American Journal of Botany 80(3): 351–359. 1993.

MORPHOLOGICAL AND GENETIC VARIABILITY IN PLANTAGO CORDATA (PLANTAGINACEAE), A THREATENED AQUATIC PLANT[1]

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Morphological and genetic variation were studied in *Plantago cordata* Lam., an imperiled aquatic plant. The existence of distinct seasonal morphs was confirmed by numerical morphological analyses of specimens from 53 populations. Plants from two North Carolina populations possessed leaves that were significantly shorter, narrower, and fewer-veined than populations in other portions of the species range. Genetic variation within and among ten populations in seven states/provinces (Illinois, Missouri, New York, North Carolina, Ohio, Ontario, and Wisconsin) was examined electrophoretically. The species is an apparent allopolyploid with fixed heterozygosity observed at 67% of the loci surveyed. Electrophoretic variation was mostly partitioned among populations. The genetic identity among populations was high except for North Carolina populations which differed at 27% of the loci surveyed.

Many factors can influence the likelihood of extinction for a given plant species such as the historical range, habitat specificity, and local abundance of individuals. Wide-ranging species with restricted habitat requirements often become endangered due to habitat destruction (Rabinowitz, 1981). Species existing as small, isolated populations can lack adequate interpopulational gene flow to maintain genetic polymorphisms and may diverge genetically (Beardmore, 1983; Chesser, 1983). Populations encountering bottlenecks may lose a significant amount of genetic variation due to inbreeding and drift (Nei, Maruyama, and Chakraborty, 1975; Frankel and Soulé, 1981; Clegg and Brown, 1983; Waller, O'Malley, and Gawler, 1987; Barrett and Kohn, 1991; Les, Reinartz, and Esselman, 1991).

The continued survival of a species may depend on the maintenance of evolutionary potential through preservation of genetic diversity throughout its range (Lewontin, 1974; Gottlieb, 1977; Beardmore, 1983; Namkoong, 1983; Carson and Templeton, 1984). Reduced genetic variation has been implicated as a factor preventing the response of rare species to environmental changes, thereby increasing their likelihood of extinction (Drury, 1974; Beardmore, 1983). Understanding the genetic composition and dynamics of small, fragmented populations is essential for developing plans for their management and recovery (Lacy, 1988).

The extent of genetic variation in the heartleaf plantain (*Plantago cordata* Lam.) has been studied previously by morphological approaches (Tessene, 1969; Primack, 1980) and common garden experiments (Meagher, Antonovics, and Primack, 1978). Tessene (1969) suggested that there was little genetic variation in *P. cordata,* and concluded that the species was adapted to a specialized biological niche. A common garden study of North Carolina populations, however, indicated that genetic variation existed both within and among populations (Meagher, Antonovics, and Primack, 1978). Primack (1980) concluded that levels of phenotypic variation in *P. cordata* were similar to those of widespread plantain species.

We have attempted to clarify patterns of variation in *Plantago cordata* by conducting further morphological and genetic analyses of the species using numerical and electrophoretic techniques. Here we report on the results of these studies and provide recommendations for the conservation and management of this imperiled plant.

MATERIALS AND METHODS

Morphological analyses — We examined 279 herbarium specimens collected throughout the range of the species. Measurements of leaf length (mm), leaf width (mm), leaf vein number, and petiole length (mm) were made on 885 leaves; 503 measurements of floral spike length (mm) were made. Data were also taken from 18 live specimens from two North Carolina populations that were studied previously, but represented by only one herbarium collection. Plants from populations in Davidson Co., North Carolina were grown from seed kindly provided by J. Antonovics and B. Best. One population (designated NCA) was collected on NC 47, 3.5 miles northwest of Denton (35°42′N, 80°10′W); a second population (NCB) was collected from the north edge of Uwharrie National Forest (35°32′N, 80°10′W). In all cases, winter and summer leaf forms were determined by their position on the plant where there is a clear shift between smaller, compact whorls of winter leaves and larger, looser rosettes of summer leaves.

Measurements from 903 leaves comprised the operational taxonomic units (OTUs) used in initial analyses.

[1] Received for publication 18 June 1992; revision accepted 10 November 1992.

The authors thank J. Antonovics, B. Best, M. Bowles, J. Brown, D. DeSteven, T. Ehlinger, S. Gunn, M. Hamoya, K. Heidmann, J. Karron, J. Knoop, M. Oldham, J. Reinartz, T. Schuck, T. Smith, D. Sheridan, F. Stearns, W. Wagner, A. Weakley, C. Williams, J. Witebsky, S. Young, and P. Zika for various assistance; the curators of herbaria (BGSU, BLH, BUT, CM, DUKE, DAO, GH, ILL, ISC, MICH, MIL, MIN, MO, NY, OS, PH, UARK, US, UWM, WIS) from which we borrowed specimens; and M. J. Spring for assistance with the illustrations. This study represents part of a M.S. thesis completed in 1991 by the senior author at the University of Wisconsin–Milwaukee.

