SPECIES RELATIONSHIPS AND GENETIC ANALYSIS OF ISOZYMES IN *ASTER OCCIDENTALIS*, *ASTER FALCATUS* AND THE ALLOTETRAPLOID *ASTER ASCENDENS*

by

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ABSTRACT

Complex species relationships within the genus *Aster* have proven a challenge to systematists for decades. This study examines these complexities with an investigation into evolutionary relationships among three species of *Aster*. *Aster occidentalis* ($x = 8$) and *Aster falcatus* ($x = 5$) are believed to be the progenitor species of the allotetraploid species *Aster ascendens* ($x = 13$). Previous morphological and cytological analysis show that *A. ascendens* resembles *A. occidentalis* in ray colour and growth habit, and *A. falcatus* in the shape of the outer phyllaries, and that the karyotype of *A. ascendens* exhibits a combination of karyotype characteristics from both putative parent species. The purposes of this study were: (1). to determine isozyme inheritance patterns and linkages in nine polymorphic loci of *A. occidentalis* and *A. falcatus* through analysis of testcrosses in each species using starch gel electrophoresis and enzyme specific staining, and (2) to determine the degree of divergence among these three species using genetic analysis and to test the hypothesis of hybrid origin. Seventeen populations of *A. occidentalis* (n=8), *A. falcatus* (n=5), and *A. ascendens* (n=13) were examined for allozyme variation at 15 polymorphic loci representing 10 enzyme systems.

Inheritance patterns for nine loci (coding for six of the ten enzyme systems studied) were determined using controlled testcrosses. Chi - square ($\chi 2$) analysis of *A. occidentalis* testcrosses revealed linkage between Adh-1 and Pgi-2 and between Mdh-1 and Pgm-2. Three additional loci (Lap-1, Tpi-1 and Tpi-2) were found to assort independently, providing markers for five linkage groups in total. Testcrosses of *A. falcatus* were not successful Linkage analysis of *A. falcatus* and *A. ascendens* would be of great value in the construction of a genetic map for these three species and would be useful in further clarification of the relationships among them.

Allozyme studies showed that *A. ascendens* and *A. occidentalis* had a higher genetic identity than *A. ascendens* and *A. falcatus.* Genetic identities for

A. ascendens and *A. occidentalis* (0.834), and for *A. ascendens* and *A. falcatus* (0.648) were intermediate to identities among populations within species and the identity between *A. occidentalis* and *A. falcatus* (0.539). *A. ascendens* shares 9 alleles with *A. occidentalis*, 5 alleles with *A. falcatus* and 34 alleles with both parents out of 66 alleles examined. Unique alleles were found in all three species. Results of this genetic analysis support findings based on previous morphological and cytological studies and suggest that hybridization either came about long enough ago to allow new alleles to appear in *A. ascendens*, or occurred more than once in different geographic areas with different alleles present in the parent species.

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DEDICATION

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INTRODUCTION

1.0. Species Relationships

An evolutionary species is a group of populations that are unified by the fact that they share a common evolutionary fate through time (Templeton, 1989). Species that are closely related within a genus share a longer common evolutionary history than more distantly related species. Determining the nature of that history is the task of the systematist: to study evolutionary relationships among taxa using the tools at hand. In conjunction with the traditional use of morphological characteristics for this purpose, cytological and, more recently, genetic characteristics have proven to be valuable indicators of relationship (Moritz and Hillis, 1990).

Many problems in plant systematics cannot be solved solely with morphological analysis. Differences among individuals, both within and between taxa, may be the result of environmental effects on phenotype, convergent evolution or common ancestry. Also, locating distinguishing morphological markers is often difficult, since most morphological traits are polygenic in nature. Morphological markers controlled by a single gene locus are uncommon, and represent a small part of the plant genome. Expression of these traits is often controlled by a recessive mutation, rendering them visible in the homozygous condition only (e.g., flower pigments or heterostyly) (Gottlieb, 1981b).

Cytological and genetic data, when used in combination with morphological data, increase the number of characters available for analysis of taxonomic and evolutionary relationships. Comparisons of findings from different data sets can strengthen the

conclusions reached using each type of analysis separately. Also, different approaches may yield different kinds of information about a group of species, so combined information is often the best, allowing us to identify groups that differ genetically but not morphologically or to combine into a single group genetically homologous groups that simply show a wide range of variability in a few key morphological characters. While not all taxa have this problem, there are species and genera in which the relationships among individuals, populations etc., established on the basis of morphology have not been sufficiently resolved, or where comparisons of morphological and cytological data do not point towards the same interpretation.

The genus *Aster* L. (Asteraceae) is one such difficult group. From the time of its description by Torrey and Gray (1841), relationships among many species have been unclear (Allen, 1984, 1986; Lamboy et al., 1991). Groupings and relationships within this genus based solely on morphological characters have been challenged when viewed in terms of cytological data (Semple and Brouillet, 1980b) but many problems still remain to be addressed. Genetic analysis for this purpose is the next logical step.

2.0. Mechanisms of Chromosomal Evolution.

Understanding how chromosomes evolve may be important for understanding the complexities of the relationships and origins of species in many groups. Examination and comparison of chromosomal morphology among species can reveal similarities and differences not visible at the morphological level but very useful in phylogenetic studies.

Physical characteristics of the chromosomes (e.g. number, size, centromere position, and secondary constrictions) are often correlated with taxonomic differences. Changes in the number or size of chromosomes reflect differences in the total amount of DNA within the genome and can affect phenotype by altering developmental rate (Sessions, 1990), or altering the types of genes present. In the present study, this understanding is important in order to be able to relate genetic data from isozyme analysis of *Aster* to previous studies on chromosomal characteristics.

Whereas many changes to chromosome structure involve a severe upset of a structural balance necessary for successful chromosome pairing and segregation and thus are lethal, viable anomalies do occur in the processes of meiosis and mitosis, and these can have important effects on the evolution of the species. Alterations in normal chromosome inheritance mainly involve chromosomal rearrangements as well as the occurrence of polyploidy and aneuploidy.

Deletions and duplications can occur by unequal crossing-over between homologous chromosomes during meiosis. In a deletion, the viability of the resulting gamete depends on the amount lost. While most are lethal, very small deletions can resemble point mutations, and thus have some evolutionary significance (Grant, 1981). Duplications, however, can lead to increased gene number. This is important in an evolutionary sense as a source of greater complexity. While mutation can alter the normal function of existing genes, duplication provides new "raw material" for genetic diversification (Grant, 1975).

Inversions occur when the chromosome breaks at two separate points and the ends rejoin after a 180° rotation, thereby reversing the order of the genes along that segment of the chromosome. Apart from the possibility of the changes in gene position affecting the viability or the phenotype of the gametes produced by an inversion, structural problems may occur during synapsis and division, such as deletions and duplications resulting from crossing over within the inversion loop. Inversion may also result in centromere loss. The loss of a centromere means the loss of the entire chromosomal fragment, and can effectively reduce the number of chromosomes in the plant genome. This reduction of chromosome number is termed aneuploid reduction and may also occur through nondisjunction or chromosome lag.

Translocation involves the movement of whole segments from one chromosome to another and functions as an important source of chromosomal change. Unequal reciprocal translocations can also result in the loss of chromosomes and will alter existing linkage groups.

A major source of change in plant chromosome number is polyploidy, a process by which the entire set of chromosomes is duplicated, resulting in multiple (three or more) sets. Polyploidy occurs in nature through somatic doubling or through the fertilization of unreduced gametes. It may take the form of autopolyploidy, in which a polyploid race arises from a single diploid ancestor, or (more commonly) as allopolyploidy, which involves the formation of an interspecific hybrid from parents with either the same or different chromosome numbers. Allopolyploidy may occur as the fusion of unreduced gametes, producing a fertile allotetraploid, or through the fusion of haploid gametes from

each parent. In the latter case, if the parents' genomes are sufficiently different, the resulting diploid hybrid may be sterile because of the inability of the chromosomes to pair successfully. Chromosome doubling may then occur, often in somatic tissues, re-establishing fertility and forming a new stable species.

Allopolyploidy, alone or in combination with chromosomal rearrangements and aneuploidy, can produce new species that are distinct from their parents, are able to reproduce sexually with normal fertility, and which can maintain heterozygous gene combinations because the number of loci present has doubled. Allopolyploids, carrying the combined genomes of both parents, have homeologous chromosomes containing these duplicate genes which may have different alleles in each parent. Because allopolyploidy still allows recombination and segregation to occur, adaptations to new environments can occur and become established in the genome with the expression of new genotypes, while the duplication provides raw genetic material for diversification and increased genetic complexity (Grant, 1981).

While these changes do occur naturally, they are not widespread in all plant groups. Frequency of polyploidy is higher in herbaceous perennials than in other angiosperms, perhaps because of certain factors related to growth habit that are requirements for polyploidy - a long life span with the possibility of vegetative regeneration and the common occurrence of natural interspecific hybridization. Woody dicots lack differing chromosomal rearrangements between related hybridizing species, whereas annuals lack the long life span (deWet, 1980).

The result of these chromosomal changes is the potential for the development of highly complicated inter and intra-species relationships that may be traceable through resulting alterations in the physical aspects of the chromosomes themselves. Chromosome banding techniques are valuable in the identification of homologous and homeologous chromosomes, which are useful in tracing species phylogeny (Sessions, 1990).

3.0. Analysis of Genetic Variation Using Isozymes

Isozyme electrophoresis has, in the past two decades, provided an extensive source of genetic markers that can characterize populations and species. These isozymes represent variable forms of enzymes, the changes being due to alterations in the structural gene(s) coding for that particular enzyme (Crawford, 1983). Structural alterations are manifest as changes in the size, charge or configuration of the proteins, causing them to migrate at different rates through a gel when an electric current is passed through it. With the use of enzyme specific stains, the phenotype appears as a characteristic pattern of bands on the gel (Crawford, 1990). This technique was developed by Hunter and Markert (1957) and since then has been applied to a wide range of studies in systematics, population genetics, mating systems, cultivar identification, inheritance and evolution. Chromosomes can be mapped with isozyme loci through analysis of linkage groups and may then serve as genetic markers for other traits. This is especially valuable for species lacking morphological markers (Moore and Collins, 1983; Weeden and Wendel, 1989).

The advantages to using isozymes are found in properties that facilitate simple, straightforward analysis. Isozymes are inherited in Mendelian fashion and are expressed

codominantly. Because of this the genotype can be inferred from the visible phenotype, without the interference of environmental effects (although there is the possibility of post-translational modification). Isozymes show complete penetrance and high levels of polymorphism, reflecting up to 30 -40% of the genetic variation present in the genome (Hamrick, 1989; Brown and Weir, 1983). Comparisons between individuals and between parent and progeny are used to determine the number of alternate forms being coded for (the number of loci) and the alleles (allozymes) occurring at each locus (Weeden and Wendel, 1989).

Caution must be taken however when using isozyme analysis. Overlapping loci, null alleles, developmental stage or artifacts (resulting from the electrophoretic process itself) can alter the apparent number of loci, or the patterns or the number of bands at a locus; interpretation from direct inspection of the bands alone is likely to be faulty. A formal genetic analysis is therefore most often used to confirm inheritance and variation through study of the segregation patterns in the progeny (Gottlieb, 1981b). It is also important to keep in mind when studying species relationships through comparative genetic analysis that there is no set amount of genetic change necessary for species divergence, nor does every gene evolve at the same rate; much depends on the organism and the genes involved. In some cases, alterations in very few genes may result in major morphological or physiological shifts (Harrison, 1991). Some genes are more important in the maintenance of species integrity than others, widening the range of genetic changes necessary for species divergence (Wiley, 1981). Comparisons between species then must take into account the type and the number of loci used in the analysis.

In a growing number of plant systematic studies, genetic differences among populations and species have been examined at the protein level using isozyme analysis in order to obtain a greater number of genetic markers representing larger portions of the genome than are available using morphological markers alone (Crawford, 1989). Applications of this technique have been mainly in the identification and maintenance of crop species varieties (Weeden and Wendel, 1989), but the use of isozyme electrophoresis in evolutionary studies of species relationships and origins is increasing (Moore and Collins, 1983; Weeden and Wendel, 1989). By examination and comparison of the number of gene loci present, and the range and frequencies of allelic forms, patterns of variation can be determined and applied to studies of the evolutionary relatedness of species, the origins of polyploid species, and the form of polyploidy involved. Isozyme data are also valuable for the construction of linkage maps for the same purposes as well as providing an aid to increase our understanding of chromosomal evolution and its effects on species relationships (Lassner and Orton, 1983; Gottlieb, 1981a). Studies using isozyme analysis to examine species relationships cover a number of different plant groups; examples are *Asplenium* (Werth et al., 1985), *Clarkia* (Gottlieb and Weeden, 1979; Allen, Gottlieb and Ford, 1991), *Coreopsis* (Cosner and Crawford, 1994), *Helianthus* (Rieseberg and Doyle, 1989; Rieseberg et al., 1991), *Limnanthes* (McNeill and Jain, 1983; Kesseli and Jain, 1984), and *Salix* (Purdy and Bayer, 1995).

In the work done on the Appalachian fern *Asplenium* by Werth, Guttman and Eshbaugh (1985), for example, the authors studied a complex made up of three diploid species and three allotetraploid derivative species. Genetic distance between diploid

species was calculated using allozyme data (alleles present and their frequencies over 15 genetic loci), and was found to be fairly high (.67 - 1.3). The allotetraploid species showed higher levels of heterozygosity (a characteristic of allopolyploids) than their diploid parents at almost all loci, showing that even though the diploid progenitor species were fairly divergent, they were still able to form viable hybrids that could be successfully associated with a particular pair of diploid parents. Unique alleles were present in all species, suggesting the hybridization had not occurred recently.

Isozyme divergence can also occur without corresponding divergence at the morphological level. Studies indicate a wide range of genetic identities among conspecific populations, especially self-pollinating species (Gottlieb, 1981b; Crawford, 1990) due to different populations of selfers becoming fixed for different alleles at a number of loci. Outcrossing species tend to show the same alleles in relatively similar frequencies.

Speciation (defined as reproductive isolation) can occur with little genetic change, as is seen in a study of two morphologically similar species of *Stephanomeria*, which had a genetic identity of 0.94 (Gottlieb, 1973). There was a single unique allele in the derivative species *S. malheurensis* but it also lacked many of the alleles of the progenitor subspp. *coronaria*. Crosses between the two yielded reduced seed set and fertility due to chromosomal structural differences. This has also been seen in studies of *Capsicum* (McLeod et al., 1983), *Coreopsis* (Crawford and Smith, 1982) and *Lycopersicon* (Rick et al., 1976).

4.0. Complexities in *Aster*

The tendency towards polyploidy and interspecific hybridization in herbaceous perennial angiosperms is exemplified by the genus *Aster* L. (Asteraceae). *Aster* is rife with complex interspecies relationships resulting mainly from a high incidence of polyploidy and interspecific hybridization. It has a range of differing base chromosome numbers, $x = 4$, 5, 7, 8 and 9 (Jones, 1980a,b; Allen, 1984, 1986; Allen, Dean and Chambers, 1983). The most common chromosome base numbers are $x = 5$, 8 and 9. *Aster concolor*, an eastern North American species, is the only species with n = 4 (Jones, 1980a), whereas $x = 7$ is found in two species within the section *Conyzopsis* (Houle and Brouillet, 1985). Note that the letter n refers to the haploid chromosome number in an individual plant (the number of chromosomes present in the gametes), while x refers to the basal chromosome number present in a group, and that multiples of that number may be present based on x. Comprised of over 250 species worldwide, most of them endemic to North America, the genus *Aster* is a widely heterogeneous group of mostly perennial herbaceous plants occurring in a variety of habitats. The flowers are grouped into heads, with pistillate ray flowers ranging in colour from white to pink to purple or blue. Disc florets vary in number, and are tubular and perfect. The bracts of the involucre are more or less overlapping with herbaceous or foliaceous tips. Asters have leafy stems with alternate leaves ranging from entire to serrated (Torrey and Gray, 1841, Douglas, 1995).

Initial treatments of *Aster* by Torrey and Gray in 1841 and later treatments by Gray (1884) assessed relationships and defined species using morphological characteristics.

Taxonomic decisions based on morphology have proven problematical (Jones, 1978, 1980b; Jones and Young, 1983), as many morphological traits show extreme levels of variability within species. Natural groupings based on morphology, however, do exist. Important defining characters include, leaf shape and arrangement, type of inflorescence, and the morphology of the involucral bracts beneath the inflorescence as described above (Jones, 1980a).

Raven et al. (1960) carried out an extensive chromosome survey of the tribe Astereae in an attempt to clarify relationships among species from a perspective other than morphology. They found chromosome numbers based on $x = 9$ to be widely distributed amongst many phylogenetic lines and associated with a woody habit, which they considered primitive in the group. For these reasons, they regarded $x = 9$ as basic for the tribe. Although counts based on $x = 5$ were reported by Raven et al. (1960), it was argued that they were the result of aneuploid loss from the $x = 9$ ancestor. This was in agreement with previous studies by Huziwara (1958).

The work of Turner et al. (1961) on *Aster* was in disagreement with the conclusions of Raven et al. (1960). They stated that the lack of many $x = 6$ and $x = 7$ species in *Aster* disproves an $x = 9$ ancestor. The size of the genus and morphological similarities among the $x = 5$, 8 and 9 groups would make the selective loss of $x = 6$ and 7 groups unlikely. If, however, the ancestral chromosome number were $x = 4$ or 5 and the genus had evolved to its present morphological level with this basal chromosome number, then subsequent cytological differentiation could occur later on through allopolyploidy and

autopolyploidy. They agreed that aneuploid loss may then have occurred from newly formed $x = 9$ groups.

