Evolution, 45(7), 1991, pp. 1641–1650

GENETIC CONSEQUENCES OF RARITY IN *ASTER FURCATUS* (ASTERACEAE), A THREATENED, SELF-INCOMPATIBLE PLANT

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Abstract.—*Aster furcatus* is a rare, self-incompatible plant with fewer than 50 known populations throughout its range. We verified self-incompatibility in *A. furcatus* by conducting experimental self- and cross-pollinations and by examining seed set in a small population comprised of a single clonal genet. We examined variation at 22 electrophoretic loci in 23 populations of *A. furcatus* from across its range in Wisconsin, Illinois, Indiana, and Missouri. Except for two rare alleles found in single individuals in three populations, all loci but one of those examined were fixed for single alleles. The only variable locus (triosephosphate isomerase, *TPI-1*) tended to exhibit genotype frequencies in Hardy-Weinberg equilibrium or with a slight excess of heterozygotes. Although overall gene diversity was extremely low, *TPI* genotype frequencies were indicative of an outcrossing plant. We examined the subpopulation genetic structure among clonal plants within one Wisconsin population in greater detail. *F* statistics indicated that much of the genetic variation at the polymorphic *TPI* locus was due to differentiation among populations. We discuss the implications of self-incompatibility and low levels of genetic variation for the evolution and conservation of *Aster furcatus* and other rare plants with similar breeding systems.

Key words.—*Aster furcatus*, Asteraceae, electrophoresis, population structure, rarity, self-incompatibility.

Received March 21, 1990. Accepted May 20, 1991.

It is important to understand the population genetic structure of rare and threatened species because genetic diversity is thought to correlate with adaptive capacity (Beardmore, 1983). Genetic events that accompany rarity in outcrossing species involve both "short-term" and "long-term" effects (Frankel, 1982). In the short term, a genetic bottleneck (following population attenuation or founder effect) results in reduction of allelic diversity and genetic variation (Nei et al., 1975; Denniston, 1978). The direct genetic impact of a single bottleneck is believed to be minimal except in cases of severe population reduction to one to two individuals (Frankel and Soulé, 1981). Small populations, however, are highly susceptible to drift, which will decrease genetic variation and allelic diversity over a few generations (Frankel and Soulé, 1981). Following a bottleneck, populations can rebuild their numbers and genetic diversity in the long term to nearly prebottleneck levels. These events essentially follow Mayr's (1954) model of founding population recovery. For outcrossing species to recover from severe bottlenecks, it is critical that they regain outcrossing potential.

Species that possess genetic incompatibility mechanisms present a unique problem for recovery from severe bottlenecks. It is important to consider these species because approximately 50% of all angiosperms are genetically self-incompatible (Nettancourt, 1977). In the sporophytic, multiallelic incompatibility system typical of the Asteraceae (Burtt, 1977; Richards, 1986), populations consist of many "mating types" that possess different genotypes at a self-incompatibility (*S*) locus (Grant, 1975). Pollen rejection occurs if the stigmatic genotype shares any *S* alleles with the pollen genotype. Although pollen is haploid, recognition of the diploid component of the donor plant is probably a function of sporophytic substances adhering to the outer

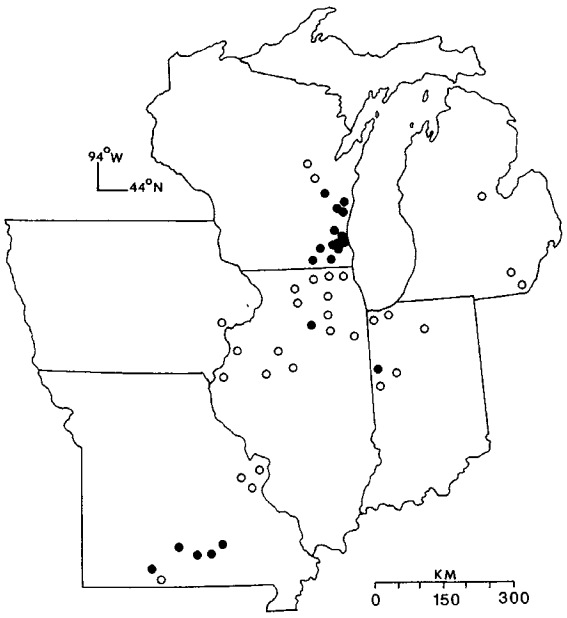

FIG. 1. Map of the midwestern United States showing the historical distribution of *Aster furcatus* populations based on specimen data (open circles) furnished by W. Lamboy, University of Illinois, and localities studied electrophoretically (closed circles). Many of the unsampled localities are no longer extant. A complete listing of specific localities and vouchers is available from the authors.

