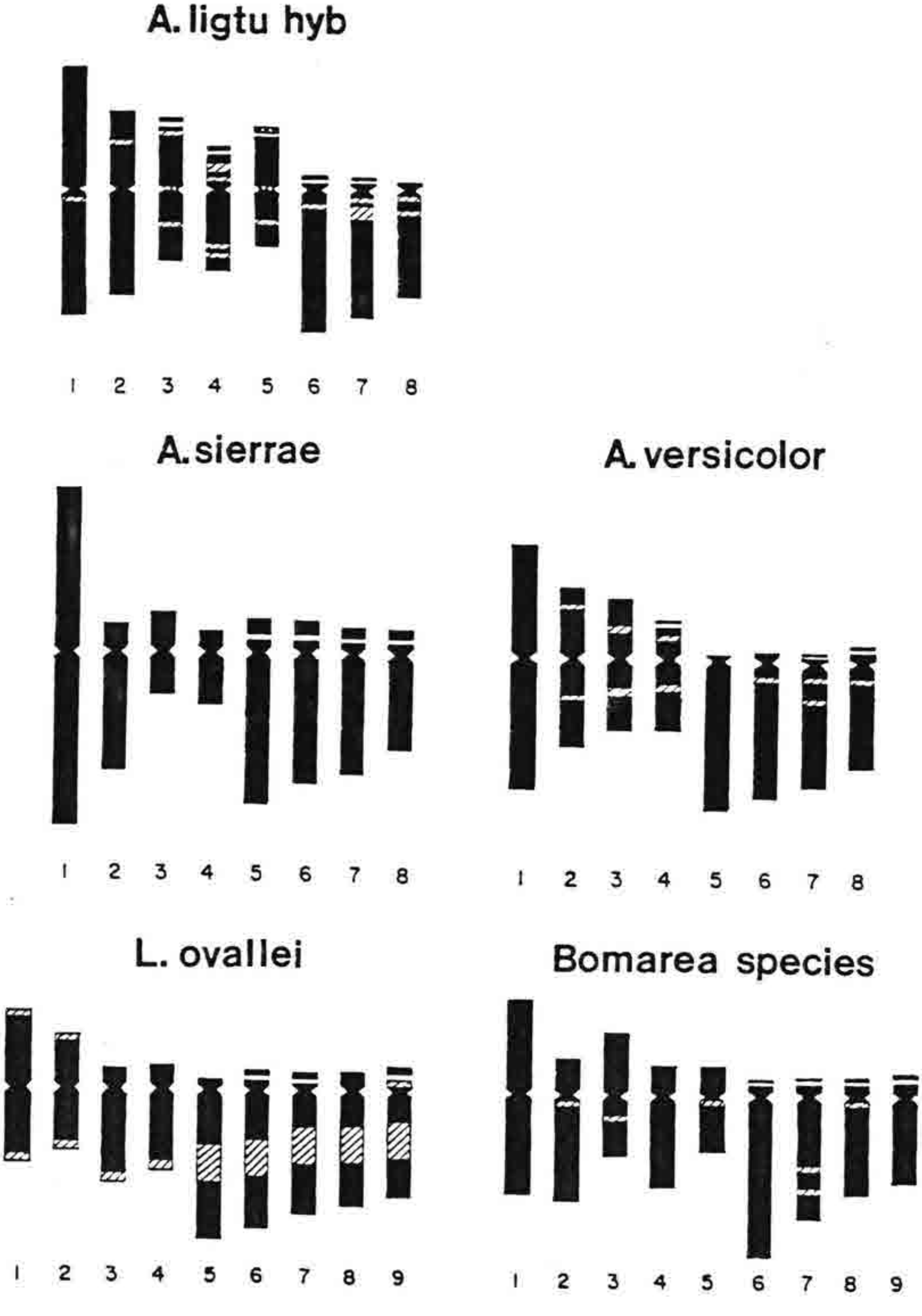
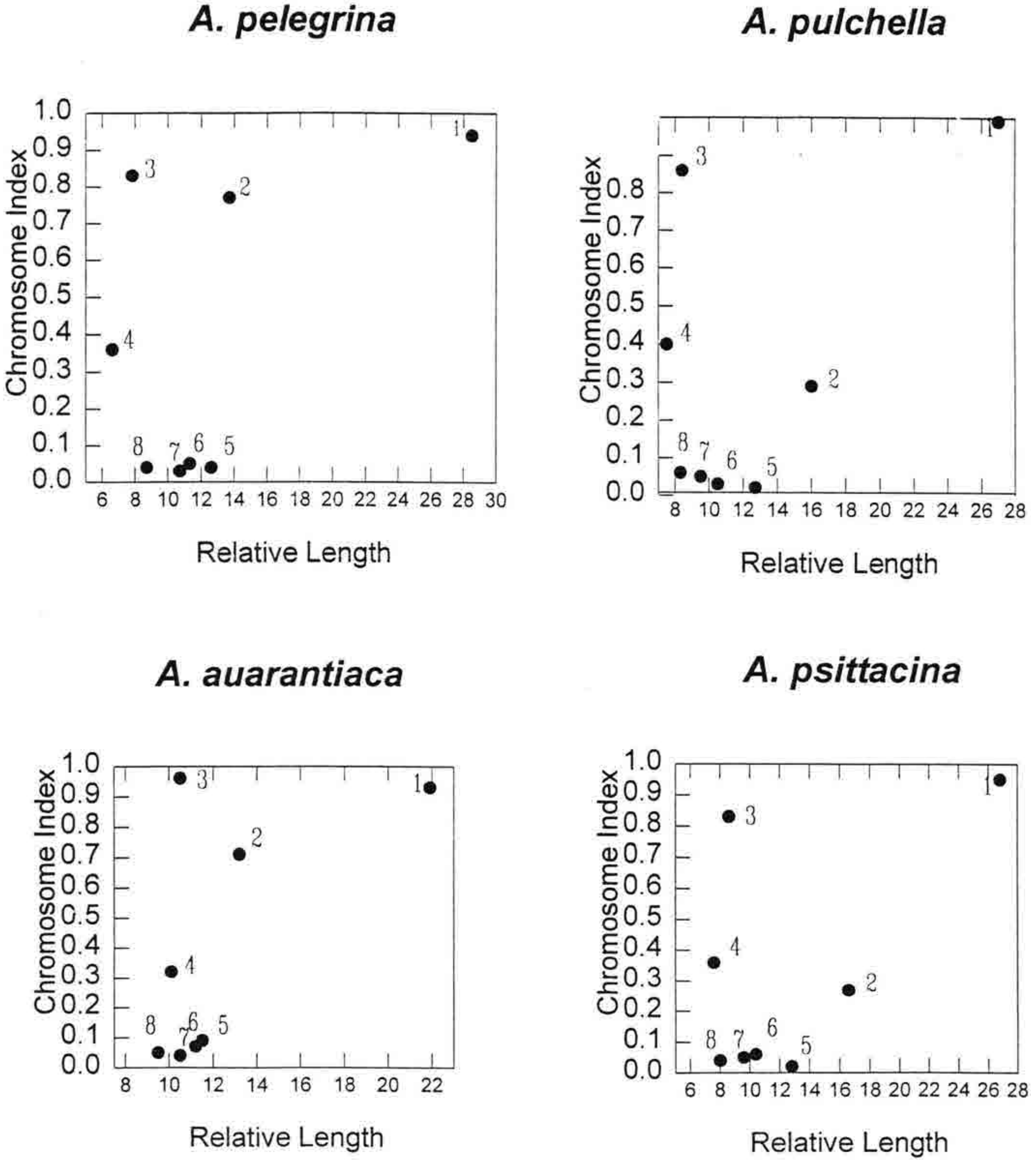