The use of chromosome counts to delineate the species and sections within the genus *Aster* has been confounded by the prevalence of polyploidy and hybridization (between species with similar chromosome numbers, and also between species with non-homologous chromosome numbers). It became obvious to researchers that further work was needed. Semple and Brouillet (1980a) proposed that *Aster* be divided into two taxonomic and phylogenetic groups, based on examination of morphology and cytology of North American species. A large group of species with chromosome counts based on $x =$ 8 and 9 would be retained in the genus *Aster*, whereas a smaller group (11 species) of $x =$ 4, 5 and 6 would be shifted to another genus, *Lasallea* Greene. *Lasallea* species were defined as belonging to a distinct morphological group, and were considered to possess a long common evolutionary history during which they could specialize, but still maintain a stable karyotype. Semple and Brouillet saw no overt similarities between the karyotype of *Lasallea* and the $x = 8$ and 9 karyotypes of *Aster* to contradict their placements. In a companion paper (1980b), Semple and Brouillet presented evidence based on chromosome morphology for their separation of *Aster* species. They examined the satellite chromosomes of 46 species of *Aster* and 9 species of the newly expanded *Lasallea* Greene (more correctly named *Virgulus* Raf. (Reveal and Keener, 1981)), and found a consistent difference in the satellite bearing chromosomes (the size of the satellite was smaller in the $x = 4$, 5 groups, and the NOR was located further away from the centromere). The Nucleolar Organizer Regions (NOR) are regions of chromosomal DNA

coding for ribosomal RNA. They can be identified as secondary restrictions in the chromosome, often located towards the end of the chromosome, and are therefore useful as markers for identification. This evidence, in conjunction with their morphological study suggested that both aneuploid reduction and allopolyploid increases produced the $x = 5$ and the $x = 9$ groups of Astereae but on different time scales.

Jones' classification of North American *Aster* (1980a) retained the $x = 4, 5$ species within *Aster*. Jones disagreed with Semple and Brouillets' splitting of the genus, citing the high occurrence of successful hybridization within the genus, and the degree of genetic similarity required for this. As well she held to the more traditional groupings based on morphological characters (i.e. as per Gray's treatment (1884)), maintaining that the separation of a group of species based on chromosome number would force further subdivisions to occur, requiring genus recognition for groups such as *Doellingeria*, *Heleastrum* and *Sericocarpus* and others based on minor morphological distinctions. Jones stressed the need for a rearrangement within the genus to provide clarity with respect to species limits and relationships. She presented 10 subgenera for *Aster*, incorporating $x = 4, 5, 7, 8$ and 9 groups (Jones, 1980a). In a companion paper, Jones (1980b) presented chromosome counts from over 250 populations of *Aster* representing 56 species and eight putative hybrids. She listed the species according to the classification system established in her previous paper (1980a), based on basic chromosome numbers and an ecological species concept. This treatment reinstates six groups to species status, reduces two others to subspecies level and assigns putative hybrid status to four taxa originally published as species.

Approaching the problem of original base chromosome number in Astereae from a different perspective, Gottlieb (1981a) pointed out that ploidal level was reflected in the multiplication of the genome, doubling the number of loci, and was not necessarily relative to the number of chromosomes. Using five species of *Machaerantha* ($x = 9$) and two species of *Aster* (an $x = 5$ and an $x = 9$), he analyzed the isozymes present in 17 enzyme systems with starch gel electrophoresis (SGE). His findings showed no evidence of duplicate loci in the $x = 9$ groups. Comparing this to other studies of known allopolyploids where gene multiplicity is seen, he rejected polyploidy as the mechanism behind the $x = 9$ groups. Aneuploid reduction from $x = 9$ to $x = 4$ or 5 would arise most likely by translocation of euchromatin along with the loss of centromeres and heterochromatin.

These interpretations of placement and phylogeny are still subject to debate. Most researchers maintain the use of a broad treatment of *Aster* based primarily on morphological characteristics. The use of chromosome counts and karyotype morphology has illuminated relationships among some species, but many problems are still unsolved. *Aster* remains a difficult genus.

5.0. Relationships Among Three Species of *Aster*.

The approximately 250 species of *Aster* in North America have been divided into different subgenera and sections by different authors. Semple and Brouillet (1980a) assigned eight subgenera and fourteen sections in *Aster*, and the eleven species they

removed were placed in three sections of *Lasallea* Greene (=*Virgulus* Raf.), based on morphological characteristics of the roots, leaves and phyllaries. Another treatment by Jones (1980a), based on chromosome number, divides *Aster* into ten subgenera and thirty sections.

Various treatments of *Aster* based on morphology and chromosome number have resulted in different placements for *A. occidentalis*, *A. falcatus* and *A. ascendens*. Jones (1980a) placed *A. occidentalis* and *A. ascendens* together with *A. bernardinus*, *A. chilensis* and *A. halli* in section *Occidentales*, subgenus *Symphyotrichum*, and *A. falcatus* along with *A. ericoides* in section *Multiflori*, subgenus *Virgulus*. Dean and Chambers grouped the five species of section *Occidentales* together with approximately 10 other species from different sections of *Symphyotrichum* into the *A. occidentalis* complex, based on intergradation of morphological characteristics and their ability to hybridize successfully (Dean and Chambers, 1983; Allen, Dean and Chambers, 1983). An alternate treatment by Semple and Brouillet (1980a,b) shifted *A. occidentalis* to subsection *Foliacei*, section *Dumosi* in the subgenus *Aster* (no mention of *A. ascendens* is made in this treatment), and removed *A. falcatus* from *Aster* entirely to the genus *Lasallea* based on chromosome number and morphology.

When Allen (1985) investigated the relationships of *Aster ascendens* (which is unique within the genus in possessing $x = 13$ chromosomes), she examined potential parents based on morphological characters, chromosome numbers and karyotype characteristics, as well as range, habitat and flowering time. Allen rejected aneuploidy as the source of the $x = 13$ chromosome base number for *A. ascendens* because of its rarity

within the *A. occidentalis* complex to which this species belonged. The most likely mechanism for the origin of this species was allopolyploidy, through a cross between an $x = 8$ and an $x = 5$ species. While allopolyploidy is a mechanism fairly common in the origin of new species in *Aster*, it is not common between species of differing chromosome numbers (Dean and Chambers 1983; Allen, 1986).

Possible $x = 8$ candidates for the *A. ascendens* progenitor species included the members of the *A. occidentalis* complex and *A. frondosus* (Allen, 1985). Of these only *A. occidentalis* showed sufficient morphological resemblance to *A. ascendens.* There are only three western N.A. species with $x = 5$ as a basal chromosome number; *A. campestris*, *A. ericoides* and *A. falcatus*. *Aster campestris* resembled *A. ascendens* in ray colour but little else and so was not considered further. *Aster ericoides* and *A. falcatus* are very similar and commonly interbreed but they are distinguished morphologically in the size of the flower heads and the arrangement of the inflorescences. Analysis of morphological characters showed greater similarity between *A. falcatus* and *A. ascendens* than between *A. ericoides* and *A. ascendens* (Allen, 1985).

Allen (1985) proposed that the parents of *A. ascendens* were *A. occidentalis* (with $x = 8$) and *A. falcatus* (with $x = 5$), species with sufficient range overlap and morphological similarity to the allotetraploid. Analysis of the karyotypes of these three species showed that *A. ascendens* was intermediate between *A. occidentalis* and *A. falcatus*, containing the satellite chromosomes of both species and an additive size distribution. While exhibiting a morphology closer to *A. occidentalis*

(i.e. in ray colour and overall growth habit), *A. ascendens* does reflect *A. falcatus* in the shape of its outer phyllaries (Allen, 1985). Diploid *A. ascendens* was reported as most likely originating as a cross between diploids through fusion of haploid gametes, which would then have undergone chromosome doubling to make the hybrid fertile (i.e. $5 + 8 = 13 \rightarrow 26$), or through the union of unreduced gametes. An alternative explanation was a cross between tetraploids, giving rise directly to a fertile diploid. Tetraploid *A. ascendens*, found mainly in the north and the east of its range, could have arisen through direct autopolyploid derivation from diploids or via a separate hybridization event between polyploid *A. occidentalis* and *A. falcatus* (Allen, 1985).

In order for this hybridization event to have occurred successfully, the degree of genetic affinity existing between *A. occidentalis* and *A. falcatus* would be expected to be high, despite the differences in chromosome number. This situation illustrates our limited understanding of the connection between chromosomal change and phylogenetic relationships, both in plant species generally and in *Aster* in particular. Clearly, new approaches are needed to give us a clearer understanding of both chromosomal evolution and hybrid speciation.

6.0. Objectives of This Study

In this study, I investigated isozyme variation in *Aster occidentalis* (n=8), *Aster falcatus* (n=5), and their proposed derivative species, the amphiploid *Aster ascendens* (n=13). These serve as an initial focus for genetic analysis of the genus *Aster*. The first

goal of this study was to determine inheritance of isozyme loci and their linkage groups in *A. occidentalis* and *A. falcatus*. Determination of the number of loci and the inheritance of individual alleles are necessary for interpreting patterns of variability within and among populations. Linkage groups between species capable of hybridization would be expected to be similar in order for hybridization to be successful. Comparison of linkage groupings between the parent species, and in the future, between these species and their hybrid derivative *A. ascendens*, should help to (a) confirm the hybrid origin of *A. ascendens*, and (b) improve our understanding of chromosomal evolution in this group. The second goal of this project was to study genetic variability in populations of all three species using isozyme electrophoresis in order to determine the degree of genetic divergence among them and to examine patterns of variation both within and among species.

MATERIALS AND METHODS

1.0 Plant Material

Three populations of *Aster occidentalis* (T. & G.) Hook. (1188, 1198, 1217) and four populations of *Aster falcatus* Lindl. in Hook. (1230, 1231, 1234, 1235) were used as sources of parental individuals for crosses made to determine inheritance and linkage groups. In total, 64 plants from *A. occidentalis* and 89 plants from *A. falcatus* were sampled for this study to be surveyed for eleven enzymes (ADH, EST, IDH, LAP, MDH, ME PGI, PGM, 6PGD, SkDH and TPI). All plants were grown from seed collected by G.A. Allen during 1987, from sites in California, Utah and Arizona. Detailed collection localities are given in Table 1 and Figure 1.

To determine population variability and population differences, samples from three *Aster* populations, *A. occidentalis* (1227), *A. falcatus* (1232) and *A. ascendens* (1207), were surveyed electrophoretically to determine genotype and allele frequencies of isozyme loci. Populations 1232 and 1207 were from Arizona, and population 1227 was from Oregon (see Table 1; Figure 1). In addition, unpublished electrophoretic data from populations assayed by G.A. Allen were combined with data from the populations I analyzed. From her original gel records, I compiled data for nine enzymes (EST, LAP, ME, MDH, 6PGD, PGI, PGM, SkDH, and TPI) from two populations of *A. occidentalis* (1223 and 1225), and six populations of *A. ascendens* (1194, 1197, 1202, 1218, 1219 and 1222). Allelic data for four enzymes (EST, ME, 6PGD and SkDH) that I had not sampled

Table 1. Collection Sites for Source Populations of *Aster ascendens*, *Aster falcatus* and *Aster occidentalis.*

Species	Collection # (Population)	Collection date	Locality and Habitat
A. ascendens	1194	Sept. 05, 1987	California, Alpine Co., Hwy. 89, W of Monitor Pass. Narrow grassy riparian zone along a small creek. elevation - 4500 ft. lat.38°09′ N long. 119°50′ W
	1197	Sept. 07, 1987	California, Inyo Co., NW of Bishop on the Pleasant Valley Dam Rd. Ungrazed, grassy meadow along tributary creek of Owens River. elevation - 4500 ft. lat. 37°23′ N long. 118°29′ W
	1202	Sept. 10, 1987	California, San Bernardino Co., off Hwy. 38 at Juniper Spring. Long narrow meadow extending down the valley from spring. elevation - 6800 ft lat. 34°13′ N long. 116°43′ W
	1207	Sept. 15, 1987	Arizona, Coconino Co., Kaibab Plateau, N of Crane Lake. in a meadow along Hwy.67. elevation - 8500 ft lat. 36°40′ N long. 112°08′30″ W
	1218	Sept. 18, 1987	Utah, Garfield Co., off hwy. leading to Bryce Canyon, in a large meadow just N of park gates and E of the hwy. elevation 7700 ft. lat. 37°40′ N long. 112°05′ W
	1219	Sept. 21, 1987	Nevada, White Pine Co., S of Hwy. 50 towards Hamilton at Round Spring. Edges of spring outflow, down to nearby creek, badly overgrazed. elevation - 6800 ft. lat. 39°18′ N long. 115°30′ W

Table 1. (cont'd).

Species	Collection # (Population)	Collection date	Locality and Habitat
A. ascendens	1222	Sept. 23, 1987	Nevada, Humboldt Co., Pine Valley Mtns., S of Denio Junction, just off Hwy. 140 on a dirt road towards Hillside Spring. Medium sized flat meadow near spring. elevation - 6100 ft. lat. 41°47′ N long. 118°39′ W
A. falcatus	1230	Oct. 22, 1987	Arizona, Yavapai Co., SW of Prescott at Indian Creek Campground. growing on road edge and along creekbed in *Pinus ponderosa* forest. elevation - approx. 7000 ft lat. 34°50′ N long. 112°50′ W
	1231	Oct. 22, 1987	Arizona, Coconino Co., Mogollon Rim in openings in *Pinus ponderosa* forest elevation - 6800 ft. lat 34°50′ N long. 111°26′ W
	1232	Oct. 23, 1987	Arizona, Coconino Co., N side of Upper Lake Mary; SW slope along ditch down a hillside in scattered *Pinus ponderosa* forest. elevation - 2100 ft. lat. 35°10′ N long. 111°60′ W
	1234	Oct. 24, 1987	Arizona, Coconino Co., Kaibab Plateau - growing in openings in *Pinus ponderosa* forest along wet, disturbed slopes. elevation - 7900 ft lat. 36°60′ N long. 112°20′ W
	1235	Oct. 24, 1987	Utah, Washington Co., New Harmony, growing along road to trailhead at meadow crossing. elevation - 5306 ft. lat. 37°28′ N long. 113°18′ W

Table 1. (cont'd).

Species	Collection # (Population)	Collection date	Locality and Habitat
A. occidentalis	1188	Sept. 05, 1987	California, El Dorado Co.,E of Corral Flat, Leek Springs, growing in damp meadow, heavily grazed. elevation -1800 ft. lat. 38°38′ N long. 120°14′ W
	1198	Sept. 07, 1987	California, Inyo Co., upper end of North Lake in a damp grassy meadow along stream near lake. elevation-9200 ft lat. 37°14′ N long. 118°37′ W
	1217	Sept. 18, 1987	Utah, Iron Co.,1.2 miles E of Cedar Breaks turnoff, Midway Creek - grassy valley along the creek. elevation - 9700 ft lat. 37°30′ N long. 112°50′ W
	1223	Sept. 24, 1987	Oregon, Lake Co., Fremont National Forest, Hwy. 140 between Adel and Lakeview, just off hwy on road to Summit Prairie along the banks of Camas Creek. elevation - 5700 ft. lat. 42°13′ N long. 120°13′ W
	1225	Sept. 25, 1987	California, Shasta Co., Hwy. 89 just N of Britton Lake at Cayton Creek crossing, meadow along the creek bank. elevation - 3300 ft lat. 41°02′ 47″ N long 121°37′ 11″ W
	1227	Sept. 26, 1987	Oregon, Deschutes Co., state Park Road to La Pine Recreation area, at crossing of Little Deschutes River, in grassy pasture on the east side elevation - 4200 ft. lat. 43°46′ N long. 121°28′ W

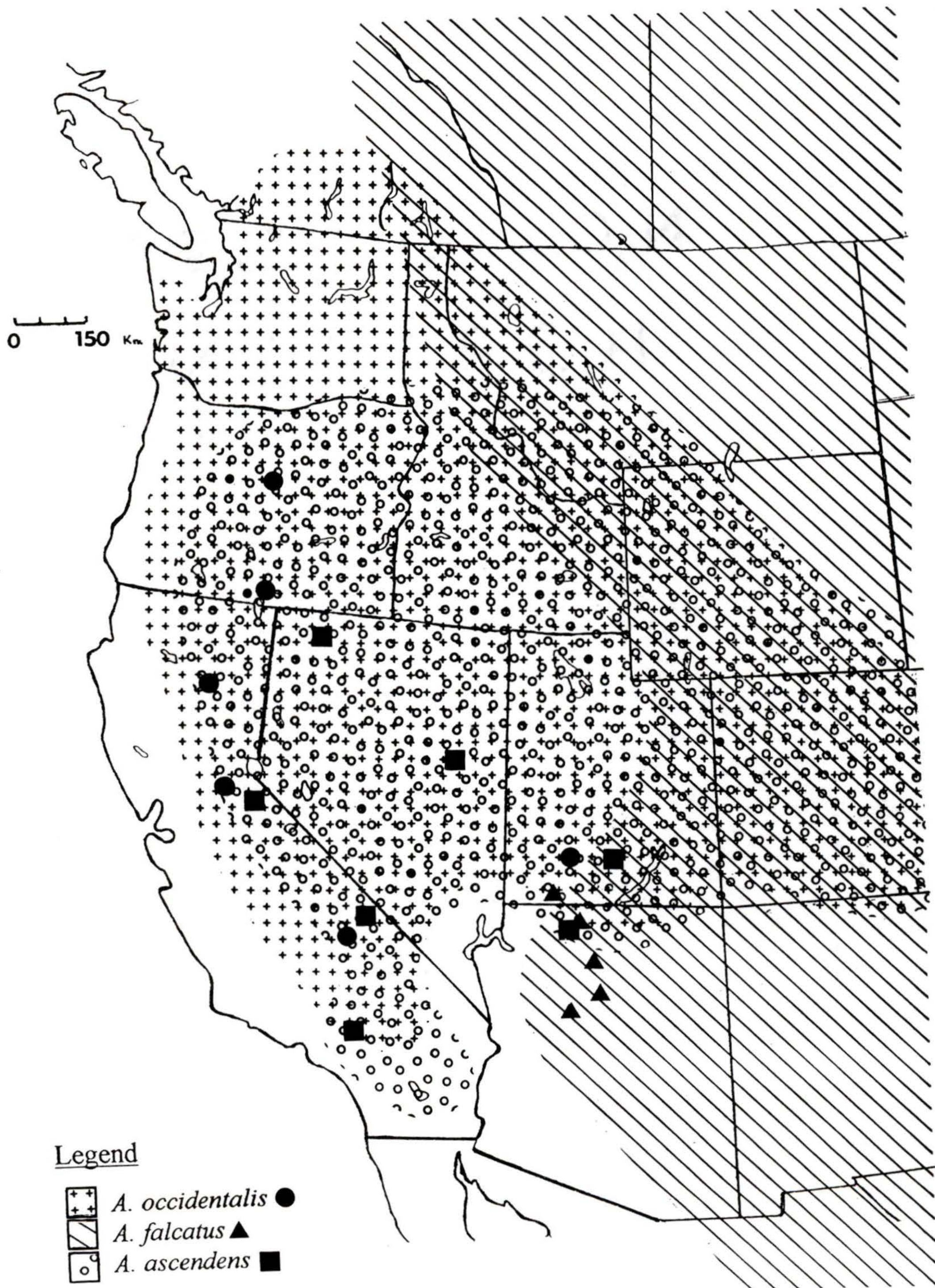


Figure 1. Map of the North American Continent Showing the Ranges and Collection Sites of *Aster ascendens*, *A. falcatus* and *A. occidentalis*.

extensively for in *A. occidentalis* (1198, 1217), and *A. falcatus* (1230, 1231, 1234, 1235) were also obtained from electrophoretic analysis by Allen (unpublished).