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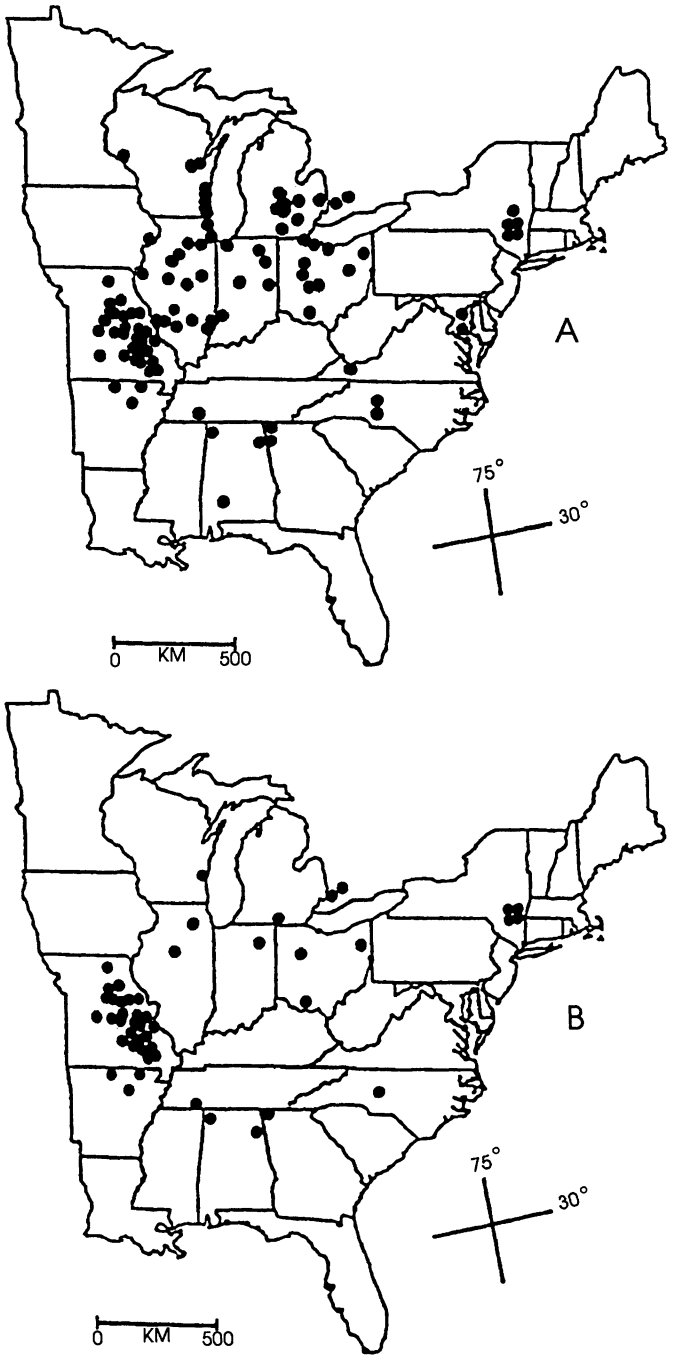

Fig. 1. Distribution maps of *Plantago cordata*. A. Since 1845. B. Current (documented as extant).

Petiole and floral spike length data were excluded because many specimens had been altered to facilitate mounting. Localities and collection dates were recorded for all OTUs. Data were analyzed using the SYSTAT software package (Wilkinson, 1988). Variables were standardized and a matrix of euclidean distances was calculated. Distance values were used in an average linkage cluster analysis (UPGMA) to produce a dendrogram. Means and ranges of morphological characters were calculated. "Whole plant" averages (rounded to the nearest 0.5 cm) were then determined for each character. We analyzed a subset of 53 plants that represented major historic populations. The resulting matrix included 95 OTUs (53 plants, divided into winter and summer leaf averages). Data were standardized and analyzed as above.

To explore other possible clustering associations, we recoded data matrix labels to identify OTUs representing NCA and NCB populations, winter morphology, and summer morphology. Data were analyzed by a one-way ANOVA with adjustment for unequal sample sizes, and a Tukey HSD post-hoc test was made. We also performed a discriminant analysis to determine clustering relationships of North Carolina OTUs and OTUs representing winter and summer morphologies.

Electrophoresis—Suitable activity for electrophoresis was obtained using young flower spikes (1.0–1.5 cm long), fresh leaves of field-collected plants (central leaves of early spring rosettes), and leaves from plants (less than 12 weeks old) raised from seed. Electrophoretic phenotypes were consistent across these tissue types. To minimize damage to field plants, only small ramets were collected (with appropriate permits).

In addition to field material collected from Illinois, Missouri, New York, Ohio, and Wisconsin, we electrophoresed seed-grown plants from two North Carolina populations (described above) and one Ontario population (Illinois plants and seeds from Ontario plants were generously provided by M. Bowles). All plants were grown and overwintered under uniform conditions in the University of Wisconsin–Milwaukee greenhouse, and were later transplanted to an outdoor coldframe. We also electrophoresed progeny (N = 22) resulting from self-pollinated spikes (Milwaukee, WI population).

Plant tissue was ground in 0.2 ml of extracting buffer (pH 7.5, 0.25 ml 2-mercaptoethanol) (Gottlieb, 1981) to which 5 mg of polyvinylpyrrolidone (MW 40,000) was added during grinding.

Horizontal starch gel electrophoresis incorporated two buffer systems and provided consistent activity and resolution for ten enzymes using standard procedures (Kephart, 1990). A total of 219 plants from ten populations was surveyed with seven to 37 plants sampled per population.

Six enzymes, aminoaspartate transaminase (AAT, EC 2.6.1.1), leucine aminopeptidase (LAP, EC 3.4.1.1), aldolase (ALD, EC 4.1.2.13), phosphoglucosisomerase (PGI, EC 5.3.1.9), triose phosphate isomerase (TPI, EC 5.3.1.1), and superoxide dismutase (SOD, EC 1.15.1.1), were resolved using a discontinuous lithium-borate system, pH 8.3 (Gottlieb, 1981); runs were made at a constant current of 50 ma. A continuous histidine-citrate system, pH 6.5 (Cardy, Stuber, and Goodman, 1981), was used to resolve isocitrate dehydrogenase (IDH, EC 1.1.1.27), phosphoglucomutase (PGM, EC 2.7.5.1), 6-phosphogluconate dehydrogenase (6-PGDH, EC 1.1.1.44), and malate dehydrogenase (MDH, EC 1.1.1.37); runs were made at a constant voltage of 100 V. Agarose overlays were used to stain for all enzymes except AAT, LAP, and MDH.