Fig. 10.1 Continued.



A. pelegrina

A. pulchella

A. auarantiaca

A. psittacina

Fig. 10.2a: Diagrammatic representation of genomes.

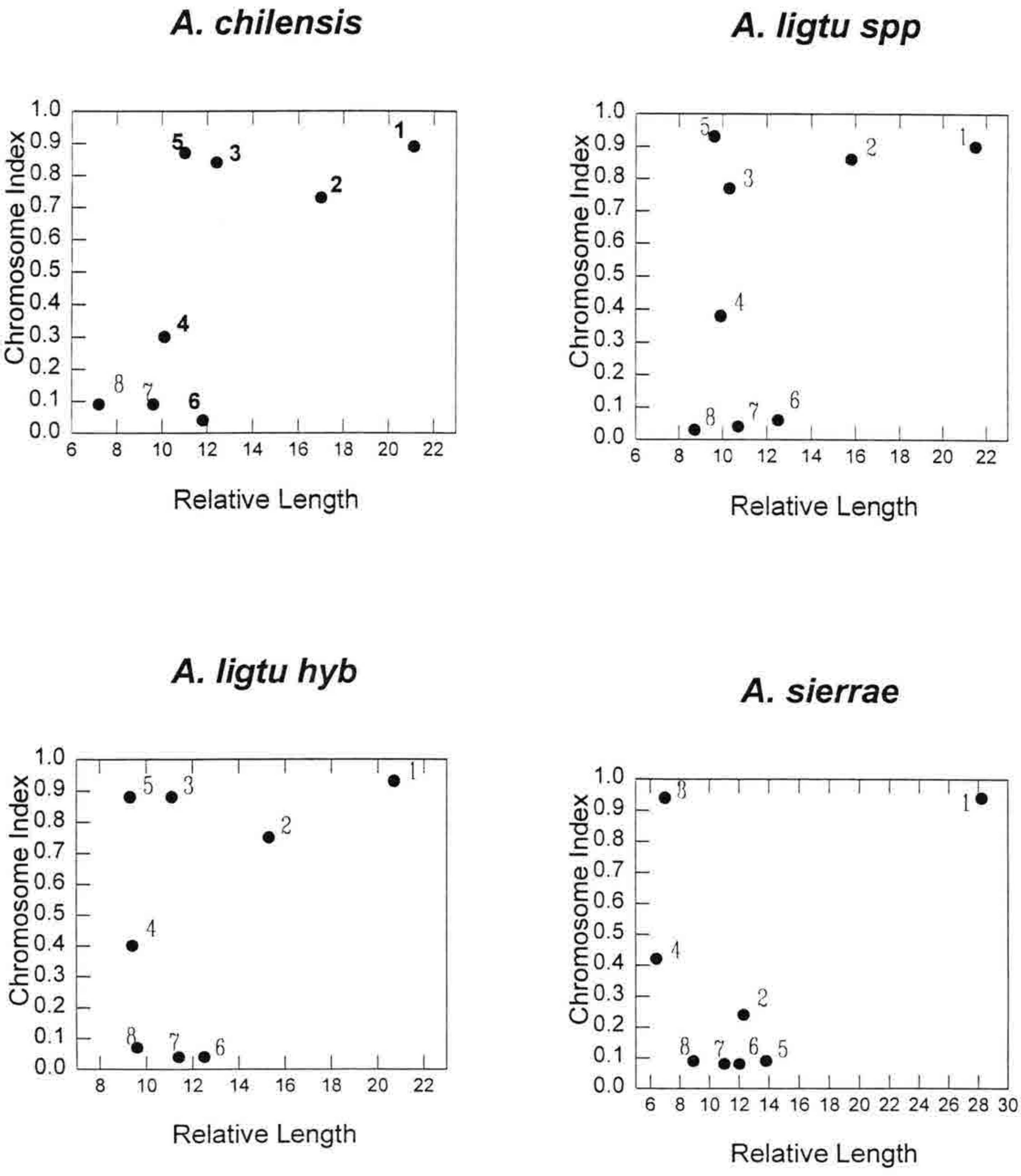


Fig. 10.2b: Diagrammatic representation of genomes.