Voucher specimens for all populations used in this study are available in the Herbarium at the University of Victoria (UVIC), Victoria, British Columbia, Canada.

2.0 Electrophoretic Analysis

2.1 Extraction Procedure

Young leaf tissue (typically the second and third leaf from the central apex) was collected for all assays with the exception of alcohol dehydrogenase (ADH). For ADH, young root tissue was used, harvested after the plants had been kept well watered for two to three days. The amount of tissue used for extraction varied from 30 - 100 mg, depending on the number of samples required. On average, 10 mg of tissue was used per sample.

The extraction buffer (modified from Soltis et al., 1983) contained 0.1 M Tris HCl (pH 7.5), 1 mM EDTA, 10 mM KCl, and 10 mM $MgCl_2.6H_20$ with 14 mM mercaptoethanol added just prior to use. A small amount of polyvinylpyrolidone (PVP) was added to the leaf tissue just before grinding, the amount dependent on the amount of tissue being sampled but on average, 80 mg/ml of extraction buffer.

Plant tissue was placed in individual wells of pre-chilled porcelain grinding plates and kept on packed ice. The PVP was added, then three to nine drops (150 - 450 ul) of the extraction buffer (dependent on the amount of leaf tissue used and the number of samples to be taken). The tissue was ground to a slurry using ice filled test tubes, and absorbed

onto Whatman #3 filter paper wicks, approximately 2mm x 10mm. Samples to be frozen for future use were grouped in sets of ten, sandwiched between two glass slides, wrapped in foil, and stored at -80° C.

2.2 Electrophoresis

Gels were prepared with 10.5% starch using a variety of buffer systems (see below). The gel mixture was heated over a Bunsen burner, swirled constantly to prevent clumping until it had thickened, turned translucent and was boiling evenly. It was then degassed using a vacuum pump and immediately poured into a gel mold. Three sizes of gel molds were used; 19.5 x 17.5cm at depths of 5mm (for two slices), 9mm (for three to four slices) and 12mm (for five slices). The gel was covered with a glass plate while still hot, and stored covered at room temperature overnight.

Samples were loaded onto the gels by inserting them within a vertical slit made approximately one third the way up the gel. Each gel held up to 32 samples. Wicks containing a tracking dye of Amaranth, Brilliant Blue G, ethanol and water (Murphy et al., 1990) were added at each end to serve as markers for the run. Cellulose sponges were used to connect the electrode buffers and the gel. Plastic wrap covered the exposed portions of the gel and it was then weighted with two or three glass plates to ensure firm contact between the gel and the conducting sponges. Packs of crushed ice were placed below and above the gel and all runs were carried out in a refrigerator at 4° C.

2.3 Buffer Systems and Run Conditions.

I assayed for eleven different enzyme systems, utilizing five different buffer systems. The enzymes Alcohol dehydrogenase (ADH) (E.C.1.1.1.1), Esterase (EST) (E.C.3.1.1.-), Leucine aminopeptidase (LAP) (E.C.3.4.11.1), Phosphoglucose isomerase (PGI) (E.C.5.3.1.9), Phosphoglucomutase (PGM) (E.C.5.4.2.2) and Triose phosphate isomerase (TPI) (E.C.5.3.1.1) used a discontinuous system composed of a Tris / citric acid gel buffer, mixed with 1/10 volume of electrode buffer, consisting of LiOH and boric acid (Soltis et al., 1983) The gels were run at 100 milliamps and maintained at a maximum of 300 V.

Malic enzyme (ME) (E.C.1.1.1.40) and Shikimate dehydrogenase (SkDH) (E.C.1.1.1.25) used a discontinuous buffering system of trisodium citrate / HCl electrode buffer at pH 7.0 and L-Histidine / HCl gel buffer at pH 7.0 (Soltis et al., 1983). The gels were run at 50 milliamps.

Malate dehydrogenase (MDH) (E.C.1.1.1.37) used a continuous free base histidine / citric acid buffer system at pH 5.7, the gel buffer at a dilution of one part electrode buffer in six parts water (Soltis et al., 1983). To increase resolution of the bands on the gel, this recipe was modified by the addition of 4% sucrose (w/v) to the starch prior to gel preparation (Wendel and Weeden, 1989). These gels were run at 35 milliamps, 250 V.

A continuous buffer system of morpholine - citrate was used for 6 - Phosphogluconate dehydrogenase (6PGD) (E.C.1.1.1.44). The gel buffer was a 1 in 15

dilution of the electrode buffer (Pleasants and Wendel, 1989). The system was run at 25 milliamps.

Isocitrate dehydrogenase (IDH) (E.C.1.1.1.42) was run using a continuous buffering system of Tris-citrate (monohydrate) at pH 8.0 (Soltis et al., 1983). The gel buffer was a 1 in 15 dilution of the electrode buffer. The gels were run at 35 milliamps.

The gels were run until the marker dye had reached the anodal boundary (approximately 10 cm from the origin). They were then removed from the casing and the excess above the leading edge cut away. The upper left corner of the gel was nicked for orientation. The cathodal strip, having previously tested negative for activity for the enzymes being assayed, was discarded. Individual horizontal slices were placed immediately into trays containing specific stains, covered and incubated at 37° C in the dark until the reactions were complete. The stains were taken from Cheliak and Pitel (1984), Soltis et al. (1983), and Wendel and Weeden (1989). Stain recipes and references are in Appendix 1.

The developed gels were sketched, then mounted and dried using porous cellophane sheets from Hoefer. The gel slice was sandwiched in between two of the cellophane sheets while under water to prevent air from getting trapped with the gel. The sheets were then placed between two plastic mounting frames, removed from the water and stretched taut on the frames before they were clipped into place using Binder clips. When dry, they were cut from the frames with a scalpel, the gel and cellulose layers having become transparent and firmly adhered together. This mounting process provides an easily stored permanent record without loss of resolution.

3.0 Screening

3.1 Parental Screening for Linkage Study

Seeds for each parent population were chosen from examination of individual banding patterns in available electrophoretic records (Allen, unpublished). I picked half-sibling seed from individuals in those populations exhibiting high variability (i.e. showing heterozygosity for multiple enzyme loci) as well as from plants in those populations not previously sampled. A minimum of forty individuals (some half-siblings, but most from different parents) per population were included in the parent populations for screening. The seeds were stratified for three or four days at 4°C, then the seed coats were nicked and removed with forceps to increase percent germination. Germinated seedlings were transferred to soil six-packs and grown under long day conditions in a controlled environment chamber (20°C for a 14 hour day, 15°C for a 10 hour night). After the second set of leaves had emerged, the seedlings were fertilized bi-weekly with dilute (50%) Miracle-Gro (a complete fertilizer with 20-20-20 NPK and micronutrients) and repotted as necessary.

Leaf material collected from *A. falcatus* (populations 1230, 1231, 1234 and 1235) and *A.occidentalis* (populations 1188, 1198, and 1217) was screened for a total of eleven enzyme systems: ADH, EST, IDH, LAP, MDH, ME, PGI, PGM, SkDH, TPI, and 6PGD. Of these, six systems showed sufficient activity, resolution and variability for use in this study; these were ADH, LAP, MDH, PGI, PGM, and TPI. Esterase (EST) showed poor resolution and was not analyzed further. Four enzymes, IDH, ME, SkDH and 6PGD were found to be monomorphic in these populations.

Genotypes for polymorphic enzymes were inferred from the banding patterns of each locus and were used to select suitable parents for the testcrosses. These were selected to cover as many of the 64 potential locus pairings as possible.

3.2 Screening for the Allelic Variability Study

Seedlings were obtained for each of the three populations; *A. ascendens* (1207), *A.occidentalis* (1227), and *A. falcatus* (1232), each seedling from a different parent plant in the original populations. A total of 19 plants were used from *A. ascendens* (1207), 39 plants from *A. occidentalis* (1227) and 26 plants from *A. falcatus* (1232). These plants were screened for the eleven enzyme systems outlined above. The loci IDH, ME, SkDH, and 6GPD were included and EST were retained in the data set as polymorphism was not a requirement for this portion of the study, and much of the Esterase was found to be interpretable. Genotypes were recorded and the allelic frequencies calculated for each population.

3.3 Cytological Analysis (Mitotic Chromosome Counts)

Some of the populations used in this study had unknown chromosome numbers (1207, 1127 and 1232); others had shown evidence (Allen, unpublished data) of containing more than one ploidy level within the population (1188) or very few individuals had been previously sampled. In order to determine the ploidy of the plants used in this study, chromosome counts were made for all plants used in the crossing experiments. This was carried out to ensure that only diploid individuals were being used for

testcrosses, as expected patterns and ratios of the progeny are based on the assumption of diploidy. The counts were also used to determine the extent of polyploidy present within the parental populations. Young, actively growing root tips were collected, prepared and stained according to methods of Allen (1984). Examples showing good separation and clear countability were photographed.

Chromosome counts were taken from 1207 (*A. ascendens*), 1227 (*A. occidentalis*) and 1232 (*A. falcatus*) in order to verify the interpretation of the allelic frequency data, as polyploidy would affect the definition of individual loci and the genotype of each locus.

4.0 Testcrosses for the Linkage Study

After the screening process was complete, the parent plants were transferred from the growth chambers to cold frames, where individual plants were divided between two pots and allowed to overwinter. In the spring, one set remained in 6" pots outside on a raised table, while the other set was transferred to a field plot.

Controlled test crosses were made between plants heterozygous for two or more loci and those homozygous for the same loci. Flower heads were tagged and bagged before the flowers were open. Pollinator exclusion bags were made from Reemay, a synthetic cloth with an irregular pore size (2μm to 36μm). This material was selected to allow air and light to reach the flowers, while excluding pollinators.

Crosses were made by rubbing the flower heads together. Because the individual florets open from the edge to the center of the head over two or three days, the process was repeated the next day to maximize the number of cross-pollinated flowers per head.

Seed heads were collected after the seed had set (usually three weeks after pollination), placed in labelled envelopes and stored at 4° C. Seed stratification and germination followed established procedure. The progeny from all testcrosses were raised to the sampling stage in growth chambers set for new seedling growth at 16°C for a 12 hour day and 13°C for a 12 hour night. The progeny from each cross were labeled according to the cross number and species, i.e. CO16 - 25 refers to Cross 16, *A. occidentalis*, individual number 25.

Assays of progeny were carried out for specific enzymes using the same electrophoretic techniques described above for parental screening. Ratios of progeny genotypes for each locus and each locus pair were recorded and compiled for analysis.

5.0 Linkage Analysis

Because isozyme banding patterns are inherited in a Mendelian fashion, results of each cross can be compared to expected ratios to determine goodness of fit. For each cross, progeny ratios were tested against an expected 1:1 ratio using $\chi 2$ analysis to determine if the ratio was that expected for a single gene locus. This was done to detect contaminant genotypes, selfing, or to determine if more than one locus could be involved. If all loci for that cross adhered to the expected Mendelian ratios then the multilocus

banding patterns from the pairs of loci were assessed using $\chi 2$ analysis, tested against an expected 1:1:1:1 ratio for independent assortment. Expected ratios would show equal numbers of progeny in both parental and both recombinant classes. For example, a parent heterozygous for two loci (1ab2ab) crossed with another homozygous for those loci (1aa2aa) would be expected to produce progeny with parental genotypes (1aa2aa, 1ab2ab) and recombinant genotypes (1aa2ab, 1ab2aa). Significant $\chi 2$ values were interpreted as indications of linkage between loci, manifested as a significant difference between the number of parental type progeny and the number of recombinant type progeny.

6.0. Determination of Self Compatibility

In order to determine whether deviations from the expected 1:1 ratios for single loci were due to contamination or selfing, I conducted a series of selfing experiments in the summer of 1995. Twenty control inflorescences from different parent plants in various populations were tagged and left open to outside pollinators, and twenty were bagged prior to opening in double thicknesses of the Reemay cloth used in the original testcrosses. Seed set was determined for plants in each group after three weeks.

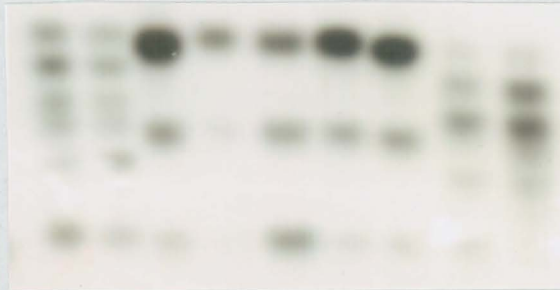
RESULTS

1.0. Inheritance of Isozymes in *Aster.*

Six of the eleven enzyme systems examined showed variability in at least one species, and could therefore be analyzed to determine the genetic basis for inheritance. Segregation of alleles for each enzyme in the progeny serve to confirm the inheritance patterns of isozyme loci - indicating the number of loci present, the quaternary structure of the enzyme (monomeric, dimeric etc.) and, where possible, whether or not the enzymes occur in the same subcellular compartment (although subcellular compartmentalization was not determined for all enzymes in this study). Isozyme loci for each enzyme were numbered sequentially beginning with the most anodal locus (i.e., Adh-1, Adh-2). Allozymes (allelic forms produced at a given locus) were lettered in the same manner (a, b, c, etc.)

Alcohol Dehydrogenase (ADH)

Root tissue revealed very high levels of ADH activity in two separate zones of the gel, designated Adh-1 and Adh-2. As expected for a locus inherited in Mendelian fashion, both parental banding patterns were visible in testcross progeny (Figure 2 for Adh-1). The minimum number of bands for this enzyme was three, the anodal band representing a homozygous Adh-1, the slowest band a homozygous Adh-2 and the middle band an interlocus heterodimer. This occurs as an interaction between the protein subunits of a dimeric enzyme when both loci are expressed in the same subcellular compartment



	1a1a	———	——— 1a1a
			——— 1a1b
heterodimer	1a2a	———	━━━ 1b1b, 1a2a
			——— 1b2a
	2a2a	———	——— 2a2a
genotypes		1aa	1ab
		2aa	2aa

Figure 2. Photograph and Zymogram of Isozyme Inheritance Patterns for Alcohol Dehydrogenase (ADH), a Dimeric Enzyme. A testcross for Adh-1 shows either three or six bands in the progeny. The slower allele of Adh-1 may overlap the interlocus heterodimer forming between Adh-1a and Adh-2a, but usually forms a much thicker band as a result and is interpretable.

(Crawford, 1983). The maximum number of bands in an Adh-1 testcross progeny was six (where Adh-2 is homozygous). The first three bands indicate heterozygosity at Adh-1, the middle band of the triplet a heteromer formed between protein subunits of the different alleles. The slowest band was identified as Adh-2 from other individuals showing homozygosity at both ADH loci, and the two intermediate bands are interlocus heterodimers representing the different protein subunit combinations occurring between each of the alleles of Adh-1 and the Adh-2 allele.

Leucine aminopeptidase (LAP)

Lap-1 was confirmed from the testcrosses as a single locus in *Aster* with low to moderate activity in leaf tissue. Each lane contained one or two bands, an indication of a monomeric enzyme (no intralocus interactions) in homozygous or heterozygous condition, respectively (Figure 3).

Malate dehydrogenase (MDH)

Three separate zones of activity were visible for MDH, representing three putative loci, showing variable levels of intensity. The Mdh-1 locus was the only variable locus in the parent populations, seen as either a single band or a triplet, characteristic of a dimeric enzyme. No interlocus heteromers were visible, indicating separate subcellular compartments for the products of each locus. Both Mdh-2 and Mdh-3 were fixed for the same alleles in all parent populations, bringing the maximum number of bands per lane to five.