pollen wall (Heslop-Harrison, 1975). Consequently, these systems may be characterized by dominance relationships (Brewbaker, 1957). Self-pollination is prevented as well as cross-pollination among individuals that share alleles at an *S* locus. An abundance of *S* alleles within most populations, however, ensures a high probability of cross-compatibility. Species possessing this mechanism are strict outcrossers (because selfing is prevented genetically) and would likely suffer from inbreeding depression if selfing was induced (Charlesworth and Charlesworth, 1987).

Negative frequency dependent selection favors rare incompatibility alleles in populations of multiallelic self-incompatible plants (Falconer, 1989), leading to many alleles having roughly equal frequencies. In plant populations with multiallelic systems, losses of *S* alleles following population crashes or founder events would directly reduce fecundity by decreasing the pool of compatible mating types. In extreme instances (e.g., one to a few founder seeds), drift may result in the loss of compatible mating types to the point where there is an insufficient number to produce seed in the recovering population (Imrie et al., 1972). Subsequent population growth following a founder event does nothing to restore the loss of rare alleles (Simberloff, 1988) including incompatibility alleles that are characterized by surprisingly low mutation rates (Lewis, 1949; cf. Wright, 1964). Although the implications of this scenario may predict rapid extinction for such species, the occurrence of rare self-incompatible plants such as *Aster furcatus* warrants further evaluation of this question. Asexual reproduction may be an important means of surviving the initial bottleneck phase for plants with genetic incompatibility mechanisms. A study of the population genetic structure of a rare self-incompatible species capable of asexual reproduction should provide insights into understanding the effects of severe genetic bottlenecks.

In this study, we examine the population structure and reproductive biology of a threatened plant, *Aster furcatus,* to ascertain the genetic consequences of rarity in a self-incompatible species capable of vegetative reproduction. Specifically, we address the questions: 1) Is incompatibility maintained in this presumably self-incompatible species? 2) What is the extent of detectable genetic variability within and among surviving populations of the species? 3) What does the population genetic structure of *Aster furcatus* reveal of its reproductive biology?

MATERIALS AND METHODS

Genetic Incompatibility

Genetic incompatibility in *Aster furcatus* was verified using two approaches. First, we conducted experimental self- and cross-pollinations using plants transplanted from a natural population (Sheboygan Falls) to the UW-M Field Station experimental garden. We evaluated compatibility by comparing seed set of bagged inflorescences pollinated only with self pollen obtained from other inflorescences on the same plant to seed set of bagged and unbagged inflorescences receiving pollen from what were assumed to be different genotypes in the population based on the spatial structure of clones. We tested for dependence of seed set on polli-

TABLE 1. Allele frequencies for 22 enzyme loci surveyed in 20 natural Missouri (MO), Indiana (IN), and Wisconsin (WI) populations of *Aster furcatus*. Only the three variable loci are presented; the remaining 19 loci were completely fixed in our samples.

Population	*N*	AAT-2[1]		ALD-1		TPI-1	
		a	b	a	b	a	b
Sheboygan Falls (WI)	26	1.0	0.0	0.0	1.0	0.40	0.60
Bachman Woods (WI)	18	1.0	0.0	0.0	1.0	0.33	0.67
Greendale Cemetery (WI)	54	0.95	0.05	0.0	1.0	0.29	0.71
Fond Du Lac (WI)	29	1.0	0.0	0.0	1.0	0.98	0.02
Cambridge Avenue (WI)	28	1.0	0.0	0.0	1.0	0.0	1.0
Perkins Property (WI)	26	1.0	0.0	0.0	1.0	0.62	0.38
Lauderdale Lakes (WI)	26	1.0	0.0	0.0	1.0	0.13	0.87
Roehl Co. Park (WI)	26	1.0	0.0	0.0	1.0	1.0	0.0
Riveredge Center (WI)	45	1.0	0.0	0.02	0.98	0.28	0.72
Kletsch Park (WI)	27	1.0	0.0	0.0	1.0	0.50	0.50
Whitnall Park (WI)	29	1.0	0.0	0.0	1.0	0.0	1.0
Jacobus Park (WI)	29	1.0	0.0	0.0	1.0	0.03	0.97
Jacobus II (WI)	18	1.0	0.0	0.0	1.0	0.17	0.83
Honey Creek (WI)	40	0.95	0.05	0.0	1.0	0.25	0.75
Piney River (MO)	28	1.0	0.0	0.0	1.0	0.45	0.55
Bryant Creek (MO)	28	1.0	0.0	0.0	1.0	1.0	0.0
Buttin Rock (MO)	27	1.0	0.0	0.0	1.0	0.35	0.65
Bay Creek Hollow (MO)	28	1.0	0.0	0.0	1.0	0.0	1.0
Belew Hollow (MO)	28	1.0	0.0	0.0	1.0	0.04	0.96
Fall Creek Gorge (IN)	21	0.84	0.16	1.0	0.0	0.48	0.52