We calculated gene frequencies for all loci and used the program GENESTAT-PC, version 2.1 (Lewis and Whitkus, 1989), to calculate Nei's gene diversity statistics and genetic identities (Nei, 1972, 1973). Two analyses were

TABLE 1. *Historic and surviving sites (by county) of* Plantago cordata *based on herbarium specimen data*

State/Province	Historic	Present	% Lost
Alabama	4	2	50
District of Columbia	1	0	100
Georgia	4	1	75
Illinois	20	2	90
Indiana	6	1	86
Iowa	1	0	100
Maryland	1	0	100
Michigan	8	1	88
Missouri	30	28	15
New York	6	3	50
North Carolina	2	1	50
Ohio	9	3	67
Virginia	2	0	100
Wisconsin	7	1	86
Ontario, Canada	3	2	33
Total	104	45	57

TABLE 2. *Morphological variation among summer and winter seasonal morphs, and North Carolina populations (NCA, NCB) of* Plantago cordata

		N	Range	Mean	(SD)[a]
Leaf length (cm)	Summer	530.0	5.0–77.0	12.5	(5.1)a
	Winter	252.0	0.8–10.0	5.8	(1.7)b
	NCA	52.0	1.5–14.0	5.6	(2.8)b
	NCB	69.0	3.5–16.5	9.4	(3.4)c
	All[b]	903.0	0.8–77.0	10.0	(5.2)
Leaf width (cm)	Summer	530.0	4.0–22.0	8.3	(3.3)a
	Winter	252.0	0.5–6.0	2.9	(1.2)b
	NCA	52.0	0.5–7.5	2.8	(1.7)b
	NCB	69.0	1.0–11.0	4.9	(2.3)c
	All[b]	903.0	0.5–22.0	6.2	(3.7)
Nerve number	Summer	530.0	5.0–13.0	7.3	(1.2)a
	Winter	252.0	1.0–9.0	5.1	(1.4)b
	NCA	52.0	1.0–7.0	4.3	(1.3)b
	NCB	69.0	3.0–7.0	5.8	(1.1)b
	All[b]	903.0	1.0–13.0	6.4	(1.7)
Spike length (cm)	Summer[b]	412.0	7.0–78.0	35.7	(11.8)
	Winter[b]	96.0	7.0–47.0	24.4	(7.8)
	All[b]	503.0	7.0–78.0	33.7	(12.0)

[a] Values (for each variable) that share the same lowercase letter are not significantly different by ANOVA.
[b] Group excluded from ANOVA.

performed; one coding putatively silenced genes, and one excluding these loci. A genetic identity matrix was used to generate a UPGMA dendrogram.

RESULTS

Distribution and morphology—Supplemented by data obtained from Nature Conservancy records (for Arkansas, Georgia, Indiana, and Tennessee), our survey of herbarium specimens enabled us to summarize the current and historic distribution of *Plantago cordata* (Fig. 1). The percent of site extirpation by county varied from 15% to 100% (Table 1). We estimate that 57% of the historic sites for the species have been lost.

The ranges, means, and standard deviations for leaf length, width, and nerve number for "summer," "winter," and North Carolina populations NCA and NCB are summarized in Table 2. Spike length is reported only for summer and winter specimens because North Carolina plants were not flowering at the time of measurement.

The length, width, and nerve number of summer leaves differed significantly from winter, NCA, and NCB categories. NCB differed significantly from summer, winter, and NCA categories for leaf length and width, and from summer for nerve number. There were no significant differences between NCA and winter categories for any of the variables (Table 2).

A UPGMA dendrogram of standardized euclidean distances (Fig. 2) clustered OTUs of NCA among those representing winter morphology, whereas NCB OTUs clustered among those representing summer morphology. The North Carolina herbarium specimen OTU clustered with greenhouse-grown OTUs from NCB. No other geographic trends in the cluster analysis were apparent.

Results of a discriminant analysis indicated that summer OTUs clustered as predicted 94% of the time, with only 2% grouping among NCB OTUs, and 4% grouping with winter OTUs. Winter OTUs were correctly classified 92% of the time with the remaining 8% grouping with NCA OTUs. Correct classifications in the discriminant analysis recognized discrete NCA OTUs in only 30% of the cases. The remaining 70% clustered with winter OTUs and none were reclassified as either summer or NCB OTUs. Only 28.5% of NCB OTUs were correctly classified; others clustered with summer (43%) and winter (28.5%) OTUs. None clustered with NCA OTUs.

Electrophoretic variation—No evidence of segregation was observed for heterozygous banding patterns at the enzyme loci AAT, IDH, MDH, PGI, PGM, and TPI in progeny derived from selfed plants or among individuals sampled in natural populations.

Enzyme phenotypes of loci for AAT-1 (Fig. 3a), ALD-1, ALD-2 (Fig. 3b), IDH-1 (Fig. 3c), LAP-1 (Fig. 3d), PGI-1 (Fig. 3g), PGM-3 (Fig. 3h), SOD-1, and SOD-2 (Fig. 3i) were monomorphic and fixed over all populations surveyed. Gene duplications (monomorphic for all populations) occurred at AAT (involving AAT-2 and AAT-3; Fig. 3a), at IDH (involving IDH-2 and IDH-3; Fig. 3c), at MDH (involving MDH-1 and MDH-2; Fig. 3e), at 6-PGDH (involving 6-PGDH-1 and 6-PGDH-2; Fig. 3f), and at TPI (involving TPI-1 and TPI-2; Fig. 3j). Duplications were evidenced by fixed heterozygosity (all loci) and heterodimer formation (except 6-PGDH, TPI) indicating subunit interaction.

Enzyme phenotypes were variable at nine loci (Table 3). An apparent duplication at MDH (involving MDH-3 and MDH-4) characterizes all populations sampled except for North Carolina plants. The phenotype is fixed, but a heterodimer is absent. Two allozymes were detected at MDH-3; a "slow" electromorph characterizes all populations sampled except NCA and NCB which are fixed for a faster electromorph. MDH-4 is apparently silenced in North Carolina plants.