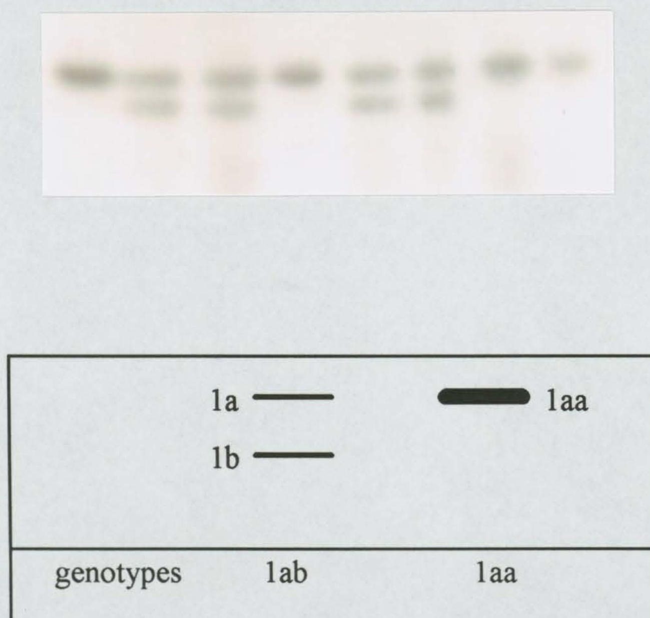
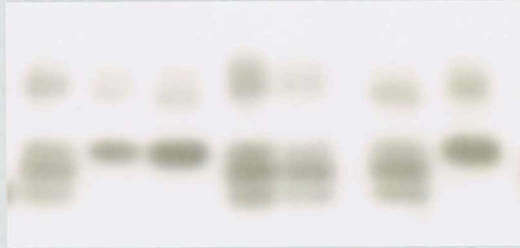


Figure 3. Photograph and Zymogram of Isozyme Inheritance Patterns for Leucine aminopeptidase (LAP), a Monomeric Enzyme.



Pgi-1	1a1a	1a1a
	1a1b	1a1b
	1b1b	1b1b
Pgi-2	2a2a	2a2a
	2a2b	
	2b2b	
genotypes	1ab	1ab
	2ab	2aa

Figure 4. Photograph and Zymogram of Isozyme Inheritance Patterns for Phosphoglucose Isomerase (PGI), a Dimeric Enzyme.

Phosphoglucose Isomerase (PGI)

Two loci of PGI activity were seen, the band numbers ranging from two to six with both loci showing high levels of activity. A two banded phenotype was interpreted as representing homozygous genotypes at both Pgi-1 and Pgi-2 and suggested different subcellular compartmentalization (Figure 4). Separation of isozyme loci into different subcellular compartments is usual for plants (Kephart, 1990). Some variation was visible at Pgi-1 in the form of very tight triplets but most bands were too close together to be separated with accuracy for testcrosses using that buffer system. Greater separation between alleles was evident at Pgi-2. These were seen as the single bands or triplets of a dimeric enzyme.

Phosphoglucomutase (PGM)

Two isozymes of PGM were observed in the parent populations. The Pgm-1 locus was invariant and showed no interlocus interactions with Pgm-2. A maximum of two bands was seen at Pgm-2, indicating monomeric structure of this enzyme. Activity levels from leaf material were consistently high.

Triose Phosphate Isomerase (TPI)

Banding patterns for TPI indicated two isozymes in separate subcellular compartments for a dimeric enzyme. In testcross progeny for a single TPI locus (Tpi-1 for example) either two bands or four bands were present, representing homozygosity for

both loci or heterozygosity at Tpi-1 and homozygosity at Tpi-2. A maximum of six bands was visible when both loci were heterozygous.

Of the eleven enzyme systems assayed, six (ADH, LAP, MDH, PGI, PGM and TPI, outlined above) were used for the linkage study as they showed evidence of polymorphism and provided adequate resolution for interpretation. For these six enzyme systems a total of twelve loci were determined, nine of them polymorphic in *Aster occidentalis* (Table 2). Population 1188 showed very high levels of heterozygosity in most loci assayed during the initial screening process. However, all eight plants sampled from population 1188 were found to be tetraploid (n=16), therefore this population was not useful for this study. In the remaining populations of *A. occidentalis* (1198 and 1217), Adh-1, Adh-2, and Tpi-2 showed lower levels of polymorphism in population 1198 than in 1217. The Lap-1 isozyme was variable in population 1217 only. Three loci, Pgi-2, Pgm-2, and Mdh-1, showed comparable and higher levels of heterozygosity in populations 1198 and 1217. For *A. occidentalis* then, of thirty-six possible locus pairs to test for linkage, multiple heterozygotes were available for twenty-two and successful crosses were made for seventeen. The decrease in the number of crosses that were possible was due also to the rarity of discernible heterozygous Pgi-1 in the parent populations. Populations 1198 and 1217 showed no evidence of polyploidy.

Populations of *Aster falcatus* displayed much less variability than *A. occidentalis* at most loci. Four of the nine loci that were polymorphic in *A. occidentalis* were monomorphic in *A. falcatus* (Adh-2, Pgi-1, Tpi-1 and Tpi-2). The Mdh-1 locus was

Table 2. Summary of Isozyme Loci Determined for *Aster occidentalis*

Enzyme	No. of Loci	Structure	Reported for Other Species*
ADH	2	dimeric	1-3 loci, dimeric
LAP	1	monomeric	2-3 loci, monomeric
MDH	3	dimeric	3 loci, dimeric
PGI	2	dimeric	2 loci, dimeric
PGM	2	monomeric	2 loci, monomeric
TPI	2	dimeric	2 loci, dimeric

*Kephart (1990)

heterozygous in five individuals out of the four *A. falcatus* populations (1230, 1231, 1234 and 1235). This decreased the number of locus pairs available for linkage analysis (from 36 to 8). A total of 39 reciprocal crosses were made for *A. occidentalis* (21 crosses and 18 repeats) and 9 crosses for *A. falcatus.* Low levels of heterozygosity were seen for Lap-1 but Pgm-2, Pgi-2 and Adh-1 were more variable. Unfortunately, the crosses that were made using *A. falcatus* did not set seed successfully due to high rainfall and humidity during late August and early September, 1994. Analysis of inheritance and linkage patterns are therefore reported only for *A. occidentalis.*

For each enzyme a $\chi 2$ test for goodness of fit was run on the progeny from each inflorescence to determine the probable number of loci (Wendel and Weeden, 1989; Rohlf and Sokal, 1981). Because isozymes are inherited codominantly and in a Mendelian fashion, expected ratios of heterozygote to homozygote for a single locus are 1:1. For each cross, all enzyme loci were tested individually (Table 3). Prior to this test, apparent loci were identified by comparing variations in the banding patterns of the parent plants. The likelihood of detecting departures from the expected ratios is dependent on sample size, larger sample sizes decreasing the chances of committing a Type 1 or a Type 2 error simultaneously (Zar, 1974). Crosses with fewer than ten individuals were considered too small a sample for accurate analysis and were pooled for each locus (Tamarin, 1991). Only crosses whose loci contained alleles segregating according to the expected 1:1 ratio were used in the final pooled results and later in linkage analysis. Some crosses did show discrepancies, either the appearance of foreign alleles or an excess of homozygotes or heterozygotes of the expected genotypes. As other crosses for these enzymes gave the

Table 3. Single Locus Goodness of Fit Test ($\chi 2 = 3.841$ at $P<.05$) for Eight Polymorphic Loci in *Aster occidentalis*. (corrected = data corrected for contamination, ⌀ = data not used in pooled results or in further analysis)

Locus	Cross	Parental Genotypes	Progeny Genotypes	Expected ratio	df	$\chi 2$	note
Adh-1	CO24-2	aa ab	2aa:7ab	1:1	1	-	
	CO28-1	aa ab	3aa:10ab	1:1	1	3.76	corrected
	CO28-2	aa ab	5aa:1ab	1:1	1	-	
	CO32-1	aa ab	18aa:5ab	1:1	1	7.3*	⌀
	CO32-2	aa ab	1aa:13ab	1:1	1	-	⌀
Pooled		**homozygous: heterozygous**	**10:18**	**1:1**	**1**	**2.28**	
Lap-1	CO16-1	cc cd	49cc:41cd	1:1	1	0.71	
	CO16-2	cc cd	23cc:17cd	1:1	1	0.90	⌀
	CO17-1	cc cd	19cc:23cd	1:1	1	0.38	⌀
	CO25-1	aa ab	4aa:5ab	1:1	1	-	corrected
	CO34-1	cc cd	2cc:3cd	1:1	1	-	corrected
	CO34-2	cc cd	0cc:3cd	1:1	1	-	
	CO35-1	cc cd	2cc:3cd	1:1	1	-	corrected
	CO35-2	cc cd	4cc:1cd	1:1	1	-	
	CO41-1	aa ab	18aa:6ab	1:1	1	6.00*	⌀
	CO41-2	aa ab	14aa:9ab	1:1	1	1.08	
	CO43-2	aa ab	21aa:13ab	1:1	1	1.88	
	CO44-1	aa ab	6aa:4ab	1:1	1	0.40	⌀
	CO44-2	aa ab	3aa:4ab	1:1	1	0.16	⌀
Pooled		**homozygous: heterozygous**	**96:78**	**1:1**	**1**	**1.86**	

Table 3 (cont'd).

Locus	Cross	Parental Genotypes	Progeny Genotypes	Expected ratio	df	χ2	note
Mdh-1	CO12-1	aa ab	2aa:7ab	1:1	1	-	
	CO12-2	aa ab	12aa:7ab	1:1	1	1.30	
	CO13-1	aa ab	27aa:18ab	1:1	1	1.80	
	CO15-1	bb ab	11bb:10ab	1:1	1	0.04	corrected
	CO16-1	aa ab	46aa:44ab	1:1	1	0.04	
	CO16-2	aa ab	29aa:11ab	1:1	1	8.10*	∅
	CO18-1	bb ab	5bb:5ab	1:1	1	0.00	∅
	CO18-2	bb ab	2bb:4ab	1:1	1	-	
	CO20-1	aa ab	24aa:16ab	1:1	1	1.60	
	CO20-2	aa ab	6aa:8ab	1:1	1	0.28	corrected
	CO24-2	aa ab	4aa:5ab	1:1	1	-	
	CO25-1	aa ab	2aa:7ab	1:1	1	-	corrected
	CO27-1	aa ab	7aa:6ab	1:1	1	0.076	
	CO27-2	aa ab	42aa:34ab	1:1	1	0.84	
	CO28-1	aa ab	6aa:7ab	1:1	1	0.076	corrected
	CO28-2	aa ab	4aa:2ab	1:1	1	-	
	CO29-1	aa ab	22aa:26ab	1:1	1	0.33	corrected
	CO29-2	aa ab	28aa:19ab	1:1	1	1.72	∅
	CO30-1	bb ab	5bb:2ab	1:1	1	-	

Table 3 (cont'd).

Locus	Cross	Parental Genotypes	Progeny Genotypes	Expected ratio	df	$\chi 2$	note
Mdh-1	CO30-2	bb ab	8bb:7ab	1:1	1	0.06	
	CO31-1	bb ab	3bb:5ab	1:1	1	-	corrected
	CO31-2	bb ab	2bb:3ab	1:1	1	-	corrected
	CO34-1	aa ab	1aa:4ab	1:1	1	-	corrected
	CO34-2	aa ab	1aa:2ab	1:1	1	-	
	CO35-1	aa ab	2aa:3ab	1:1	1	-	corrected
	CO35-2	aa ab	3aa:2ab	1:1	1	-	
	CO36-1	bb ab	2bb:6ab	1:1	1	-	corrected
	CO36-2	bb ab	4bb:2ab	1:1	1	-	corrected
	CO38-1	bb ab	14bb:3ab	1:1	1	7.11*	∅
	CO38-2	bb ab	12bb:14ab	1:1	1	0.154	
	CO41-1	aa ab	13aa:11ab	1:1	1	0.16	∅
	CO41-2	aa ab	12aa:11ab	1:1	1	0.04	
	CO43-2	aa ab	17aa:16ab	1:1	1	0.03	
Pooled		**homozygous: heterozygous**	**289:278**	**1:1**	**1**	**0.213**	
Pgi-1	CO2	aa ab	11aa:15ab	1:1	1	0.614	
Pooled		**homozygous: heterozygous**	**11:15**	**1:1**	**1**	**0.614**	

Table 3 (cont'd).

Locus	Cross	Parental Genotypes	Progeny Genotypes	Expected ratio	df	χ2	note
Pgi-2	CO2	aa ab	10aa:16ab	1:1	1	1.38	
	CO3	aa ab	29aa:26ab	1:1	1	0.163	
	CO12-1	aa ab	6aa:3ab	1:1	1	-	corrected
	CO12-2	aa ab	13aa:6ab	1:1	1	2.48	
	CO13	aa ab	27aa:18ab	1:1	1	1.80	
	CO14-1	aa ab	26aa:6ab	1:1	1	9.14*	∅
	CO14-2	aa ab	5aa:9ab	1:1	1	1.14	∅
	CO15-1	aa ab	9aa:12ab	1:1	1	0.42	corrected
	CO16-1	aa ab	36aa:54ab	1:1	1	3.60	
	CO16-2	aa ab	23aa:19ab	1:1	1	0.38	∅
	CO17	aa ab	4aa:6ab	1:1	1	7.7*	∅
	CO18-1	aa ab	12aa:30ab	1:1	1	0.40	∅
	CO18-2	aa ab	1aa 4ab	1:1	1	-	
	CO19-1	aa ab	1aa:2ab	1:1	1	-	corrected
	CO19-2	aa ab	1aa:4ab	1:1	1	-	corrected
	CO20-1	aa ab	19aa:21ab	1:1	1	0.10	
	CO20-2	aa ab	9aa:5ab	1:1	1	1.14	corrected
	CO24-2	aa ab	6aa:3ab	1:1	1	-	
	CO26-1	aa ab	17aa:8ab	1:1	1	3.24	corrected

Table 3 (cont'd).

Locus	Cross	Parental Genotypes	Progeny Genotypes	Expected ratio	df	χ2	note
Pgi-2	CO27-1	aa ab	9aa:4ab	1:1	1	1.92	
	CO27-2	aa ab	44aa:32ab	1:1	1	1.89	
	CO28-1	aa ab	7aa:6ab	1:1	1	0.076	corrected
	CO28-2	aa ab	4aa:2ab	1:1	1	-	
	CO29-1	aa ab	24aa:24ab	1:1	1	0.00	corrected
	CO30-1	aa ab	2aa:5ab	1:1	1	-	corrected
	CO30-2	aa ab	10aa:5ab	1:1	1	1.67	
	CO31-1	aa ab	5aa:3ab	1:1	1	-	corrected
	CO31-2	aa ab	2aa:3ab	1:1	1	-	corrected
	CO32-1	aa ab	18aa:5ab	1:1	1	7.3*	∅
	CO32-2	aa ab	8aa:6ab	1:1	1	-	∅
	CO43-2	aa ab	15aa:19ab	1:1	1	0.47	
Pooled		**homozygous: heterozygous**	**307:300**	**1:1**	**1**	**0.08**	
Pgm-2	CO14-1	dd cd	0dd:28cd	1:1	1	28*	∅
	CO14-2	dd cd	10dd:4cd	1:1	1	2.57	∅
	CO15-1	dd cd	11dd:10cd	1:1	1	0.04	corrected
	CO16-1	ab bc	44ab:46bc	1:1	1	0.04	
	CO16-2	ab bc	29ab:11bc	1:1	1	7.71*	∅

Table 3 (cont'd).

Locus	Cross	Parental Genotypes	Progeny Genotypes	Expected ratio	df	χ2	note
Pgm-2	CO18-1	bb ab	6bb:4ab	1:1	1	0.40	∅
	CO18-2	bb ab	2bb:4ab	1:1	1	-	
	CO19-1	bb ab	0bb:3ab	1:1	1	-	corrected
	CO19-2	bb ab	2bb:3ab	1:1	1	-	corrected
	CO24-2	dd cd	6dd:3cd	1:1	1	-	
	CO25-1	dd cd	5dd:4cd	1:1	1	-	corrected
	CO28-1	dd cd	3dd:10cd	1:1	1	3.76	corrected
	CO28-2	dd cd	3dd:3cd	1:1	1	-	
	CO30-1	dd cd	5dd:2cd	1:1	1	-	corrected
	CO30-2	dd cd	8dd:7cd	1:1	1	0.06	
	CO31-1	dd cd	3dd:5cd	1:1	1	-	corrected
	CO31-2	dd cd	4dd:1cd	1:1	1	-	corrected
	CO34-1	dd bd	2dd:3bd	1:1	1	-	corrected
	CO34-2	dd bd	2dd:1bd	1:1	1	-	
	CO35-1	dd bd	2dd:3bd	1:1	1	-	corrected
	CO35-2	dd bd	3dd:2bd	1:1	1	-	
	CO36-1	dd bd	4dd:4bd	1:1	1	-	corrected
	CO36-2	dd bd	6dd:0bd	1:1	1	-	corrected
	CO38-1	dd bd	11dd:6bd	1:1	1	1.47	∅
	CO38-2	dd bd	14dd:12bd	1:1	1	0.154	
Pooled		**homozygous: heterozygous**	**129:126**	**1:1**	**1**	**0.035**	

Table 3 (cont'd).

Locus	Cross	Parental Genotypes	Progeny Genotypes	Expected ratio	df	χ2	note
Tpi-1	CO3	aa ab	32aa:23ab	1:1	1	1.47	
	CO14-1	bb ab	9bb:19ab	1:1	1	3.57	∅
	CO14-2	bb ab	2bb:12ab	1:1	1	7.14*	∅
	CO15-1	aa ab	12aa:9ab	1:1	1	0.43	corrected
	CO17	aa ab	26aa:16ab	1:1	1	2.38	∅
	CO18-1	aa ab	3aa:4ab	1:1	1	-	∅
	CO18-2	aa ab	3aa:3ab	1:1	1	-	
	CO19-1	bb ab	2bb:1ab	1:1	1	-	corrected
	CO19-2	bb ab	5bb:0ab	1:1	1	-	corrected
	CO20-1	bb ab	22bb:18ab	1:1	1	0.40	
	CO20-2	bb ab	4bb:10ab	1:1	1	2.57	corrected
	CO24-2	aa ab	3aa:6ab	1:1	1	-	
	CO25-1	bb ab	5bb:4ab	1:1	1	-	corrected
	CO26-1	aa ab	15aa:10ab	1:1	1	1.00	corrected
	CO28-1	aa ab	3aa:10ab	1:1	1	3.76	corrected
	CO28-2	aa ab	2aa:4ab	1:1	1	-	
	CO30-1	aa ab	2aa:5ab	1:1	1	-	corrected
	CO30-2	aa ab	9aa:6ab	1:1	1	0.60	corrected
	CO31-1	aa ab	2aa:6ab	1:1	1	-	corrected
	CO31-2	aa ab	3aa:2ab	1:1	1	-	corrected

Table 3 (cont'd).