[1] Sample size for *AAT-2* is 20 (19 for Fall Creek Gorge); sample size equals *N* for all other loci.

nation treatment using log likelihood ratio tests (Sokal and Rohlf, 1982).

Second, we intensively studied seed production within one population (Kletsch Park) that we believed to be a single clone/genet on the basis of electrophoretic examination (all examined individuals represented one genotype, all were heterozygous for *TPI-1*, and all ramets occurred within one small (20 m^2) area). In this population we examined seed set in over 20,000 florets from 551 inflorescences of 37 individuals evenly sampled within the population. Percent seed set was determined for each individual stem, as well as for the population. Total population size and estimated population seed output were calculated from density data obtained by direct count of stems in three evenly spaced ½ m^2 quadrats.

Electrophoresis—Sampling

We examined electrophoretically 872 individuals representing 23 populations of *Aster furcatus* (Table 1; Fig. 1). An intensive survey was made of one population (Sheboygan Falls) in conjunction with transplantation to remove these plants from a highway construction site. We mapped the locations of 14 major and 29 minor population subdivisions at this site based on discontinuities in the distribution of ramets, and transplanted 685 individual ramets to our experimental garden. A subsample of plants from all subdivisions (317 individuals) was obtained from the garden specimens and examined electrophoretically. Three individuals (representing three different Illinois populations) were grown from rhizomes furnished by Warren Lamboy, University of Illinois. The remainder of material for electrophoretic analysis was collected from living plants in natural populations. At the remaining 19 sites, one fresh, young leaf was removed from each ramet selected in spatially stratified subsamples of the populations (18–54 ramets). Refrigerated leaves kept moist in plastic bags retained excellent enzyme activity for at least seven days.

Electrophoresis—Methods

For all samples, a piece of leaf material (approximately 2–3 cm^2 devoid of midrib tissue) was ground in a chilled mortar with pestle in a pH 7.5 extracting buffer (Gott-

lieb, 1981). Following centrifugation, supernatants were absorbed onto paper wicks and loaded into 12% starch gels. Two drops of 50% sucrose solution added to remaining supernatants prepared samples for polyacrylamide gel electrophoresis.

Aldolase (ALD, EC 4.1.2.13), amylase (AMY, EC 3.2.1.1), glutamate dehydrogenase (GDH, EC 1.4.1.2), leucine aminopeptidase (LAP, EC 3.4.11.1), phosphoglucoisomerase (PGI, EC 5.3.1.9), superoxide dismutase (SOD, EC 1.15.1.1), and triosephosphate isomerase (TPI, EC 5.3.1.1) were resolved using a pH 8.3 lithium-borate buffer system (Gottlieb, 1981). Isocitrate dehydrogenase [$NADP^+$] (IDH, EC 1.1.1.42), malate dehydrogenase (MDH, EC 1.1.1.37), 6-phosphogluconate dehydrogenase (6-PGDH, EC 1.1.1.44), and phosphoglucomutase (PGM, EC 2.7.5.1) were resolved using a pH 6.5 histidine citrate system (Cardy et al., 1981). Aspartate aminotransferase (AAT, EC 2.6.1.1) was resolved on polyacrylamide using a tris-glycine discontinuous buffer system (Davis, 1964).

Staining procedures for all enzymes followed Soltis et al. (1983) using agarose overlays for ALD (and SOD), IDH, PGI, TPI, 6-PGDH, and PGM. Genetic interpretations of banding patterns were inferred from known isozyme numbers in diploid plants and the active subunit structure of each enzyme (Crawford, 1983).