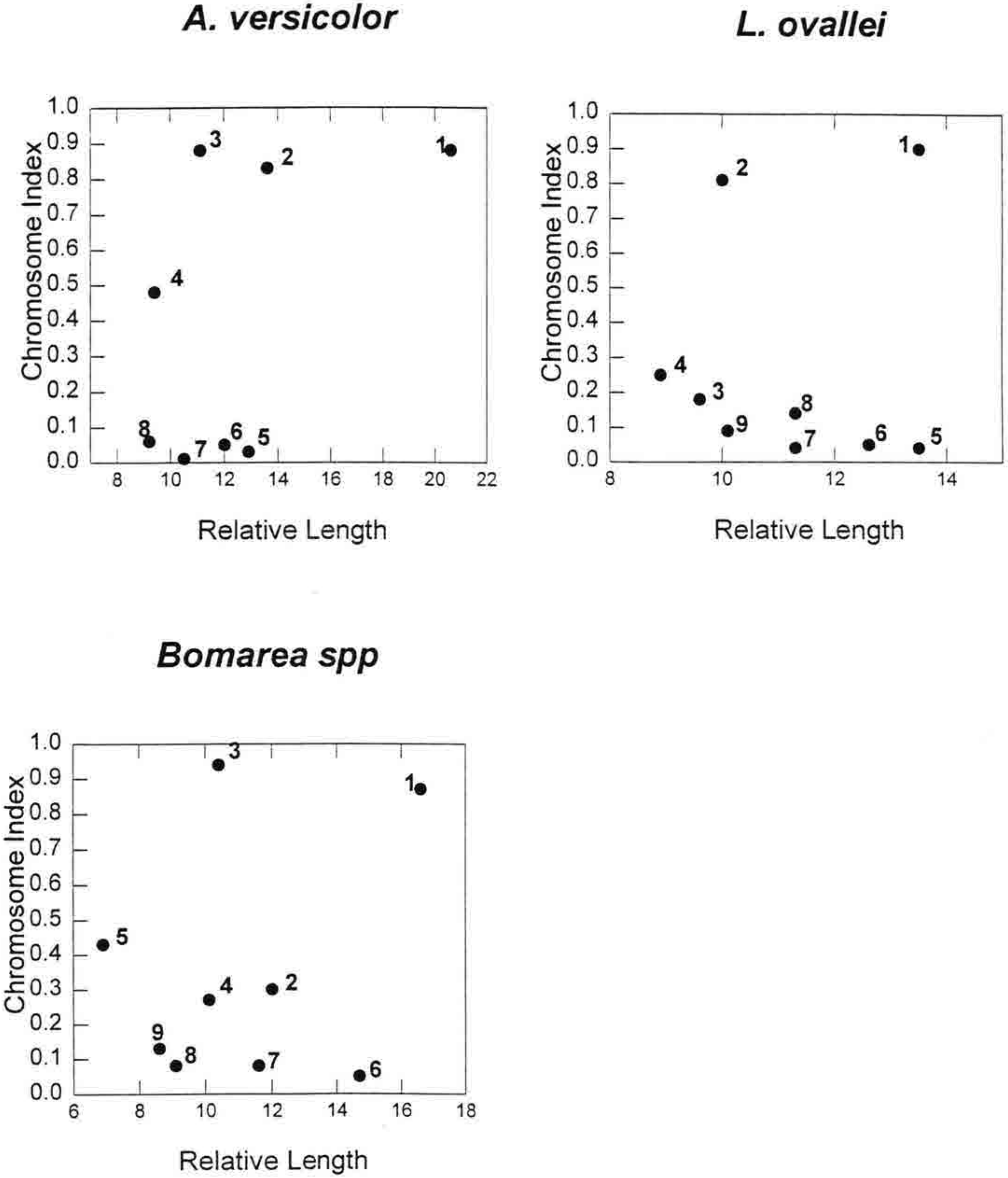


Fig. 10.2c: Diagrammatic representation of genomes.

of the shorter arm of the chromosome as well as a narrow band at the centromere and another narrow band distal to the centromere on the longer arm.

Chromosomes of pair number 3 had similar shapes for both species but the relative lengths were different. One chromosome of this pair in *A. ligtu* carried a small satellite. The heterochromatic banding patterns for both pairs in both species were similar.

Pair number 4 of *A. ligtu* was submetacentric in shape and had a relative length of 9.9%. The submetacentric chromosome pair of *A. chilensis* was pair number 5 and it had a relative length of 10.1%, close to that of the same shaped pair in *A. ligtu*. There was some similarity in Giemsa banding patterns with both pairs having a wide heterochromatic band at the distal end of the short arm and a fainter band on the long arm.

The final chromosome pair of the first group of the genome of each species was pair number 5 of *A. ligtu* and pair number 4 of *A. chilensis* and were metacentric in shape. These chromosome pairs were of different sizes, 9.6% (*A. ligtu)* and 11.0% (*A. chilensis)*. The heterochromatic bands were quite similar with the exception of the wide band present at the distal end of one arm of the chromosomes of *A. ligtu* which was not observed in the other species.

The three acrocentric pairs of chromosomes were of different lengths in the two species. All three pairs carried satellites in *A. chilensis* but only two pairs had satellites in *A. ligtu*. The heterochromatic banding pattern was similar in both species for pair number 6 but there were marked differences in the patterns for pairs number 7 and 8.

There are some similarities between these two species and some differences. It is likely that they are in fact biotypes of the same species or it may be that one is an old hybrid of the other. The habitats in which *A. ligtu* is known to grow covers a wide area of Peru between 32° and 38° south and from the coast inland along the river valleys. This wide range of habitat and the isolation of some populations may have resulted in the development of different biotypes of the same original species. The morphological characteristics of *A. ligtu* and *A. chilensis* are the same.