Phenotypes at the 6-PGDH locus are complex (Fig. 3f), and dosage effects resulting from tetraploidy were apparent. A duplication involving 6-PGDH-3 and 6-PGDH-4 is polymorphic with two allozymes observed at each locus

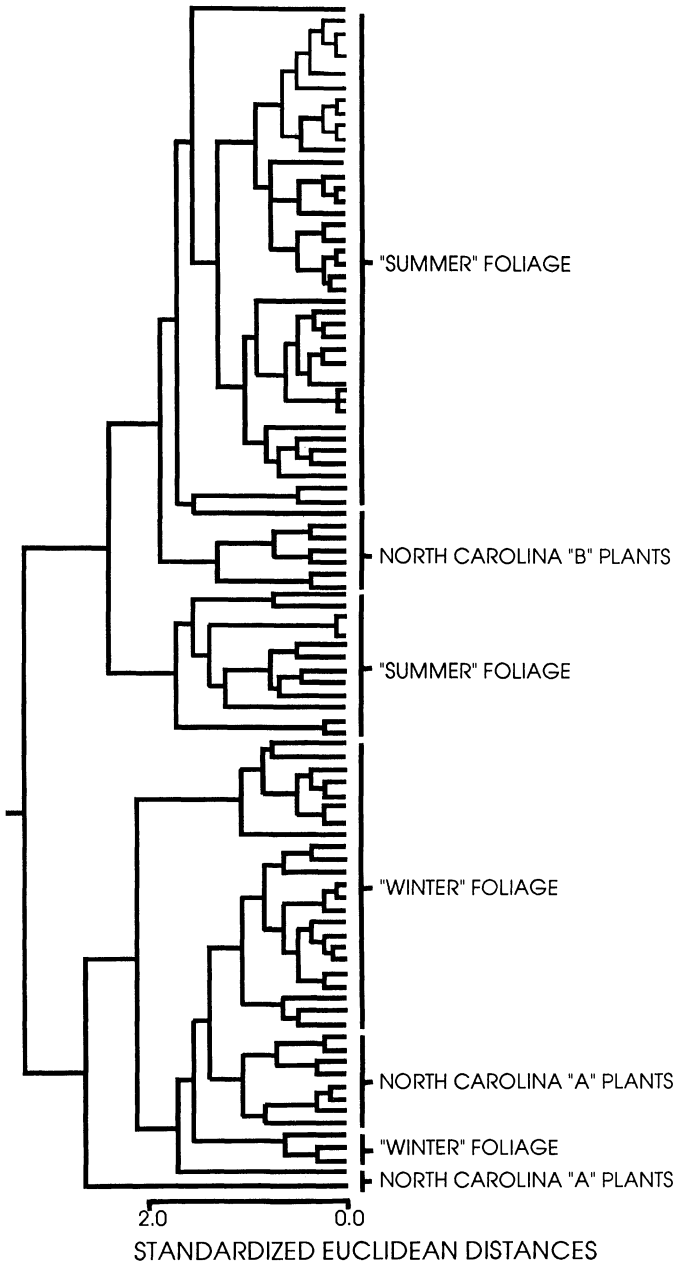

Fig. 2. UPGMA dendrogram of morphological data showing clustering of summer, winter, and North Carolina OTUs (NCA and NCB populations) in *Plantago cordata*.

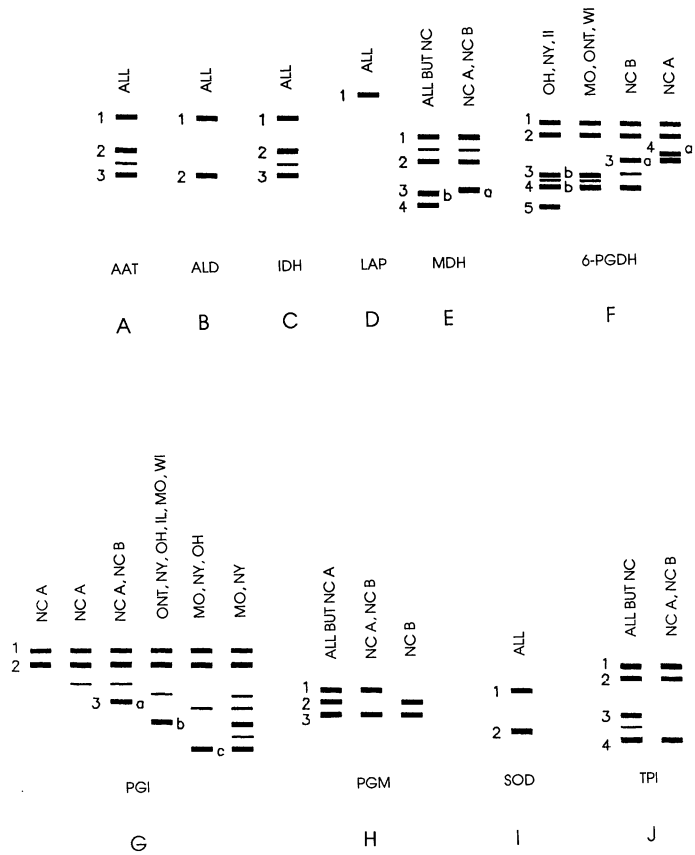

Fig. 3. Isozyme (numbers) and allozyme (letters) phenotypes (thick bands) observed for 30 enzyme loci (abbreviations explained in text) in *Plantago cordata* populations studied (IL = Illinois, MO = Missouri, NY = New York, NCA = North Carolina population A, NCB = North Carolina population B, OH = Ohio, ONT = Ontario, Canada, WI = Wisconsin). Thin bands indicate observed heterodimers.

(Fig. 3f). North Carolina populations possess the "fast" electromorph for 6-PGDH-3, whereas the slower electromorph was fixed in all other populations sampled. NCA also possesses a novel "fast" electromorph for 6-PGDH-4, whereas other populations sampled were fixed for the slower phenotype. A heterodimer occurs between isozymes of 6-PGDH-3 and 6-PGDH-4 in all cases, but is difficult to discern in NCA plants (Fig. 3f). Heterozygosity at 6-PGDH-3 and 6-PGDH-4 is fixed. A fifth locus (possibly representing posttranslational modification) fixed for one allele was consistent but observed only in plants from Illinois, New York, and Ohio.

