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CHEMICAL, CYTOLOGICAL AND GENETIC CONSIDERATIONS OF THE POSSIBLE HYBRID ORIGIN OF ASTER BLAKEI (PORTER) HOUSE

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CHEMICAL, CYTOLOGICAL AND GENETIC CONSIDERATIONS OF THE POSSIBLE HYBRID ORIGIN OF ASTER BLAKEI (PORTER) HOUSE

by

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ABSTRACT

CHEMICAL, CYTOLOGICAL AND GENETIC CONSIDERATIONS
OF THE POSSIBLE HYBRID ORIGIN OF
ASTER BLAKEI (PORTER) HOUSE

by

L. Michael Hill

It was the purpose of this investigation to consider the possible hybrid origin of *Aster Blakei* from *A. acuminatus* Michx. and *A. nemoralis* Ait. by utilizing cytological, genetic and chemical techniques.

The cytological techniques involved a study of the mitotic and meiotic chromosomes of all three taxa by the smear method. The study revealed the chromosome number of *A. acuminatus*, *A. nemoralis* and *A. Blakei* to be 2N = 18. *A. Blakei* formed bivalents in Prophase I. The F_1 hybrids of the cross *A. nemoralis* x *A. acuminatus* demonstrated regular meiosis most of the time. Viability of pollen was revealed by staining in aniline blue in lactophenol. *A. Blakei* collected from one natural source revealed 79% stainable pollen while *A. Blakei* collected from a second natural source scored 97% stainable pollen. The F_1 hybrids had 89-90% stainable pollen. Overall, the cytological evidence indicated that *A. Blakei* was fertile, but not as fertile as *A. nemoralis* or *A. acuminatus*.

Chemical studies were conducted using thin-layer chromatography of unidentified phenolic compounds. These compounds were used as markers in detecting hybridization in

nature. The results demonstrated that *A. Blakei* was intermediate between *A. acuminatus* and *A. nemoralis*.

The genetic techniques involved crosses between the putative parents, *A. nemoralis* and *A. acuminatus*. The resulting hybrids were then backcrossed to their parents. Crosses were also made within the F_1 population. Other crosses were made between *A. Blakei* collected from a natural source and the parental taxa. The study confirmed that the hybrid of *A. acuminatus* and *A. nemoralis* is attainable and is indistinguishable from *A. Blakei*. Crosses between the parental taxa and *A. Blakei* resulted in some progeny which were similar to *A. Blakei*. This suggested that the range of variation of *A. Blakei* might contain both backcross and hybrid types. *A. Blakei*, *A. acuminatus* and *A. nemoralis* are all interfertile. The F_1 hybrids of *A. acuminatus* and *A. nemoralis* are also interfertile.

The evidence suggests that *A. Blakei* is a natural hybrid of *A. acuminatus* and *A. nemoralis*. Introgression was demonstrated under greenhouse conditions and in one natural population.

CHAPTER I

INTRODUCTION

The genus *Aster* is large and polymorphic. It is widely recognized to be a genus in which some species boundaries are blurred and variable (Anderson, 1929: Shinners, 1941: Rosendahl and Cronquist, 1949). The polymorphic nature of many species is believed to be caused by aneuploidy, polyploidy and hybridization (Solbrig, 1967). Other species exhibit considerable phenotypic plasticity (Van Faasen, 1971).

Fernald (1950) submits that most of the variability in the genus is caused by hybridization. It is most prevalent in the Section EUASTER Gray, and this is where experimental studies have been focused (Wetmore and Delisle, 1939; Avers, 1953a; Uttal, 1962). However, the work of Avers on the Heterophylli series demonstrated that species amalgamation was not extensive. She cited many barriers which would prohibit extensive hybridization (Avers, 1953b) and concluded that most of the variation was caused by introgressive hybridization in local populations.

Recent studies have revealed that intergeneric and interspecific hybridization might be occurring in the Section ORTHOMERIS T. and G. Hybrids between an unknown species of *Solidago* and *Aster ptarmicoides* (Nees) T. and G. have been reported by Yeo (1971). Pike (1970) accumulated morphological and geographical evidence which indicated that *Aster Blakei* (Porter) House was an intermediate of *A. acuminatus* Michx.

and *A. nemoralis* Ait. This evidence also suggested that introgression might be occurring in the direction of *A. nemoralis*. He determined that these taxa cannot be identified unless hybridity is considered.

The present investigation considers the possible hybrid origin of *A. Blakei* utilizing chemical, genetic and cytological techniques. The chemical evidence is based on thin-layer chromatography of unidentified phenolic compounds. This technique has been useful in hybridization studies in other genera (Alston and Turner, 1962; Carter and Brehm, 1969; Walker, 1969; Belzer and Owenby, 1971). Abrahamson and Solbrig (1970) have suggested that phenolics might not be useful in considering the taxonomy of the Heterophylli series of *Aster*. The genetic evidence is based on a chemical and morphological analysis of an F_1 population produced by crossing *A. nemoralis* with *A. acuminatus*. The cytological evidence is based on observations of mitotic and meiotic chromosomes of all three taxa and the F_1 population.

CHAPTER II

REVIEW OF THE LITERATURE

The Evolutionary Significance of Hybridization in Plants

Hybridization is defined by Stebbins (1969) as the crossing between individuals belonging to populations which have different adaptive norms. This definition is not limited to hybridization between species, but also includes hybridization between sub-species, varieties and ecotypes. In essence, hybridization is the reversal of evolutionary divergence (Grant, 1971.

Principles of Hybridization. The F_1 offspring of a cross are usually the morphological, chemical and ecological intermediates of their parents (Solbrig, 1970). The F_2 progeny will show a wide range of morphological and physiological variability. Clausen and Hiesey (1958) demonstrated that the F_2 of a cross between foothill and subalpine ecotypes of *Potentilla glandulosa* showed extremely variable ranges of tolerance when transplanted into three environments differing in altitude. No two plants behaved or looked alike. This variation is due to recombination. The array of variation in an F_2 population is inhibited by the degree of linkage that exists in the genetic material of that population (Anderson, 1949). Other methods of regulating recombination are discussed by Grant (1958).

The offspring of an interspecific cross are usually semi- to completely sterile because of irregularities at meiosis. Exceptions exist in that some genera have fertile, cytologically normal hybrids between recognized species (Sax, 1935; Stebbins, 1945). These exceptions make the application of a strict genetic criteria of species differentiation impossible unless there are drastic revisions of present classifications.

Hybridization and Introgression. A partially fertile hybrid will cross with its parents, other hybrids, or undergo selfing. The resultant parental, hybrid, backcross and recombinant progeny is called a hybrid swarm (Grant, 1971). These are new genotypes which can be acted on by natural selection (Stebbins, 1959). Repeated backcrossing can eventually result in the phenomenon known as introgressive hybridization. Anderson (1949) first used this term and defined it as gene flow between species as a consequence of successful hybridization. Introgression can be an aid in speciation. This is shown by examples in *Zea* (Stebbins, 1971) and *Helianthus* (Solbrig, 1970). The methods of detecting introgression at the morphological level are discussed by Anderson (1949), and the application of these techniques have recently been demonstrated by Shah, *et al* (1970) in *Saccharum*. Examples of introgression in animals are discussed by Mayr (1963).

Hybrids and Habitats. Hybrids may encounter difficulty in competing with their parents in the parental habitat. Hybrids will adjust to intermediate or "hybrid" habitats usually made available by the disturbance of man (Anderson, 1948). Thus, the formation of hybrid, backcross and recombinant progeny within hybrid or recombinational habitats will insure the temporary survival of the new genotypes. Recent examples of the invasion of disturbed habitats by hybrids and introgressants are found in Flavaria (Long and Rhamstine, 1968), Solanum (Ugent, 1970) and Senecio (Chapman and Jones, 1971).

Hybridization and Speciation. Other than the phenomenon of introgression, hybridization can lead to speciation in additional ways. One of the best known methods is allopolyploidy. An allopolyploid is the result of chromosome doubling in a species hybrid (Stebbins, 1947). The new alloploid is fertile due to the presence of homologous chromosomes. Because of the ploidy level difference, reproductive isolation results. Examples are discussed by Grant (1971) and Solbrig (1970).

Speciation by transgressive segregation may occur. Hybrid progeny produce characteristics which exceed the limits of variation found in either of the parental types. Segregation of the genetic factors responsible for reproductive isolation is certain to occur in any progeny derived from a fertile interspecific hybrid (Stebbins, 1959). Transgressive segregation has been considered to be important in the evolution of the genus Canna (Khoshoo and Mukherjee, 1970).

Some hybrids compensate for sterility by reproducing vegetatively or apomictically. Such complexes are morphologically uniform, occupy a definite geographical area, and are morphologically differentiated from related species. They are termed microspecies by Grant (1971). Examples are discussed by Grant and Grant (1971) in Opuntia and by Stebbins (1959) in Elymus.

A final example of speciation by hybridization is the phenomenon known as recombinational speciation. This can result from the hybridization of species that are isolated by a chromosomal sterility barrier composed of two or more separable segmental rearrangements. The hybrid can give rise to one or more new homozygous recombination types for the segmental rearrangements. These new types will be fertile themselves, but reproductively isolated from the parents. Examples are discussed by Grant (1971).

Other than speciation, there are other consequences of hybridization. Hybridization can cause a perfection of isolating mechanisms (Mayr, 1970), or it can cause an increase in genetic variability (Stebbins, 1969). Knobloch (1972) advocates that biologists should think more positively about hybridization as a major force in producing variation. He cites 20,682 examples of interspecific and 2,993 examples of intergeneric hybrids in the plant kingdom. 2,242 of these latter hybrids occur in the Compositae.

It would seem then, that hybridization is a positive force in evolution as long as variation is generated by its occurrence. It must occur at times when new habitats are made available allowing the maintenance of hybrid, backcross and recombinant types. Natural selection will determine the success of the new genotypes.

Chromosome Evolution in Aster

Chromosome numbers in Aster. The genus *Aster* contains 200-250 species (Van Faasen, 1971). Chromosome numbers have been reported for 123 species, sub-species and varieties. Numbers for North and South American (New World) asters have been reported by Wetmore and Delisle (1939); Clausen, *et al* (1940); Avers (1953a, 1957); Huziwara (1941, 1958, 1962a), Darlington and Wylie (1956); Raven, *et al* (1960); Van Faasen (1963), Solbrig, *et al* (1964, 1969); Nelson (1966); Jones (1968), Hill and Rogers (1970), Kovanda (1972) and Strother (1972). The numbers are summarized in Fig. 1.

Numbers for Japanese, Chinese, Siberian and European (Old World) asters have been reported by Tahara and Shimotomai (1926); Morinaga and Fukushima (1931); Shimotomai and Huziwara (1941); Huziwara (1953, 1957a, 1957b, 1962b, 1965); Darlington and Wylie (1956) and Hsu (1967). The numbers are summarized in Fig. 2.

The more frequent numbers in Old World asters are 2N = 18, 36 and 54. This suggests that the basic number of this genus is X = 9 in Old World asters. Polyploidization then resulted in the numbers 2N = 36, 54 and 72. The more frequent numbers for New World asters are 2N = 18 and 36, with lower frequencies of 2N = 10, 16, 26, 32, 40, 46, 48, 50, 54, 64 and 72. The New World asters thus have much variation in chromosome number. Many workers (Huziwara, 1958; Raven, *et al*, 1960; Solbrig, *et al*, 1964, 1969) interpret this variation to be due to a reduction of the basic number of X = 9 to secondary base

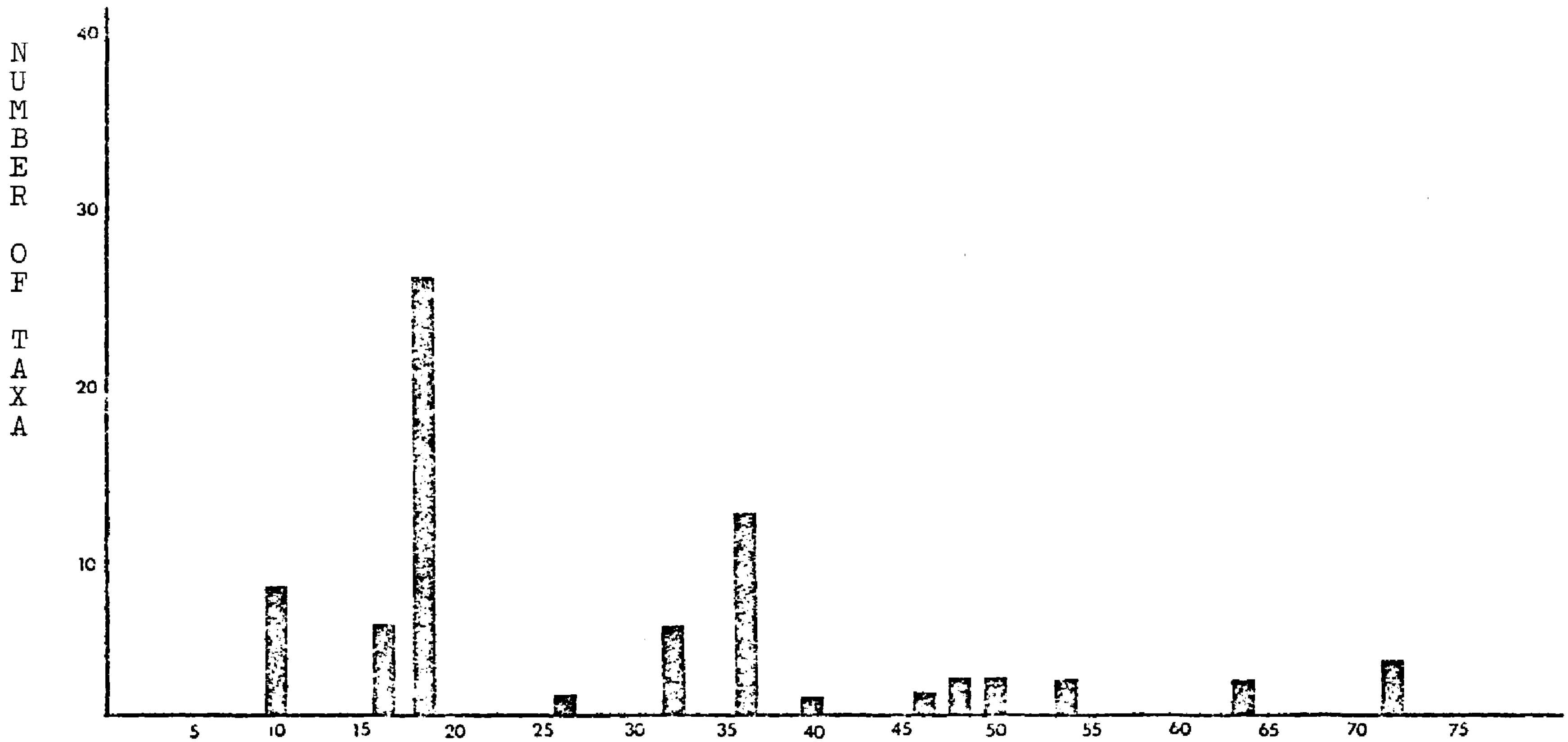

Fig. 1. Frequency of somatic chromosome numbers in New World asters.