A. ligtu hybrid is probably the result of a cross between *A. ligtu* species and *A. haemantha* (Gulmon and Mooney, 1980). A comparison of the individual chromosomes of *A. ligtu* species and *A. ligtu* hybrid revealed that at least one chromosome of each pair in *A. ligtu* hybrid had the same size and shape as one of each pair of the species. The common chromosomes in the hybrid and the species respectively were 1 & 1, 3 & 3, 6 & 5, 8 & 8, 9 & 9, 11 & 11, 14 & 13 and

16 & 15. The heterochromatic bands on these chromosomes also indicated that these chromosomes were of the same origin.

Interestingly, the chromosomes of each pair of *A. ligtu* hybrid had almost exactly the same heterochromatic bands even though the lengths of these chromosomes were often different within each pair. This suggests that, if the other chromosome of each pair was derived from *A. haemantha*, then the latter species must have a similar karyotype to *A. ligtu* species. No true *A. haemantha* species were available for analysis at this time but future study may be able to determine whether the genomes of this species and *A. ligtu* are closely related.

Similarly, *A. psittacina* and *A. pulchella* are thought to be different names for the same species (Hannibal, 1941; Stinson, 1942; Robinson, 1963). Chromosome sizes, shapes and satellite association were similar for all pairs of both of the species. No heterochromatic bands were revealed on the chromosomes of *A. psittacina* using the present technique, whereas some scattered and faint banding was found on the chromosomes of *A. pulchella*. Conclusions on the actual homology of the chromosomes could not be determined at this time since the lack of bands on one species may have resulted from the developmental stage of the cells examined and not be due to any inherent differences. However, it would appear from the high degree of similarity of the two genomes that these two names are in fact just different designations for the same species.

Chromosome numbers in the cultivars varied from diploid (2n=2x=16) to hypertetraploid (2n=4x+1=33). The karyotypes of all cultivars showed some similarities as well as differences but all were rather complicated.

Two of the three diploid cultivars had eight chromosomes which were metacentric, submetacentric or subtelocentric in shape and eight acrocentric chromosomes. This was the same distribution as many of the species. The third diploid cultivar, `Canaria', was somewhat different as it had nine chromosomes in the first group and only seven acrocentric chromosomes in the second group. One subtelocentric chromosome, chromosome 7, had a ratio of 6.9, which was svery close to being classified as an acrocentric chromosome.

Five of the acrocentric chromosomes in `Canaria' and `Eureka' carried satellites which were generally small but occasionally slightly larger than the small short arm of the chromosome. `Orchid' had only four acrocentrics which carried satellites.

There were eleven triploid cultivars included in this study and one, `Orange Beauty', which was hypertriploid. All of these cultivars had twelve chromosomes ingroup one which were metacentric, submetacentric or subtelocentric. Except for `Rosita' the cultivars all had three large metacentric chromosomes. `Rosita' had only two large metacentrics. There were also 4, 5 or 6 medium or small metacentrics in the first group of the genome. The remaining chromosomes in the first group were submetacentric or subtelocentric. All triploid cultivars except `Red Surprise' had one or two medium-sized subtelocentric or submetacentric chromosomes, none of which carried satellites. A tiny submetacentric or subtelocentric chromosome was found in all cultivars except `Mona Lisa' and `Monika'. None of these carried a satellite except for `Orange Beauty' which had a small satellite on the short arm. `Monika' and `Mona Lisa' had a slightly larger subtelocentric chromosome which had a small satellite.

There were twelve acrocentric chromosomes in each of the triploid cultivars and thirteen in the hypertriploid `Orange Beauty'. There were from one up to nine acrocentric chromosomes in the triploid cultivars which were carrying satellites which were generally small but were sometimes larger than the short arms of the chromosomes. The range in relative length of these chromosomes was similar for all triploid cultivars.

The karyotype of the tetraploid and near-tetraploid cultivars was somewhat more complicated than for the triploids and diploids. There were 16 chromosomes in group one for six of the tetraploid cultivars but only 14 in `Neva' and `Alnba'. All the genomes except `Neva' had only two large metacentrics. A tiny submetacentric was observed in all of these cultivars with those in `Neva' and `Alnba' carrying small satellites. `Arizo' and `Neva' also had a medium-small submetacentric which carried a small satellite. A tiny metacentric chromosome was also found in all the tetraploid cultivars except for `Alnba' and `Neva'.

The number of acrocentric chromosomes in these cultivars varied from 16 in `Jubilee', `Orego', `Arizo' and `Texas', 15 in the hypotetraploid `Luciana', 18 in `Neva' and `Alnba' and 17 in the hypertetraploid `Rosario'. There were two satellited acrocentric chromosomes in `Rosario' and `Jubilee' with up to seven in `Alnba'.

Most of the *Alstroemeria* cultivars studied were sterile. This is thought to be due to interspecific hybrid sterility at the diploid level or because one of the parents was an unknown spontaneous tetraploid which resulted in the development of sterile, triploid seedlings (Broertjes and Verboom, 1974). Examination of the individual chromosomes of each cultivar revealed that while some were clearly homologous, most were not. Observation of these cultivars during meiosis is needed to determine the level of pairing for each of them. It should be noted that although pairing was fairly normal in *A. pelegrina rosea*, with eight bivalents, fertility was only 45%

(Stephens et al., 1993). Thus, even with very low fertility there may be more regular pairing in the cultivars than would be expected.