Locus	Cross	Parental Genotypes	Progeny Genotypes	Expected ratio	df	χ2	note
Tpi-1	CO36-1	aa ab	3aa:5ab	1:1	1	-	corrected
	CO36-2	aa ab	1aa:5ab	1:1	1	-	corrected
	CO38-1	aa ab	1aa:12ab	1:1	1	9.30*	∅
	CO38-2	aa ab	15aa:11ab	1:1	1	0.60	
	CO41-1	bb ab	17bb:7ab	1:1	1	4.16*	∅
	CO41-2	bb ab	13bb:10ab	1:1	1	0.39	
	CO43-2	aa ab	12aa:22ab	1:1	1	2.94	
Pooled		**homozygous: heterozygous**	**168:170**	**1:1**	**1**	**0.011**	
Tpi-2	CO15-1	aa ab	1aa:11ab	1:1	1	0.46	corrected
	CO18-1	aa ab	3aa:7ab	1:1	1	1.60	∅
	CO18-2	aa ab	2aa:4ab	1:1	1	-	
	CO43-2	aa ab	14aa:20ab	1:1	1	1.05	
Pooled		**homozygous: heterozygous**	**26:35**	**1:1**	**1**	**1.32**	

expected ratios for independent assortment at a single locus, these departures were most likely the result of contamination from an outside pollen source. In crosses containing foreign alleles (alleles not present in the parental genotype), those plants were removed from the progeny base and a $\chi 2$ test carried out on the remaining progeny. For those not segregating in a 1:1 ratio, with an excess in one category or the other in any of the loci for which crosses were made , no accurate correction could be made, so the entire progeny set was excluded from further analysis. In order to determine whether or not these excesses were due to selfing rather than contamination from pollen carrying the same alleles, a series of selfing experiments was carried out in the summer of 1995.

1.1. Self Compatibility in *Aster*

Selfing experiments completed in the summer of 1995 showed seed set in the twenty control inflorescences from different parental plants averaging 63% (n=2580). Average seed set in the test group (bagged inflorescences) was 0.71% (n = 2654), suggesting self pollination was not the contributing factor in the testcross progeny and that anomalies in the segregation ratios were most likely a result of contamination from neighbouring plants carrying the same alleles as the parents involved in the testcross. Of 133 single locus $\chi 2$ tests covering eight loci from six enzymes, 102 were used in the final analyses.

2.0. Linkage Groups in *A. occidentalis*

To determine the linkage patterns present within the eight isozyme loci available, $\chi 2$ analysis was used to detect significant deviation from the 1:1:1:1 ratio expected for pairs of unlinked, independently assorting genes. Crosses that showed a 1:1 ratio for individual loci were tested in pairwise fashion for linkage. Crosses with fewer than 20 progeny were not examined independently due to the effects of small sample size on accuracy, but were pooled with others in their class and retested (Table 4, 5). Of the 17 locus pairs tested for linkage, two deviated significantly from expected ratios. One locus pair, Adh-1/Pgi-2, had a $\chi 2$ value of 9.703, greater than the critical value of 7.841 (at df=3, P=.05). Another pairing, Mdh-1/Pgm-2, had a pooled $\chi 2$ value of 48.78. Three additional loci, Lap-1, Tpi-1 and Tpi-2, assorted independently with each other and all other loci. The Adh-2 locus could not be placed due to the lack of successful testcrosses with other enzymes. Both Mdh-2 and Pgi-1 assorted independently of Adh-1 and Pgi-2 but could not be tested with respect to the other loci. In total, five prospective linkage groupings were established in *A. occidentalis*.

3.0. Genetic Analysis of Allelic Variability in *A. occidentalis, A. ascendens* and *A. falcatus*

I surveyed eleven enzymes coding for 20 loci using starch gel electrophoresis (SGE) for populations 1227 (*A. occidentalis*), 1207 (*A. ascendens*) and 1232 (*A. falcatus*); Adh-1, Adh-2, Est-1, Est-2, Idh-1, Idh-2, Lap-1, Me-1, Mdh-1, Mdh-2, Mdh-3, Pgi-1, Pgi-2, 6Pgd-1, 6Pgd-2, Pgm-1, Pgm-2, Skdh-1, Tpi-1, and Tpi-2. Of these, five loci were found to be monomorphic in all

Table 4. Progeny Ratios and $\chi 2$ Analysis for Individual Testcrosses in *Aster occidentalis*

$\chi 2 = 7.841$ at $P<.05$., H = heterozygous genotype, F = homozygous for fast allele, S = homozygous for slow allele

Progeny Phenotypes: H/H = heterozygous at both loci, H/F = heterozygous at first locus, homozygous at second etc

Locus Pair	Cross	Progeny Phenotypes									Expected	$\chi 2$
		H/H	H/F	H/S	F/H	S/H	F/F	F/S	S/F	S/S	Ratios	(df=3)
Adh-1/Mdh-1	CO24	4	3		1		1				1:1:1:1	-
	CO28-1	6	4		1		2				1:1:1:1	-
	CO28-2	0	1		2		3				1:1:1:1	-
Adh-1/Pgi-2	CO24-2	2	5		1		1				1:1:1:1	-
	CO28-1	2	8		3		0				1:1:1:1	-
	CO28-2	0	1		2		3				1:1:1:1	-
Adh-1/Pgm-2	CO24-2	1	6		2		0				1:1:1:1	-
	CO28-1	8	2		2		1				1:1:1:1	-
	CO28-2	0	1		3		2				1:1:1:1	-
Lap-1/Mdh-1	CO16-1	19	25			22			24		1:1:1:1	0.925
	CO25-1	4	1		3		1				1:1:1:1	-
	CO34-1	3	0			1			1		1:1:1:1	-

Table 4 (cont'd).

Locus Pair	Cross	H/H	H/F	H/S	F/H	S/H	F/F	F/S	S/F	S/S	Expected Ratios	$\chi 2$ (df=3)
Lap-1/Mdh-1	CO34-2	2	1			0			0		1:1:1:1	-
	CO35-1	1	2			2			0		1:1:1:1	-
	CO35-2	1	0			1			3		1:1:1:1	-
	CO41-2	5	4		6		8				1:1:1:1	1.51
	CO43-2	6	7		10		10				1:1:1:1	1.54
Lap-1/Pgi-2	CO16-1	26	15			28			21		1:1:1:1	4.48
	CO43-2	7	6		12		9				1:1:1:1	2.46
Lap-1/Pgm-2	CO16-1	17		24		29			20		1:1:1:1	3.58
	CO25-1	3		2	2			2			1:1:1:1	-
	CO34-1	2		1	1			1			1:1:1:1	-
	CO34-2	1		1	0			0			1:1:1:1	-
	CO35-1	3		0	0			2			1:1:1:1	-
	CO35-2	0		1	2			2			1:1:1:1	-

Table 4 (cont'd).

Locus Pair	Cross	Progeny Phenotypes									Expected Ratios	$\chi 2$ (df=3)
		H/H	H/F	H/S	F/H	S/H	F/F	F/S	S/F	S/S		
Lap-1/Tpi-1	CO25-1	2		3	2			2			1:1:1:1	-
	CO41-2	6		3	4			10			1:1:1:1	4.98
	CO43-2	10	3		12		9				1:1:1:1	5.29
Lap-1/Tpi-2	CO43-2	7	6		13		8				1:1:1:1	3.40
Mdh-1/Pgi-2	CO12-1	2	5		1		1				1:1:1:1	-
	CO12-2	6	0		1		12				1:1:1:1	-
	CO13	6	12		12		15				1:1:1:1	3.8
	CO16-1	27	17		27		19				1:1:1:1	3.7
	CO20-1	12	4		9		15				1:1:1:1	6.6
	CO20-2	3	5		2		4				1:1:1:1	-
	CO24	1	4		2		2				1:1:1:1	-
	CO27-1	3	3		1		6				1:1:1:1	-
	CO27-2	11	23		21		21				1:1:1:1	4.6

Table 4 (cont'd).

Locus Pair	Cross	H/H	H/F	H/S	F/H	S/H	F/F	F/S	S/F	S/S	Expected Ratios	χ2 (df=3)
Mdh-1/Pgi-2	CO28	1	8		7		3				1:1:1:1	-
	CO29-1	11	15		13		9				1:1:1:1	1.66
	CO43-2	10	6		9		8				1:1:1:1	1.05
Mdh-1/Pgm-2	CO15	7		3	3			8			1:1:1:1	4.90
	CO16-1	38	6		8		38				1:1:1:1	42.78*
	CO18-2	4		0	0			2			1:1:1:1	-
	CO24-2	1		4	2			2			1:1:1:1	-
	CO25	2		5	2			0			1:1:1:1	-
	CO28	6		3	7			3			1:1:1:1	2.67
	CO30	7		2	2			11			1:1:1:1	10.34*
	CO31	5		3	1			4			1:1:1:1	-
	CO34	4		2	0			2			1:1:1:1	-
	CO35	1		4	4			1			1:1:1:1	-
	CO36	4		4	1			6			1:1:1:1	-
	CO38	11		3	0			11			1:1:1:1	14.6*

Table 4 (cont'd).

Locus Pair	Cross	H/H	H/F	H/S	F/H	S/H	F/F	F/S	S/F	S/S	Expected Ratios	$\chi 2$ (df=3)
		Progeny Phenotypes										
Mdh-1/Tpi-1	CO20-1	7		9	11			13			1:1:1:1	2.00
	CO20-2	6		2	4			2			1:1:1:1	-
	CO24	4	1		2		2				1:1:1:1	-
	CO25	3		4	1			1			1:1:1:1	-
	CO28	7		2	7			3			1:1:1:1	-
	CO36	6	2			4			2		1:1:1:1	-
	CO38-1	6	8			5			7		1:1:1:1	-
	CO41-2	4		7	6			6			1:1:1:1	0.822
	CO43-2	9	7		13		4				1:1:1:1	5.168
Mdh-1/Tpi-2	CO43-2	7	9		12		5				1:1:1:1	3.24
Pgi-1/Pgi-2	C02	6	5		4		11				1:1:1:1	3.55

Table 4 (cont'd).

Locus Pair	Cross	Progeny Phenotypes									Expected Ratios	$\chi2$ (df=3)
		H/H	H/F	H/S	F/H	S/H	F/F	F/S	S/F	S/S		
Pgi-2/Pgm-2	CO16-1	28	26		18		18				1:1:1:1	3.68
	CO19-1	2		0	1			0			1:1:1:1	-
	CO19-2	3		1	0			1			1:1:1:1	-
	CO24	1		2	2			4			1:1:1:1	-
	CO28	6		2	7			4			1:1:1:1	-
Pgi-2/Tpi-1	CO3	13	13		10		19				1:1:1:1	3.10
	CO15	6	6		3		6				1:1:1:1	1.28
	CO18-2	3	1		0		2				1:1:1:1	-
	CO19-1	1		1	1			0			1:1:1:1	-
	CO19-2	0		4	0			1			1:1:1:1	-
	CO20-1	12		9	6			13			1:1:1:1	2.10
	CO20-2	4		1	6			3			1:1:1:1	-
	CO24	2	1		4		2				1:1:1:1	-
	CO26	3	5		7		10				1:1:1:1	4.28
	CO28	5	3		9		2				1:1:1:1	-
	CO43-2	12	7		10		5				1:1:1:1	6.21

Table 4 (cont'd).

Locus Pair	Cross	H/H	H/F	H/S	F/H	S/H	F/F	F/S	S/F	S/S	Expected Ratios	$\chi2$ (df=3)
Pgi-2/Tpi-2	CO15	7	5		4		5				1:1:1:1	-
	CO18-2	3	1		1		1				1:1:1:1	-
	CO43-2	12	7		8		7				1:1:1:1	2.93
Pgm-2/Tpi-1	CO19-1	2		1		0				0	1:1:1:1	-
	CO19-2	0		3		0				2	1:1:1:1	-
	CO24	1	2			5			1		1:1:1:1	-
	CO25	3	1			1			4		1:1:1:1	-
	CO28	10	3			4			2		1:1:1:1	-
	CO36-1	2	2			3			1		1:1:1:1	-
	CO36-2	0	0			5			1		1:1:1:1	-
	CO38	6	6			5			9		1:1:1:1	-
Tpi-1/Tpi-2	CO15	5	4		6		6				1:1:1:1	0.052
	CO18-2	3	0		1		2				1:1:1:1	-
	CO43-2	13	9		7		5				1:1:1:1	4.11

$\chi2$ critical value = 7.841 at $P<.05$ H = heterozygous genotype F = homozygous for fast allele

S = homozygous for slow allele (e.g. H/H =heterozygous at first and second locus)

Table 5. Pooled Progeny Ratios for Testcrosses in *A. occidentalis*

Locus Pair	Progeny Categories				Expected	$\chi2$
	Hom/Hom	Hom/Het	Het/Hom	Het/Het	Ratios	(df=3)
Adh-1/Mdh-1	6	4	8	10	1:1:1:1	2.85
Adh-1/Pgi-2	4	6	14	4	1:1:1:1	9.703*
Adh-1/Pgm-2	3	7	9	9	1:1:1:1	3.42
Lap-1/Mdh-1	47	45	40	41	1:1:1:1	0.751
Lap-1/Pgi-2	30	40	21	33	1:1:1:1	5.99
Lap-1/Pgm-2	27	34	30	26	1:1:1:1	1.32
Lap-1/Tpi-1	21	18	9	18	1:1:1:1	4.89
Lap-1/Tpi-2	8	13	6	7	1:1:1:1	3.40
Mdh-1/Pgi-2	115	105	102	93	1:1:1:1	2.37
Mdh-1/Pgm-2	88	30	39	90	1:1:1:1	48.78*
Mdh-1/Tpi-1	25	38	31	39	1:1:1:1	3.87
Mdh-1/Tpi-2	5	12	9	7	1:1:1:1	3.24
Pgi-1/Pgi-2	11	4	5	6	1:1:1:1	3.55
Pgi-2/Pgm-2	27	28	31	40	1:1:1:1	3.32
Pgi-2/Tpi-1	63	56	51	58	1:1:1:1	1.29
Pgi-2/Tpi-2	13	13	13	22	1:1:1:1	3.97
Pgm-2/Tpi-1	20	23	18	24	1:1:1:1	1.07
Tpi-1/Tpi-2	13	14	13	21	1:1:1:1	2.93

populations (Idh-1, Idh-2, Mdh-2, Mdh-3, and Pgm-1). I compiled allelic data from populations previously surveyed for these three species (Allen, unpublished) and combined it with the above populations for the remaining 15 polymorphic loci. Data were not available for all populations for Est-1, Est-2, Adh-1 or Adh-2. Variation was assessed for 11 polymorphic loci; Lap-1, Me-1, Mdh-1, 6Pgd-1, 6Pgd-2, Pgi-1, Pgi-2, Pgm-2, Skdh-1, Tpi-1 and Tpi-2. Allele frequencies for all isozyme loci are in Appendix 2. Table 6 shows alleles present for all loci analyzed of the three species.

To obtain allele frequencies for *A. ascendens* loci, considerations were made to deal with the amphiploid nature of this species (Lap-1 is given as an example). For Lap-1, the genotype of the plants in this species represents the four alleles carried for each locus. The problem arises when the sample exhibits a heterozygous banding pattern. A two banded pattern that would be interpreted as cd in a diploid, for example, could be the result of three possible allele combinations in an amphiploid: cccd, ccdd, or cddd. To estimate allele frequencies in the *A. ascendens* populations, the decision as to which combination was used depended on the frequencies of the alleles involved in the populations of *A. occidentalis* and *A. falcatus*. If allele c had a higher overall frequency than d in either *A. occidentalis* or *A. falcatus*, then the genotype was designated for *A. ascendens* as a 3:1 dosage for c, cccd. If both c and d alleles were close in frequency, the *A. ascendens* samples were designated ccdd, etc.. Further studies involving testcrosses for these enzymes in *A. ascendens* are required before these genotypic frequencies can be

Table 6. Occurrence of Alleles in All Populations of *A. occidentalis*, *A. ascendens*, and *A. falcatus* (■= most frequent allele, + = allele present)

Population		Adh-1				Adh-2			Est-1				
		a	b	c	d	a	b	c	a	b	c	d	e
A. occidentalis	1198		■	+		■						■	+
	1217	+		■		■				+	+	■	+
	1223	not sampled				not sampled			not sampled				
	1225	not sampled				not sampled						■	+
	1227	+	■	+		■					+	■	
A. ascendens	1194	not sampled				not sampled					+	+	
	1197	not sampled				not sampled			not sampled				
	1202	not sampled				not sampled					+	■	
	1207		■	+	+	■	+	+	+	+	+	■	
	1218	not sampled				not sampled					■	+	
	1219	not sampled				not sampled				+	+	■	+
	1222	not sampled				not sampled			not sampled				
A. falcatus	1230			+	■			■	not sampled				
	1231			+	■			■	+	■			
	1232			+	■			■		■		+	
	1234			+	■			■	+	■	+	+	
	1235			+	■			■	+	■			

Table 6 (cont'd).

Population		Est-2				Lap-1								
		a	b	c	d	a	b	c	d	e	f	g	h	i
A. occidentalis	1198		■	+				■						
	1217		■					■		+				
	1223	not sampled						■	+	+				
	1225		■	+				■	+	+	+			
	1227	+	+	■			+	+	+	■				
A. ascendens	1194	not sampled					+	■		+				
	1197	not sampled				■		+						
	1202		■		+	■	+	+						
	1207	+		■	+		+	■	+	+				
	1218		■	+		■	+	+	+	+				
	1219		■	+	+	■	+	+	+					
	1222	not sampled					+	■	+	+				
A. falcatus	1230	not sampled				+		+	+		■	+		
	1231			■	+	+				+		■	+	+
	1232			+	■	+	■	+	+	+	■	+		
	1234		+	■	+	+				+	+	■		+
	1235		■	+			+	+			■			

Table 6 (cont'd).