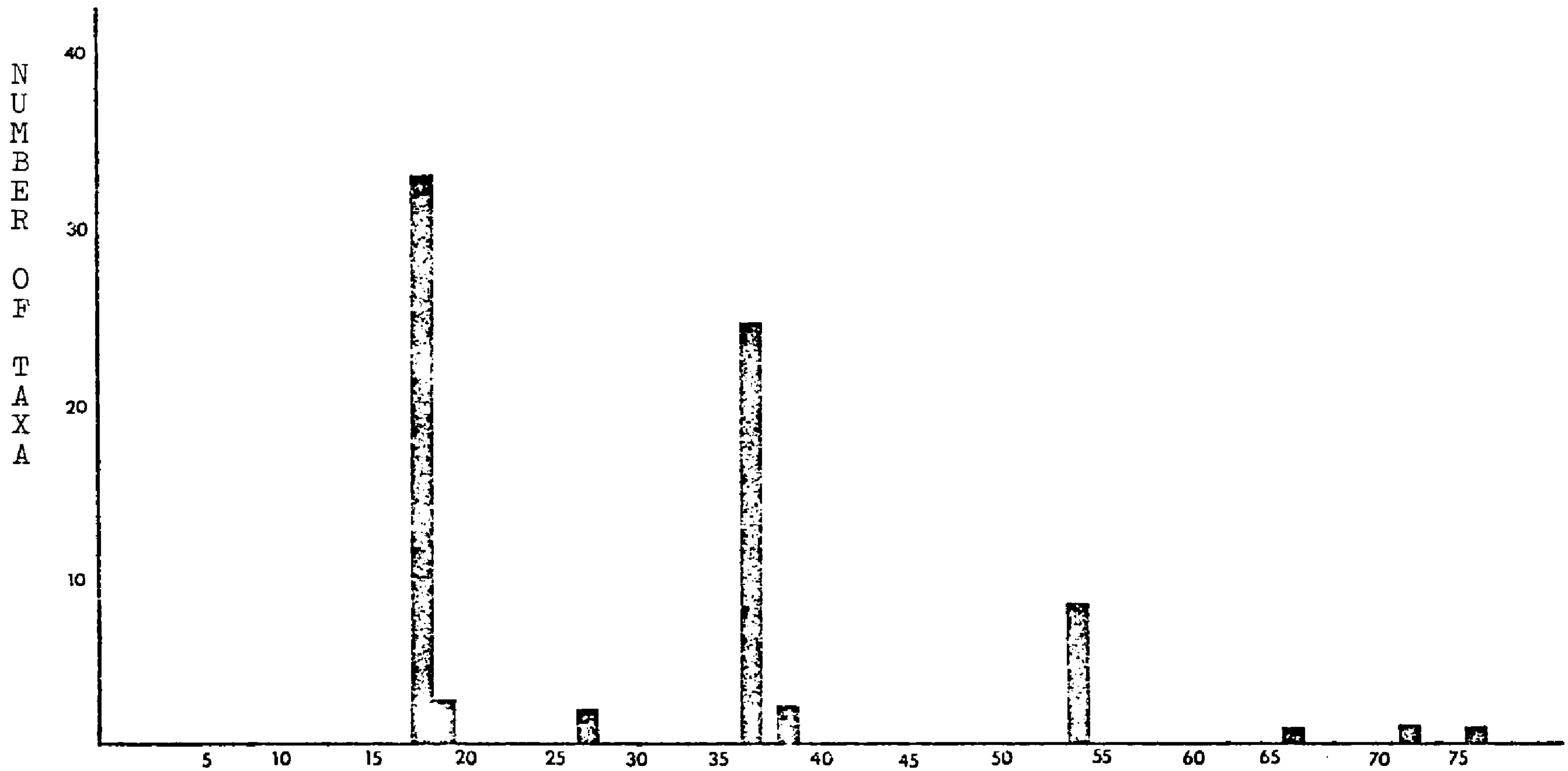

Fig. 2. Frequency of somatic chromosome numbers in Old World asters

numbers of X = 4 and 5. Subsequent polyploidization can thus give rise to 2N = 18, 36 and 72 from X = 9, 2N = 10, 20 and 40 from X = 5, and 2N = 16, 32 and 64 from X = 4. This reduction in base number is attributed to aneuploidy (Huziwara, 1959) or to massive chromosomal rearrangements caused by catastrophic evolution (Solbrig, 1967).

These data thus suggest the primitive base number of Old and New World taxa to be X = 9, with secondary base numbers of X = 4 and 5 in the New World group only. Some workers disagree with this interpretation (Turner, et al, 1961a, 1961b, Turner, 1970). This group affirms that the basic numbers for the genus were X = 4 and 5 which gave rise to the numbers X = 8, 9 and 10 by allopolyploidy. Subsequent polyploidizations resulted in the present numbers. To support their theory, Turner and his co-workers note that if reduction from X = 9 occurred, then species with X = 6 and 7 should be present. Asters with X = 6 or 7 are not present today. Raven, et al (1960) suggest that species with X = 6 or 7 were selected against. Turner, et al (1961a) replied by noting species with X = 5, 8 and 9 were very closely related morphologically. Thus those ancestral taxa with X = 6 and 7 were also related morphologically. They wondered why species with X = 6 or 7 would be selected against while those with X = 5 and 8 would not.

On the other hand, Solbrig, et al (1964) cite the preponderance of species with X = 9 among shrubbery and perennial plants covering the many habitats available to these species. They further note that species with chromosome numbers of X = 4 or 5 did not have morphological characters which correlated with the primitive characters of the Compositae proposed by Cronquist (1955).

Since chromosome numbers for only half of the genus have been reported, more data are needed to resolve the problem. As it stands now, the primitive basic number of the genus is open to question. It is either X = 9, or X = 4 and 5.

Polyploidy in _Aster_ obviously occurs. Most of the determinations have been made by Huziwara. He summarizes (Huziwara, 1967) that most of the polyploids are considered to be allopolyploids consisting of two genomic complements of chromosomes rather than of four or six. He does not cite evidence. Studies by Avers (1953a) and Clausen, _et al_ (1940) on some polyploid New World asters have revealed that meiosis is regular and characterized by the formation of typical bivalent configurations. This formation suggests that these are allopolyploids. However, the true nature of most of the polyploids in this genus awaits further experimental verification.

Aneuploidy has been documented in only one species by Huziwara (1957b) and Matsuda (1966, 1967a, 1967b). The species is _A. ageratoides_ Turcz., which is morphologically variable. These workers attribute this variation to aneuploidy.

Chromosome Morphology. Karyotypic studies have been carried out exclusively by Huziwara on some Old World (Huziwara, 1957a, 1957b, 1962a, 1962b, 1965) and New World asters (Huziwara, 1958, 1965). The data reported in these papers summarized in

Table 1, include average chromosome lengths as well as the total form percent (TF%)

Table 1. Average chromosome lengths and total form percent (TF%) of Old and New World asters.

Origin	Median Lengths (Microns)	TF%
Old World	5.5	42.0
New World	3.1	33.0

Total form percent has been defined by Huziwara (1959) as the ratio in percent of the total sum of the short arm lengths to the total sum of chromosome lengths. The TF% is a measure of symmetry of the chromosome complements of any species of interest.

The summary in Table 1 shows that the Old World species have complements which are more symmetrical and larger than New World species. Thus, New World species have evolved further since asymmetry goes hand in hand with evolutionary advancement (Stebbins, 1950; Swanson, 1957). Therefore, New World asters are somewhat removed from Old World asters phylogenetically. A reason for this has been suggested by Stebbins in a letter to Huziwara (1958). He suggested that more diverse habitats are present in the New World which would favor progressive evolution. Such habitat diversity might also be one of the reasons for the variation in chromosome numbers prevalent in the New World group.

In conclusion, the genus *Aster* possesses chromosome numbers which center around X = 9 or multiples thereof. This is not the case in New World asters, which appear to possess numbers based on X = 4 and 5, as well as X = 9. The determination of the primitive base number of the genus has not been resolved. Arguments for and against suggested primitive base numbers are reviewed, and it appears that more data are needed to resolve the question. Polyploidy occurs in Old and New World asters, most of which is believed to be allopolyploidy. Experimental evidence is needed to verify this suggestion. Aneuploidy has been documented in only one taxon of Old World origin. Karyotypic studies have revealed that New World asters have evolved farther than Old World asters. The genus is as complex at the cytological level as it is at the morphological level.

Phenolics and Hybridization

Phenolics and Their Use in Biochemical Systematics. Phenolics are secondary compounds which are of small molecular weight. They do not serve as an energy source but are a part of the biology of the organism as it adapts to the environment (Alston and Turner, 1966). Levin (1971) discusses their functions which involves two main areas. They can serve as insect attractants by determining flower color, or in protecting plants from diseases. This latter function has recently been questioned by Challice and Westwood (1972). Phenolics may also be involved in growth regulation (Galston, 1969).

Alston (1967) classifies phenolics as either simple or compound. Simple phenolics are closely linked to the amino acid phenylalanine through the shikkimic acid pathway (Alston and Turner, 1966). The enzyme which mediates this linkage is phenylalanine deaminase. However, this role has been questioned by Swain and Williams (1970). Compound phenolics, commonly known as the flavonoids, are formed from simple phenolics by acetoacetyl condensation (Harborne, 1965). In ultraviolet light, simple phenolics fluoresce whitish blue to blue, and the flavonoids fluoresce yellow to dark yellow (Alston, 1967).

General techniques for the preliminary separation of phenolics are discussed in reviews edited by Gėissman (1962) and Harborne (1964). Data and techniques on the reaction of phenolics with various spray reagents are available (Smith, 1960; Block, et al, 1958). Nuclear magnetic resonance (Mabry, 1969) and ultraviolet spectral data (Jurd, 1962) are also available. Further data of this type have recently been summarized by Mabry, et al, (1970).

Phenolics exhibit structural variability, wide distribution throughout the plant kingdom, stability in ordinary handling and extraction and ease of identification (Harborne, 1967). The stability of phenolics can be maintained in oven-dried material (Lorenz and Schulz-Schaeffer, 1964) and in herbarium specimens (Widen and Britten, 1971). If dried material is used, Bate-Smith and Harborne (1971) suggest that herbarium specimens be checked with fresh tissue whenever possible.

There is some controversy among some authors on the reliability of phenolics in taxonomic studies. Some workers feel that phenolics are overly subject to environmental influence and that data should be interpreted with caution (Ball, et al, 1967). Variation can exist between developmental stages (Schwarze, 1959; Asker and Frost, 1970) and between months of the year (Yap and Reichardt, 1964; Taylor, 1971) as well as between populations within a species (Brunesberg, 1965; Crawford, 1970). Other papers (Buzzati-Traverso, 1953; Kirk, et al, 1954) indicate phenolics are not subject to environmental influences such as diet, age of tissue, etc. Controlled experiments by McClure and Alston (1964) on the duckweed Sphirodela oligorrhiza showed no variation of phenolics due to environment. A different set of controlled experiments with the same species by Ball, et al (1967) were in agreement. But similar experiments on a closely related species of duckweed did reveal differences. In a recent study by Parks, et al (1972), the experiments of Ball and his co-workers were repeated, with different results. Parks and his collaborators worked with fourteen inbred stocks of cotton from four species. These were grown in five different localities ranging from the Mojave Desert to Raleigh, North Carolina. Under these five environments, the petal phenols showed no variation. However, the leaves were variable in their phenolic profile.

This kind of variation is dependent upon many factors. However, Alston (1967) points out that it is impossible to carry out a significant chemical study without considering the nature and extent of variation. Neither does the fact that such variation exists diminish the importance of the chemical data. He further asserts that the major challenge in biochemical systematics is to learn to take advantage of variation rather than being overly concerned about the existence of such variation.

With respect to hybridization studies, Alston (1965) comments that the presence of a chemical marker indicates the presence of a specific marker gene in the population. In local populations, hybridization studies can be performed using phenolics as markers since one is not concerned with variation *per se* (Ball, *et al*, 1967). Levin (1971) points out that the extent to which phenolics may contribute to the solution of evolutionary problems depends upon their biological value to the plant. The greater their value, the more reliable will be their presence in a population. The arguments against the use of phenolics in taxonomic work mainly refer to their use in showing relationships and affinities. They do not criticize the use of phenolics as markers in hybridization studies (Ball, *et al*, 1967; Runemarck, 1968).

Phenolics in the Detection of Hybridization. The first paper which dealt with the chemical detection of hybridization in nature was published by Turner and Alston (1959). Their work with natural hybrids of *Baptisia laevicaulis* x *B. viridis* showed the hybrids to contain a summation of the species-specific compounds present in both parents. Some hybrids contained a new compound which they thought might represent a "hybrid" compound.

These same workers applied chromatography in the analysis of a hybrid swarm of a trispecies population of *B. laevicaulis*, *B. leucanta* and *B. viridis* (Alston and Turner, 1962). Their morphological analysis suggested that *B. viridis* was acting as a bridge between the other two species. The chemical evidence countered that no mixing of *B. laevicaulis* and *B. leucanta* genomes was occurring. Thus the data suggested that the majority of all hybrids were F_1 hybrids of *B. laevicaulis* x *B. viridis* and of *B. leucanta* x *B. viridis*. The authors felt that the chemical evidence was too strong to discount. Again, the hybrid chemical profile was a summation of the parental chemical profiles. This ultimately has become a principle of such studies: The phenolic compounds present in natural hybrids represent the summation of the species-specific phenolic compounds found in the parental taxa.

This principle has been employed time and time again with positive results. Smith and Levin (1963) used phenolic markers to confirm reticulate evolution in *Asplenium* by establishing the simultaneous presence of three genomes in a single species. Levin (1966) was able to demonstrate hybridization in *Phlox* using chemical data that were more clear cut than morphological analysis alone. Positive confirmation of natural hybridization has been obtained in such genera as *Saxifraga* (Jaworska and Nybom, 1967), *Dicentra* (Fahselt and Owenby, 1968) *Tragopogon* (Belzer and Owenby, 1971) and *Prunus* (Olden and Nybom, 1968), to name a few. Even intergeneric hybridization between *Lychnis* and *Silene* has been confirmed by phenolic studies (Crang and Dean, 1971).

Of course, not all results have been positive. Clausen (1963) could distinguish between two birch species chemically, but could not chemically identify known morphological hybrids. Garber and Stromonaes (1964) found similar results in Collinsia. Parents were distinguishable on the basis of chemistry, but chromatography of hybrids did not indicate parentage. These negative results are infrequent.

Phenolics have been valuable in confirming allopolyploid origins. Stebbins, et al (1963) demonstrated that the tetraploid species Viola quercetorum possessed phenolics that were a summation of those present in V. purpurea subsp. purpurea and V. aurea subsp. mohavensis. This evidence combined with morphological, geographical, and ecological data led to the conclusion that the former taxon was of alloploid origin. Similar results were found in Avena (Rajhathy, et al, 1971), but were not as clear cut as in the former case.

Chemical data are more meaningful when the structure and identity of phenolics are known. For instance, artifacts of hydrolysis during the preparation of extracts can be counted. This could multiply the number of presumptive differences on a false basis (Alston, 1965). If identity is known, such a problem would not occur. Fahselt and Owenby (1968) demonstrated that either hydrolyzed or unhydrolyzed phenols will show positive results in detecting hybridization in Dicentra. Similar results have been positive in detecting hybridization in Coprosma (Taylor, 1964). Alston (1965) states that it also helps to know enzyme specificity and genetic regulation of phenolics which would allow the postulation of the evolution of these chemicals in a taxon. Bohm and Glennie (1971) recently correlated the structure of phenolics with phylogeny. A primitive chemical, or simple phenolic, was usually found in a morphologically primitive genus. Advanced chemicals, such as flavonoids, were found in more advanced genera.

The formation of a hybrid compound actually represents a compound termed as novel, or nonparental (Alston, 1967). Examples are documented by Alston, et al (1965). Such novel compounds were suspected by Turner and Alston (1959) in Baptisia hybrids. Levy and Levin (1971) determined in Phlox allotetraploids that novel compounds were less complex than their parental derivatives. Novel compounds were thus suggested to be precursors of parental compounds. They proposed that hybridity and subsequent polyploidy may have repressed or suppressed the activity of certain ancestral genes responsible for the production of glycosidating enzymes. Such enzymes function in the addition of sugar molecules to the phenolic backbone (Harborne, 1965). They implied that phenolic glycosidation occurs in a stepwise fashion. Most steps were under single gene control. Such a proposal awaits verification in other genera.