The cultivar `Orchid' was derived from a cross between *A. violaceae* and either *A. haemantha* or *A. aurantiaca* through the process of mutagenesis. `Canaria' was then derived from `Orchid' also through mutagenesis. Comparison of the chromosomes of each of the cultivars revealed many similarities between them as well as between the cultivars and *A. aurantiaca.*

Chromosome 2 of both cultivars and chromosome 1 of *A. aurantiaca* had approximately the same size and shape (Table 10.1). No heterochromatic bands were present on the chromosome in `Orchid' or in *A. aurantiaca*, however, small bands were present on the distal ends of the chromosome in `Canaria'.

Chromosome 4 of `Canaria' was similar to chromosome 4 of *A. aurantiaca.* The heterochromatic bands of both of these chromosomes were the same. Neither chromosome 3 or 4 of `Orchid' appeared to match either of the chromosomes of pair 2 of *A. aurantiaca.* However, the total relative lengths of chromosomes 3 and 4 for `Orchid' and `Canaria' were almost the same. This suggests that mutagenic treatment may have caused the exchange of chromosome arms between these two chromosomes. If the relative lengths of the long arms and the short arms of each chromosome are considered the chromosomes of `Canaria' could have been created by the exchange of the short arm of chromosome 3 of `Orchid' with the long arm of chromosome 4. Since the heterochromatic bands of `Canaria' and *A. aurantiaca* were located on the short arm of chromosome 4 these were still able to be used to identify this chromosome in both plants.

The relative lengths of the long and short arms of chromosomes 5 and 6 of `Orchid' and `Canaria' were exactly the same. These chromosomes were subtelocentric in shape. A chromosome of a similar total relative length in *A. aurantiaca*, chromosome 7, was also subtelocentric in shape. Heterochromatic bands on the distal ends of the long arm of the chromosome in the species were in the same location as the heterochromatic bands on the long arms of the chromosomes in the cultivars. The band at the distal end of the short arm were present on the distal ends of the chromosome in `Canaria'.

Table 10.1. Relative lengths of the long arms and short arms of *A. aurantiaca*, 'Canaria' and 'Orchid'.

Chromosome	Arm	*A. aurantiaca*	Canaria	Orchid
1	Long	6.0	6.2	6.5
	Short	5.3	5.3	6.0
2	Long	5.4	6.0	6.2
	Short	5.3	5.3	4.7
3	Long	4.2	5.4	5.0
	Short	3.0	4.2	3.5
4	Long	3.6	3.1	5.0
	Short	2.5	2.9	2.9
5	Long	2.8	4.5	4.6
	Short	2.6	1.1	1.1
6	Long	2.6	4.1	4.1
	Short	2.5	1.1	1.0
7	Long	4.9	3.2	1.8
	Short	1.2*	0.5	1.6
8	Long	3.6	1.7	2.1
	Short	1.2	1.5	0.8
9	Long	5.8	1.9	6.5
	Short	0.8*	0.8	0.8*
10	Long	5.6	6.4	6.1
	Short	0.2*	0.4*	0.2*
11	Long	5.4	6.4	6.0
	Short	0.3	0.1*	0.3*
12	Long	5.1	6.1	6.0
	Short	0.4	0.2*	0.3*
13	Long	5.2	5.7	6.0
	Short	0.3	0.2*	0.2
14	Long	5.0	5.1	4.7
	Short	0.1	0.2	0.3
15	Long	4.6	4.7	4.2
	Short	0.3*	0.1*	0.2
16	Long	4.5	4.2	3.5
	Short	0.1*	0.2	0.1

*Satellites are present on these arms

Chromosome 4 of `Canaria' was similar to chromosome 4 of *A. aurantiaca*. The and the satellite it carried in the species were not observed in the cultivars. If this is the same chromosome in both the species and the cultivars then the elimination of part of the chromosome may be the result of the mutagenic treatment of the species used to create the cultivars.

Chromosome 9 in `Orchid', chromosome 11 in `Canaria' and chromosome 9 in *A. aurantiaca* all had the same total relative length. The shape of these chromosomes was slightly different however. Comparison of the heterochromatic bands on the long arms of the chromosomes indicated that they were very similar.

Chromosome 14 of `Canaria' and chromosome 13 of `Orchid' had almost the same total relative length as chromosome 14 of *A. aurantiaca*. The relative lengths of the long arms and the short arms of each of these chromosomes were also very similar to one another. The heterochromatic banding pattern on the chromosome of *A. aurantiaca* was also observed on the chromosome of `Canaria' but there were no heterochromatic bands revealed on the chromosome of `Orchid'.

The final chromosome in the cultivars that appeared to be similar to one from *A. aurantiaca* was chromosome 16 of `Canaria' and chromosome 15 of `Orchid'. This chromosome in both of the cultivars had almost the same total relative length as chromosome 16 of aurantiaca. The heterochromatic banding patterns, however, did not match. There was a wide heterochromatic band in the middle of the long arm of the chromosomes of *A. aurantiaca* but only a thin heterochromatic band was found at the proximal end of the long arm of `Orchid' and no bands were observed on `Canaria'. The homology of these chromosomes, therefore, cannot be determined from the present study.

`Eureka' is thought to be composed of two non-homologous genomes (Hang and Tsuchiya, 1988). Isozyme banding patterns (part 1 of this study) indicated that the two possible parents of the species studied were *A. pelegrina* and *A. aurantiaca*. Comparison of the genomes and banding patterns of the two species and the cultivar indicated that both species may have contributed chromosomes to the cultivar (Table 10.2). There are a number of chromosomes in the first group of the genome of the cultivar that cannot be attributed to either of the species, however.

Table 10.2. Relative lengths of long arms and short arm of *A. pelegrina* and `Eureka'.