Complex phenotypes were also observed for PGI (Fig. 3g). PGI-2 was fixed in all populations sampled and represents a duplication involving PGI-3. Three allozymes were detected for PGI-3. The fastest electromorph at PGI-3 was unique to North Carolina plants, but it was null in several individuals from NCA; some individuals exhibited the heterodimer and others lacked evidence of the allele entirely (Fig. 3g). This pattern most likely represents the silencing of the PGI-3 locus in some NCA individuals. Fixed heterozygosity and heterodimer formation characterize this gene duplication in plants from NCA and NCB that do not possess null phenotypes. Populations sampled from Ontario, Illinois, and Wisconsin were fixed for the intermediate electromorph at PGI-3 and exhibited fixed heterozygosity with PGI-2. Populations from Missouri, New York, and Ohio were polymorphic for an intermediate and a slower electromorph at PGI-3 (Table 3). The slow electromorph was common in New York (freq. = 0.8), and rarer in Missouri (freq. = 0.09–0.32) and Ohio (freq. = 0.1) (Table 3). Several New York and Missouri plants exhibited six-banded "hybrid" heterozygous phenotypes at PGI-2/3 that possessed both slow and intermediate allozymes and all (three) respective interacting heterodimers (Fig. 3g).

All populations were fixed at PGM-1 except for NCB where several individuals lacked expression of this locus.

TABLE 3. *Allelic frequencies at variable loci for ten* Plantago cordata *populations.*[a] *Sample size (N) indicated*

Locus/ Alleles		OH (30)	MOL (7)	MOB (22)	WIM (14)	WIO (23)	ONT (25)	ILL (3)	NY (30)	NCA (23)	NCB (37)
MDH-3	*a*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.000	1.000
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.0	0.0
MDH-4	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.0[b]	0.0[b]
6-PGDH-3	*a*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.000	1.000
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.0	0.0
6-PGDH-4	*a*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.000	0.0
	b	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.0	1.000
6-PGDH-5	*a*	1.000	0.0[b]	0.0[b]	0.0[b]	0.0[b]	0.0[b]	1.000	1.000	0.0[b]	0.0[b]
PGI-3	*a*	0.0	0.0[d]	0.0	0.0	0.0	0.0	0.0	0.0	0.909[c]	1.000
	b	0.900	0.680	0.910	1.000	1.000	1.000	1.000	0.200	0.0	0.0
	c	0.100	0.320	0.090	0.0	0.0	0.0	0.0	0.800	0.0	0.0
PGM-1	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.945[c]
PGM-2	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.0[b]	0.675[c]
TPI-3	*a*	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	0.0[b]	0.0[b]

[a] OH = Adams Co., Ohio; MOL = Lincoln Co., Missouri; MOB = Boone Co., Missouri; WIM = Milwaukee Co., Wisconsin; WIO = Ozaukee Co., Wisconsin; ONT = Middlesex Co., Ontario; ILL = Tazwell Co., Illinois; NY = Columbia Co., New York; NCA = Denton, North Carolina; NCB = Uwharrie National Forest, North Carolina.

[b] Locus silenced in all individuals sampled.

[c] Locus silenced in some individuals sampled.

[d] $N = 11$.

PGM-2 was fixed in all populations other than North Carolina. NCA lacked expression of PGM-2 entirely, and NCB contained individuals that both possessed and lacked expression of the locus. PGM-1 and PGM-2 putatively represent a gene duplication that has been silenced in North Carolina populations (this variation does not represent segregation because the heterozygous pattern remained fixed in all selfed progeny). Dosage effects were apparent at both PGM-1 and PGM-2.

TPI-3 was absent in North Carolina populations, but present as a duplication in the eight other populations surveyed where it interacts to form a heterodimer with TPI-4. The lack of expression of TPI-3 in North Carolina populations is attributed to gene silencing.

Allelic frequencies at variable loci detected in the surveyed populations of *Plantago cordata* are summarized in Table 3. Gene silencing occurred at five loci in North Carolina populations, but was not observed in other sampled populations (with the possible exception of 6-PGDH-5). Null phenotypes were consistent and repeatable when individuals were resampled.

A UPGMA dendrogram (Fig. 4) of genetic identities clusters North Carolina populations apart from the remaining eight populations surveyed. Northern populations are very similar ($I = 1.00$–0.96), whereas their identity to North Carolina populations is reduced to 0.79. Although the reduced genetic identity of North Carolina populations is due in part to several silenced loci, it remains substantially lowered even when calculated in exclusion of silenced loci ($I = 0.9936$ for northern populations, $I = 0.9583$ for North Carolina populations, $I = 0.8737$ for northern X North Carolina populations).

Gene diversity statistics (Table 4) indicate that most of the observed electrophoretic variation is partitioned between populations ($D_{ST} = 0.0793$; $G_{ST} = 0.8651$), with little variation within populations ($H_S = 0.0124$). Total gene diversity (H_T) for *Plantago cordata* is 0.0916. When loci including null alleles are excluded from the analysis, the gene diversity statistics change only slightly: $D_{ST} = 0.0488$, $G_{ST} = 0.7804$, $H_S = 0.0137$, and $H_T = 0.0625$.

DISCUSSION

Distribution—The decline of *Plantago cordata* is generally attributed to urbanization (Tessene, 1969; Meagher, Antonovics, and Primack, 1978; Morgan, 1980; Alverson, 1981; Kurz and Bowles, 1981; Bowles and Apfelbaum, 1989). *Plantago cordata* is associated with the occurrence of limestone and dolomitic bedrock (Tessene, 1969) and calcareous clays. Typically, it grows in shallow, clear streams, in springs within cracks of the bedrock, or in gravel (Tessene, 1969). Plants from Milwaukee Co., Wisconsin grow in a silty stream with a mucky bottom over limestone bedrock (Stromberg and Stearns, 1989). Plants in Ozaukee Co. Wisconsin, Missouri, and Ohio occur in more typical sites. These streams are gravel-bottomed, are covered by a thick deciduous canopy, and support few other aquatic plants. A similar habitat is reported for North Carolina populations (Meagher, Antonovics, and Primack, 1978). Plants from Middlesex, Ontario, Canada, grow in calcareous woodland depressions (M. Bowles, personal communication, 1988). Plants in Arkansas and Tennessee grow in calcareous "Black Belt" clay (S. Gunn, Alabama State Conservation and Natural Resources Department, personal communication, 1989). In New York, heart-leaved plantains exist in a different habitat. Here, plants are in open sunlight, occur in slightly brackish water, and are inundated at high tide. Many plants are buried deeply by sand and possess a fine deposit of silty sand on their surfaces.