Phenolics in Population Analysis. As mentioned earlier in this review, natural hybridization can result in the formation of hybrid swarms which consist of parental, hybrid, backcross and recombinant progeny (Grant, 1971). Some plant biosystematists are interested in these swarms. Within these populations, genotypes may arise which are adaptively superior if a new habitat emerges. Knowledge of population structure in a hybrid swarm may allow for predictions concerning the evolutionary future of the population.

Analysis of hybrid swarms have already been mentioned in this review with reference to the work of Alston and Turner (1962). Four-way hybridization within Baptisia has been analyzed by these workers (Alston and Turner, 1963). The six possible hybrids from four species were all chromatographically distinguishable. All hybrids represented a summation of parental chemical profiles. Introgressants (backcrosses) were identified as representing a summation of a few hybrid and mostly parental compounds. In this manner, these workers were able to demonstrate the extent of introgression. They noted that chemical introgressants occurred peripherally to areas of hybridization.

In a subsequent analysis of two Baptisia species (McHale and Alston, 1964), it was noted that backcross types were very difficult to distinguish from selfed hybrids. It was proposed that a plant having essentially a complement of the chemical markers of species A and only a few chemical markers of species B might reasonably be judged as backcross types to A. Coupled with the morphological data, the chemical data were used to group a given population into parental, hybrid and backcross specimens. Neither morphological or chromatographic evidence was regarded as infallible in the interpretation of the genetic origin of a particular specimen. It was concluded that the plants did not fall into discrete categories. The overall structure of the population was portrayed as very complex. Coupled with morphology, chromatography more clearly defined population structure. Principles necessary to define how specific chromatography could be awaited the results of chromatographic studies of synthetic hybrids. No results have since been reported.

A recent study by Crawford (1972) addresses itself to a chemical and morphological study of parents, F_1 hybrids, first generation backcrosses and F_2 hybrids synthesized in the greenhouse. The original cross was between two varieties of Coreopsis mutica DC. F_1 hybrids were variable in leaf morphology but uniform chemically. This supports the hypothesis that flavonoids are more useful than morphology in determining whether particular plants are F_1 hybrids. One new spot occurred in the F_1 that was suspected to be a "hybrid" compound. But a comparison of natural and synthetic hybrids did not help in specifying which of the natural hybrids were F_1's. Morphological analysis of backcross progeny showed many to be still indistinguishable from the recurrent parent. However, all contained at least one compound from the non-recurrent parent. Comparisons of natural and synthetic backcrosses showed it was easier to define the origin of natural backcrosses on the basis of flavonoid chemistry. F_2's were variable both chemically and morphologically.

Problems of identification similar to those of McHale and Alston (1964) have been encountered by Levin in studying natural populations of Liatris (Levin, 1967a) and Phlox (1967b), and by Baetecke and Alston (1968) in Baptisia. Such problems apparently have not been encountered by Carter and Brehm (1969) in *Iris* or by Hanover and Wilkinson (1970) in *Picea*. All of these workers have noted the presence of phenolic compounds characteristic of the non-recurrent parent in areas peripheral to hybridization between hybrids and the recurrent parent. These individuals were defined as introgressant progeny of unknown generations.

In conclusion, phenolics used as chemical markers in hybridization studies have been valuable in demonstrating the simultaneous presence of parental genomes in a hybrid. Inheritance of parental compounds is additive in hybrids. In some instances, phenolics have been useful in determining population structure. A comparison of the chemistry, morphology and genetics of natural and synthetic hybrid swarms would be the best approach. Chemical evidence should not be considered alone but should always be included with the known morphological, cytological, genetic and ecological evidence.

CHAPTER III

MATERIALS AND METHODS

Horticulture. Nineteen specimens of *A. nemoralis*, ten specimens of *A. Blakei* and twelve specimens of *A. acuminatus* were removed from populations in New Hampshire and Maine. These specimens were potted in Jiffy Mix, which is a commercial mixture of peat and vermiculite. The pots were kept in a warm propagation house which was maintained at an average of 24°C. day and night. When cold treatment was necessary, the plants were moved to a room maintained at an average of 10°C. day and night. The cold treatment lasted from the latter part of November to mid-January of each year. Sometimes temperatures in both houses would fluctuate above the averages on warm days. No artificial lighting was used. Voucher specimens of the plants referred to above are recorded in the Appendix from #293-378. They have been deposited in the Herbarium of the University of New Hampshire. The Appendix also contains the history of the progeny from the genetic study and includes records on asters collected from the natural populations referred to in the next section.

Seeds were stored at room temperature until they were subjected to a six to seven week cold treatment at 8°C. During this period they were stored in plastic Petri dishes containing moist filter paper. After this cold period, seeds were placed into small pots containing Jiffy Mix and covered with a thin layer of ground sphagnum. The pots were kept in the warm propagation house enclosed in plastic bags. The plastic bags were removed approximately ten days after seed germination.

Taxonomy and Collection. Specimens were collected from three main areas. The first collection of twenty-five specimens came from the southern shore of Lake Ossipee in New Hampshire. A second collection of sixteen plants came from the southern shore of Lake Winnisquam in New Hampshire. A final collection of fifty-four plants came from Great Wass Island in Washington County, Maine. This latter collection consisted of a total of fourteen specimens of *A. nemoralis* and *A. acuminatus* in discrete colonies and forty specimens of *A. nemoralis*, *A. Blakei* and *A. acuminatus* collected at Ponds Point on the eastern tip of the island facing the Gulf of Maine. *A. acuminatus* was collected in colonies from a wooded area of high elevation. *A. nemoralis* was collected in colonies from a small bog located in the center of the island. These asters are clonal. Their stoloniferous habit is a species characteristic (Fernald, 1950). The asters grew in well-defined clones at Lake Ossipee and Lake Winnisquam. One ramet was sampled from each clone at these locations. The clones were not well defined at Great Wass Island. Sampling at this location was carried out with an eye for morphological diversity throughout the area of the population.

These specimens as well as those cultivated in the greenhouse were scored using the hybrid index designed by Pike (1970). The technique is based on the original suggestion of Anderson (1936). Ten separate characters were found by Dr. Pike to differ between the two putative parental species

(Table 2). An example will illustrate how the index was used. An arbitrary score value of 0 was assigned to the extreme condition of flower color found in *A. nemoralis*. A score value of 2 was assigned to the contrasting condition of flower color in *A. acuminatus*. A score value of 1 was assigned to the intermediate condition of flower color in *A. Blakei*. Score values were similarly assigned for the other characters listed in Table 2. Some characters exhibited more than 3 ranges of variation. For instance, leaf number exhibited 7 ranges of variation, and score values were assigned from 0 in *A. nemoralis* to 6 in *A. acuminatus* with intermediate values assigned to *A. Blakei*. Each character was then examined and the appropriate score was assigned. The total of the scores determined for the ten characters was the index value for each plant. These were then plotted on a histogram to demonstrate the frequency of parental and hybrid specimens.

Cytology. For root-tip studies, 0.002M 8-oxyquinoline was used as a pre-treatment for 60-80 minutes at room temperature. The root tips were then stained and squashed in acetoorcein according to the method of Huziwara (1957a). Cover slips were smeared with Mayers albumin and dried over a flame to permit adhering of the preparation to the coverslip. Permanent mounts were made according to the technique of McClintock (1929) with the following modifications: the slide and coverslip were separated in 10% acetic acid using the method of Celeraier (1956); the coverslip was then passed through changes of 1:1, 1:3, and 1:9 acetic alcohol, two changes of 95% ethyl alcohol and the slide and coverslip were recombined in diaphane.

Table 2. Morphological characters employed in the hybrid index and their specifics in each taxon (From Pike, 1970).

Morphological Character	Morphological Characterization		
	A. nemoralis	A. Blakei	A. acuminatus
# of leaves	35-100	20-34	0-19
ratio of leaf measurements (length/width)	10-7	6.9-3.3	3.32-0
distance (mm.) between median internodes	1-8	9-23	24-30 or more
leaf margin	revolute	median	very flat
leaf margin	scabrous	median	hairy
leaf margin	entire	tip gland or small serrate	strongly serrate
# bracts per peduncle	4 or above	2-3	1-0
# heads per influorescence	solitary		multiple
ligule color	blue-violet	median	white
zebra hairs	none	sparse	abundant

For meiotic studies, flower buds were fixed in 1:3 acetic alcohol at 8°C. Flowers were dissected in a small vial containing 70% alcohol. Each flower suitable for analysis was placed on a slide in a drop of acetocarmine stain. The acetocarmine was prepared according to the suggestion of Sax (1931). Permanent slides were made according to the procedure described previously. Flowers were stored in 70% ethyl alcohol at 8°C.

Chromosomes were observed under oil at 1125x. Photographs were taken with a Kodak camera set on a Spencer (A.O.) microscope with the preparation under oil at 1455x.

Chemistry. Fresh basal leaves of each specimen to be studied were removed and shredded by hand into a jar containing 10 ml. of 1% 1N HCl in methyl alcohol. This extraction procedure was suggested by Turner and Mabry (1964). The sample jars were stored in the dark at room temperature for forty-eight hours. The extract was then poured off and concentrated to approximately one milliliter under warm air from a hand drier. The samples were stored in a small vial at 8°C.

Both paper and thin-layer chromatography was attempted. By comparison, it was found that paper chromatography gave poor resolution. Spots were hard to define and too close to each other. Paper chromatography required a very large amount of the extract in order to give results. Thin-layer chromatography required only a small amount of extract and resolution was excellent.

Samples were spotted on thin-layer sheets of cellulose with a layer thickness of 0.10 mm. 10 microliters of the sample were spotted on the left hand corner of a cellulose plate. Warm air applied to the area minimized the spreading of the spot.

Each plate was then placed into a developing tank containing the first solvent system: n-butyl alcohol, glacial acetic acid and water (6:1:2). Development took 6-8 hours. Each plate was then dried overnight and the following morning placed into the second solvent system: 10% acetic acid containing .1 gram of sodium acetate per 100 ml. of acid. Development in this system took 2-2½ hours.

After drying, the finished plate was viewed under visible and ultraviolet light. Colors of each spot under both conditions were recorded. These colors were again recorded after exposure to ammonia vapor in a fume hood. The plates were subsequently treated with spray reagents recommended for use by Block, et al (1958). The sprays were: 1% alcoholic ferric chloride; 1% aqueous basic lead acetate; 1% aqueous lead acetate; 1% aqueous sodium carbonate; 1% alcoholic aluminum chloride; Benedicts reagent and diazotized sulfanilic acid. The latter reagent was prepared and administered according to the specifications of Smith (1960). The purpose of the spray reagents was to distinguish between spots and to determine whether or not the spots were phenolics. It was discovered that 1% aqueous lead acetate gave good distinctive colors under long-wave ultraviolet light. Each spot was labeled with a number for identification on the basis of their specific colors, color reactions, and location on the plate. The location was determined by the Rf value, defined as the distance the spot moves from the point of origin divided by the distance traveled by the solvent front from the point of origin (Smith, 1960). The relative locations of these spots to each other were also used as a criterion of identification. No attempts were made to identify the chemical structure of these compounds.

Pollen stainability. Pollen grains from flowers of the asters cultivated in the greenhouse were stained in aniline blue in lactophenol. The stain was prepared according to the schedule of Sass (1959). The first 200 grains were scored for stainability under 10x magnification. The grains that stained dark blue were scored as being fertile. The grains which did not stain were scored as infertile.

Genetics. Interspecific crosses between specimens of *A. acuminatus* and *A. nemoralis* were conducted in the fall of 1970 and spring of 1971. Fruits from these crosses were then tested for fertility. The fruit of these asters is an achene, defined as a small, dry, one-celled, one-seeded indehiscent fruit (Harrington and Durrell, 1957). Plump achenes were determined to be fertile because a germination test was positive. Flat achenes were determined to be infertile since a germination test proved negative.

Backcrosses were conducted between the F_1 hybrids of the 1970 progeny and their parents in the spring of 1971. Crosses were also conducted between available specimens of *A. Blakei*, *A. acuminatus* and *A. nemoralis* brought into the greenhouse from various parts of New Hampshire and Maine.

Sib-matings between the F_1's of the 1970 progeny were also conducted. Crosses were performed in an insect free warm propagation house by rubbing the heads of two plants together. Crosses were made when most of the flowers in the heads were open and shedding pollen.

CHAPTER IV

RESULTS AND DISCUSSION

Chemistry and Morphology. The first chemical survey conducted on these asters was based on a collection of twenty-three specimens. This survey included twelve specimens of *A. nemoralis*, eight specimens of *A. acuminatus* and three speciment of *A. Blakei*. These plants were in three stages of development: eight specimens were in a state of vegetative growth before any buds had formed, six specimens had unopened flower buds and nine specimens were flowering. Although the chromatographs were not sprayed with any phenol-detecting reagents, the resultant spots were probably phenolics because their fluorescence under ultraviolet light was characteristic of phenolics according to the description of Alston (1967). The results of this survey are given in Table 3 with respect to the mean number of fluorescent spots. These means indicated that there were differences in the number of phenolic spots at different stages of development. An analysis of variance (Table 4) demonstrated that these differences were significant. It was decided that any comparison of these taxa at the chemical level should be made at a standard stage of development. All further comparisons were made when the plants were flowering because the complete hybrid index of Pike (1970) could then be used in relating morphology with chemistry.

Table 3. Mean number of fluorescent spots produced in three developmental stages of a greenhouse population of *Aster acuminatus*, *A. Blakei* and *A. nemoralis*.

Stage of Development	Mean Number of Fluorescent Spots
Before Buds	11.5
Unopened Buds	18.3
Opened Flowers	16.7

Table 4. Analysis of variance of the number of fluorescent spots over three developmental stages in a greenhouse population of *Aster acuminatus*, *A. nemoralis* and *A. Blakei*.

Source	DF	MS	F
Treatment	2	98.159	4.843*
Error	20	20.267	
Total	22		

*Significant at .05 level

The morphological analyses of specimens collected from Lake Winnisquam, Lake Ossipee and Great Wass Island are summarized in Figure 3. All of these specimens were flowering when collected. Plants which scored 0-4 were designated A. nemoralis, 8-19 were A. Blakei and 25-31 were A. acuminatus (Pike, 1970). The population at Lake Winnisquam was essentially a variable population of A. Blakei (Fig. 3a). The population at Lake Ossipee indexed as either A. nemoralis or A. Blakei (Fig. 3b). The population at Great Wass Island consisted of the parental taxa in discrete colonies (Fig. 3c), and all three taxa together in a local population at Ponds Point (Fig. 3d). Fig. 3e summarizes the data in Fig. 3c and 3d.