Allelic frequencies were used to calculate genetic identities and distances (Nei, 1972), gene diversity statistics, and *F* statistics. Total gene diversity in all populations sampled (H_T) and gene diversity within individual populations (H_S) (Nei, 1973; Nei and Chesser, 1983) were calculated using version 2.1 of GENESTAT-PC (Lewis and Whitkus, 1989). We calculated *F* statistics using the program DIPLOID.FOR in Weir (1990). The proportion of polymorphic loci (*P*, where a locus was defined as polymorphic if the frequency of the most common allele was <0.95), mean number of alleles at polymorphic loci (K_p), mean number of alleles/locus (K), and observed heterozygosity (H_o, scored by direct count) were calculated. Deviation of heterozygote frequencies from those expected at polymorphic enzyme loci was assessed using the disequilibrium coefficient (D_A) (Weir, 1990). Significance of departures from Hardy-Weinberg equilibrium was tested using Fisher's Exact Test (Weir, 1990) using tables published in Vithayasai (1973). The Sheboygan Falls population was spatially divided into several large, clonal patches. We calculated disequilibrium coefficients for each large patch in the Sheboygan Falls population, and for each of 20 individual populations. In the latter analysis, the number of individuals and observed genotypes in the Sheboygan Falls population were recalculated to reflect the minimum possible number of genets per patch (based on electrophoretic data) to reduce bias imparted from resampling clonally derived ramets. In the Sheboygan Falls population, ramet genotypes for *TPI-1* were mapped in patches, and phenotype frequencies were assessed for deviation from expected (Hardy-Weinberg) values within large scale patches. The three Illinois populations were excluded from statistical analyses because of inadequate sample sizes.

Results

Genetic Incompatibility

Seed set in bagged, cross-pollinated heads (50.7%) was somewhat greater than seed set in heads that were open-pollinated by insects in the research garden (42.7%; $G = 5.59$, $P < 0.05$) indicating that open, insect pollinated heads may have been slightly pollen limited. Seed set in bagged, self-pollinated heads was greatly reduced (0.74%; $G = 2998$, $P < 0.001$), demonstrating a functional, genetic self-incompatibility mechanism. Seed set of individual ramets ranged from 0–1.2%, and there was an overall estimated seed set of only 0.35% in an open-pollinated, putative clonal, natural population (Kletsch Park). Average stem density at this site was 144 stems/m^2, which yields an estimate of 2,880 stems within the 20 m^2 site. With an average floret number/stem of 549, the number of florets in the population approached 1.5 million in 1989. The average seed set of 0.35% would have produced approximately 5,500 (selfed) seeds in this clonal population in 1989.

Genetic Variation

Twelve enzymes, putatively coded by 22 genetic loci, were resolved by electropho-

TABLE 2. Sample sizes (N), proportion of polymorphic loci (P), mean number of alleles per polymorphic locus (K_P), mean number of alleles per locus (K), and mean observed loci heterozygous per individual (H_O) in 20 populations of *Aster furcatus* from Wisconsin (WI), Missouri (MO), and Indiana (IN).

Population	N	P	K_P	K	H_O
Sheboygan Falls (WI)	317	0.046	2.000	1.046	0.022
Bachman Woods (WI)	18	0.048	2.000	1.048	0.016
Greendale Cemetery (WI)	54	0.091	2.000	1.091	0.021
Fond Du Lac (WI)	29	0.000	—	1.046	0.002
Cambridge Avenue (WI)	28	0.000	—	1.000	0.000
Perkins Property (WI)	26	0.046	2.000	1.046	0.040
Lauderdale Lakes (WI)	26	0.046	2.000	1.046	0.012
Roehl Co. Park (WI)	26	0.000	—	1.000	0.000
Riveredge Center (WI)	45	0.046	2.000	1.091	0.025
Kletsch Park (WI)	27	0.046	2.000	1.046	0.046
Whitnall Park (WI)	29	0.000	—	1.000	0.000
Jacobus Park (WI)	29	0.046	2.000	1.046	0.003
Jocobus II (WI)	18	0.046	2.000	1.046	0.015
Honey Creek (WI)	40	0.091	2.000	1.091	0.023
Piney River (MO)	28	0.046	2.000	1.046	0.028
Bryant Creek (MO)	28	0.000	—	1.000	0.000
Buttin Rock (MO)	27	0.046	2.000	1.046	0.022
Bay Creek Hollow (MO)	28	0.000	—	1.000	0.000
Belew Hollow (MO)	28	0.046	2.000	1.046	0.003
Fall Creek Gorge (IN)	21	0.091	2.000	1.091	0.030