Historically, *Plantago cordata* has been recorded in 16 states and one Canadian province. Since the earliest record of the plant in 1791, populations have been extirpated in much of the Midwest, although relatively recent lo-

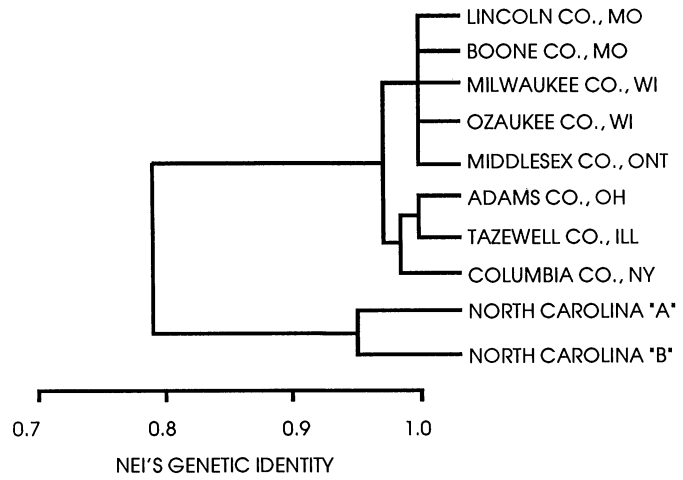

Fig. 4. UPGMA analysis of genetic identities showing relationships among ten *Plantago cordata* populations studied.

calities have been discovered in Arkansas and Tennessee. Herbarium records (Tessene, 1969) indicate that although the heartleaf plantain was widespread historically, it was usually not abundant at any one site.

Our examination of herbarium records documents the extirpation of approximately 57% of the historic localities of *Plantago cordata.* The largest proportion of losses has occurred in the Midwest, a region that has experienced extensive agricultural development. Tessene (1969) attributed the disappearance of the species to siltation, pollution, stream rerouting, logging, and site conversion to cattle pasture. One site (Adams Co., OH) has been fenced to prevent cows from trampling on the plants (J. Knoop, The Nature Conservancy, personal communication, 1988). In contrast to streams that no longer support populations of the plant, sites where *P. cordata* continues to thrive are able to clear quickly of silt after hard rains. The removal of *P. cordata* from consideration as a federally endangered species (B. Harrison, U.S. Fish and Wildlife Service, personal communication, 1989) is disturbing in light of these high losses of populations. Even in Missouri, the state with the most populations, 15% of the sites are extirpated.

Morphological variation—We observed a range in leaf length for plants with "summer" morphology wider than that reported by Tessene (1968, 1969) or Bassett (1967, 1973). Tessene's (1968, 1969) reported range for leaf width was also narrower than what we observed. Nerve numbers reported by Tessene agree closely with our observations (Table 2), although they also represent a narrower range.

Clearly, *Plantago cordata* has two, distinct foliage morphologies corresponding to winter and summer rosettes. Extensive phenotypic variation is evidenced by the wide ranges associated with leaf dimensions. Major groupings in the cluster analysis (Fig. 2) reflect the two seasonal forms; however, few other associations are evident. OTUs identified by geographic location, season of collection, last frost date for the region, and day length showed no correlation with the clustering topology.

North Carolina plants from NCA and NCB are distinct morphologically and also differ from other populations

TABLE 4. *Gene diversity statistics (after Nei and Chesser, 1983), for variable loci and all loci in* Plantago cordata

Locus	H_T	H_S	D_{ST}	G_{ST}
MDH-3	0.3200	0.0000	0.3200	1.0000
MDH-4	0.3200	0.0000	0.3200	1.0000
6-PGDH-3	0.3200	0.0000	0.3200	1.0000
6-PGDH-4	0.1800	0.0000	0.1800	1.0000
6-PGDH-5	0.4200	0.0000	0.4200	1.0000
PGI-3	0.4957	0.1143	0.3815	0.7695
PGM-1	0.0710	0.0615	0.0094	0.1326
PGM-2	0.3019	0.1950	0.1069	0.3542
TPI-3	0.3200	0.0000	0.3200	1.0000
All loci	0.0916	0.0124	0.0793	0.8651

surveyed. NCB plants are more similar to other populations, whereas NCA individuals are smaller overall. In greenhouse-grown progeny from these populations, the distinctive morphologies were apparent even in the seedling stage. Greenhouse-grown plants raised from seed collected in Wisconsin and Ontario did not produce these unusual phenotypes. The herbarium specimen OTU from North Carolina clustered with NCB greenhouse plants.

NCA plants resembled winter rosette plants from other regions (Fig. 5b, d) and grouped mainly with them in cluster analyses, whereas NCB plants resembled summer plants (Fig. 5a, c) and grouped with them in our numerical analyses. NCA plants differ from winter plants, however, by their lack of leaf dentition and their pattern of venation (Fig. 5b, d). Using herbarium specimens to characterize morphological variation in *Plantago cordata* has been useful but not entirely satisfactory due to the inability to ascertain the environmental component of the observed variation. Primack (1980) examined reproductive characteristics; however, some of those features such as seed number may have been subject to environmental influences. Common garden studies using plants collected from all available sites may provide a better method for examining morphological variation within and among populations. Although our morphological analyses did not show discrete differences for all populations studied, they support previous reports of genetic variation in North Carolina plants (Meagher, Antonovics, and Primack, 1978).