Population		Me-1				Mdh-1			6Pgd-1		
		a	b	c	d	a	b	c	a	b	c
A. occidentalis	1198	+	■				■	+	■		
	1217	+	■	+	+		+	■	■	+	
	1223	■				+	■	+	■		
	1225	■					■	+	■	+	
	1227	■				+	■	+	■	+	
A. ascendens	1194	+	■	+			■	+	■	+	
	1197	+	■				■	+	■		+
	1202	+	■			+	■	+	■	+	
	1207	■				■	+	+	■		
	1218	+	■	+			■	+	■	+	
	1219	■	+			+	■		■	+	
	1222	+	■				■	+	■		
A. falcatus	1230	+	■				■			■	
	1231		■	+			■	+		■	
	1232		■	+			■	+		■	
	1234	+	■			+	■			■	
	1235		■			+	■			■	

Table 6 (cont'd).

Population		6Pgd-2				Pgi-1		
		a	b	c	d	a	b	c
A. occidentalis	1198		■			■	+	
	1217		■				■	
	1223		■			+	■	+
	1225		■				■	+
	1227		■			+	■	+
A. ascendens	1194		■			+	■	+
	1197		■				■	+
	1202	+	■	+			■	
	1207		■	+	+	+	■	+
	1218	+	■	+		+	■	+
	1219	+	■				■	
	1222		■				■	
A. falcatus	1230		■				■	
	1231		■				■	
	1232		■				■	
	1234		■			+	■	
	1235		■				■	+

Table 6 (cont'd).

Population		Pgi-2						Pgm-2						
		a	b	c	d	e	f	a	b	c	d	e	f	g
A. occidentalis	1198		■	+						+	■			
	1217		■	+						+	■			
	1223		■	+						+	■			
	1225	+	■	+		+				+	■			
	1227		+	■	+					■	+		+	
A. ascendens	1194		■	+					+	+	■		+	
	1197		■	+					+	+	■		+	
	1202	+	■	+					+	+	■	+	+	
	1207	+	■	+				+	+	+	■			
	1218		■	+	+	+		+	+	+	■			
	1219		■	+					+	■	+		+	+
	1222	+	■	+		+			+	+	■		+	+
A. falcatus	1230		+	■					+	+	■			
	1231		+	■			+		+	■	+			
	1232	+	+	■		+			+	■	+			
	1234			■		+			+	■	+			
	1235		+	■						■	+			

Table 6 (cont'd).

Population		Skdh-1			Tpi-1				Tpi-2			
		a	b	c	a	b	c	d	a	b	c	d
A. occidentalis	1198	■				■	+			■		
	1217	■	+		+	+	■			■	+	
	1223	■			+	■	+			■	+	
	1225	■				■	+			■		
	1227	■				■	+	+		■		
A. ascendens	1194	■			+	■	+		+	■	+	
	1197	+	■	+		+	■			■	+	
	1202	■	+			+	■			■	+	
	1207	■	+			■	+			■	+	
	1218	+	■	+		■	+			■	+	
	1219	■	+			■	+		+	■	+	
	1222	■	+		+	■	+			■	+	+
A. falcatus	1230	+	■				■			■		
	1231		■				■			■		
	1232		■	+			■			■		
	1234		■				■		+	■		
	1235		■				■			■		

confirmed, and thus the allele frequencies and genetic identity statistics reported for *A. ascendens* are estimates.

For most of the isozymes, the most common allele was the same over all populations within each species. In *A. occidentalis,* population 1227 differed more often with respect to the most common allele and the alleles present than the other populations of that species. *Aster falcatus* populations were far more uniform, with the exception of the alleles of Lap-1, where the number of alleles was higher and the most common allele varied among populations. Comparisons between species showed a closer relationship between *A. occidentalis* and *A. ascendens* than between *A. ascendens* and *A. falcatus* in terms of allele type and distribution (Table 6).

Over the seventeen populations involved in this portion of the study, a total of 66 alleles were observed for 15 polymorphic loci. Of these, 34 alleles were common to all three species. Fifteen alleles were common to two species only; 9 to *A.occidentalis* and *A. ascendens*, 5 to *A. falcatus* and *A. ascendens*, and 1 allele to *A. occidentalis* and *A. falcatus*. The remaining 17 alleles were unique to one species; 4 were found only in *A. occidentalis*, 4 were seen only in *A. falcatus* and 9 appeared only in *A. ascendens*.

4.0. Genetic Variation in *A. occidentalis, A. ascendens* and *A. falcatus*

Mean heterozygosity per locus, average number of alleles per locus, percent polymorphic loci (using .95 and .99 criteria), and interpopulational and interspecies

Table 7. Genetic Variability Measured Across 11 Isozyme Loci in Three Species of *Aster; A. occidentalis, A. ascendens* and *A. falcatus*

Species	Population	Average heterozygosity per locus unbiased (SE)	Average number of alleles per locus (SE)	Percent Polymorphic Loci .95	.99
Aster	1198	0.235 (.069)	1.55 (.16)	54.5	54.5
occidentalis	1217	0.238 (.054)	2.09 (.25)	72.73	81.82
	1223	0.216 (.066)	2.00 (.27)	54.5	63.64
	1225	0.199 (.065)	2.00 (.33)	63.64	63.64
	1227	0.297 (.078)	2.27 (.33)	63.64	63.64
Pooled Populations		0.237	1.98	61.80	65.45
Aster	1194	0.248 (.057)	2.36 (.28)	72.73	81.82
ascendens	1197	0.246 (.065)	2.00 (.19)	63.64	81.82
	1202	0.266 (.063)	2.64 (.39)	72.73	90.91
	1207	0.303 (.074)	2.27 (.33)	72.73	72.73
	1218	0.369 (.064)	2.73 (.24)	81.82	100
	1219	0.315 (.064)	2.36 (.36)	81.82	81.82
	1222	0.256 (.069)	2.45 (.39)	63.64	72.73
Pooled Populations		0.286	2.40	72.73	83.12
Aster	1230	0.152 (.069)	1.82 (.38)	45.45	45.45
falcatus	1231	0.128 (.065)	1.91 (.39)	27.27	45.45
	1232	0.126 (.068)	2.27 (.56)	27.27	54.5
	1234	0.117 (.044)	2.00 (.36)	45.45	63.64
	1235	0.113 (.052)	1.55 (.21)	36.36	45.5
Pooled Populations		0.127	1.91	36.36	50.90

genetic identities (Nei, 1972) were calculated on 11 polymorphic loci using the BIOSYS-1 program (Swofford and Selander, 1981) (Table 7). *Aster ascendens* populations were characterized by high levels of genetic variability, whereas *A. occidentalis* was slightly lower. *Aster falcatus*, in comparison, showed very low levels of variability over its populations and less variation among populations.

Genetic Identities (*I*) were calculated for all possible pairs of populations within and between species (Appendix 3). Within species, genetic identities between populations were highest in *A. falcatus*, all greater than or equal to 0.938 and averaging 0.959. Populational comparisons within *A. ascendens* were slightly lower overall with a mean of 0.861, but over a wider range. Genetic identities for population 1207 were markedly and consistently lower than for any of the other comparisons. *Aster occidentalis* populations had higher genetic identities than *A. ascendens* with an average of 0.873 and also showed a wider range of genetic identities than *A. falcatus*.

The highest mean genetic identities between species were between *A. occidentalis* and *A. ascendens* (0.834). A lower mean of 0.648 was calculated for *A. falcatus* and *A. ascendens* but the lowest genetic identity was determined for *A. occidentalis* and *A. falcatus* (0.539) (Table 8).

Table 8. Means and Ranges of Genetic Identities (Nei, 1972) Calculated for Each Species Combination of *A. occidentalis*, *A. ascendens* and *A. falcatus*.

Species	Species		
	A. occidentalis	*A. ascendens*	*A. falcatus*
A. occidentalis	0.873 (0.795 - 0.987)	0.834 (0.698 - 0.954)	0.539 (0.470 - 0.647)
A. ascendens	—	0.861 (0.684 - 0.983)	0.648 (0.395 - 0.817)
A. falcatus	—	—	0.959 (0.938 - 0.988)

DISCUSSION

1.0. The Genetic Basis of Isozyme Banding Patterns

Isozyme analysis in plant systematic studies has been very successful in providing insights into evolutionary relationships among taxa. High levels of polymorphism within each locus can provide a wealth of potential genetic markers not available through morphological analysis. Interpretation of isozyme number, structure, and mode of inheritance from direct counts of the number of bands present, however, can cause errors in interpretation (Weeden and Wendel, 1989). Many early studies using isozymes dealt with few, highly polymorphic enzymes (e.g. esterases, peroxidases) chosen specifically because of the high number of bands present in the phenotype. This can cause misinterpretations of the levels of variability actually present in the genome. Electrophoretic banding patterns are dependent on enzyme structure, the ploidy of the individual and its genotype (Gottlieb, 1981b). Additional factors, such as overlapping loci, null alleles (non active allele forms) and procedural artifacts can alter band number. Therefore in order to reach a more accurate assessment of genetic variability, a formal genetic analysis (involving the determination of progeny ratios) is needed, using testcrosses to determine the number of loci and their structure. Without this information, use of isozymes for studying species origins and relationships is more likely to lead to erroneous conclusions (Crawford, 1989; Weeden and Wendel, 1989).

Despite the high possibilities of misinterpretation without genetic analysis, relatively few formal studies of isozyme inheritance have been done using a reasonable

number of loci (10 or more) and a wider variety of enzymes. Most of the 35 taxa summarized by Weeden and Wendel (1989) covered this way are crop species, conifers or ferns; fewer wild plant groups have been examined. Examples of some of the latter category in dicots include *Clarkia xantiana* (Gottlieb, 1984), *Limnanthes* (McNeill and Jain, 1983), *Stephanomeria diegensis* (Gallez and Gottlieb, 1982) and *Turnera ulmifolia* (Shore and Barrett, 1987). Formal genetic analysis has been carried out on other groups since then, including *Vaccinium* section *Cyanococcus* (van Heemstra et al., 1991), *Stellaria longpipes* and *Stellaria longifolia* (Cai and Chinnappa, 1989) and *Cicer arietinum* (Gaur and Slinkard, 1990). In these studies at least 10 enzyme loci were examined electrophoretically and the genetic basis for the banding patterns determined using analysis of controlled testcrosses. Information from this type of genetic analysis has been used in other studies on species origin or species delimitation, where knowledge of the genetic basis for the loci used was essential (e.g. *Vaccinium* - Bruederle and Vorsa, 1994). Other studies using formal genetic analyses as a comparative reference have been done (e.g. Small et al., 1992), though without evidence based on testcross progeny segregation, these findings can only be taken as inferences (Weeden and Wendel, 1989). While isozyme number is believed to be highly conserved (Gottlieb, 1982; Crawford, 1989), changes can occur due to the developmental stage of the plant, the type of tissue, gene duplication, gene silencing or ploidy, and thus gene number should not be presumed invariant.

In *Aster*, published information on isozymes is limited to two species (*A. riparius* and *A. hydrophilus*) analyzed by Gottlieb (1981a), and a study of seven species in *Aster*

section *Biotia* examining phenetic relationships (Lamboy et al., 1991). Neither of these studies used formal genetic analysis to determine the genetic basis for isozyme banding patterns and in both studies not many plants were surveyed. In a genus such as *Aster*, a more extensive type of study on the genetic level is needed in order to provide a basis for assessing genetic variation and genetic divergence among populations.

1.1. Number of Isozyme Loci Determined in *Aster occidentalis*

Of the six enzyme systems analyzed (with 12 loci), patterns of inheritance for nine polymorphic loci were determined using genetic analysis. The remaining three putative loci (Pgm-1, Mdh-2, Mdh-3) were monomorphic and therefore could not be analyzed through crossing experiments. The number of loci found for each enzyme is summarized in Table 2 along with numbers of loci for these enzymes reported from other studies, compiled by Kephart (1990). The only deviation from the numbers of loci found in other studies is found in LAP. My analysis showed a single monomeric locus for this enzyme, while Kephart reports two or three monomeric loci. However, my findings for LAP are consistent with those of Gottlieb (1981a), Lamboy et al. (1991), and Allen (unpublished). This suggests that either additional loci are present in *Aster* but don't produce enough enzyme to be picked up on the gel, or that a single locus for this enzyme in *Aster* is normal.

In *Aster occidentalis*, I found three loci for MDH, with possibly a fourth which showed rarely and faintly. Only the Mdh-1 locus was polymorphic; heterozygotes showed the triplet characteristic of a dimeric enzyme (Table 2). While three loci have been found

by Kephart (1990), Allen (unpublished), and Weeden and Wendel (1989); Gottlieb (1981a) shows four loci for this enzyme. The faintness of the fourth isozyme in my survey may be due to the developmental stage of the tissue or the buffers used in the assay (Kephart, 1990).

Two loci were found for each of PGI, PGM and TPI, with Pgm-1 the only monomorphic locus. Both PGI and TPI are dimeric, while PGM is monomeric (Table 2). This is consistent with reports for these enzymes in *Aster* by Lamboy et al. (1991), Gottlieb (1981a), and Allen (unpublished) for *Aster occidentalis* and *A. falcatus* and a range of additional species outside this genus (Kephart, 1990).

Alcohol dehydrogenase (ADH) has not been investigated in any previous isozyme analyses of *Aster*. Kephart (1990) lists ADH as a dimeric enzyme with one to three loci, all showing expression in the cytosol. The two loci found for ADH in *Aster occidentalis* fit within the range of loci found in other species, including the bands corresponding to heterodimers formed when the gene products are expressed in the same subcellular compartment (Figure 1, Table 2). This consistency in isozyme number for each enzyme is predictable for enzymes with well defined biochemical roles (i.e. the Krebs cycle). Those enzymes that are less specific (i.e. Peptidase, Peroxidase, Esterase) may vary to a greater extent (Weeden and Wendel, 1989).

Although no segregation data were available for *Aster falcatus*, the banding patterns were the same as those of *A. occidentalis*, implying the same mode of inheritance. Inheritance of isozyme loci in *A. occidentalis* and *A. falcatus* shows the same number of loci in both species for the six enzymes used for linkage analysis and the additional four

(EST, ME, 6PGD and SkDH) used later for allozyme analysis. No evidence of duplication or loss of loci was found.

1.2. Linkage Groups in *Aster occidentalis*

Linkage analysis in *A. occidentalis* using eight polymorphic loci revealed locus pairs for two linkage groups and single locus markers for three more (Table 5). The Pgi-2 and Adh-1 loci showed more evidence of recombination (a weaker linkage) than Pgm-2 and Mdh-1 suggesting they are further apart on the chromosome than Pgm-2 and Mdh-1 (Table 3). Three other loci, Tpi-1, Tpi-2 and Lap-1, were inherited independently of each other and the other two linkage groups. Since the number of linkage groups expected is dependent upon and equal to the number of chromosome pairs for that species, *A. occidentalis* would be expected to contain three additional linkage groups in addition to those found in this study. Plants heterozygous for additional loci and improvement of protocols to increase the resolution of different enzyme systems (i.e., 6PGD, SkDH, ME, AAT etc.) is needed to identify gene loci in additional linkage groups and expand this linkage map. *Aster falcatus*, with five pairs of chromosomes would be expected to show five linkage groups. This species would be expected to contain largely the same loci as *A. occidentalis*, however, in order for successful hybridization to occur (Waples, 1988).

In allopolyploidy, the combination of two separate genomes results in homeologous chromosomes, yielding a karyotype of dissimilar chromosomes. Pairing does not occur between these homeologues, but does occur between homologous chromosomes, thus conserving the original alleles from both parents (Crawford, 1990).

The combining of two haploid gametes of differing chromosome complement would most likely lead to reduced fitness. Synapsis would not be able to occur in meiosis because of structural difficulties (i.e. differences in the number of chromosomes, their sizes, or the type or order of the genes), and the resulting hybrid would likely be sterile. However, this problem can be avoided if the hybrid is the result of the fusion of unreduced gametes, or if somatic chromosome doubling occurs to provide homologues for each chromosome. Thus while some degree of genetic inequality between parental genomes can be tolerated in amphiploids, higher levels of homology between genomes increase the chance of a successful hybrid.

Aster ascendens (n=13) is a fertile allotetraploid hybrid derivative of *A. occidentalis* and *A. falcatus,* thought to have originated either by fusion of unreduced gametes or somatic doubling after haploid gamete fusion (Allen, 1985). This hybrid should therefore show the allopolyploid characteristic of duplicate loci (seen as fixed heterozygosity), as it would receive these from both *A. occidentalis* and *A. falcatus* (Crawford, 1990). The linkage groups would therefore be expected to show combinations of the parental types. For example, *A. occidentalis* shows linkage between Pgi-2 and Adh-1 while Tpi-1 is inherited independently. If *A. falcatus* were to show linkage between Adh-1 and Tpi-1 but not with Pgi-2, then *A. ascendens* would be expected to show linkages between Pgi-2 and Adh-1 and between Adh-1 and Tpi-1 but not between Tpi-1 and Pgi-2. Determination of linkages would follow the same format as I have followed for the diploid species; it would however involve examination of several different cross types and would require different parental genotypes at each locus. For example, to

determine the inheritance patterns and linkage described above, I would need *A. ascendens* plants both heterozygous (for different parental alleles) at all combinations of these three loci and plants homozygous (again for different alleles) for these loci in order to determine which banding patterns come from which parental portion of the *A. ascendens* genome.

2.0. Comparison of Allelic Variation In *A. occidentalis, A. falcatus* and *A. ascendens.*

Analysis of genetic variation based on allele frequencies in a total of ten populations of *A. occidentalis* and *A. falcatus* (see Results) revealed many similarities in the genomes of these two species. *Aster occidentalis* and *A. falcatus* share 61% of the 57 alleles determined for 15 polymorphic loci (they share alleles at 14 of the 15 loci) for these two species. At 10 of these loci, however, different alleles are common in *A. occidentalis* and in *A. falcatus*. These differences in frequencies may account in part for the low genetic identity between the two species (Table 8). These results suggest that some genetic homology does exist between the two species but there is evidence of continuing genetic divergence (assuming the alleles found are representative). In addition to these differences in allele frequency, the two species were found to be completely divergent at the Adh-2 locus and shared few alleles at the Lap-1 locus.