resis. Five of 20 populations surveyed were completely fixed at all loci. Two alleles for *AAT-2* were observed in three populations (Greendale, Honey Creek, Fall Creek Gorge); two alleles for *ALD-1* were observed in the Riveredge population (Table 1). In all of these populations, the rare *AAT-2* or *ALD-1* alleles were present in only one to three sampled individuals. The Fall Creek Gorge, Indiana population was fixed for the rare *ALD-1* allele. Two alleles for *TPI-1* were observed in 15 populations; the remaining loci were fixed for one allele in all other populations. Heterozygosity was detected only at *TPI-1*. Variation at isozyme loci in *Aster furcatus* is very low with $P = 0.000$ to 0.091, $K_p = 2.00$, $K = 1.000$ to 1.091, and $H_o = 0.000$ to 0.046 (Table 2). Gene diversity statistics (Table 3) indicate that total genetic diversity across all sampled loci is very low ($H_T = 0.027$). Much of the genetic variation at the polymorphic *TPI-1* locus is due to differentiation among populations ($F_{ST} = 0.430$). Within populations, there tends to be an excess of heterozygosity ($F_{IS} = -0.254$).

Disequilibrium coefficients measuring departure from Hardy-Weinberg equilibrium in subpopulations at the Sheboygan Falls site span a range from -0.250 to $+0.059$ and 4 of the 14 patches were fixed for a single allele (Table 4). Overall, there was significant differentiation among patches at the *TPI* locus ($F_{ST} = 0.279$) and there was an excess of heterozygosity within patches ($F_{IS} = -0.3061$). Disequilibrium coefficients calculated for populations ranged from -0.250 to $+0.068$ and deviated significantly from expected values at only three sites (Table 5). All but two of the populations not fixed for one *TPI* allele had an excess of heterozygotes. Nine of the 20 populations had both TPI alleles but lacked one of the homozygous genotypes in our sample (Table 5). Mean genetic identities are high among 14 Wisconsin populations ($I = 0.990$), 5 Missouri populations ($I = 0.985$), and among all 20 populations ($I = 0.986$).

DISCUSSION

Breeding System.—Multiallelic, sporophytic, self-incompatibility is common in the family Asteraceae (Brewbaker, 1957; Burtt, 1977; Richards, 1986) and has been found in all 11 species of *Aster* surveyed by Fryxell (1957). The very low seed set in self-pollinated heads and within an isolated clone of *Aster furcatus* is evidence for a strong

TABLE 3. Gene diversity and F statistics for 20 Wisconsin (WI), Missouri (MO), and Indiana populations of *Aster furcatus*. The Indiana population is included only in the all populations (ALL) group. H_T is the total gene diversity in all populations and H_S is gene diversity within populations (Nei, 1973). F statistics and their standard deviations were calculated by jackknifing over populations according to the methods in Weir (1990). All loci other than *TPI-1* had insufficient variation to reliably estimate F statistics.

Locus group	H_S	H_T	F_{IT} (SD)	F_{ST} (SD)	F_{IS} (SD)
TPI-1 (WI)	0.2703	0.4574	0.236 (0.217) ns	0.406 (0.157)*	−0.288 (0.140)*
TPI-1 (MO)	0.2091	0.4659	0.680 (0.403) ns	0.716 (0.357)*	−0.132 (0.098) ns
TPI-1 (ALL)	0.2670	0.4630	0.286 (0.165) ns	0.430 (0.123)**	−0.254 (0.102)*
ALL (WI)	0.0131	0.0216			
ALL (MO)	0.0095	0.0212			
ALL (ALL)	0.0133	0.0268			

NS, F statistic not significantly different than 0.0, $P > 0.05$.
* $0.01 < P < 0.05$.
** $P < 0.01$.

genetic self-incompatibility mechanism in this species as well. Many outcrossing, self-incompatible plants also reproduce vegetatively, allowing for facultative generation of variation (via outcrossing) or uniformity (via vegetative reproduction) (Briggs and Walters, 1984). With *A. furcatus* capable of producing variable progeny through outcrossing and uniform progeny through vegetative reproduction, it is paradoxical that the species is threatened with extinction.