Electrophoretic variation—Electrophoresis has been used widely by biologists studying rare species (e.g., Karron, 1987; Waller, O'Malley, and Gawler, 1987; Lesica et al., 1988; Kephart, 1990; Les, Reinartz, and Esselman, 1991). Primack (1980) suggested using enzyme electrophoresis to examine the extent of genetic polymorphism in *Plantago cordata.* Electrophoresis can provide estimates of existing genetic variation and its partitioning within and among populations at local and regional levels. Levels of allozyme variation have also been correlated with breeding systems and geographic range (Hamrick, 1989), allowing for investigation of these aspects in *P. cordata.*

The chromosome number of *Plantago cordata* is $2n = 24$ (Tessene, 1969). With $2n = 12$ known for several plantain species (Tessene, 1969), *P. cordata* is presumably tetraploid. Sixty-seven percent of enzyme loci resolved for *P. cordata* exhibited fixed heterozygosity. Intergenic

hybrids could be distinguished from simple heterozygotes because the allelic markers did not exhibit independent assortment (Gottlieb, 1974). The high level of fixed heterozygosity in *P. cordata* indicates that it is an allopolyploid.

Although the species is strongly protogynous, flowers with mature anthers and receptive stigmas can occur simultaneously on the same spike. Concurrent sex expression also results from different stages of maturity among spikes produced on single individuals. Along with self-compatibility, these factors allow for self-pollination. The ability to self is characteristic of allopolyploids, whereas autopolyploids are generally self-incompatible and outcrossing (Barrett and Shore, 1989). It is difficult to elucidate possible diploid progenitors of the heartleaf plantain because all species in section *Palaeopsyllium* are tetraploid (Bassett, 1967, 1973), none has overlapping flowering times (Harper, 1944; Tessene, 1968), none is aquatic, and none closely resembles the species morphologically (Bassett, 1967, 1973; Tessene, 1968, 1969).

The pattern of genetic variation in *Plantago cordata* is summarized by gene diversity statistics. The low value for H_S (0.0124) indicates little within-population variation in *P. cordata,* whereas the high value of G_{ST} (0.8651) indicates that variation is partitioned mainly between populations. Gene diversity in *P. cordata* should be compared to plants that are sexual, wind pollinated, seasonal, and synchronous in phenology, and long-lived polycarps, but there is a scarcity of gene diversity statistics for other such polyploids. Total diversity ($H_T = 0.0916$) is slightly lower than found by Bayer (1989) for polyploid species of *Antennaria* ($H_T = 0.125$). These *Antennaria* species are outcrossing (dioecious), sexual, insect pollinated, long-lived perennials (Bayer, 1989). Both polyploid *Antennaria* and polyploid *P. cordata* have higher overall G_{ST} values, but lower H_S and H_T values than most diploid species with similar life histories (Loveless and Hamrick, 1984). The high G_{ST} value for *P. cordata* suggests that this species has a much lower level of interpopulational gene flow than many wind-pollinated, outcrossing species (Loveless and Hamrick, 1984). This result may be a consequence of the extremely low reproductive output of *P. cordata* as Primack (1979) reported.

Selfing in *Plantago cordata* (by geitonogamy) may be beneficial in diffuse populations. Ensured seed set by selfing could be advantageous for plants spaced widely along creek banks. "Hybrid" heterozygous phenotypes at PGI-2/3 indicate evidence of outcrossing in Missouri and New York populations, or at least of sexual recombination among genets. Plants in both populations are more densely aggregated, a factor that may promote outcrossing. Interestingly, greenhouse-grown plants with these hybrid PGI phenotypes did not produce flowers at the same rate as the other plants, appeared smaller, and were collected originally from drier, rockier sites.

The observed lack of variation for plants existing north of the glacial boundary indicates founder effects and genetic bottlenecks resulting from known as well as suspected population crashes. Populations from Missouri, Wisconsin, and Ontario cluster with a genetic identity of 1.00 (Fig. 4). These populations occur west of the Appalachians, and other than Missouri, are north of the glacial boundary. Ohio, Illinois, and New York populations group in a second cluster, with the latter (the most different of the plantain habitats) showing the lowest affinity to other northern populations. The slow allozyme for PGI-3 is common in New York but rare or absent in other populations, a probable consequence of drift.

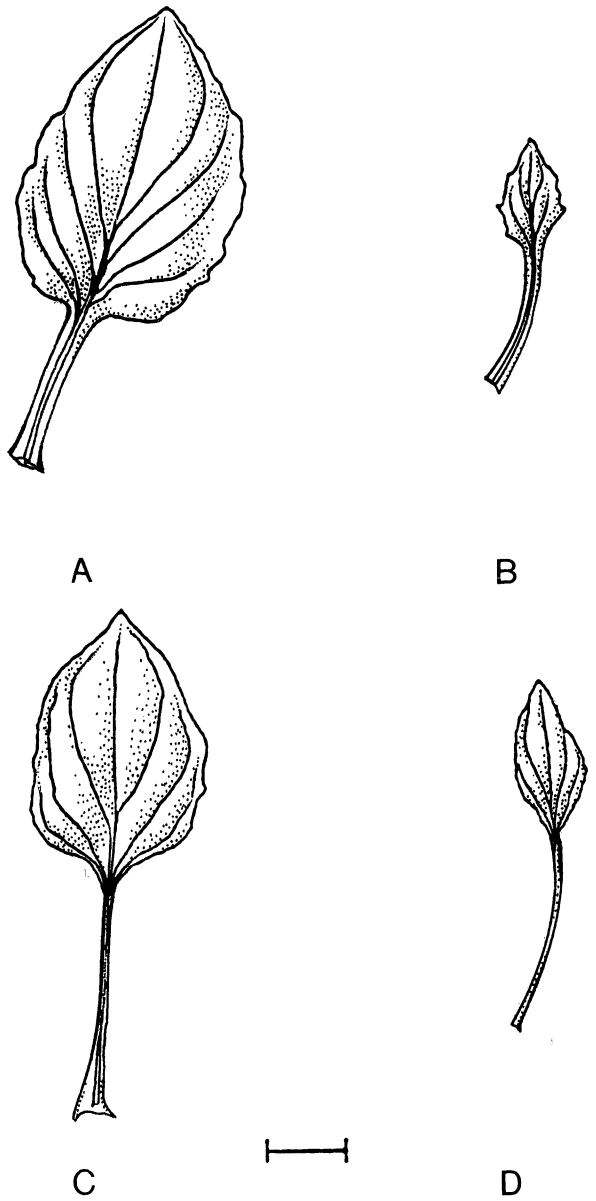

Fig. 5. Representative leaf morphology in *Plantago cordata.* A. Summer foliage. B. Winter foliage. C. North Carolina B population foliage. D. North Carolina A population foliage. A–D drawn from greenhouse specimens (voucher data available on request). Bar = 4 cm.