Chromatographs of these specimens yielded numerous spots which were identified as phenolic compounds by the following criteria: the extraction and separation solvents were specifically selected to remove and isolate phenolics on a chromatograph (Siekel, 1962); the use of diazotized sulfanilic acid as a test for the presence of phenolics gave positive results on some of these spots; the colors under ultraviolet light before and after exposure to ammonia vapor yielded fluorescence reactions in all spots similar to those suggested by Alston (1967) for phenolics; and the color reactions of these spots with various spray reagents as suggested by Block, et al (1958) yielded positive results in detecting the presence of phenolics on a chromatograph. A total of twenty-eight spots were chosen as diagnostic of these asters. These were selected because of their high frequency of

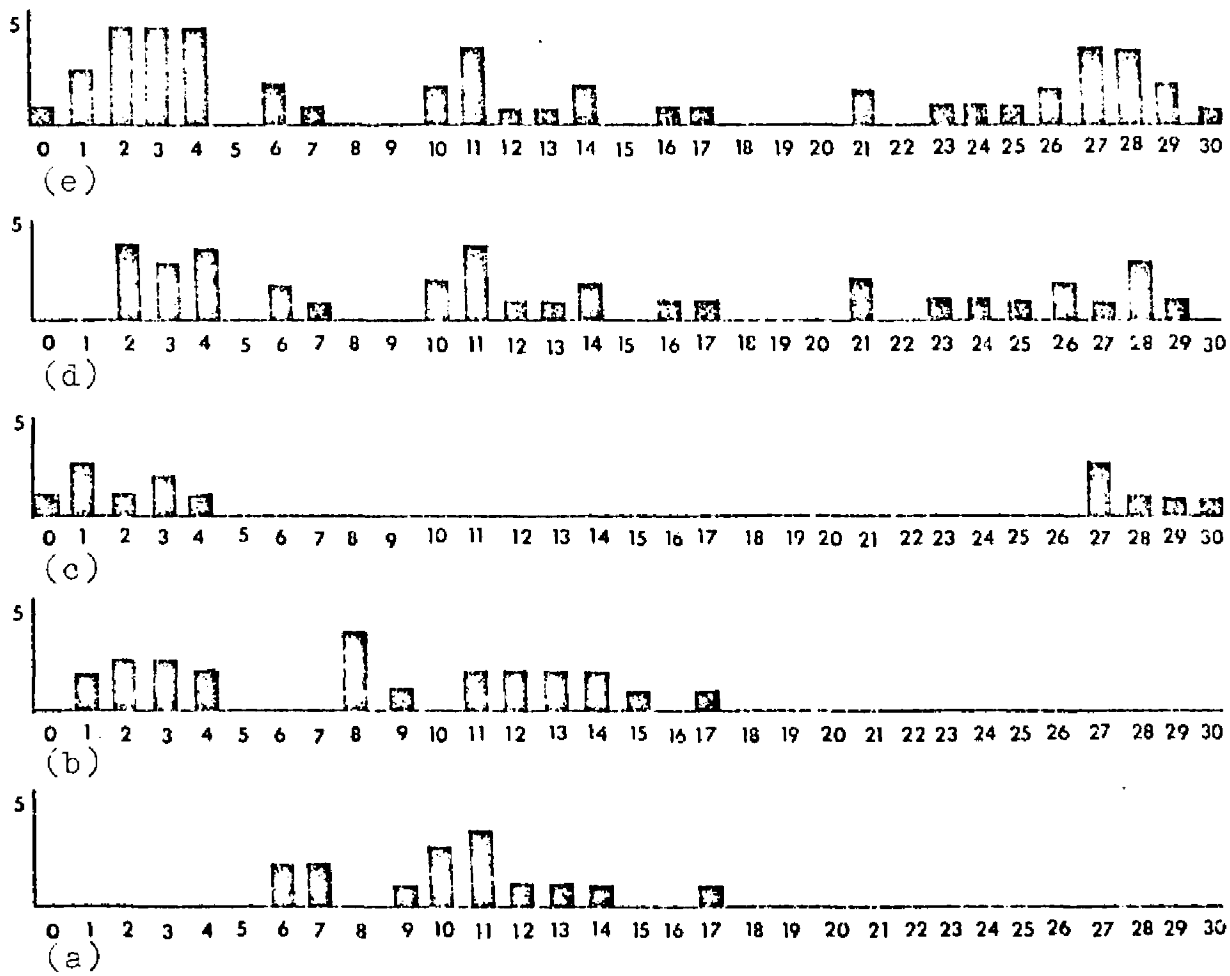

Fig. 3. Morphological hybrid index of Asters at (a) Lake Winnisquam (b) Lake Ossipee and (c,d,e) Great Wass Island

occurrence or because of their diagnostic value in identifying the parental taxa. The data on these spots are summarized in Tables 5 and 6.

The results of the chemical analysis of these populations indicated that the parental taxa could be identified on a chemical basis alone. This was verified by the evidence on Great Wass Island. A. nemoralis possessed four compounds which were species-specific. These are compounds # 1, 2, 3 and 4 of the chemical profile of A. nemoralis given in Fig. 4. A. acuminatus possessed three compounds which were species-specific. These compounds numbered 26, 27 and 28 in the chemical profile of this taxon pictured in Fig. 5. A histogram of the frequency of occurrence of phenolics in the parental taxa in discrete colonies is demonstrated in Figure 6. Twenty-one compounds were common to both parents. The remaining seven phenolics clearly separate the parental species.

A similar analysis of the Ponds Point population on Great Wass Island reveals that the parental taxa maintain a similar chemical integrity (Fig. 7a,c). A. Blakei contained a summation of compounds specific to both parents but did not contain any new species-specific compounds itself (Fig. 7b). This observation is in agreement with the generalizations of several authors discussed earlier in the literature review. The phenolic compounds present in natural hybrids represent the summation of species-specific phenolic compounds found in the parental taxa. The morphological (Fig. 3d) and chemical (Fig. 7b) evidence therefore suggests that A. Blakei at Ponds Point originated as a hybrid of A. nemoralis and A. acuminatus.

Table 5. Rf values, colors and color reaction with 1% lead acetate of the compounds diagnostic of Aster nemoralis, A. acuminatus and A. Blakei.

#	Rf values		Colors[1]				Pb acetate	
	BAW[2]	HOAc[3]	UV	UV-NH_3	VSB[4]	VSB-NH_3	VSB	UV
1	.45	.46	BrAb	Br	LY	IY	IY	BrAb
2	.58	.10	BlAb	BlAb	-	IY	IY	BlAb
3	.28	.23	BlAb	BlAb	-	IY	IY	BlAb
4	.44	.17	BrAb	BrAb	LY	DY	IY	DBrAb
5	.71	.19	Wb	YGr	-	Y	IY	YGr
6	.55	.23	Wb	Wb	-	-	-	Wb
7	.66	.07	Db	Db	-	-	-	bBl
8	.71	.34	WY	YGr	-	-	IY	YG
9	.43	.37	P	P	-	-	-	P
10	.42	.41	BrAb	BrAb	LY	DY	Y	DBrAb
11	.72	.67	YGr	Y	-	-	-	-
12	.59	.61	Yb	Yb	-	LY	Y	b
13	.52	.55	YGr	Y	-	-	-	-
14	.40	.49	Wb	Wb	-	-	-	-
15	.59	.73	YGr	Y	-	LY	Y	b
16	.44	.58	b	b	-	-	-	b
17	.48	.66	Y	Y	-	-	-	Y
18	.47	.74	Y	Y	-	-	-	Y
19	.41	.66	Y	Y	-	-	-	Y
20	.32	.62	Wb	Y	-	-	-	WY
21	.27	.63	Wb	Y	-	-	-	WY
22	.72	.65	Wb	Y	-	-	-	WY
23	.33	.78	Y	Y	-	-	-	WY
24	.28	.79	Y	Y	-	-	-	WY
25	.73	.53	YGr	Y	-	-	-	WY
26	.51	.20	BrAb	BrAb	LY	LY	-	-
27	.54	.47	BrAb	BrAb	LY	DY	LY	Y
28	.63	.28	BrAb	BrAb	LY	DY	IY	RBrAb

[1]Key: Ab = absorbing. All other spots are fluorescing.
Bl = black, b = blue, Br = brown, D = dark,
Gr = green, I = ivory, L = light, P = pink,
R = reddish, W = whitish, Y = yellow

[2]n-butyl alcohol, glacial acetic acid, water (6:1:2).

[3]10% acetic acid containing .1 gm. sodium acetate.

[4]VSB = visible

Table 6. Color[1] reactions with various spray reagents of the compounds diagnostic of *Aster nemoralis*, *A. acuminatus*, and *A. Blakei*.

#	SuA[2]	$FeCl_3$	Na_2CO_3		Benedict		$AlCl_3$		B Pb Ac[3]	
			VSB[4]	UV	VSB	UV	VSB	UV	VSB	UV
1	-	-	Y	BrAb	IY	-	-	Y	IY	BrAb
2	-	-	IY	LBrAb	LY	LBrAb	-	Y	IY	BrAb
3	-	-	IY	LBrAb	LY	LBrAb	-	Y	IY	BrAb
4	YBr	Gr	Y	BrAb	LY	BrAb	LY	Y	IY	Br
5	YBr	bBl	LY	YGr	LY	DBrAb	-	Wb	IY	DBrAb
6	-	-	-	Wb	-	Wb	-	b	-	Wb
7	-	-	-	YBr	-	Db	-	b	-	bBl
8	YBr	bBl	IY	YGr	LY	Br	-	Wb	IY	Br
9	-	-	-	P	-	P	-	P	-	P
10	YBr	Gr	Y	DBrAb	LY	BrAb	Y	Y	LY	DBrAb
11	-	-	-	Wb	-	Wb	-	Wb	-	Wb
12	YBr	bBl	Y	WY	LY	Br	-	Wb	IY	DBr
13	-	-	-	Wb	-	Wb	-	Wb	-	Wb
14	-	-	-	WY	-	-	-	Wb	-	Wb
15	YBr	bBl	-	WY	IY	lBr	-	Wb	-	Wb
16	-	-	-	b	-	b	-	b	-	b
17	-	-	-	WY	-	-	-	Wb	-	Y
18	-	-	-	WY	-	-	-	Wb	-	Y
19	-	-	-	WY	-	-	-	Wb	-	Y
20	-	-	-	WY	-	-	-	Wb	-	Y
21	-	-	-	WY	-	-	-	Wb	-	YGr
22	-	-	-	WY	-	-	-	Wb	-	YGr
23	-	-	-	WY	-	-	-	Wb	-	YGr
24	-	-	-	WY	-	-	-	Wb	-	YGr
25	-	-	-	WY	-	-	-	Wb	-	YGr
26	YBr	-	LY	Y	Y	BrYAb	IY	Y	IY	OY
27	YBr	Gr	Y	Y	LY	Y	LY	BrY	LY	Y
28	YBr	Gr	Y	BrAb	LY	BrAb	LY	Y	IY	BrAb

[1]Key: Ab = absorbing. All other spots are fluorescing. Bl = black, b = blue, Br = brown, D = dark, Gr = green, I = ivory, L = light, O = orange, P = pink, R = reddish, W = whitish, Y = yellow.

[2]SuA = diazotized sulfanilic acid.

[3]B Pb Ac = 1% basic lead acetate.

[4]VSB = visible.

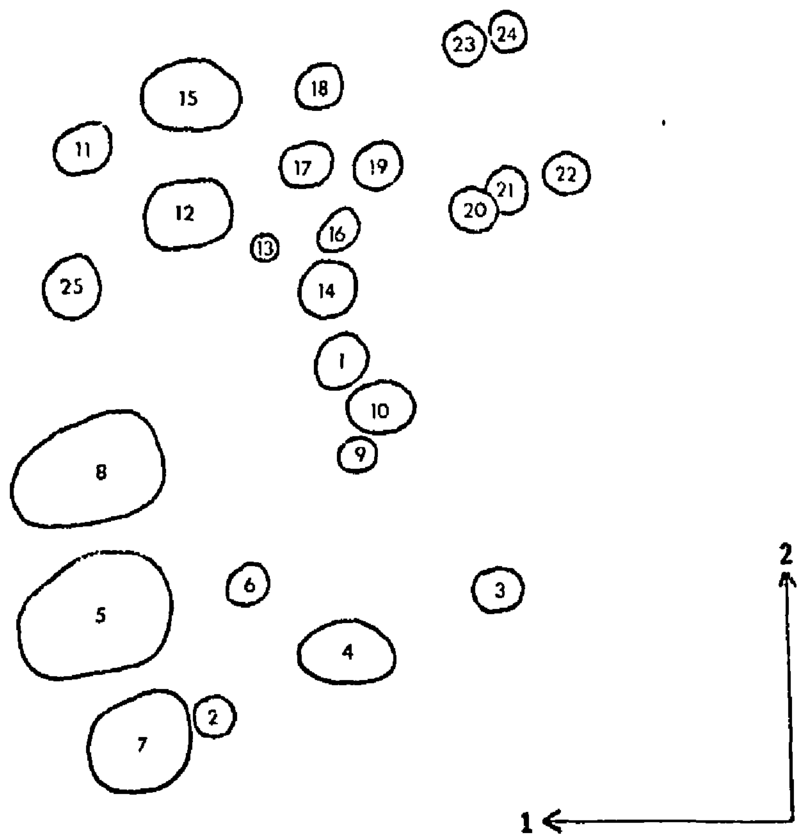

Fig. 4. Chemical phenolic profile of <u>Aster nemoralis</u>. Solvent System 1: n-butyl alcohol, glacial acetic acid, water (6:1:2). Solvent System 2: 10% acetic acid containing 0.1 gm. sodium acetate per 100 ml.

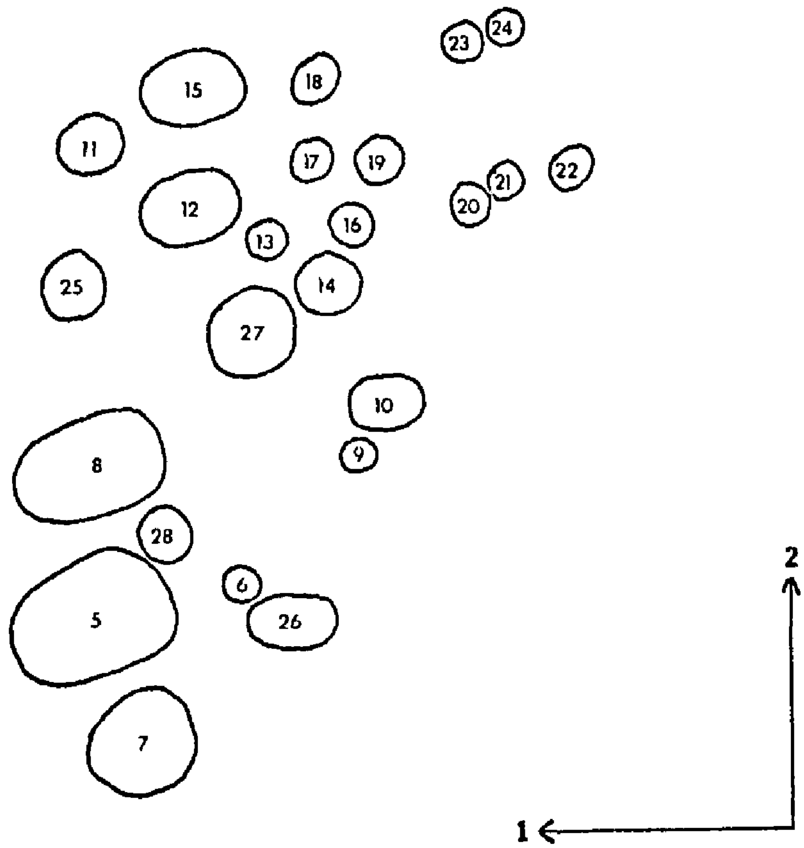

Fig. 5. Chemical phenolic profile of <u>Aster acuminatus</u>. Solvent System 1: n-butyl alcohol, glacial acetic acid, water (6:1:2). Solvent System 2: 10% acetic acid containing 0.1 gm. sodium acetate per 100 ml.