For *Aster furcatus,* a major impact of severe bottlenecks may be the scarcity of different "mating types" due to the loss of incompatibility alleles that typically occur in low frequencies. In other Asteraceae, small, isolated populations of *Carthamus* contain only six to eight alleles (Imrie et al., 1972), fewer than those usually reported for self-incompatible plants. One population of *Hymenoxys acaulis* var. *glabra* (Asteraceae) was found to consist entirely of a single mating type (De Mauro, 1989, unpubl. in Barrett and Kohn, 1991). In comparison, popula-

TABLE 4. Deviations from expected heterozygote frequencies at the triosephosphate isomerase locus (*TPI-1*) in 14 subpopulations of *Aster furcatus* (Sheboygan Falls population). The disequilibrium coefficients (D_A), measuring departures from Hardy-Weinberg equilibrium, and their standard errors were computed following Weir (1990). Significance of D_A was tested using tables in Vithayasai (1973). F statistics and their standard deviations were calculated by jackknifing over subpopulations according to the methods in Weir (1990).

Subpopulation (N)	Phenotype frequency			D_A(SE)	Significance of D_A
	AA	AB	BB		
1 (4)	0	0	1.000	0 (0)	—
2 (11)	0	1.000	0	−0.250 (0)	NS
3 (22)	0.955	0	0.045	0.043 (0.0086)	*
4 (22)	0	0.727	0.273	−0.132 (0.0074)	**
5 (30)	0	0.900	0.100	−0.202 (0.0045)	**
6 (11)	0	0.818	0.182	−0.167 (0.0143)	NS
7 (72)	0.042	0.180	0.778	0.024 (0.0022)	NS
8 (30)	0	1.000	0	−0.250 (0)	**
9 (44)	0.227	0.545	0.227	−0.23 (0.0057)	NS
10 (14)	1.000	0	0	0 (0)	—
11 (4)	0	0	1.000	0 (0)	—
12 (21)	0.333	0.381	0.286	0.059 (0.0115)	NS
13 (4)	0	0	1.000	0 (0)	—
14 (28)	0.179	0.571	0.250	−0.037 (0.0088)	NS

F_{IT} (SD) = 0.0672 (0.256) NS
F_{ST} (SD) = 0.2787 (0.132)*
F_{IS} (SD) = −0.3061 (0.175) NS

NS, $P > 0.05$.
* $0.01 < P < 0.05$.
** $P < 0.01$.

TABLE 5. Deviations from expected heterozygote frequencies at the triosephosphate isomerase locus (*TPI-1*) in 20 populations of *Aster furcatus*. The disequilibrium coefficients (D_A), measuring departures from Hardy-Weinberg equilibrium, and their standard errors were computed following Weir (1990). Significance of D_A was tested using tables in Vithayasai (1973). *H*, expected frequency of heterozygotes.

Population	*N*	Phenotype frequency			*H*	D_A (SE)	*P*
		AA	AB	BB			
Sheboygan Falls	26	0.231	0.346	0.423	0.481	0.068 (0.0091)	NS
Bachman Woods	18	0.167	0.333	0.500	0.444	0.056 (0.0127)	NS
Greendale Cemetery	54	0.056	0.463	0.481	0.409	−0.027 (0.0035)	NS
Fond Du Lac	29	0.966	0.034	0.0	0.034	−0.000 (0.0001)	NS
Cambridge Avenue	28	0.000	0.000	1.000	0.0	0 (0)	NS
Perkins Property	26	0.115	0.885	0.0	0.493	−0.196 (0.0054)	**
Lauderdale Lakes	26	0.0	0.269	0.731	0.233	−0.018 (0.0023)	NS
Roehl Co. Park	26	1.000	0.0	0.0	0.0	0 (0)	NS
Riveredge Center	45	0.0	0.556	0.444	0.401	−0.077 (0.0031)	*
Kletsch Park	27	0.0	1.000	0.0	0.500	−0.250 (0)	**
Whitnall Park	29	0.0	0.0	1.000	0.0	0 (0)	NS
Jacobus Park	29	0.0	0.069	0.931	0.067	−0.001 (0.0003)	NS
Jacobus II	18	0.0	0.333	0.667	0.278	−0.028 (0044)	NS
Honey Creek	40	0.0	0.500	0.500	0.375	−0.062 (0.0031)	NS
Piney Creek	28	0.143	0.607	0.250	0.494	−0.056 (0.0085)	NS
Bryant Creek	28	1.000	0.0	0.0	0.0	0 (0)	NS
Buttin Rock	27	0.111	0.481	0.407	0.456	−0.013 (0.0083)	NS
Bay Creek Hollow	28	0.0	0.0	1.000	0.0	0 (0)	NS
Belew Hollow	28	0.0	0.071	0.929	0.069	−0.001 (0.0003)	NS
Fall Creek Gorge	21	0.142	0.667	0.191	0.499	−0.084 (0.0112)	NS