Aster occidentalis populations were polymorphic for all but two loci, Adh-2 and 6Pgd-2. The Adh-2 locus was fixed for the a allele and 6Pgd-2 was fixed for the b allele. *Aster falcatus* was fixed for the c allele at Adh-2, the b allele at 6Pgd-1, the b allele at 6Pgd-2, and the c allele at Tpi-1 (Table 6). This is reflected in calculations of mean

heterozygosity, average number of alleles per locus, and the percentage of polymorphic loci, where *A. falcatus* populations had markedly lower values than *A. occidentalis* for all these indices of variability (Table 7). Reviews of intrapopulational allozyme variation indicate that plant species generally maintain high levels of variability within populations. A survey by Hamrick et al. (1979) compiled information on over 100 species, showing that the average species had 37% polymorphic loci, 1.69 alleles per locus and 0.141 mean heterozygosity per individual (Hamrick, 1989). Perennial outcrossers such as *Aster occidentalis* and *Aster falcatus* are expected to show higher levels of variability than selfers, which would likely become fixed for a smaller number of alleles within a single population. The levels of variation in *A. occidentalis* are high, with mean heterozygosity pooled over five populations at 0.237, 1.98 alleles per locus and 65% polymorphic loci. *Aster falcatus* is much closer to the average values cited by Hamrick for these measures, with 0.127 mean heterozygosity, 1.91 alleles per locus and 51% polymorphic loci. A possible explanation for the difference may be related to geography. Collection sites for all five *A. falcatus* populations are all located in the southwest corner of the range of the species, while those for *A. occidentalis* are more widely distributed over its range (Figure 1). This would mean that only a portion of the range of *A. falcatus* was represented. References cite northern populations of *A. falcatus* as mainly hexaploid (Jones, 1978) and the possibility exists that diploid populations of *A. falcatus* are rare relicts, representing a small part of the variation present in this species in the past (Allen, personal communication).

Aster ascendens, suggested by Allen (1985) to be an allotetraploid hybrid between *A. occidentalis* and *A. falcatus*, shares a number of alleles with each of its putative parents. Thirty-four of the 35 alleles shared between *A. occidentalis* and *A. falcatus* are also found in *A. ascendens*. This indicates genetic homology between the parent species and provides support for the relationship of *A. ascendens* to *A. occidentalis* and *A. falcatus*.

Isozyme data reveal evidence of allopolyploidy in *A. ascendens*. Disomic inheritance is present in all polymorphic loci. Multiple banded phenotypes were present for some individuals in *A. ascendens* populations for a majority of the loci. For example, Lap-1 (a monomer) showed some individuals with three and four bands, suggesting gene duplication at this locus. Similar examples were found in LAP, MDH, 6PGD, PGI, PGM, and SkDH. Interpretation of these multibanded phenotypes as evidence of duplicate loci is supported by the high levels of mean heterozygosity in *A. ascendens*, the high percentage of polymorphic loci and the higher number of alleles per locus (Table 5). The reason this fixed heterozygosity is not seen throughout each population to a greater degree is likely a result of the amount of genomic overlap between *A. occidentalis* and *A. falcatus*. Where both parents share the same alleles at a particular locus, the hybrid would show the same pattern, appearing monogenic (Werth, 1989).

Nine alleles are found only in *A. occidentalis* and *A. ascendens*, at the Pgm-2, 6Pgd-1, Tpi-1, Tpi-2, Est-1, Est-2, Adh-1 and Adh-2 loci (Table 4). Five alleles are common to *A. falcatus* and *A. ascendens* at Lap-1, Skdh-1, Tpi-2, Adh-1 and Adh-2 loci. The presence of alleles in the hybrid that are found in only one parent, such as these 14

alleles in *A. ascendens*, are valuable markers providing evidence of ancestry from these species and are characteristic of allopolyploid species, in which the addition of the genomes preserves the original parental alleles (Crawford, 1990; Finkeldey, 1992).

Examination of individual populations, in terms of the presence or absence of particular alleles as well as their frequencies (Table 6, Appendix 2), reveals that some populations of *A. ascendens* are more homologous with particular *A. falcatus* populations, for one or two loci (i.e., Lap-1 in 1218 is closer to *A. falcatus* than to *A. occidentalis*), but most are intermediate or closer to *A. occidentalis.* Population 1202 of *A. ascendens*, for example, shares its most common alleles with *A. occidentalis* for 11 loci.

These results indicate a closer overall relatedness between *A. occidentalis* and *A. ascendens* than between *A. ascendens* and *A. falcatus*, but with sufficient homology and enough parental markers to support a hybrid origin for *A. ascendens* from these two species.

3.0. Comparison of Genetic Identities Among Populations of *A. occidentalis*, *A. falcatus*, and *A. ascendens*

Comparison of genetic identities calculated among populations of *A. occidentalis* and *A. falcatus* (Appendix 3) show higher identities among *A. falcatus* populations (all > 0.93) than among *A. occidentalis* populations (lowest is 0.795). Whereas Gottlieb (1981b) quotes expected genetic identities among conspecific populations to be 0.90 or higher on average, a wider array of values has been seen in many studies, ranging from 0.65 to 1.0 (Crawford, 1983). The lowest genetic identity within *A. occidentalis*

populations between populations 1217 and 1227 (I = 0.795). These populations were collected at opposite ends of the species range and may represent a geographic difference in allele type and/or frequency.

Inter-specific genetic identities between *A. occidentalis* and *A. falcatus* don't show too great a range (0.47 - 0.647) and while the average of 0.539 is lower than cited as a general norm of 0.67 for congeneric species (Gottlieb, 1981b), this too fits within a wide range of values reported, 0.48 - 0.97 (Crawford, 1983). This supports keeping *A. falcatus* within the genus *Aster* rather than moving it to *Lasallea* (= *Virgulus*) as suggested by Semple and Brouillet (1980b), although comparative genetic identities with other species in the *Lasallea* species group would be useful.

Comparisons of genetic identity between *A. ascendens* populations are also within the range of values seen in other conspecific populations (Gottlieb, 1981b). Population 1207 of *A. ascendens* however, differs consistently from all other *A. ascendens* populations, yielding the lowest genetic identities within each comparative grouping. A more precise method of determining allele frequencies in polyploids is needed to determine whether these identity values are accurate. The method of determining allele frequencies in polyploids most often used involves assigning genotype based on the intensity of the banding patterns (Kephart, 1990), a method I found to be difficult to apply, as band intensity also varied with storage time and samples. The use of a maximum likelihood estimation for allele frequencies of *A. ascendens*, while not necessarily accurate, treats all populations consistently. Testcrosses in *A. ascendens*, using the process described above

to identify each allele and its parental source, may help to increase the accuracy of allele frequency assessment.

Genetic identity between *A. occidentalis* and *A. ascendens* was higher overall than between *A. falcatus* and *A. ascendens* (Table 8). *Aster falcatus* and *A. ascendens* show genetic identities within the range of reported values for congeneric species (Crawford, 1983) but in the lower range, because of the consistently low genetic identities between *A. ascendens* population 1207 and the *A. falcatus* populations. *Aster occidentalis* comparisons with *A. ascendens* are closer in some cases to values seen between conspecific populations. Population 1194 of *A. ascendens*, for example, has genetic identity values ranging from 0.819 - 0.954. In two cases these represent higher genetic affinity between species than between populations of *A. occidentalis* when compared to itself (Appendix 3). When comparing within *A. occidentalis* and *A. ascendens*, however, population 1207 of *A. ascendens* does not stand apart as being consistently lower in genetic identity. This suggests that the differences seen in the genetic identities of *A. ascendens* and *A. falcatus* may be due to differences in the frequencies of some of the alleles in population 1207 of *A. ascendens* rather than because of differences in the identity of the alleles present.

In addition to the shared alleles among the three species then, genetic identity comparisons support the theory of hybrid origin of *A. ascendens* from *A. occidentalis* and *A. falcatus*, as *A. ascendens* is intermediate in genetic identity and is closer in identity to either of its parents than they are to each other (Table 8).

4.0. Comparison of Isozymes with Morphological and Cytological Differences

Aster occidentalis and *A. falcatus* show distinct differences in a number of morphological characters (Allen, 1985; Jones, 1978, 1980b). For example, *A. occidentalis* averages 3 to 30 flower heads per stem, arranged around the stem, while *A. falcatus* carries from 10 to 80 flower heads per stem, secund. The ray flowers differ in colour and number per head; *A. occidentalis* is violet, with 15-40 per head while *A. falcatus* is white, with 7-20 per flower head. *Aster occidentalis* has more disc florets per head (30-80) than *A. falcatus* (4-15). The involucral bracts differ significantly in shape and length between the two species, as do the shape and length of the cauline leaves.

Cytological analysis supports placing *A. occidentalis* and *A. falcatus* in different sections or subgenera (Jones, 1980a,b; Allen, 1984; Semple and Brouillet, 1980b). The base chromosome number in *A. occidentalis* is n = 8 and its karyotype is fairly symmetrical, with very little difference in size among the chromosomes. The NOR is located near the end of the short arm on one of the larger chromosomes. *Aster falcatus* (n= 5), shows a greater difference in the size between chromosomes and the NOR is located very close to the terminal end of the short arm of the largest chromosome (Semple and Brouillet, 1980b; Allen, 1985).

The genetic resemblance in isozyme patterns between *A. ascendens* and its parent species *A. occidentalis* and *A. falcatus* is also shown in morphological and chromosomal characters of *A. ascendens*. *Aster ascendens* and *A. occidentalis* are very similar morphologically in most populations and overlap geographically to a large extent.

They are differentiated mainly by slightly larger flower heads in *A. occidentalis* and a difference in the size and shape of the phyllaries the phyllaries of *A. ascendens* resemble those of *A. falcatus.* The karyotype is a combination of the features of both *A. occidentalis* and *A. falcatus* (Allen, 1985). Genetic analysis of these three species of *Aster* thus supports the interpretation obtained through examination of morphological and cytological information.

5.0. Previous Work Showing *A. ascendens* to be an Allotetraploid

Aster ascendens is placed in the *Aster occidentalis* complex, a group of rhizomatous, perennial asters composed of a chromosome base number of $x = 8$ (Allen, 1985). Earlier records of chromosome counts varied for *A. ascendens*, perhaps for this reason. Jones (1980 a,b), for example, listed chromosome counts from a number of sources including = 8, 13, 16, 18, 24, 27, 32 and 36. Jones (1980a) remarked on the morphological similarity of *A. ascendens* to other species of the *A. occidentalis complex*, especially *A. occidentalis*. The potential for misidentification evidently exists, and the chromosome counts based on x = 8 here may be counts from *A. occidentalis* instead. Characteristic chromosome numbers for this species however are accepted as n = 13 and 26 (Dean, 1966; Dean and Chambers, 1983). Allen (1985) first suggested allopolyploidy between two species with different chromosome numbers as a probable origin for *A. ascendens.* A chromosome count based on x = 13, not previously seen in *Aster*, was possible through aneuploidy or polyploidy, and although allopolyploidy involving different chromosome numbers is not common in *Aster*, it is more common than aneuploid

reduction (Allen, 1985). Examination of 20 morphological characters for *A. ascendens, A. occidentalis* and two species (*A. falcatus* and *A. ericoides*) with n= 5, supported the proposed hybrid origin of *A. ascendens* from *A. occidentalis* and *A. falcatus*. Karyotypes were distinct in the parental species but *A. ascendens* showed a combination of karyotypic characters (i.e., symmetry and NOR location) (Allen, 1985).

6.0. Evidence for the Origin of Hybrid Species.

Studies of progenitor and derivative plant species have examined the probable origins of many types of hybrids - diploid hybrids, allopolyploids, and autopolyploids. These studies have shown certain characteristics that can be used to identify the type of hybrid relationship and the likely parent species. Allopolyploids, for example are found to exhibit highly differentiated chromosomes (unless the parent's karyotypes were very similar), fixed heterozygosity at many loci (this is somewhat dependent on the disparity of the parental genomes), alleles found in both parent species (i.e. if the parents are fixed for a different allele at a particular locus, both of these should be in the hybrid) and few, if any unique alleles in the hybrid (Crawford, 1990).

In *Tragopogon* spp. Roose and Gottlieb (1976), showed that 40% of the total parent loci tested were fixed for different alleles, indicating a high degree of genetic divergence. The hybrid tetraploid species showed additive banding patterns for these enzymes, indicating that despite the degree of genetic divergence, successful hybridization had occurred through allopolyploidy. Gottlieb (1973) studied *Stephanomeria diegensis*, a diploid hybrid, and its parent species, *S. exigua* and *S. virgata*, for the presence of the

hybrids alleles in the parent species. Isozyme analysis revealed very few differences between the progenitor species although they are different in both morphology and karyotype, but all the alleles of *S. diegensis* were present in its parents and it did not contain any unique alleles. In Finkeldeys' (1992) study of *Paulownia* species in Taiwan, the theory of *P. taiwaniana* hybrid origin from 2 other endemic species was tested using isozyme electrophoresis. *Paulownia taiwaniana* exhibited the characteristics of an allopolyploid hybrid; for those loci where the parents were fixed for different alleles, *P. taiwaniana* showed fixed heterozygosity, and where the parents were homozygous for the same allele, so was the hybrid. Another example is the work of Ashton and Abbott (1992) on determining the origin of *Senecio vulgaris*, believed to be of autotetraploid derivation. Isozyme analysis showed fixed heterozygosity at a number of loci, disomic inheritance and evidence of an additional locus and a unique allele. This suggested allopolyploid origin, rather than autopolyploid to the authors, who put forth the original autotetraploid progenitor as one of the parents.

Such studies show that use of genetic analysis can be highly successful in clarifying such complex species relationships and origins, but that a single technique has the potential to be misleading if used without reference to other information. The differences between prospective parental species may be distinct at the morphological level and less apparent at the genetic level or visa versa. In the case of *Aster ascendens*, morphological, chromosomal and genetic evidence all support the same interpretation. The use of genetic analysis provides additional support for allotetraploidy in *A. ascendens* because the genetic characteristics for allopolyploidy are present and the levels of heterozygosity, the number

of alleles and the percent of polymorphic loci are at levels predictable for a polyploid species. Isozyme analysis has provided valuable markers present in each parent species and the hybrid, again suggesting a combination of *A. occidentalis* and *A. falcatus* genomes in *A. ascendens.* Genetic identities place *A. ascendens* between *A. falcatus* and *A. occidentalis* and are consistent with the morphological similarity existing between *A. ascendens* and *A. occidentalis.*

Some new questions have been raised by this study. Will the differences in the genetic identities of population 1207 when compared to other populations of *A. ascendens* and to *A. falcatus* remain when more accurate allele frequencies are determined? If so, is this, along with the large range of genetic identities within *A. ascendens* an indication of multiple origins for this hybrid species? In a recent study of *Tragopogon* allotetraploids *T. mirus* and *T. miscellus* by Soltis et al. (1995), electrophoretic evidence suggests that these two allotetraploid species have originated by multiple polyploidy events, evidenced by the appearance of several distinct genotypes in different hybrid populations coming from different combinations of the parental chloroplast genomes and repeat types of ribosomal RNA genes. The authors suggest that this may allow polyploids to maintain high levels of segregating variation, and that these multiple hybridization events are far more common than previously believed. The unique alleles found in each *Aster* species (Me-1e, Pgi-2d, Tpi-1d and Adh-1a in *A. occidentalis*, Lap-1g,h,i and Pgi-2f in *A. falcatus*, and Pgm-2a,e,g, 6Pgd-1c, 6Pgd-2a,c,d, Tpi-2d and Adh-2b in *A. ascendens*) suggest that either hybridization took place far enough in the past to allow for accumulation of new alleles through mutation, or that the species *A. ascendens* is the product of multiple

hybridization events between *A. occidentalis* and *A. falcatus* in different parts of their ranges where the alleles may be slightly different. The large number of unique alleles in *A. ascendens* can certainly be explained by the mutation of the "raw material" provided by gene duplication, although morphological variations in different populations noted by Allen (1985) support the genetic evidence of multiple origins. To examine the possibility of multiple origins for *A. ascendens*, more populations need to be analyzed for a greater number of loci.

While the close relationship between *A. occidentalis* and *A. ascendens* is clear from all levels of study, the amount of genetic information supporting *A. falcatus* as the other parent of *A. ascendens* is not so large as to be wholly convincing as yet. While there is genetic similarity as discussed above, the low genetic identities of the *A. falcatus* populations to *A. ascendens* population 1207 raise some questions. If however there were multiple origins of *A. ascendens* at a time when more diploid populations of *A. falcatus* were present throughout its range (therefore carrying additional alleles not seen here) or indeed if other diploid populations of *A. falcatus* exist with varying genotypes, the 1207 population could be explained as the result of a hybridization event between *A. occidentalis* and one of these now vanished populations. Although this is supposition, crossing experiments between *A. occidentalis* and *A. falcatus* and between *A. ascendens* and *A. falcatus* would further clarify whether or not *A. falcatus* is a parent species. The use of additional loci to develop a linkage map for all three species would be valuable to this end as well. Use of fluorescent probes to mark individual loci would be helpful in tracing the location of these loci on the chromosomes and if used in conjunction with

controlled testcrosses may be of use in determining allele placement in the polyploid species. Genomic markers can also be obtained using restriction fragment length polymorphism's (RFLP) and would examine this at a different level still (Crawford, 1990). Examination of *A. ericoides* using these methods would aid in clarification as well, by addressing the possiblilty of this species (very closely related to *A. falcatus*) as an alternate parent. While these questions still remain to be answered, the determination of isozyme inheritance patterns and linkage groups in *A. occidentalis* and the analysis of genetic variability within and among these three species in this study has confirmed the interpretation of banding patterns for nine isozyme loci in *Aster*, and has provided additional support for the hybrid origin of *A. ascendens* from the diploid species *A. occidentalis* and *A. falcatus* and thus has supplied a solid foundation for future work in this complex genus.