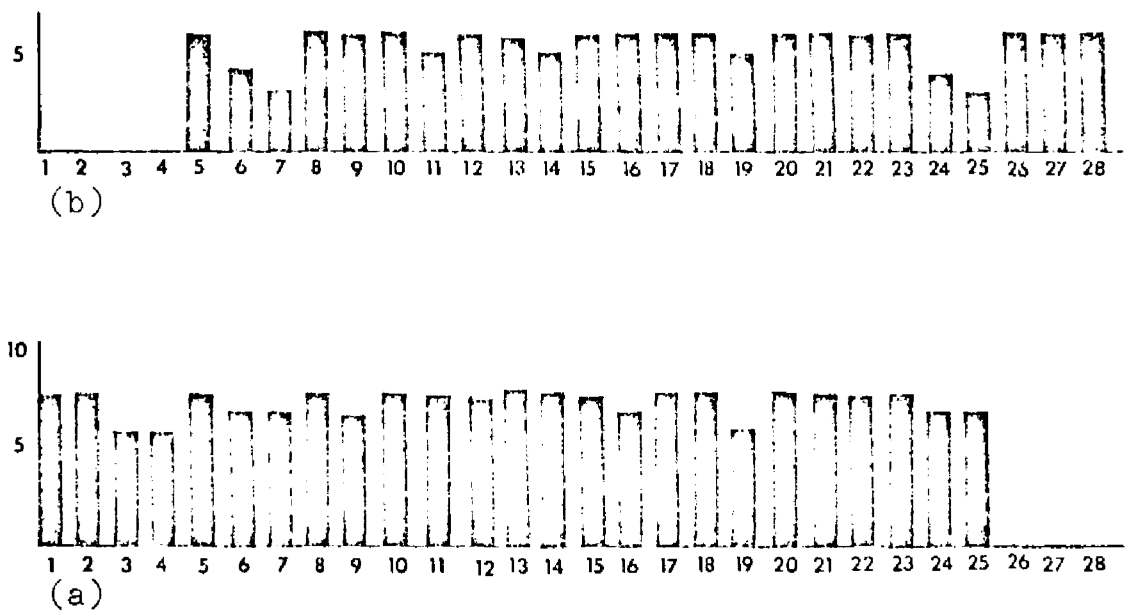

Fig. 6. Frequency of occurrence of phenolics characteristic of (a) Aster nemoralis and (b) A. acuminatus in discrete colonies on Great Wass Island.

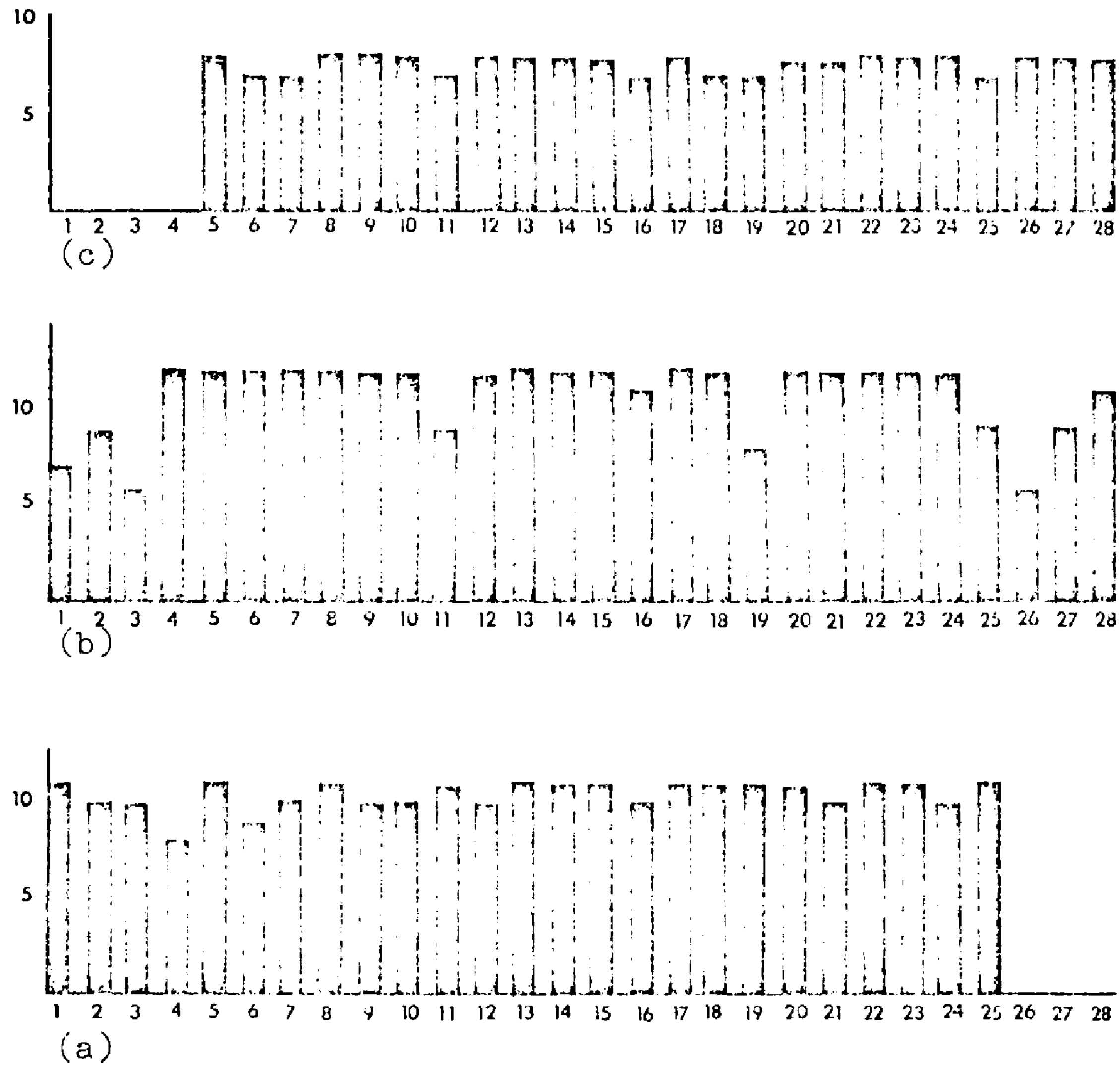

Fig. 7. Frequency of occurrence of phenolics characteristic of (a) Aster nemoralis, (b) A. Blakei and (c) A. acuminatus at Ponds Point, Great Wass Island.

It is clearly a chemical and morphological intermediate of the parental taxa.

The presence of parental compounds in a hybrid should represent the presence of parental genomes in the hybrid (Alston, 1967). A similar analysis of parental asters and their F_1 hybrid synthesized in the greenhouse is summarized in Fig. 8. Here, the hybrids contained a summation of the phenolics specific to both parents. However, some of the phenolics detected in the greenhouse asters were lower in frequency when compared with the frequency of occurrence of compounds in asters from Ponds Point (Fig. 7). This is noticeable in the F_1 hybrids in the greenhouse (Fig. 8b). Compounds 1, 2 and 3 are very low in frequency when compared with A. Blakei at Ponds Point (Fig. 7b). This might have been caused by a qualitative reduction in phenolics caused by the absence of pathogens and other predators in the homogeneous environment of the greenhouse. Levin (1971) cites numerous examples of the increase in quantity and quality of phenolics in response to predators and pathogens. When these organisms attack plants on a seasonal basis, a quantitative and qualitative decrease in phenolics is noticed when the predators or pathogens are absent. Also, a certain environmental parameter such as light or temperature might be present (or absent) in the greenhouse that would affect the synthesis of some of the phenolics.

The results of the chemical analysis of F_1 and parental taxa in the greenhouse indicate that the appearance of compounds #4, 26, 27, and 28 has a genetic basis. This is inferred from

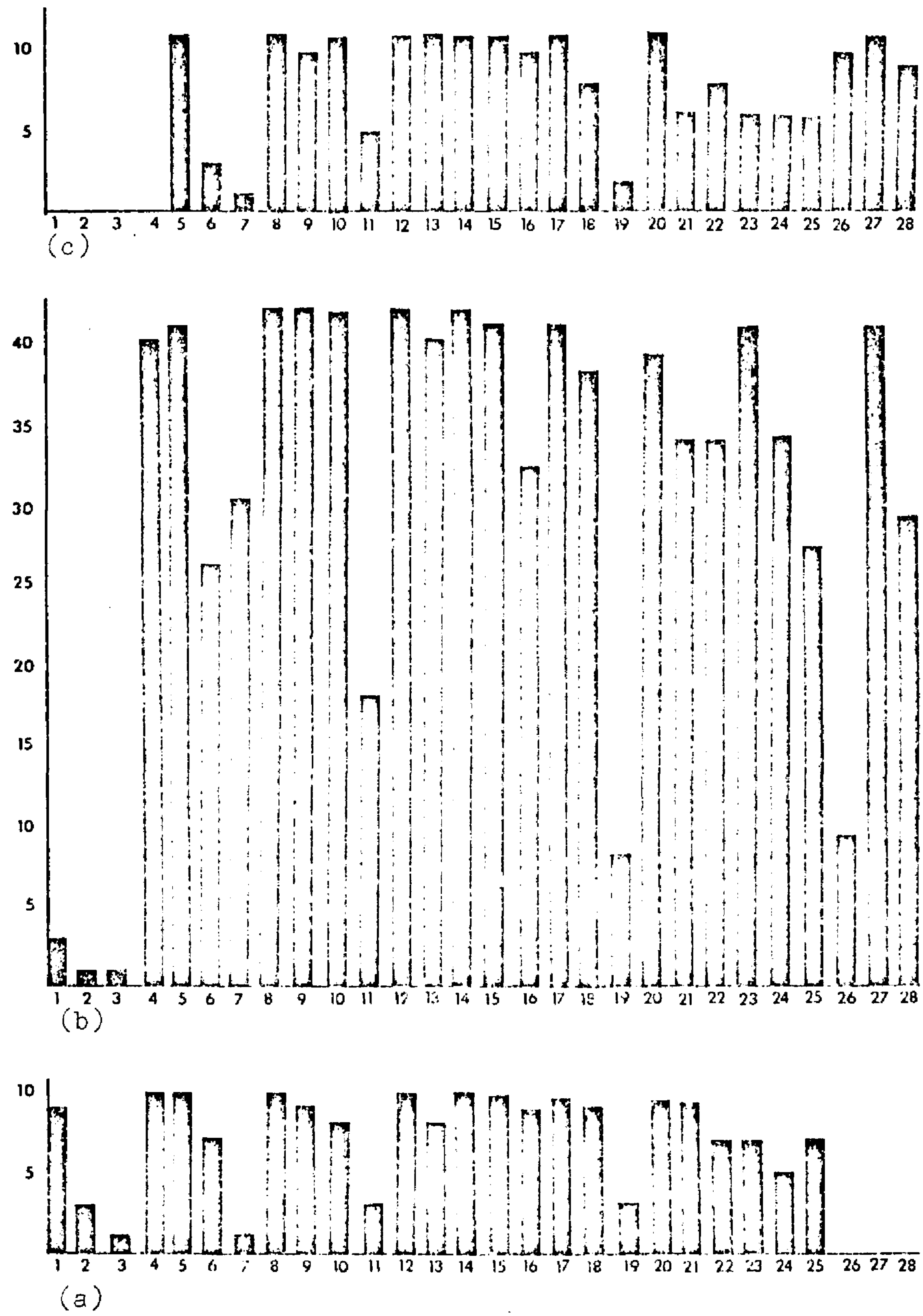

Fig. 8. Frequency of occurrence of phenolics characteristic of greenhouse specimens of (a) *Aster nemoralis* (c) *A. acuminatus* and (b) their F_1 hybrid.

the high frequency of occurrence of these phenolics in the F_1 hybrids (Fig. 8b). Most of the F_1 hybrids came from A. nemoralis female parents, and all contained one or another parental phenol, especially from A. acuminatus. A chemical analysis of these F_1's under natural conditions might show higher frequencies of parental phenolics #1, 2 and 3.

A comparison of the phenolics present in A. Blakei at Ponds Point, Lake Winnisquam and Lake Ossippee was undertaken. These results are summarized in Fig. 9 and demonstrate the essentially hybrid nature of the latter two Lake populations (Fig. 9a,b). Variation exists between these two populations at the chemical level. Compound #1 was absent from both Lake populations. Compound #4 was absent from the Lake Winnisquam population. There were also differences in the frequencies of A. acuminatus spots (#26, 27 and 28 in Fig. 9) and A. nemoralis spots (#1, 2, 3 and 4 in Fig. 9) between the three populations. In spite of this variation, both the morphological (Fig. 3a, b, and d) and chemical (Fig. 9) evidence suggest that these populations are essentially hybrids at Lake Winnisquam and Lake Ossipee.

There was evidence for introgression at Lake Ossipee. A relationship between chemical and morphological data was constructed according to a method devised by Levin (1967a). This relationship is given in Table 7. High frequencies of A. acuminatus compounds #26 and 28 were found in plants which indexed as A. nemoralis. Specimens identified as A. Blakei contained only A. acuminatus phenols. This suggests that introgression into A. nemoralis has been occurring at Lake

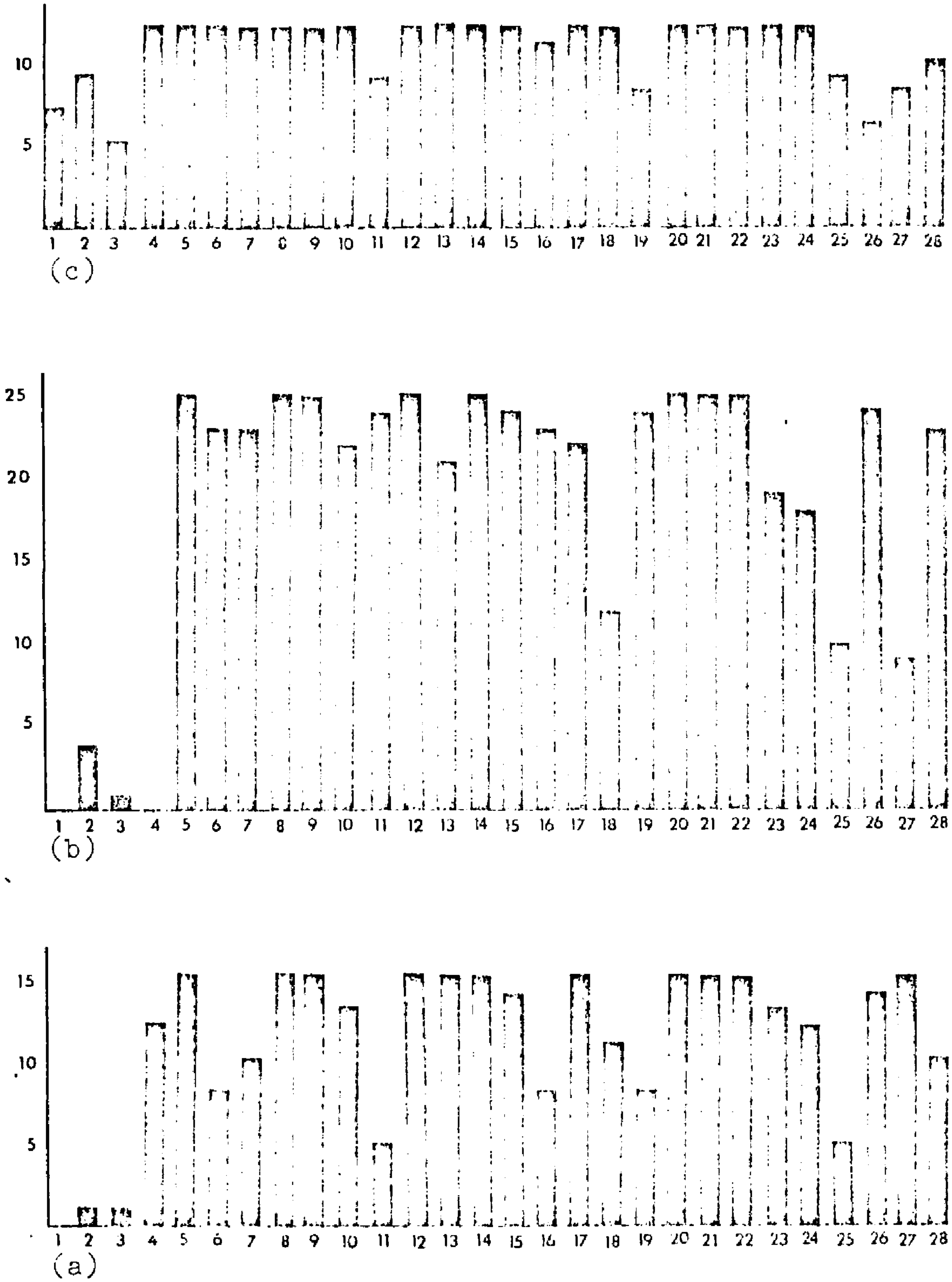

Fig. 9. Frequency of occurrence of phenolics characteristic of Aster Blakei at (a) Lake Winnisquam (b) Lake Ossipee and (c) Great Wass Island.