NS, $P > 0.05$.
* $0.01 < P < 0.05$.
** $P < 0.01$.

tions of multiallelic self-incompatible plant species that have not experienced bottlenecks are typically observed to contain many *S* alleles. East and Yarnell (1929) detected 15 allelomorphs in a population of only 16 individuals of *Nicotinana alata*. Populations of *Trifolium* species can contain up to 175 different *S* alleles, reaching levels of 93% allelic difference at the *S* locus (Lewis, 1949; Whitehouse, 1950). A summary of sporophytic multiallelic self-incompatible plants reported a range of 13-32 *S* alleles/population (Karron et al., 1990). In many natural populations such as clover, the abundance of different *S* alleles results in nearly 100% cross-compatibility (Atwood, 1944; Whitehouse, 1950). A notable exception occurs in the rare endemic *Oenothera organensis* which contains at least 45 different *S* alleles (Emerson, 1940) but only an estimated 5,000 surviving individuals (Levin et al., 1979).

Unlike species dispersed by multiple-seeded fruits (Karron et al., 1990), the single-seeded achenes of *Aster* promote founder populations that originate with only two *S* alleles. Several of the widely spaced populations representing the historical distribution of *Aster furcatus* (Fig. 1) may have been founded by single fruits, the only apparent method of long-distance dispersal in the species. The large number of populations in our sample which had both *TPI-1* alleles, but lacked one of the homozygous genotypes, suggests that many populations were founded by one or a small number of individuals, were sustained by vegetative growth of those individuals, and underwent little or no sexual reproduction within the populations. Sexual reproduction within populations would rapidly produce both homozygous genotypes. As Baker (1955) indicated, self-incompatibility creates a formidable barrier to sexual reproduction in such instances. In the case of *A. furcatus*, founding populations would be likely to develop initially as extensive clones due to self-incompatibility. The very small level of self-compatibility observed in one clonal population allowed for the production of approximately 5,500 selfed seeds in 1989, the only seed produced for dispersal. In this

respect, selfing may be an important mode of sexual reproduction in *A. furcatus* despite its genetic incompatibility. Self-incompatible species such as *A. furcatus,* however, predictably carry a high genetic load which would be expressed during occasional inbreeding and establishment of selfed seeds. The long lifespan of *A. furcatus* genets, achieved through their ability to grow clonally, has undoubtedly helped to forestall extinction of the species.

Although self-incompatible plants encountering severe bottlenecks would experience attenuated seed set by loss of *S* alleles, Schemske and Lande (1985) observed that few species are fixed completely for outcrossing, and selection for selfing may arise following population bottlenecks or colonization events (Lewis, 1973; Berry and Calvo, 1989). Indeed for some Asteraceae, increased self-incompatibility (up to 60% seed set) has been observed in individuals of "self-incompatible" montane species having small population sizes and pollinator limitation (Berry and Calvo, 1989). More intensive studies of *A. furcatus* populations are necessary to ascertain the extent of, and variation in, self-compatibility in this species.

Population structure.—Our electrophoretic study of *Aster furcatus* demonstrated widespread fixation, and that a high proportion of the existing genetic diversity was interpopulational (Tables 1, 2, 3). Although unusual for an outcrossing, self-incompatible species (Loveless and Hamrick, 1984), we interpret this pattern to be the result of genetic bottlenecks associated either with the early history of the species or a subsequent catastrophic population reduction. The loss of alleles and high fixation are attributable to drift and inbreeding accompanying early phases of recovery from one or more bottlenecks. At *TPI-1* (the only sampled locus displaying heterozygosity), however, most populations have genotype frequencies expected for an outcrossed plant. Only three of 19 populations (Table 5) deviated significantly from expected heterozygote frequencies at this locus. Heterozygote frequencies at *TPI-1* indicate that what sexual reproduction exists in extant populations is mostly due to outcrossing.