Summary

1. Complexities in the genus *Aster* L. (Asteraceae) have been attributed to high levels of polyploidy and interspecific hybridization, and to the presence of different basal chromosome numbers, making it difficult to determine species relationships and limitations for many groups within *Aster*. Morphological and chromosomal evidence from previous studies place *Aster occidentalis* (x = 8) and *Aster falcatus* (x = 5) as the progenitor species of the allotetraploid *A. ascendens* (x = 13). I used starch gel electrophoresis to determine the degree of genetic divergence among these species of *Aster* and to test the hypothesis of hybrid origin.

2. I carried out isozyme analysis of progeny from testcrosses in *A. occidentalis* to determine the inheritance patterns and the linkage groups of nine polymorphic loci representing six enzyme systems. Five linkage groups were revealed for *A. occidentalis*, providing the first step towards construction of a genetic map for this species.

3. Testcrosses were made for *A. falcatus* to determine inheritance patterns and linkage groups for this species as well, but they did not set seed successfully. Additional work in determining linkage groups for *A. falcatus* and *A. ascendens* will provide a basis for assessing the relationships among these species, which may be valuable in determining relationships of major species groups in the genus *Aster*.

4. I carried out isozyme analysis on one population each of *A. occidentalis*, *A. falcatus*, and *A. ascendens* , and analyzed allele frequency data for these populations as well as unpublished data available for fourteen additional populations. Data were obtained for fifteen polymorphic loci covering ten enzyme systems. Of the 66 alleles found, *A.*

ascendens shares 9 alleles with *A. occidentalis*, 5 alleles with *A. falcatus* and 34 alleles with both parent species. Unique alleles were also found in all three species.

5. Neis' genetic identities between *A. occidentalis* and *A. falcatus* were fairly low (0.539), but are within the range (0.28 - 0.99, mean 0.67) for congeneric species. Genetic identities between *A. ascendens* and *A. occidentalis* (0.834) and between *A. ascendens* and *A. falcatus* (0.648) were somewhat higher. These findings support the hypothesis that *A. ascendens* originated through hybridization between *A. occidentalis* and *A. falcatus*, and is an allopolyploid derived from these two species.

6. The presence of unique alleles in *A. ascendens* suggests that (a) hybridization occurred far enough in the past to allow new alleles to appear, or (b) that the appearance of *A. ascendens* was the result of multiple hybridization events. Analysis of chloroplast DNA from different populations of all three species may provide insight into the possibility of multiple origins.

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APPENDIX 1

Stain Recipes Used For Isozyme Electrophoresis of *Aster* spp.

(adapted from Cheliak and Pitel, 1984; Soltis et al., 1983, and Weeden and Wendel, 1989)

Note: Volumes given are for single gels.

Alcohol Dehydrogenase (ADH)

0.1 M Tris-HCl buffer, pH 8.0 .... 75 ml

95% ETOH .... 3 ml

NAD .... 30 mg

MTT .... 25 mg

PMS .... 1.5 mg

Esterase (EST)

0.1 M Phosphate buffer, pH 6.0 .... 50 ml

α - napthyl acetate .... 25 mg

Fast Blue RR Salt .... 50 mg

DISSOLVE acetate and Fast blue RR Salt in 1 ml of acetone before adding to the phosphate buffer. PRESOAK the gel in 50 ml of 0.1M Phosphate buffer at pH 6.0 for 7 - 10 minutes before transferring to the stain.

Isocitrate Dehydrogenase (IDH)

0.1 M Tris-HCl buffer, pH 8.0 .... 50 ml

1.0 M $MgCl_2$ .... 2.5 ml

Isocitric acid .... 100 mg

NADP .... 5 mg

MTT .... 8 mg

PMS .... 1 mg

Leucine Aminopeptidase (LAP)

0.1 M Phosphate buffer, pH 6.0 .... 50 ml

L-leucyl-β-napthylamide .... 15 mg

Black K Salt .... 25 mg

DISSOLVE the napthylamide in 1 ml of dimethylformamide before adding to the stain buffer. PRESOAK the gel in 0.1 M Phosphate buffer, pH 6.0, for 7 - 10 minutes before transferring to the stain.

Malic Enzyme (ME)

0.1 M Tris-HCl buffer, pH 8.0 .... 50 ml

DL-malic acid .... 420 mg

0.1 M $MgCl_2$ .... 2.5 ml

NADP .... 5 mg

MTT .... 5 mg

PMS .... 1 mg

ADD malic acid to the Tris-HCl buffer, then titrate to pH 7.2 with NaOH. PRESOAK the gel in 0.1 M Tris-HCl buffer, pH 7.5 for 7-10 minutes before transferring to stain.

Malate Dehydrogenase (MDH)

0.1 M Tris-HCl buffer, pH 8.0 .... 50 ml

DL-malic acid .... 420 mg

EDTA (sodium salt) .... 19 mg

NAD .... 15 mg

MTT .... 5 mg

PMS .... 1 mg

ADD malic acid to the Tris-HCl buffer, then titrate to pH 7.2 with NaOH. PRESOAK the gel in 0.1 M Tris-HCl buffer, pH 7.5 for 7-10 minutes before transferring to stain.

Phosphogloucose Isomerase (PGI)

0.1 M Tris-HCl buffer, pH 8.0 .... 50 ml

0.1 M $MgCl_2$ .... 2.5 ml

Fructose-6-Phosphate .... 20 mg

Glucose-6-PDH .... 20 U

NADP .... 5 mg

MTT .... 4 mg

PMS .... 1 mg

Phosphoglucomutase (PGM)

0.1 M Tris-HCl buffer, pH 8.0 .... 50 ml

0.1 M $MgCl_2$ .... 2.5 ml

α-D-glucose-1-phosphate (sodium salt) .... 50 mg

α-D-glucose-1,6-diphosphate (potassium salt) .... 0.5 mg

Glucose-6-PDH .... 20 U

NADP .... 5 mg

MTT 5mg

PMS .... 1 mg

ADD approx. 5 mg L-Histidine to the buffer.

Shikimate Dehydrogenase (SkDH)

0.1 M Tris-HCl buffer, pH 8.5 .... 75 ml

Shikimic acid .... 37.5 mg

NADP .... 7.5 mg

MTT .... 7.5 mg

PMS .... 2.25 mg

PRESOAK the gel in 0.1 M Tris-HCL buffer, pH 8.5 for 7-10 minutes before transferring to the stain.

6-Phosphogluconate Dehydrogenase (6-PGD)

0.1 M Tris-HCl buffer, pH 8.0 .... 50 ml

1.0 M $MgCl_2$ .... 1 ml

Ba salt of 6-Phosphogluconate.. 20 mg

NADP .... 5 mg

MTT... 5 mg

PMS .... 1 mg

PRESOAK the gel in 0.1 M Tris-HCl buffer, pH 8.5 for 7 -10 minutes before transferring to the stain.

Triose Phosphate Isomerase (TPI)

0.1 M Tris-HCl buffer, pH 8.0 .... 75 ml

EDTA (sodium salt).. 28.5 mg

Na_2 arsenate .... 345 mg

DHAP .... 7.5 mg

Glyceraldehyde 3-PDH .... 300 U

NAD .... 30 mg

MTT... 6 mg

PMS .... 2 mg

APPENDIX 2. Alleles Frequencies in All Populations of *A. occidentalis*, *A. ascendens*, and *A. falcatus*

Population		Adh-1				Adh-2			Est-1				
		a	b	c	d	a	b	c	a	b	c	d	e
A. occidentalis	1198												
	1217												
	1223	not sampled				not sampled				not sampled			
	1225	not sampled				not sampled							
	1227												
A. ascendens	1194	not sampled				not sampled							
	1197	not sampled				not sampled				not sampled			
	1202	not sampled				not sampled							
	1207												
	1218	not sampled				not sampled							
	1219	not sampled				not sampled							
	1222	not sampled				not sampled				not sampled			
A. falcatus	1230									not sampled			
	1231												
	1232												
	1234												
	1235												

APPENDIX 2. Allele Frequencies of *Aster* Populations for Loci Used in Analysis of Allelic Variation

Population		Lap-1								
		a	b	c	d	e	f	g	h	i
A. occidentalis	1198	.000	.000	1.00	.000	.000	.000	.000	.000	.000
	1217	.000	.000	.823	.000	.177	.000	.000	.000	.000
	1223	.000	.000	.700	.200	.100	.000	.000	.000	.000
	1225	.000	.000	.667	.150	.050	.133	.000	.000	.000
	1227	.000	.013	.300	.100	.587	.000	.000	.000	.000
A. ascendens	1194	.000	.206	.779	.000	.015	.000	.000	.000	.000
	1197	.683	.000	.317	.000	.000	.000	.000	.000	.000
	1202	.648	.072	.278	.000	.000	.000	.000	.000	.000
	1207	.000	.263	.447	.158	.079	.000	.000	.000	.000
	1218	.533	.000	.283	.100	.083	.000	.000	.000	.000
	1219	.629	.065	.258	.048	.000	.000	.000	.000	.000
	1222	.000	.250	.641	.078	.031	.000	.000	.000	.000
A. falcatus	1230	.500	.000	.155	.017	.000	.241	.086	.000	.000
	1231	.286	.000	.000	.000	.018	.000	.571	.036	.089
	1232	.041	.338	.203	.027	.027	.338	.054	.000	.000
	1234	.068	.000	.000	.000	.045	.136	.727	.000	.000
	1235	.000	.162	.100	.000	.000	.738	.000	.000.	.000

APPENDIX 2 (cont'd).

Population		Me-1				Mdh-1			6Pgd-1		
		a	b	c	d	a	b	c	a	b	c
A. occidentalis	1198	.303	.697	.000	.000	.000	.762	.237	1.00	.000	.000
	1217	.075	.863	.050	.013	.000	.397	.603	.988	.012	.000
	1223	1.00	.000	.000	.000	.150	.750	.100	1.00	.000	.000
	1225	1.00	.000	.000	.000	.000	.895	.105	.950	.050	.000
	1227	1.00	.000	.000	.000	.141	.474	.385	.887	.113	.000
A. ascendens	1194	.076	.879	.045	.000	.000	.700	.300	.882	.118	.000
	1197	.183	.817	.000	.000	.000	.979	.021	.929	.000	.071
	1202	.204	.796	.000	.000	.011	.795	.193	.953	.047	.000
	1207	1.00	.000	.000	.000	.944	.000	.056	1.00	.000	.000
	1218	.071	.893	.036	.000	.000	.983	.033	.567	.433	.000
	1219	.759	.241	.000	.000	.000	1.00	.000	.806	.194	.000
	1222	.088	.912	.000	.000	.000	.819	.181	1.00	.000	.000
A. falcatus	1230	.179	.821	.000	.000	.000	1.00	.000	.000	1.00	.000
	1231	.000	.967	.033	.000	.000	.975	.025	.000	1.00	.000
	1232	.000	.974	.026	.000	.000	.962	.038	.000	1.00	.000
	1234	.096	.904	.000	.000	.075	.925	.000	.000	1.00	.000
	1235	.000	1.00	.000	.000	.051	.949	.000	.000	1.00	.000

APPENDIX 2 (cont'd).

Population		6Pgd-2				Pgi-1		
		a	b	c	d	a	b	c
A. occidentalis	1198	.000	1.00	.000	.000	.783	.262	.000
	1217	.000	1.00	.000	.000	.000	1.00	.000
	1223	.000	1.00	.000	.000	.016	.790	.194
	1225	.000	1.00	.000	.000	.000	.833	.167
	1227	.000	1.00	.000	.000	.250	.700	.050
A. ascendens	1194	.000	1.00	.000	.000	.015	.882	.103
	1197	.000	1.00	.000	.000	.000	1.00	.000
	1202	.047	.930	.023	.000	.000	1.00	.000
	1207	.000	.842	.158	.000	.289	.632	.079
	1218	.033	.783	.200	.000	.233	.750	.017
	1219	.065	.935	.000	.000	.000	1.00	.000
	1222	.000	1.00	.000	.000	.000	1.00	.000
A. falcatus	1230	.000	1.00	.000	.000	.000	1.00	.000
	1231	.000	1.00	.000	.000	.000	1.00	.000
	1232	.000	1.00	.000	.000	.000	1.00	.000
	1234	.000	1.00	.000	.000	.050	.950	.000
	1235	.000	1.00	.000	.000	.000	.962	.038

APPENDIX 2 (cont'd).

Population		Pgi-2						Pgm-2						
		a	b	c	d	e	f	a	b	c	d	e	f	g
A. occidentalis	1198	.000	.613	.387	.000	.000	.000	.000	.000	.488	.512	.000	.000	.000
	1217	.000	.738	.262	.000	.000	.000	.000	.213	.000	.788	.000	.000	.000
	1223	.000	.656	.344	.000	.000	.000	.000	.000	.479	.521	.000	.000	.000
	1225	.033	.600	.217	.000	.150	.000	.000	.000	.244	.756	.000	.000	.000
	1227	.000	.375	.613	.013	.000	.000	.000	.000	.500	.463	.000	.038	.000
A. ascendens	1194	.000	.853	.147	.000	.000	.000	.000	.029	.309	.618	.000	.044	.000
	1197	.000	.829	.171	.000	.000	.000	.000	.227	.227	.545	.000	.000	.000
	1202	.093	.852	.056	.000	.000	.000	.000	.221	.174	.558	.012	.012	.023
	1207	.237	.737	.026	.000	.000	.000	.029	.176	.088	.706	.000	.000	.000
	1218	.000	.850	.067	.000	.083	.000	.017	.083	.400	.517	.000	.000	.000
	1219	.000	.661	.339	.000	.000	.000	.000	.016	.419	.484	.000	.048	.032
	1222	.039	.684	.276	.000	.000	.000	.000	.014	.459	.419	.000	.068	.041
A. falcatus	1230	.000	.071	.929	.000	.000	.000	.000	.014	.319	.667	.000	.000	.000
	1231	.000	.100	.887	.000	.000	.013	.000	.025	.613	.363	.000	.000	.000
	1232	.050	.050	.833	.000	.067	.000	.000	.039	.895	.066	.000	.000	.000
	1234	.000	.000	.975	.000	.025	.000	.000	.013	.825	.162	.000	.000	.000
	1235	.000	.113	.887	.000	.000	.000	.000	.000	.675	.325	.000	.000	.000

APPENDIX 2 (cont'd).

Population		Skdh-1			Tpi-1				Tpi-2			
		a	b	c	a	b	c	d	a	b	c	d
A. occidentalis	1198	1.00	.000	.000	.000	.718	.282	.000	.000	1.00	.000	.000
	1217	.925	.075	.000	.012	.321	.667	.000	.000	.869	.131	.000
	1223	1.00	.000	.000	.016	.938	.047	.000	.000	.969	.031	.000
	1225	1.00	.000	.000	.000	.925	.075	.000	.000	1.00	.000	.000
	1227	1.00	.000	.000	.000	.725	.262	.013	.000	1.00	.000	.000
A. ascendens	1194	1.00	.000	.000	.044	.647	.309	.000	.015	.985	.000	.000
	1197	.113	.806	.081	.000	.463	.538	.000	.000	.975	.250	.000
	1202	.981	.019	.000	.000	.444	.556	.000	.000	.944	.056	.000
	1207	.684	.316	.000	.000	.711	.289	.000	.000	1.00	.000	.000
	1218	.345	.552	.103	.000	.514	.486	.000	.000	.986	.014	.000
	1219	.717	.283	.000	.000	.581	.419	.000	.048	.919	.016	.000
	1222	.838	.162	.000	.014	.716	.270	.000	.000	.973	.014	.014
A. falcatus	1230	.054	.946	.000	.000	.000	1.00	.000	.000	1.00	.000	.000
	1231	.000	1.00	.000	.000	.000	1.00	.000	.000	1.00	.000	.000
	1232	.000	.986	.014	.000	.000	1.00	.000	.000	1.00	.000	.000
	1234	.000	1.00	.000	.000	.000	1.00	.000	.038	.962	.000	.000
	1235	.000	1.00	.000	.000	.000	1.00	.000	.000	1.00	.000	.000

APPENDIX 3. Genetic Identities (Nei, 1972) Between Populations of *Aster occidentalis*, *Aster ascendens* and *Aster falcatus*

Species	*Aster occidentalis*					*Aster ascendens*							*Aster falcatus*				
Pop.	1198	1217	1223	1225	1227	1194	1197	1202	1207	1218	1219	1222	1230	1231	1232	1234	1235
1198	**																
1217	.867	**															
1223	.877	.807	**														
1225	.866	.818	.987	**													
1227	.846	.795	.945	.925	**												
1194	.922	.954	.876	.880	.819	**											
1197	.765	.830	.750	.762	.698	.848	**										
1202	.846	.921	.832	.845	.795	.941	.909	**									
1207	.763	.752	.872	.857	.845	.706	.703	.757	**								
1218	.812	.822	.748	.762	.710	.877	.952	.910	.684	**							
1219	.822	.816	.913	.918	.879	.870	.903	.927	.795	.892	**						
1222	.912	.926	.880	.874	.827	.983	.882	.935	.756	.891	.892	**					
1230	.567	.647	.529	.555	.574	.636	.794	.682	.465	.817	.745	.668	**				
1231	.539	.607	.482	.489	.534	.612	.764	.643	.409	.798	.695	.657	.968	**			
1232	.553	.595	.488	.487	.536	.620	.733	.616	.405	.777	.674	.675	.942	.959	**		
1234	.525	.562	.474	.470	.537	.574	.709	.587	.395	.747	.661	.629	.938	.988	.963	**	
1235	.546	.604	.483	.497	.529	.616	.735	.617	.416	.774	.668	.662	.955	.948	.982	.947	**

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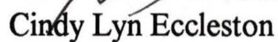
Author 

Cindy Lyn Eccleston

April 24, 1996