Chrom-some	Arm	*A. pelegrina*	Orchid
1	Long	7.5	6.9
	Short	7.3	6.4
2	Long	7.2	6.1
	Short	6.5	5.8
3	Long	4.0	4.8
	Short	3.3	3.5
4	Long	3.8	2.9
	Short	2.7	2.8
5	Long	2.3	4.6
	Short	1.8	1.2
6	Long	2.0	4.0
	Short	1.7	1.2
7	Long	2.5	1.8
	Short	0.8	1.5
8	Long	2.4	2.1
	Short	0.8	0.8
9	Long	6.1	6.6
	Short	0.2*	0.3
10	Long	5.9	6.0
	Short	0.3*	0.3*
11	Long	5.5	5.7
	Short	0.3	0.2*
12	Long	5.2	5.3
	Short	0.3	0.2*
13	Long	5.3	5.1
	Short	0.2*	0.2*
14	Long	5.0	4.9
	Short	0.2*	0.1*
15	Long	4.2	4.2
	Short	0.2	0.2
16	Long	4.0	3.5
	Short	0.2	0.3

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*Satellite carried on this arm

Chromosome 1 of `Eureka' had a similar relative length to chromosome 2 of *A. pelegrina'*. The relative length of the shorter arms were almost the same for both plants but the longer arm of the cultivar had a shorter relative length than the longer arm of the species. There were no heterochromatic bands on these chromosomes for either the species or the cultivar.

Chromosome 10 of `Eureka' had the same relative length as chromosome 9 and 10 of *A. pelegrina'*. However, since there was a wide heterochromatic band in the middle of the long arm of the cultivar chromosome, with a narrow band proximal to this, it was unlikely to have originated from *A. pelegrina* since it had no heterochromatic bands on these chromosomes. Chromosome 9 of *A. aurantiaca* had a slightly longer relative length than chromosome 10 of the cultivar. The heterochromatic banding pattern was the same for both of the chromosomes. This suggests that *A. aurantiaca* may possibly have been the contributor of this chromosome to the cultivar but no firm conclusion can be drawn at this time.

There was close similarity in the shape, relative length and presence of satellite between chromosome 11 of the cultivar and chromosome 10 of *A. aurantiaca*. The distribution of heterochromatic bands was also very similar on both chromosomes.

Chromosome 12 of `Eureka' was the same relative length as chromosome 12 of *A. pelegrina* and almost the same size as chromosome 11 of *A. aurantiaca*. Since there was a narrow heterochromatic band in the proximal third of the long arm of the cultivar chromosome it is most likely that this chromosome may be attributed to *A. aurantiaca*. The relative lengths of the long arm and the short arm of this species was very similar to those of the cultivar. There was a satellite present on the short arm of the cultivar chromosome, however, that was not observed on the chromosome in the species.

The next chromosome in `Eureka', number 13, had about the same relative length as chromosome 14 of *A. pelegrina*. The relative lengths of the long arm, short arm and the presence of satellites for each of these chromosomes was almost the same. There were no heterochromatic bands on either chromosome. This suggests that *A. pelegrina* may have been the contributing species for this chromosome in the cultivar.

Chromosome 14 of `Eureka' had similar relative lengths for the long arm and the short arm of chromosome 14 of *A. aurantiaca*. However, there was a satellite observed on the short arm of the cultivar that was not present in the species. The thin heterochromatic band at the proximal end of the short arm of the chromosome in the cultivar did not match the banding pattern of the chromosome in the species. It is unlikely that this species was responsible for chromosome 14 of the cultivar.

The final chromosome of the cultivar that may have been related to one of the species was number 15. It was almost the same length and shape and chromosome 15 of *A. pelegrina*. Furthermore,there were no heterochromatic bands on either of these chromosomes.

Although it is possible that both *A. pelegrina* and *A. aurantiaca* may have contributed chromosomes to the cultivar `Eureka' it seems unlikely that these were the only species contributors.

The cultivars `Carmen' and `Marina' are both `Parigo' hybrids which were developed from the progeny of a three-way cross with *A. aurantiaca* as the first parent (Goemans, 1962). The other parents of the cross were not revealed by the English breeder. Isozyme characterization of these cultivars revealed that the other species involved in their development may have been *A. haemantha, A. magnifica ssp maxima* and either *A. pelegrina* for `Carmen' or *A. psittacina* for `Marina'. Neither *A. pelegrina* or *A. psittacina* revealed any heterochromatic bands using the present techniques. *A. aurantiaca* had very distinctive bands on many of it's chromosomes.

Chromosome 1 of `Carmen' had the same relative lengths of long arm and short arm as chromosome 2 of *A. psittacina* (Table 10.3). There were no heterochromatic bands present on this chromosome which further supported the hypothesis that the cultivar chromosome may be related to chromosome 2 of the species.

Chromosomes 18 and 19 of `Carmen' had the same relative lengths of long arm and short arm as chromosomes 11 and 13 respectively of *A. pelegrina* and none of these chromosomes had heterochromatic bands.

Chromosomes 4 and 9 of `Marina' were almost the same size and shape as chromosomes 3 and 9, respectively, of *A. psittacina.* There were no heterochromatic bands on any of these chromosomes further supporting the possibility that these chromosomes were homologous.

Chromosomes 14 and 15 of `Marina' appeared to match chromosome 10 of *A. psittacina* and chromosome 10 of *A. pelegrina*, respectively, but comparison of their heterochromatic banding patterns suggests that they are not homologous.

Table 10.3. Relative length of long arms and short arms of *A. psittacina*, \`Marina' and \`Carmen'.