The mean number of flower heads per shoot on plants grown under a 50% shade lattice in our research garden was only 40% of that of plants grown in the full sun (383 full sun vs. 154 50% shade) (Reinartz and Les, unpubl.). This result is pertinent because most natural populations are found in heavily shaded forest habitats where we found the mean number of flower heads per shoot to range only from 1.1 to 1.9. In contrast, the mean number of heads per shoot in patches of *A. furcatus* from sunny field locations ranges from 12 to 35. Many of the populations we studied had some patches found in the full sun (forest edges, disturbances, etc.) where most of the seed was produced.

In the Sheboygan Falls population, patch 14 existed in a sunny field habitat just outside the forest edge. Patch 12 was partly shaded and at the forest edge. Nearly all of the seed produced in the Sheboygan Falls population in 1988 originated from these two patches. Because genotype frequencies and heterozygote frequencies for *TPI-1* did not differ from expected in these patches, we conclude that these sun patches represent several different genets. As we sampled patches further into the woods from the forest edge, however, deviations from expected genotype frequencies indicated that many of the forest patches represented single, clonally spread, genets. These shaded patches probably originated from seed produced in the sun patches.

Genotype frequencies in the Sheboygan Falls population do not differ significantly from Hardy-Weinberg equilibrium. Within this population, clonal growth undoubtedly influences the population structure at fine (subpopulational) scale comparisons, yet the genotype frequencies of the population as a whole (and most other populations as well) approximated equilibrium conditions. Significant deviations from Hardy-Weinberg equilibrium were characteristic of clonal populations such as Kletsch Park where all plants sampled were heterozygous at *TPI-1,* and likely represented resampled ramets of a single genet (Table 5). Although at one locus (*TPI-1*), a major influential component of the total genetic variation reflects interpopulational differences (Table 3), the overall genetic identity of populations is extremely high, an indication of their rela-

tively recent dispersion. We conclude that all the populations we sampled have originated fairly recently from a common homogeneous gene pool.

The importance of maintaining a rich pool of *S* alleles in self-incompatible species is obvious. Although it is a common practice to study only the allelic variation component of imperiled plant populations (typically by electrophoretic techniques), a result of low variation may lead to inappropriate conservation recommendations. Low electrophoretically detectable genetic variation and homozygosity may suggest that it is unnecessary to conserve a diverse sample of individuals from different populations because of their apparent similarity. In multiallelic incompatible species such as *Aster furcatus,* the overall low genetic variability reveals little of the allelic diversity at the *S* locus. For such species, it may be advisable to introduce new *S* alleles into some genetically depauperate populations in order to increase the number of mating types and promote the restoration of outcrossing potential in the population. This practice has been implemented successfully in at least one case involving *Hymenoxys acaulis* var. *glabra* (De Mauro, unpubl., in Barrett and Kohn, 1991). Low levels of seed set observed in experimentally crossed plants of *Aster furcatus* (50.7%, this study) and several *Espeletia* species (Berry and Calvo, 1989) may reflect an inadequate pool of *S* alleles necessary for attainment of 100% seed set in recovering populations.

It is evident from this discussion that observed low levels of electrophoretically detectable genetic variation in rare plants may not adequately reflect the evolutionary importance of preserving a large number of individuals within and among populations of self-incompatible species. In such instances, the extermination of even a few populations or individuals within populations may destroy rare *S* alleles essential for the recovery of full fecundity of individuals in a population.

Acknowledgments

The authors thank D. Crawford, A. Jones, and W. Lamboy for their helpful advice on various aspects of this project. We thank B. Weir for advice on statistical analysis and for providing a copy of his program DIPLOID.FOR. We are grateful to R. Barloga, R. Dolan, N. Harriman, J. Kline, L. Leitner, L. Nelson, S. Remley, J. Wallace, and M. Wallace for field related assistance, and to T. Schuck for his care of greenhouse material. This work was supported by a grant to J.A.R. and D.H.L. from the Wisconsin Department of Transportation through recommendation of S. Holtz and the Wisconsin Department of Natural Resources.

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