Table 7. Frequency of occurrence of compounds diagnostic of *Aster nemoralis* and *A. acuminatus* in relation to morphology in the population at Lake Ossipee, New Hampshire.

Index #	Compound Number 2	3	26	27	28
1	50		100		100
2	33	33	100		100
3	67		67		100
4			50		100
8			75	25	75
9			100	100	100
11			100	50	100
12			100	100	100
13			100	100	100
14			100	100	100
15			100	100	100
17			100		

Ossipee. A pictorialized scatter diagram was constructed according to the methods of Anderson (1949). The diagram was determined from the data on serrate vs. entire leaf margins, revolute vs. flat leaf margins, zebra hairs, flower color and leaf number (Fig. 10). Zebra hairs and serrate leaf margins, which are characters of *A. acuminatus*, were present in many plants indexed as *A. nemoralis*. This provides morphological evidence for the introgression of *A. acuminatus* into *A. nemoralis* at Lake Ossipee.

A. acuminatus was not present in the population at Lake Ossipee. The habitat is a disturbed one, which is located in an area of land development. There are woody habitats which could have supported *A. acuminatus*, but new roads have cut off drainage. Many of these areas are thus too moist and swampy

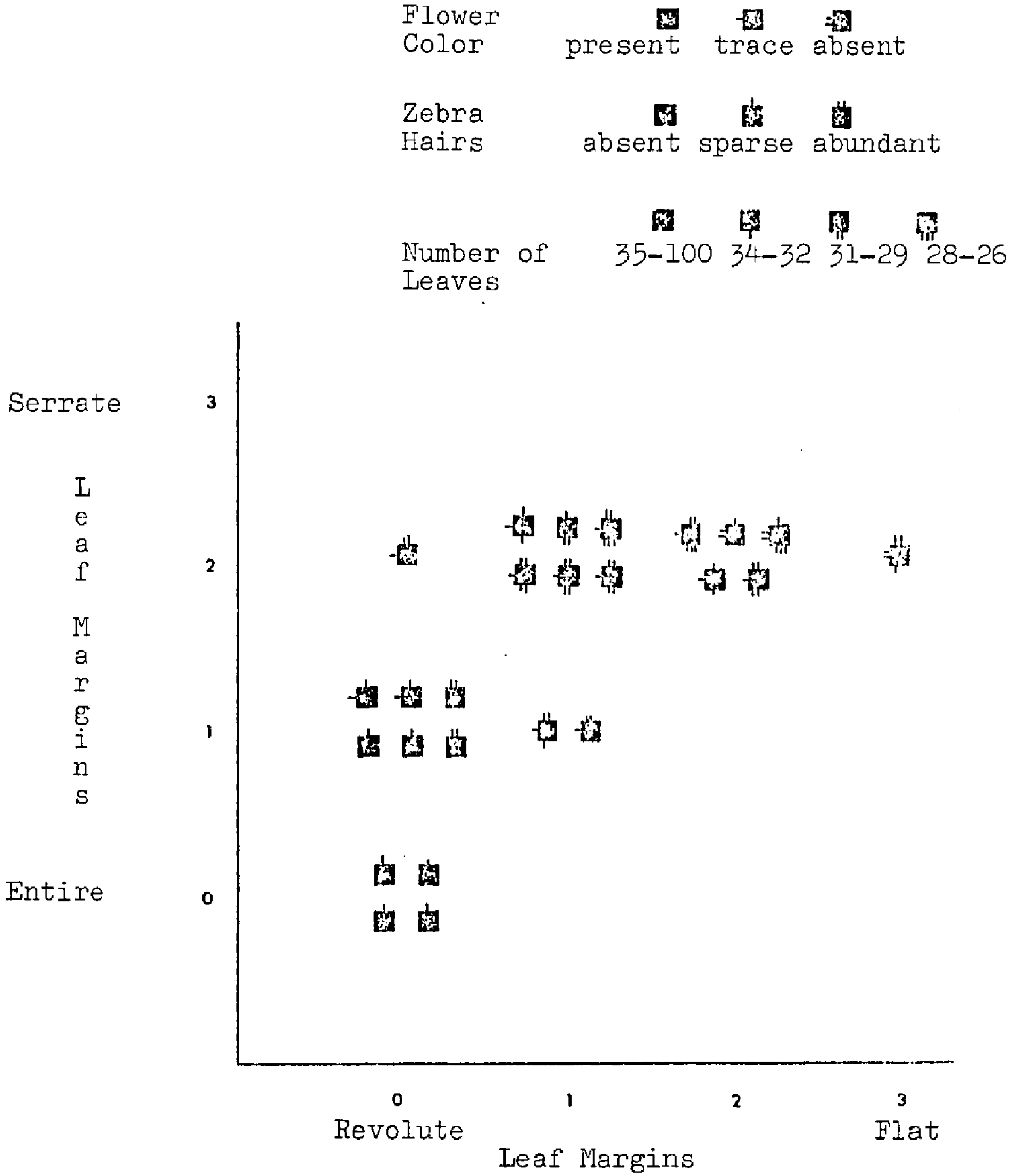

Fig. 10. Pictorialized scatter diagram of Asters at Lake Ossipee.

to sustain A. acuminatus. It is suggested that the disturbance removed this taxon, leaving A. Blakei to cross with A. nemoralis. The result is the variable population found there today. There was no chemical or morphological evidence for introgression in the populations at Lake Winnisquam or Great Wass Island. The herbarium specimens which gave the results discussed in this section are numbered 1-136 in the Appendix.

Cytology. The chromosome numbers of these taxa were determined to be 2N = 18. This was based on counts of mitotic and meiotic chromosomes in asters from five geographic locations. A total of forty-three specimens gave good counts. These are listed throughout the Appendix. The chromosome numbers of A. Blakei and A. nemoralis were new and were reported in the literature (Hill and Rogers, 1970). The number for A. acuminatus was in agreement with the number reported for this taxon by Nelson (1966). These specimens were not karyotyped. A representative plate of the mitotic chromosomes of A. nemoralis is shown in Fig. 11. A plate of the meiotic chromosomes of A. Blakei shows nine bivalents (Fig. 12).

The behavior of the meiotic chromosomes of all three taxa were compared. Both parental taxa formed nine bivalents at meiosis. No irregularities were observed. Pairing in A. Blakei was regular although loose associations were occasionally noted (Fig. 12). Some bridges and lagging occurred in Anaphase I (Fig. 13). 239 plates of meiotic stages were observed. 93% showed nine bivalents, and the remaining few showed irregularities referred to above. Similar observations

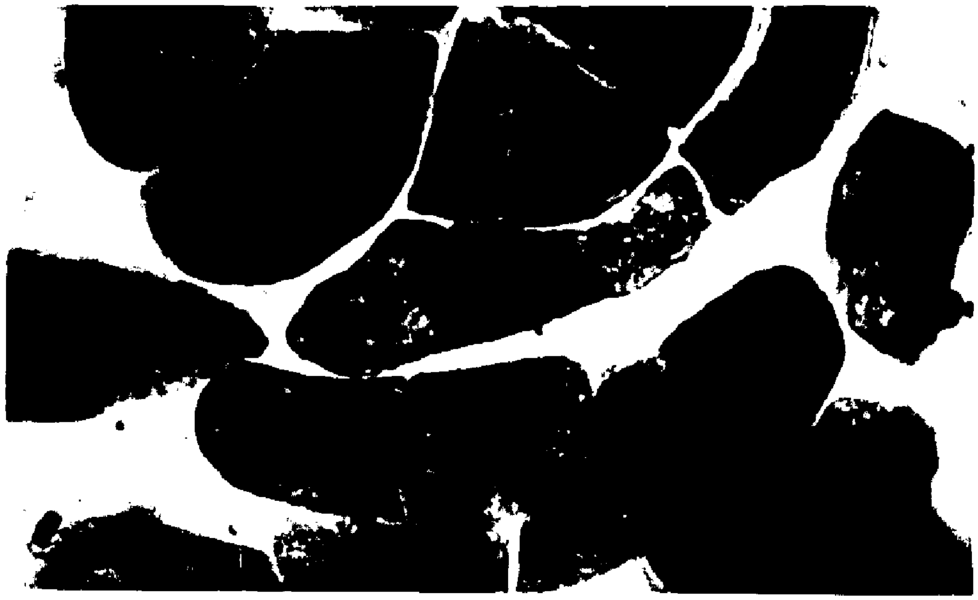

Fig. 11. Mitotic chromosomes of _Aster_ _nemoralis_, Metaphase.

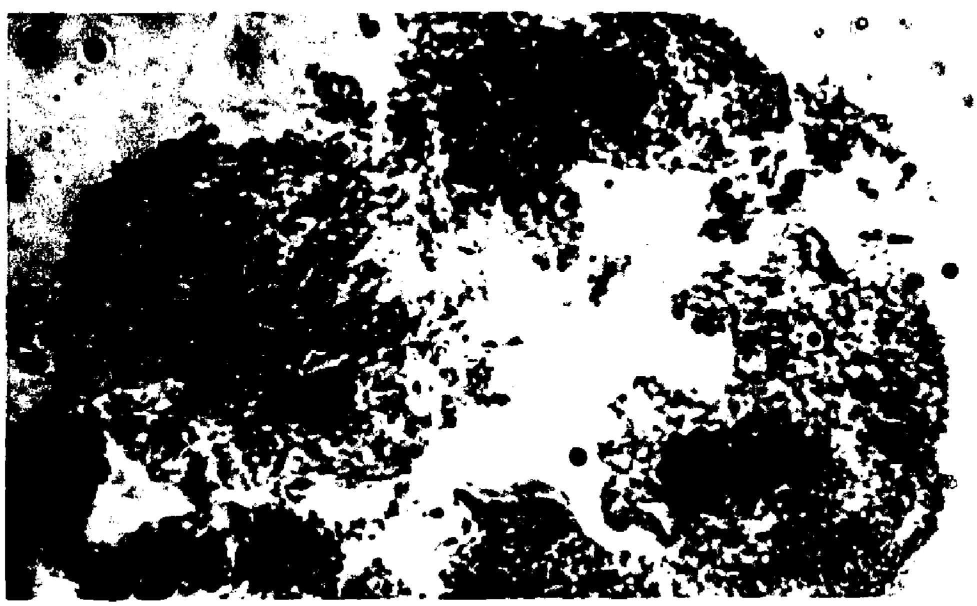

Fig. 12. Meiotic chromosomes of _Aster_ _Blakei_, Metaphase I. Loose associations are indicated by the arrow.

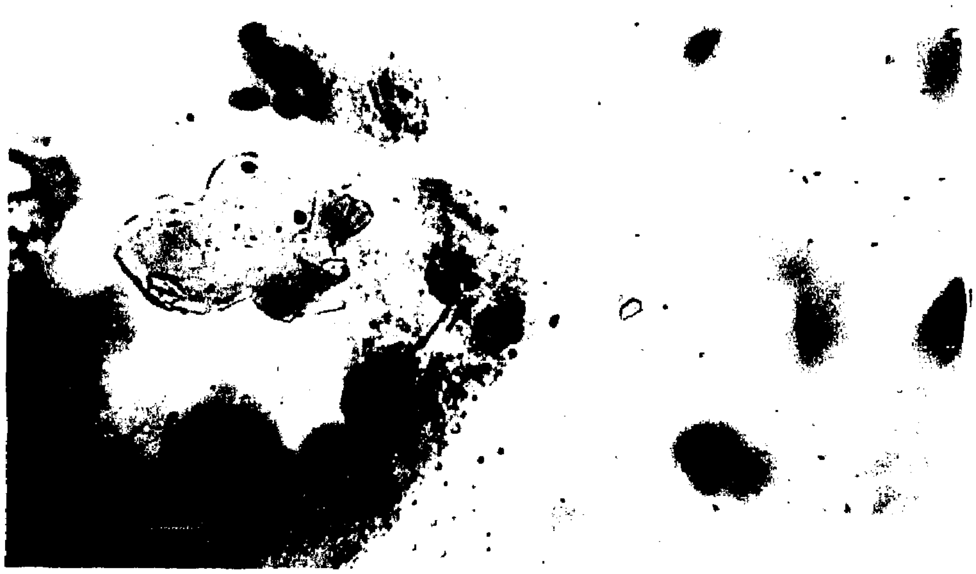

Fig. 13. Meiotic chromosomes of Aster Blakei, Anaphase I.

were noted in the F_1 hybrid population. Other suspected species hybrids in the genus exhibit the same meiotic behavior (Avers, 1953a; Wetmore and Delisle, 1939).

Pollen stainability was determined for all three taxa. The results are given in Table 8. All of the hybrids studied were the F_1 hybrids in the greenhouse. The mean stainability was 96% for nine specimens of A. nemoralis, 97% for ten specimens of A. acuminatus and 90% for forty-one specimens of their F_1 hybrid. The stainability for these hybrids was repeated a year later and 89% stainability resulted. Pollen stainability was thus lower in the F_1's than in their parents. An analysis of variance showed that the lower percentage of pollen stainability in the F_1's was significant (Table 9). Five specimens of A. Blakei from Lake Ossipee exhibited a mean pollen stainability

Table 8. Pollen stainability (%) of *Aster nemoralis*, *A. acuminatus* and their F_1 hybrid.

A. nemoralis	F_1 Hybrid			*A. acuminatus*
89	67	91	91	97
96	73	76	87	98
96	99	87	90	91
98	85	94	97	99
99	78	98	99	97
98	89	99	99	95
90	85	87	99	97
97	85	99	98	98
98	92	99	97	100
	86	86	93	97
	81	100	89	
	77	94	91	
	93	95	78	
	98	88		

Table 9. Analysis of variance of pollen stainability (%) in *Aster nemoralis*, *A. acuminatus* and their F_1 hybrid.

Source	dF	MS	F
Treatment	2	264.554	5.214 *
Error	57	50.735	
Total	59		

*Significant at .05 level

of 79%, while five specimens of A. Blakei from Gould Pond in Milton, New Hampshire, scored a mean stainability of 97%. It should be emphasized that pollen stainability reflects only the presence of nuclear material in pollen grains. It is a relative measure of viability. The representative specimens which gave these data are #96-136 for the F_1 hybrid, #293-312 for A. Blakei collected from Lake Ossipee and Gould Pond, #313-321 for A. nemoralis and #351-360 for A. acuminatus.

The observations of the behavior of meiotic chromosomes in the F_1 hybrid and natural specimens of A. Blakei coupled with the data on pollen stainability suggests that A. Blakei is fertile, but not as fertile as A. nemoralis and A. acuminatus. This is in agreement with the observations of Wetmore and Delisle (1939) and Avers (1953a). The hybrids they studied were fertile. The reasons for fertility in hybrids of Aster or any other taxon are always hard to determine. Grant (1971) suggests that the formation of partly to completely fertile hybrids in nature is because the parental species have not yet evolved reproductive barriers. Such species are usually referred to as semi- or incipient species. These asters might represent an example of plant populations which would be morphologically differentiated enough to be taxonomically identified as different species, yet they would be genetically similar with no isolating mechanisms existing between them.