Chromosome	Arm	*A. psittacina*	Marina	Carmen
1	Long	7.2	6.8	6.7
	Short	6.8	6.7	5.9
2	Long	6.6	6.5	6.3
	short	6.3	5.9	5.9
3	Long	6.8	6.2	6.2
	Short	1.7	5.7	5.5
4	Long	6.3	6.7	4.6
	Short	1.8	1.8	4.0
5	Long	2.5	4.4	5.9
	Short	2.1	3.1	1.8
6	Long	2.2	4.4	4.8
	Short	1.8	2.6	2.1
7	Long	2.9	4.8	3.3
	Short	1.1	1.8	2.7
8	Long	2.7	3.0	3.2
	Short	1.0	2.7	1.8
9	Long	6.5	2.4	2.5
	Short	0.2	1.8	2.0
10	Long	6.2	2.9	1.7
	Short	0.1	0.8	1.6
11	Long	5.0	2.2	2.1
	Short	0.3	0.7	1.0
12	Long	4.8	1.6	2.1
	Short	0.2*	1.4	0.7
13	Long	4.6	6.5	7.2
	Short	0.2*	0.3	0.3
14	Long	4.5	6.2	6.5
	Short	0.2	0.1	0.1*
15	Long	4.0	6.0	6.3
	Short	0.2	0.3*	0.3*
16	Long	3.6	5.7	5.9
	Short	0.1*	0.2*	0.3*
17	Long		5.4	5.6
	Short		0.3	0.3
18	Long		5.3	5.5
	Short		0.4	0.3
19	Long		5.0	5.2
	Short		0.1	0.3*

Table 10.3. Relative length of long arms and short arms of *A. psittacina*, `Marina' and `Carmen' cont'd.

Chromosome	Arm	*A. psittacina*	Marina	Carmen
20	Long		4.6	4.8
	Short		0.1	0.1
21	Long		4.3	4.3
	Short		0.2*	0.3
22	Long		4.0	3.8
	Short		0.1*	0.2
23	Long		3.5	3.8
	Short		0.1*	0.1*
24	Long		3.2	3.2
	Short		0.2*	0.4

*Satellites carried on these arms.

Chromosome 18 of `Marina' appeared to be homologous to chromosome 11 of *A. aurantiaca* with similar relative lengths and heterochromatic regions.

Chromosomes 20 and 23 of `Marina' were of similar relative size and shape to chromosomes 14 and 16, respectively, of *A. psittacina*. The lack of distinct heterochromatic regions on any of these chromosomes further supported the possibility of homology between these pairs of chromosomes.

There were several chromosomes that were common to both `Marina' and `Carmen' and this indicated the close relationship between these cultivars. The parental species which contributed these chromosomes could not be determined from the present study but may be deduced from further analysis of other species. The chromosomes which were of the same relative length for long and short arms and had the same heterochromatic patterns were numbers 2, 3, 11, 16, 18 and 23 for both cultivars.

Although *A. aurantiaca* is known to be one of the parents of these cultivars few close matches could be found between the chromosomes of the cultivars and those of *A. aurantiaca*. Many of the chromosomes of the cultivars exhibited the unique banding patterns of the species. However, the differences in measurements of the chromosome arm lengths may have been due to experimental artifact or to mutagenic treatment of the cultivars during their development.

The cultivar `Appelbloesem` was developed through X-ray mutation of the cultivar `King Cardinal' (Micke et al., 1985). A comparison of the chromosomes of these cultivars revealed that many of them were of the same relative lengths and shape and had the same heterochromatic banding pattern. Chromosomes 4, 7, 8, 14, 17 and 21-24 for each cultivar, however, had different lengths and banding patterns. For chromosomes 4 this difference in heterochromatic bands could be explained by the inversion of the short arm of the chromosome. For chromosome 7 the missing heterochromatic band at the distal end of the long arm of `Appelbloesem' may be due to the breakage of part of the chromosome arm when subjected to X-ray mutation. Chromosomes 14 and 16 of `Appelbloesem' had heterochromatic bands which could have been created by the reciprocal translocation of the distal half of the long arms of the same chromosomes in `King Cardinal'.

Isozyme analysis (part 1) suggested that *A. aurantiaca* and *A. haemantha* may be possible parents of these cultivars. Comparison of the chromosomes of the cultivar and *A. aurantiaca* revealed some similarities (Table 10.4). Chromosomes could be paired from the cultivar `King Cardinal' and the species based on their relative lengths, shapes and heterochromatic banding patterns. These pairs were for chromosomes 3 and 1, 7 and 4, 13 and 9, 14 and 9, 16 and 11, and 21 and 16 of the cultivar and the species respectively.

Comparison of the chromosomes of many of the other cultivars with those of the species also revealed the similarities between them. It was possible to identify many chromosomes in the cultivars to determine which species were possible contributors of these chromosomes to the cultivars. With the addition of information on other species more of the chromosomes may be attributed to specific species.

The presence of specific unique chromosomes in the cultivars `Orange Beauty' and `Rosita' were proposed to be useful in the identification of the parental species which contributed them (Hang and Tsuchiya, 1988). They may have originated from *A. pelegrina* (Stephens, et al., 1993). Comparison of the banding patterns of chromosomes 11 of `Orange Beauty' and 12 of `Rosita' to chromosomes 7 and 8 of *A. pelegrina* revealed that the chromosomes in the two cultivars had satellites which were heterochromatic and there was a narrow heterochromatic band near the centromere of the chromosome in `Orange Beauty'. There were no satellites on the chromosome of *A. pelegrina* in the plant included in this study but satellites were noted on the same chromosome of other plants of this species (Stephens et al., 1993). No heterochromatic bands were revealed on the species chromosomes.

Table 10.4. Relative length of long arm and short arm of `King Cardinal'

Chromosome	Arm	Length	Chromosome	Arm	Length
1	Long	7.8	2	Long	6.1
	Short	6.6		Short	6.0
3	Long	5.9	4	Long	5.3
	Short	5.5		Short	3.4
5	Long	6.2	6	Long	4.7
	Short	1.8		Short	1.7
7	Long	3.7	8	Long	3.1
	Short	2.8		Short	2.8
9	Long	2.3	10	Long	3.0
	Short	2.0		Short	0.7
11	Long	2.3	12	Long	1.6
	Short	0.9		Short	1.4
13	Long	6.6	14	Long	6.1
	Short	0.3		Short	0.3
15	Long	5.8	16	Long	5.5
	Short	0.3		Short	0.2
17	Long	5.4	18	Long	5.3
	Short	0.1		Short	0.1
19	Long	4.8	20	Long	4.7
	Short	0.4*		Short	0.3
21	Long	4.6	22	Long	4.2
	Short	0.2		Short	0.2
23	Long	3.9	24	Long	3.8
	Short	0.2		Short	0.2

*Satellite carried on this arm

The chromosome in Rosita' seemed to be completely homologous to that of *A. pelegrina* while that in `Orange Beauty' was slightly different and could not be unambiguously identified as originating in *A. pelegrina*.