Genetics. Crosses made between A. nemoralis and A. acuminatus in the fall of 1970 resulted in seed set that was higher when A. nemoralis was the female parent (Table 10).

This was repeated in the spring of 1971 using parents from a wider geographic source. The reason for this result probably

Table 10. Seed set of the cross *Aster nemoralis* x *A. acuminatus* during two flowering seasons.

Season	Female Parent	Total Flowers Examined	Good Seed	Seed Set (%)
fall, 1970	*A. nemoralis*	1180	205	17.4
	A. acuminatus	1191	30	2.1
spring, 1971	*A. nemoralis*	2426	302	12.5
	A. acuminatus	2297	26	1.1

rests in the presence of a maternal barrier to pollination and fertilization in *A. acuminatus*.

The F_1's produced from the fall 1970 cross were cultivated in May, 1971, and collected in May and June of 1972. Results of a morphological analysis according to the techniques of Pike (1970) are represented in Fig. 14a. Most of these specimens were intermediate between their parents, and fell within the range assigned to *Aster Blakei* by Pike (1970). Representative specimens of *A. Blakei* collected from Lake Ossipee and F_1 hybrids from the cross *A. nemoralis* x *A. acuminatus* are demonstrated together in Fig. 15 and 16. The morphological similarities are quite obvious. Many of the F_1 hybrids resembled some of the specimens of *A. Blakei* collected from Gould Pond, Lake Winnisquam and Great Wass Island. It was noticed that some morphological characters were more variable than others. A Coefficient of Variation (CV) was calculated

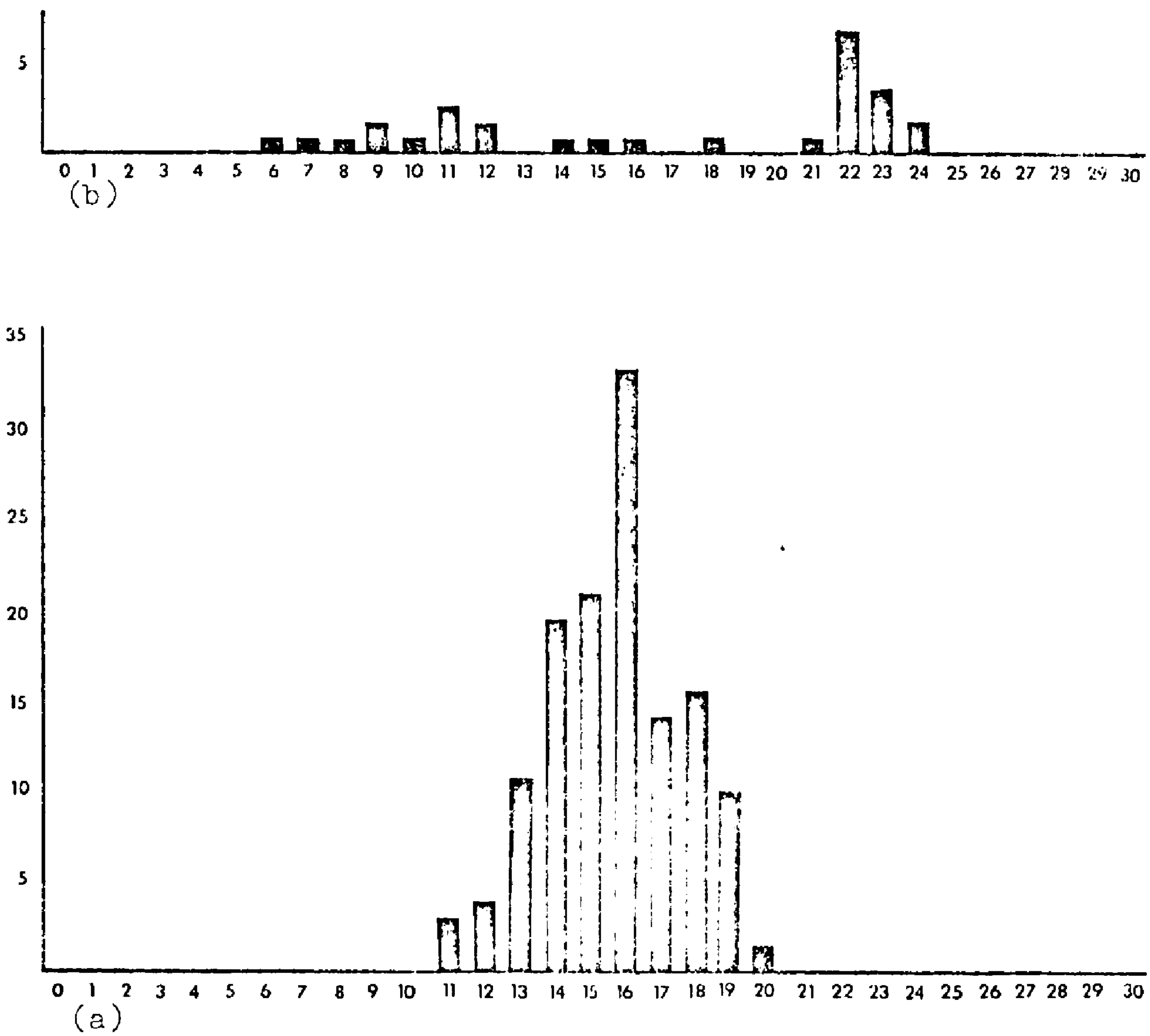

Fig. 14. Morphological hybrid index of the (a) F_1 hybrids and (b) progeny from the crosses of _Aster Blakei_ with _A. nemoralis_ and _A. acuminatus_. Specimens with index numbers of 6-15 resulted from crosses between _A. Blakei_ and _A. nemoralis_; specimens with index numbers 16-24 resulted from crosses between _A. Blakei_ and _A. acuminatus_.

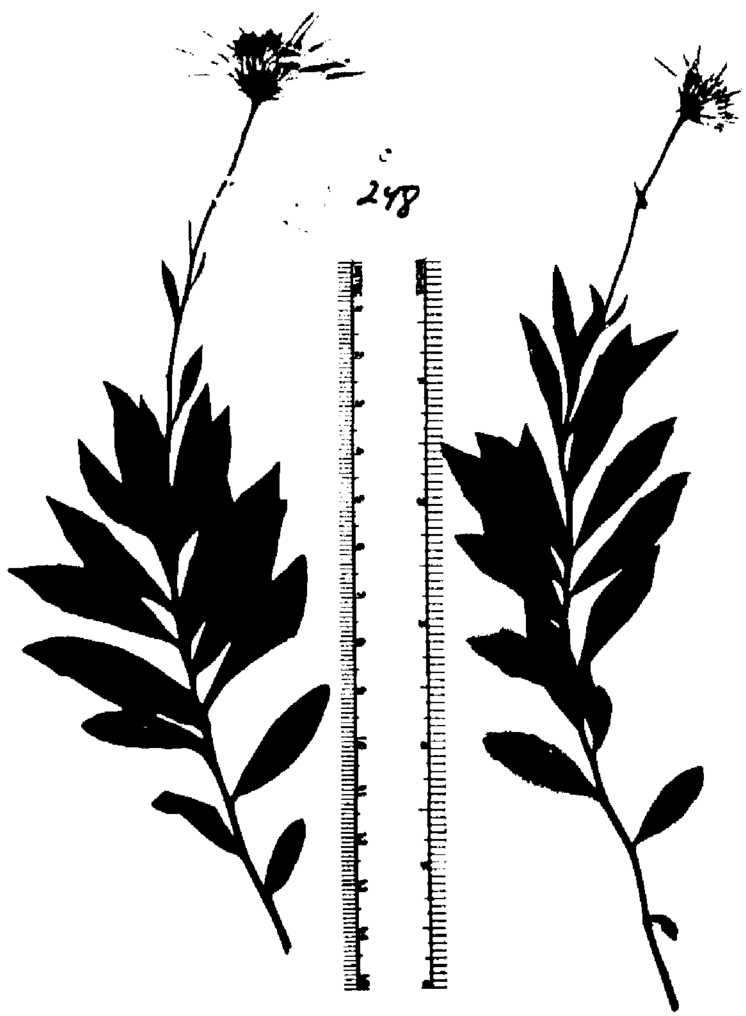

Fig. 15. Aster Blakei collected from Lake Ossipee and an F_1 hybrid of the cross A. nemoralis x A. acuminatus. The F_1 hybrid is shown on the left. The tag number represents the identification of the F_1 hybrid in the Appendix.

Fig. 16. Aster Blakei collected from Lake Ossipee and an F_1 hybrid of the cross A. nemoralis x A. acuminatus. The F_1 hybrid is shown on the left. The tag number represents the identification of the F_1 hybrid in the Appendix.

for each character. This calculation utilized the formula suggested by Sokol and Rehlf (1969). The results of this analysis are in Table 11. Each statistic was determined from

Table 11. Coefficient of variation (CV) in percent of the ten morphological characters of an F_1 population of *Aster nemoralis* x *A. acuminatus*.

Morphological Character	s[1]	Y[2]	CV
Leaf number	0.999	5.821	17.2
Ratio of leaf size	0.652	3.018	21.6
Internode length	0.438	1.256	34.9
Revoluteness of leaf margins	0.489	2.863	17.1
Scabrous leaf margins	0.432	2.131	20.3
Serrate leaf margins	0.203	2.970	6.8
Bracts subtending peduncle	0.747	1.994	37.4
Number of heads	0.444	1.268	35.0
Ligule color	0.204	1.982	10.3
Zebra hairs	0.461	2.304	20.0

[1]Standard Deviation

[2]Mean

index numbers for 168 F_1 hybrid specimens (#96-136 and #166-292 in the Appendix). The most variable characters were bract number, number of heads, and internode length. It was assumed that each variate being compared was continuous. Ligule color may be continuous, but genetic tests would be needed to confirm this. Discontinuous variates cannot be compared with continuous variates (Simpson and Roe, 1939). In all, the F_1 hybrids were morphologically uniform for some characters and variable for others.

A limited number of crosses between *A. Blakei* and the parental taxa resulted in seed set (Table 12). *A. Blakei* in

Table 12. Seed set of crosses between the parental taxa and *Aster Blakei*, Fall 1970

Female Parents	Total Flowers Examined	Good Seed	Seed Set(%)
A. acuminatus x	162	39	24.0
A. Blakei	107	36	33.6
A. nemoralis x	134	44	32.8
A. Blakei	111	20	18.0

this case was from nature and of unknown generation. Twenty-nine specimens that were preserved from this population exhibit ranges of variation that fell outside the limits of the parental taxa but inside the limits of *A. Blakei* (Fig. 14b). The specimens which gave these results are #137-165 in the Appendix. This is admittedly a small sample, but it appears that the range of variation for *A. Blakei* suggested by Pike (1970) might contain both backcross and hybrid types. The range of *A. Blakei*, which runs from index numbers of 8-19, would include backcross types from *A. nemoralis* running from 8-10 and F_1's from 11-20. Backcrosses to *A. acuminatus* fell between the ranges of this parent and the hybrid.

Backcrosses between the parents and their F_1 hybrid resulted in good seed set. These have been planted and seeds have germinated. The data on these crosses are given in Table 13. It was evident that maternal barriers in A. acuminatus

Table 13. Seed set and seed germination from the cross between the parental taxa and their F_1 hybrid, Spring 1971.

Female Parents	Total Flowers Examined	Good Seed	# Germ.	Seed Set(%)	% Germ.
A. acuminatus x	1267	345	158	27	46
F_1 Hybrid	1213	476	310	39	65
A. nemoralis x	1594	428	217	27	51
F_1 Hybrid	1306	225	111	17	49

that existed for A. nemoralis pollen did not exist for pollen from the F_1. The relatively adequate percentages of germination for all of these crosses do not indicate any genetic crossing barrier.

The crosses made within the hybrid population also resulted in good seed set. These were sib-matings and intraspecific crosses. The data on seed set and seed germination are presented in Table 14. The hybrids set viable seed, although germination tended to be lower in seed from sib crosses.

Table 14. Seed set and seed germination from the crosses made within the F_1 population, Spring, 1971.

Type of Cross	Total Flowers Examined	Good Seed	# Germ.	Seed Set(%)	% Germ.
Intrasp.	784	303	124	41	41
Sib.	863	357	79	39	22

The results on seed set and seed germination indicates that all three taxa can intercross. There is a partial barrier to hybridization when A. acuminatus is the female parent, but this barrier breaks down as backcrossing proceeds. Pike (1970) has noted that some hybrid swarms of these asters show a morphological skew toward A. nemoralis. The data presented in Table 10 show crossing between A. acuminatus and A. nemoralis tends to favor A. nemoralis as the female parent. If this occurred in nature, there will be more seeds of the F_1 produced on the A. nemoralis parent and backcrosses will therefore be more numerous in the wetter habitat of A. nemoralis. The wetter habitats will tend to select the A. nemoralis backcrosses in preference to A. acuminatus backcrosses.

Reproductive Biology. The influorescence, or head, of these asters were composed of two types of flowers. The outer margins of the heads contained pistillate ray flowers which had a strap-shaped corolla on one side. The central main body of the head contained perfect disc flowers with a tubular corolla. In the disc flowers, stamens were fused by their anthers to form a cylinder around the style. The stigma emerged from within the cylinder before the anthers shed pollen. The stigma was thus ready to receive pollen from other sources before pollen became available from the same flower. The time between stigma emergence and anther dehiscence was not determined. All three asters maintained a stoloniferous habit and were perennials. Seed never set in heads that were undisturbed. Seed did not set in any heads of asters from natural sources when selfing was attempted. This inferred that the specimens of _A_. _nemoralis_, _A_. _acuminatus_ and _A_. _Blakei_ collected from nature and utilized in this study were self-incompatable. This was in agreement with the results of Avers (1953a), Wetmore and Delisle (1939) and Uttal (1962) on asters of the Section EUASTER Gray.

CHAPTER V

CONCLUSIONS

A. Blakei is a morphological and chemical intermediate of A. acuminatus and A. nemoralis. The chemical and genetic evidence coupled with the morphological data supports the thesis of Pike (1970) that A. Blakei is of hybrid origin. Crosses between A. nemoralis and A. acuminatus have yielded hybrids which fell within the range of A. Blakei morphologically and chemically. This is further evidence for the hybrid origin of this taxon. Gene exchange from A. nemoralis to A. acuminatus and vice versa has been demonstrated in the green house. This evidence suggests that these organisms have the capability to undergo introgression. There is morphological and chemical evidence for introgression from A. acuminatus into A. nemoralis in at least one location in nature (Lake Ossipee).

The range of morphological variation assigned to A. Blakei by Pike (1970) might contain both hybrid and backcross specimens. A chemical and morphological study of the backcross progeny now being cultivated might clarify the exact nature of A. Blakei as either a hybrid or stabilized introgressant.

A. Blakei is a fertile hybrid which can backcross with A. acuminatus and A. nemoralis. It can also intercross with itself and produce recombinant progeny. The fitness and vigor of the backcross and recombinant progeny remain to be determined. The behavior of the meiotic chromosomes of *A. Blakei* appears to be relatively normal. The chromosome number for *A. Blakei*, *A. nemoralis* and *A. acuminatus* is 2N = 18. Isolation between *A. nemoralis* and *A. acuminatus* might occur by hybrid breakdown, selection against backcross and recombinant progeny, or by physical habitat separation. Possibly, many of these progeny would be selected against unless hybrid or recombinational habitats were available for them to invade.

It is suggested that *A. nemoralis* and *A. acuminatus* are semi-species in the sense of Grant (1971). They remain as good biological species when allopatric, but cross when the opportunity is presented in sympatry.

CHAPTER VI

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APPENDIX

The following table contains a record of the herbarium specimens which serves as a voucher for the work done in this study. Explanations of abbreviations are listed at the end of the table on page 83. These specimens have been deposited in the Herbarium of the University of New Hampshire.

#	Name	Chromosome Number	F.P.	M.P.	Source	Date
1	A.B.		N.A.	N.A.	Lake	9/70
2	"		"	"	Winnisquam	"
3	"		"	"	" "	"
4	"		"	"	" "	"
5	"		"	"	" "	"
6	"		"	"	" "	"
7	"		"	"	" "	"
8	"		"	"	" "	"
9	"		"	"	" "	"
10	"		"	"	" "	"
11	"		"	"	" "	"
12	"		"	"	" "	"
13	"		"	"	" "	"
14	"		"	"	" "	"
15	"		"	"	" "	"
16	"		"	"	" "	"
17	A.B.		"	"	Lake	9/70
18	A.N.		"	"	Ossipee	"
19	A.B.		"	"	" "	"
20	"		"	"	" "	"
21	"		"	"	" "	"
22	"		"	"	" "	"
23	"		"	"	" "	"
24	"		"	"	" "	"
25	A.N.		"	"	" "	"
26	A.B.		"	"	" "	"
27	"		"	"	" "	"
28	A.N.		"	"	" "	"
29	"		"	"	" "	"
30	A.B.		"	"	" "	"
31	"		"	"	" "	"
32	A.N.		"	"	" "	"
33	A.B.		"	"	" "	"
34	"		"	"	" "	"

Appendix (Cont.)

#	Name	Chromosome Number	F.P.	M.P.	Source	Date
35	A.B.		N.A.	N.A.	Lake	9/70
36	"		"	"	Ossipee	"
37	A.N.		"	"	" "	"
38	"		"	"	" "	"
39	"		"	"	" "	"
40	"		"	"	" "	"
41	"		"	"	" "	"
42	A.A.		"	"	Ponds	8/71
43	A.N.		"	"	Point,	"
44	A.N.		"	"	Great	"
45	A.B.		"	"	Wass	"
46	"		"	"	Island	"
47	A.N.		"	"	" "	"
48	A.B.		"	"	" "	"
49	"	N = 9	"	"	" "	"
50	"		"	"	" "	"
51	"		"	"	" "	"
52	A.A.		"	"	" "	"
53	A.B.		"	"	" "	"
54	A.A.		"	"	" "	"
55	A.B.	N = 9	"	"	" "	"
56	"		"	"	" "	"
57	"		"	"	" "	"
58	A.A.		"	"	" "	"
59	A.B.		"	"	" "	"
60	A.A.		"	"	" "	"
61	A.B.		"	"	" "	"
62	A.A.		"	"	" "	"
63	A.N.		"	"	" "	"
64	A.A.		"	"	" "	"
65	"		"	"	" "	"
66	A.N.		"	"	" "	"
67	A.B.		"	"	" "	"
68	A.N.		"	"	" "	"
69	A.A.		"	"	" "	"
70	A.B.		"	"	" "	"
71	"		"	"	" "	"
72	"		"	"	" "	"
73	A.N.		"	"	" "	"
74	A.B.		"	"	" "	"
75	"		"	"	" "	"
76	A.N.		"	"	" "	"
77	A.B.		"	"	" "	"
78	A.N.		"	"	" "	"

Appendix (Cont.)

#	Name	Chromo- some Number	F.P.	M.P.	Source	Date
79	A.N.		N.A.	N.A.	" "	8/71
80	A.B.		"	"	" "	"
81	A.A.		"	"	Woods	"
82	"		"	"	" "	"
83	A.N.		"	"	Bog	"
84	"		"	"	"	"
85	"		"	"	"	"
86	"		"	"	"	"
87	A.A.	N = 9	"	"	Woods	"
88	"		"	"	" "	"
89	A.N.		"	"	Bog	"
90	"		"	"	"	"
91	"		"	"	"	"
92	A.A.		"	"	Woods	"
93	"		"	"	" "	"
94	A.N.		"	"	Bog	"
95	"		"	"	"	"
96	F_1		#327	#358	Seed	6/72
97	Hyb.		"	"	"	"
98	"		"	"	"	"
99	"		#325	#359	"	"
100	"	N = 9	"	"	"	"
101	"	N = 9	"	"	"	"
102	"	N = 9	#321	#360	"	"
103	"		"	"	"	"
104	"	N = 9	"	"	"	"
105	"	N = 9	"	"	"	"
106	"	N = 9	"	"	"	"
107	"	N = 9	"	"	"	"
108	"		"	"	"	"
109	"	N = 9	"	"	"	"
110	"		#315	#358	"	"
111	"		#328	#358	"	"
112	"		"	"	"	"
113	"	N = 9	"	"	"	"
114	"		"	"	"	"
115	"		"	"	"	"
116	"		"	"	"	"
117	"		"	"	"	"
118	"		"	"	"	"
119	"		#325	#359	"	"
120	"	N = 9	"	"	"	"
121	"	N = 9	"	"	"	"
122	"		"	"	"	"

Appendix (Cont.)

#	Name	Chromo-some Number	F.P.	M.P.	Source	Date
123	F_1	N = 9	#325	#359	Seed	6/72
124	Hyb	N = 9	"	"	"	"
125	"	N = 9	"	"	"	"
126	"		"	"	"	"
127	"		"	"	"	"
128	"	N = 9	#329	#360	"	"
129	"	N = 9	#317	"	"	"
130	"		"	"	"	"
131	"		"	"	"	"
132	"		#318	"	"	"
133	"		"	"	"	"
134	"		"	"	"	"
135	"		"	"	"	"
136	"	N = 9	#358	#315	"	"
137	Back-		#325	#295	"	6/71
138	cross		"	"	"	"
139	"		#297	#351	"	"
140	"		"	"	"	"
141	"		#295	#325	"	"
142	"		#301	#351	"	"
143	"		#325	#295	"	"
144	"		#351	#301	"	"
145	"		"	"	"	"
146	"		#297	#351	"	"
147	"		#351	#301	"	"
148	"		"	"	"	"
149	"		#325	#295	"	"
150	"		"	"	"	"
151	"		#351	#301	"	"
152	"		#297	#323	"	"
153	"		#325	#295	"	"
154	"		"	"	"	"
155	"		#351	#301	"	"
156	"		#325	#295	"	"
157	"		#295	#325	"	"
158	"		#297	#351	"	"
159	"		#325	#295	"	"
160	"		#351	#301	"	"
161	"		"	"	"	"
162	"		#295	#325	"	"
163	"		#351	#301	"	"
164	"		"	"	"	"
165	"		"	"	"	"

Appendix (Cont.)

#	Name	Chromo- some Number	F.P.	M.P.	Source	Date
166	F_1		#327	#358	Seed	6/72
167	Hyb.		"	"	"	"
168	"		"	"	"	"
169	"		"	"	"	"
170	"		"	"	"	"
171	"		"	"	"	"
172	"		#325	#359	"	"
173	"		"	"	"	"
174	"		"	"	"	"
175	"		"	"	"	"
176	"		#321	#360	"	"
177	"		"	"	"	"
178	"		"	"	"	"
179	"		"	"	"	"
180	"		"	"	"	"
181	"		"	"	"	"
182	"		"	"	"	"
183	"		"	"	"	"
184	"		"	"	"	"
185	"		"	"	"	"
186	"		"	"	"	"
187	"		"	"	"	"
188	"		"	"	"	"
189	"		"	"	"	"
190	"		"	"	"	"
191	"		"	"	"	"
192	"		"	"	"	"
193	"		"	"	"	"
194	"		"	"	"	"
195	"		"	"	"	"
196	"		"	"	"	"
197	"		"	"	"	"
198	"		"	"	"	"
199	"		#315	#358	"	"
200	"		"	"	"	"
201	"		"	"	"	"
202	"		"	"	"	"
203	"		"	"	"	"
204	"		"	"	"	"
205	"		#328	"	"	"
206	"		"	"	"	"
207	"		"	"	"	"
208	"		"	"	"	"

Appendix (Cont.)

#	Name	Chromosome Number	F.P.	M.P.	Source	Date
209	F_1 Hyb.		#328	#358	Seed	6/72
210	"		"	"	"	"
211	"		"	"	"	"
212	"		"	"	"	"
213	"		"	"	"	"
214	"		"	"	"	"
215	"		"	"	"	"
216	"		"	"	"	"
217	"		"	"	"	"
218	"		"	"	"	"
219	"		"	"	"	"
220	"		"	"	"	"
221	"		"	"	"	"
222	"		"	"	"	"
223	"		"	"	"	"
224	"		#325	#359	"	"
225	"		"	"	"	"
226	"		"	"	"	"
227	"		"	"	"	"
228	"		"	"	"	"
229	"		"	"	"	"
230	"		"	"	"	"
231	"		"	"	"	"
232	"		"	"	"	"
233	"		"	"	"	"
234	"		"	"	"	"
235	"		"	"	"	"
236	"		"	"	"	"
237	"		"	"	"	"
238	"		"	"	"	"
239	"		"	"	"	"
240	"		"	"	"	"
241	"		"	"	"	"
242	"		"	"	"	"
243	"		"	"	"	"
244	"		"	"	"	"
245	"		"	"	"	"
246	"		"	"	"	"
247	"		"	"	"	"
248	"		"	"	"	"
249	"		"	"	"	"
250	"		"	"	"	"
251	"		"	"	"	"
252	"		"	"	"	"

Appendix (Cont.)

#	Name	Chromo-some Number	F.P.	M.P.	Source	Date
253	F_1		#325	#359	Seed	6/72
254	Hyb.		"	"	"	"
255	"		#317	#360	"	"
256	"		"	"	"	"
257	"		"	"	"	"
258	"		"	"	"	"
259	"		"	"	"	"
260	"		"	"	"	"
261	"		"	"	"	"
262	"		"	"	"	"
263	"		"	"	"	"
264	"		#318	"	"	"
265	"		"	"	"	"
266	"		"	"	"	"
267	"		"	"	"	"
268	"		"	"	"	"
269	"		"	"	"	"
270	"		"	"	"	"
271	"		"	"	"	"
272	"		"	"	"	"
273	"		"	"	"	"
274	"		"	"	"	"
275	"		"	"	"	"
276	"		"	"	"	"
277	"		"	"	"	"
278	"		"	"	"	"
279	"		"	"	"	"
280	"		"	"	"	"
281	"		"	"	"	"
282	"		"	"	"	"
283	"		"	"	"	"
284	"		"	"	"	"
285	"		#359	#317	"	"
286	"		"	"	"	"
287	"		#358	#315	"	"
288	"		"	"	"	"
289	"		"	"	"	"
290	"		"	"	"	"
291	"		#325	#359	"	"
292	"		"	"	"	"

Appendix (Cont.)

#	Name	Chromo-some Number	F.P.	M.P.	Source	Date
293	A.B.		N.A.	N.A.	Lake	8/69
294	"		"	"	Ossipee	"
295	"		"	"	" "	"
296	"		"	"	" "	"
297	"	N = 9	"	"	" "	"
298	"		"	"	" "	"
299	"	N = 9	"	"	" "	"
300	"		"	"	" "	"
301	"	N = 9	"	"	" "	"
302	"		"	"	" "	"
303	"	2N = 18	"	"	Gould	7/70
304	"		"	"	Pond,	"
305	"	2N = 18	"	"	Milron	"
306	"		"	"	" "	"
307	"	2N = 18	"	"	" "	"
308	"		"	"	" "	"
309	"	2N = 18	"	"	" "	"
310	"		"	"	" "	"
311	"	2N = 18	"	"	" "	"
312	"		"	"	" "	"
313	A.N.	2N = 18	"	"	Bay of	9/69
314	"		"	"	Fundy	"
315	"		"	"	" "	"
316	"		"	"	" "	"
317	"		"	"	" "	"
318	"		"	"	" "	"
319	"		"	"	" "	"
320	"		"	"	" "	"
321	"	2N = 18	"	"	" "	"
322	"		"	"	" "	"
323	"	N = 9	"	"	" "	"
324	"		"	"	" "	"
325	"	N = 9	"	"	" "	"
326	"		"	"	" "	"
327	"	N = 9	"	"	" "	"
328	"		"	"	" "	"
329	"		"	"	" "	"
330	"		"	"	" "	"
331	"	2N = 18	"	"	Gould	"
332	"		"	"	Pond	"
333	"		"	"	" "	"
334	"		"	"	" "	"
335	"	2N = 18	"	"	" "	"

Appendix (Cont.)

#	Name	Chromosome Number	F.P.	M.P.	Source	Date
336	A.N.		N.A.	N.A.	Gould	7/70
337	"		"	"	Pond	"
338	"		"	"	" "	"
339	"		"	"	" "	"
340	"		"	"	" "	"
341	"	2N = 18	"	"	" "	"
342	"		"	"	" "	"
343	"	2N = 18	"	"	" "	"
344	"		"	"	" "	"
345	"		"	"	" "	"
346	"		"	"	" "	"
347	"	2N = 18	"	"	" "	"
348	"		"	"	" "	"
349	"		"	"	" "	"
350	"		"	"	" "	"
351	A.A.		"	"	Lubec	8/69
352	"		"	"	Maine	"
353	"	N = 9	"	"	" "	"
354	"		"	"	" "	"
355	"	2N = 18	"	"	" "	"
356	"		"	"	" "	"
357	"		"	"	" "	"
358	"		"	"	" "	"
359	"		"	"	" "	"
360	"		"	"	" "	"
361	"		"	"	Gould	7/70
362	"		"	"	Pond	"
363	"		"	"	" "	"
364	"		"	"	" "	"
365	"		"	"	" "	"
366	"		"	"	" "	"
367	"	2N = 18	"	"	" "	"
368	"		"	"	" "	"
369	"		"	"	" "	"
370	"		"	"	" "	"
371	"		"	"	" "	"
372	"		"	"	" "	"
373	"		"	"	" "	"
374	"		"	"	" "	"
375	"	N = 9	"	"	" "	"
376	"		"	"	" "	"
377	"		"	"	" "	"
378	"		"	"	" "	"

Explanation of Abbreviations

A.A. = *Aster acuminatus* Michx.

A.B. = *Aster Blakei* (Porter) House

A.N. = *Aster nemoralis* Ait.

F.P. = Female parent

M.P. = Male Parent

N.A. = Not applicable

BIOGRAPHICAL DATA

Name in Full: Lynn Michael Hill

Date of Birth: August 12, 1941

Place of Birth: Washington, D. C.

Secondary Education: Venice High School, Venice, Florida

Collegiate Institutions attended	Dates	Degrees
Alabama College	1959-1963	B.S.
Tennessee Technological University	1963-1965	M.S.
The University of Tennessee, Knoxville	1965-1966	
The University of New Hampshire	1969-	

Honors or Awards: Florida Boys State, 1957; Undergraduate Research Assistantship, 1962; Delta Theta Pi, 1962; Beta Beta Beta, 1967; Phi Sigma, 1970; Sigma Xi, 1972.

Publications: Hill, L. Michael, and O. M. Rogers. 1970. Chromosome numbers of _Aster Blakei_ and _A. nemoralis_. Rhodora 72:437-438.

Positions Held	Dates
Laboratory Instructor of Biology and Anatomy and Physiology, Tennessee Technological University, Cookeville, Tennessee	1963-1965
Instructor, Department of Biological Sciences, The University of Tennessee at Martin, Martin, Tennessee	1966-1969
Graduate Research Assistant, Department of Plant Science, Nesmith Hall, The University of New Hampshire, Durham, New Hampshire.	1969-1972