The study confirmed that chromosomes may be accurately identified based on the relative lengths of their long and short arms, their shapes and their heterochromatic banding patterns. Once these chromosomes have been identified it may be possible to follow their distribution within a cultivar following hybridization. However, when mutagenic agents were used to create new cultivars changes occurred in some of the chromosomes. It may be possible to deduce these changes from the changes in the banding patterns of the chromosomes in some instances.

Chapter 11

CONCLUSION

The objectives of this study were to uniquely identify and characterize the species and cultivars of *Alstroemeria* as well as species in *Bomarea* and *Leontochir* and to provide data that may be useful in determining the probable parents of the commercially grown cultivars of *Alstroemeria*. Two approaches were used to attain these objectives. The first was to utilize the polymorphism known to exist within the enzyme systems of many plants, through the characterization of the isozyme banding patterns of these plants. The second was to carry out an extensive cytological study of the materials in order to identify each of them with respect to the chromosome complement and the Giemsa banding patterns of each of the chromosomes or chromosome pairs.

Some of the species used in this study involved plants which had different color types and others involved materials which are thought to carry different names even though they are the same species.

Isozyme analysis revealed that the two color types of *A. pelegrina* were no different in enzyme polymorphism and should be considered to be genetically similar. Earlier karyotype analysis of this species (Stephens et al., 1993) revealed that there were no cytological differences between them. The two color variants of *A. aurantiaca* showed some variation in isozyme patterns. Only PGM exhibited exactly the same zymogram. The other enzyme systems had some bands in common for each of the color variants and some that were unique for each. This study suggests that there may be differences among different populations of *A. aurantiaca* and investigation of plants from different sources should to be undertaken.

A. psittacina and *A. pulchella* are generally considered to be different names for the same species and the similarity in isozyme banding patterns and karyotypes supports this idea. The presence of heterochromatic bands on chromosomes of *A. pulchella* but not on *A. psittacina* may indicate that there may be some differences between them that have not been revealed in the present study. Alternatively, the differences may be related to the experimental technique and further investigation is needed to clarify this.

Similarly, *A. ligtu* and *A. chilensis* are thought to be synonymous. However, slight differences were identified for these two species with regard to isozyme banding patterns, karyotypes and heterochromatic bands. It is likely that these species may be closely related or are possibly biotypes of a single species.

A. ligtu hybrids are the result of a cross between *A. ligtu* and *A. haemantha*. Isozyme analysis revealed that there is a good relationship between *A. ligtu* species and hybrids as well as between *A. haemantha* hybrid and *A. ligtu* hybrid. The karyotype analysis and Giemsa banding patterns of the chromosomes indicated that many of the chromosomes in the hybrid originated in *A. ligtu* species. The proposed parentage for the *A. ligtu* hybrids is supported by the results of this research.

Unique chromosomes have been identified in some of the species which can then be found in the cultivated varieties. These include the chromosomes of pairs number 3 and 4 of *A. aurantiaca* which could be identified in the complements of the cultivars `Canaria', `Orchid', `King Cardinal' and `Appelbloessem' as well as several of the acrocentric chromosome pairs. Giemsa banding was also used to determine whether chromosomes from *A. pelegrina*, `Orange Beauty' and `Rosita', which had been previously proposed to be of the same origin, were actually homologous. The results indicate that the only the chromosome in `Rosita' could be clearly identified as originating from *A. pelegrina*..

Using these two approaches (isozyme and karyotype analysis) in the investigation of the species of *Alstroemeria* it is possible to uniquely identify each one of them. This information can be used to determine the possible parentage of the many cultivars currently grown. Once this information is ascertained a breeding program can be initiated which will be more efficient in the production of new cultivars for the United States.

There is much confusion as to the relationship between species which were collected and named by different groups over many years. In some instances the same name has been given to different plant species and on other occasions the same species has been given different names. The present approach to identification of the species should enable collectors to accurately identify their material.

Similarly, the accurate identification of cultivars for patent applications and for the protection of the breeder is very important. Using isozyme characterization and cytological analysis the unique identification of each cultivar is possible.

Using only three isozyme systems it is possible to identify all of the species used in this study while only two isozyme systems are needed to separate all 23 cultivars studied. Chromosomes which are morphologically similar among different species can be distinguished from one another based on their Giemsa banding patterns. Chromosomes which are of the same origin can be identified in the cultivars.

The genera *Bomarea* and *Leontochir* are closely related to the genus *Alstroemeria*. Examination of the karyotype of each genera revealed many similarities between them although the obvious difference was in the chromosome number of each. The former two species could be easily distinguished from all of the *Alstroemeria* species based on their isozyme patterns. However, there were many isozyme bands which were common to *Alstroemeria* and either one or both of the other genera or which were close enough to be allozymes of the same loci. The distribution of the acrocentic and the smaller metacentric and submetacentric chromosomes as shown in the diagrammatic representation of the genomes was very similar for all three genera.

The present study provides an approach which can be used to further the understanding of the relationships between these three genera and of the relationship of the species to the cultivars. When more species have been studied and their unique characteristics revealed, it will be possible to use this information to unravel the identity of the putative parents of the European cultivars. The information available through these approaches at least allows the unique identification of species and cultivars. It may also be used to determine the possible parents of many of the cultivars included in this study.

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