Plants from the North Carolina populations studied show slight genetic differentiation ($I = 0.96$) but are quite distinct from other populations. These populations contain several novel alleles, null alleles, and silenced loci (Table 3). Gene silencing is considered to be a derived condition, requiring time for gradual divergence (Ohno, 1970; Kephart, 1990). These results indicate that the North Carolina sites that we sampled represent fairly old populations.

It has been suggested that species of restricted habitats might possess limited genetic variation (Stebbins, 1942; Babbel and Selander, 1974). This prediction has been verified by studies of the rare species *Howellia aquatilis* (Lesica et al., 1988), *Pedicularis furbishiae* (Waller, O'Malley, and Gawler, 1987), *Pinus torreyana* (Ledig and Conkle, 1983), and others. *Eucalyptus caesia,* a rare Australian species, exhibits a pattern of genetic variation similar to *Plantago cordata* with slight variation within populations and greater variation among populations (Moran and Hopper, 1983).

Although the extent of *Plantago cordata*'s range has not declined significantly, the loss of populations within its range places this species at considerable risk of extinction. This plantain's unique habitat requirements may lead to further losses if streams and adjoining marshes and uplands are not protected from continued cultural disturbance.

Tessene's conclusion of low genetic variation in *Plantago cordata* could warrant a conservation plan that protects multiple sites with a low likelihood of disturbance. Accordingly, perturbations in one population could be ameliorated by founders taken from any of the other sites. This approach has been suggested for *Howellia aquatilis,* an endangered aquatic plant species lacking detectable (electrophoretic) genetic variation (Lesica et al., 1988). Conversely, the conclusion by Meagher, Antonovics, and Primack (1978) of significant levels of genetic variation in *P. cordata* warrants conservation efforts that emphasize the preservation of genetic variation whenever possible (Karron, 1991). In light of our studies verifying the existence of substantial regionally distributed genetic variation in this species, the latter conservation approach is more reasonable. With over 57% of sites for *P. cordata* extirpated, and extensive among-population heterogeneity in extant populations, it is likely that a significant amount of genetic variation has already been lost in this species. It is imperative that erosion of existing genetic variation in this species be prevented. The two, smaller populations in North Carolina represent sites of primary concern with unique genetic composition. Populations growing along the Hudson River in New York should also be studied further. These are the only heartleaved plantains known to grow in brackish conditions, tolerate tidal inundation, and thrive in full sun. Potentially, this population may contain important adaptive genes.

LITERATURE CITED

ALVERSON, W. S. 1981. Status report of *Plantago cordata* Lam. in Wisconsin. Department of Natural Resources, Madison, WI.

BABBEL, G. R., AND R. K. SELANDER. 1974. Genetic variation in edaphically restricted and widespread plant species. *Evolution* 28: 619–630.

BARRETT, S. C. H., AND J. R. KOHN. 1991. Genetic and evolutionary consequences of small population size in plants: implications for conservation. *In* D. A. Falk and K. E. Holsinger [eds.], Genetics and conservation of rare plants, 6–11. Oxford University Press, Oxford.

———, AND J. S. SHORE. 1989. Isozyme variation in colonizing plants. *In* D. E. Soltis and P. S. Soltis [eds.], Isozymes in plant biology, 106–126. Dioscorides Press, Portland, OR.

BASSETT, I. J. 1967. Taxonomy of *Plantago* L. in North America: sections *Holopsyllium* Pilger, *Palaeopsyllium* Pilger, and *Lamprosantha* Decne. *Canadian Journal of Botany* 45: 565–577.

———. 1973. The plantains of Canada. Monograph No. 7. Canada Department of Agriculture, Ottawa, Ontario.

BAYER, R. J. 1989. Patterns of isozyme variation in western North American *Antennaria* (Asteraceae: Inuleae). I. Diploid and polyploid species of the section *Alpinae. American Journal of Botany* 76: 679–691.

BEARDMORE, J. A. 1983. Extinction, survival, and genetic variation. *In* C. M. Schonewald-Cox, S. M. Chambers, B. MacBryde, and L. Thomas [eds.], Genetics and conservation, 125–151. Benjamin/Cummings, Menlo Park, CA.

BOWLES, M. L., AND S. I. APFELBAUM. 1989. Effects of land use and stochastic events on the heart-leaved plantain (*Plantago cordata* Lam.) in an Illinois stream system. *Natural Areas Journal* 9: 90–100.

CARDY, B. J., C. W. STUBER, AND M. M. GOODMAN. 1981. Techniques for starch gel electrophoresis of enzymes from maize (*Zea mays* L.). Institute of Statistics Mimeograph Series No. 1317. North Carolina State University, Raleigh, NC.

CARSON, H. L., AND A. R. TEMPLETON. 1984. Genetic revolutions in relation to speciation phenomena: the founding of new populations. *Annual Review of Ecology and Systematics* 15: 97–131.

CHESSER, R. K. 1983. Genetic variability within and among populations of the blacktailed prairie dog. *Evolution* 37: 320–331.

CLEGG, M. T., AND A. H. D. BROWN. 1983. The founding of plant populations. *In* C. M. Schonewald-Cox, S. M. Chambers, B. MacBryde, and L. Thomas [eds.], Genetics and conservation, 216–228. Benjamin/Cummings, Menlo Park, CA.

DRURY, W. H. 1974. Rare species. *Biological Conservation* 6: 162–169.

FRANKEL, O. H., AND M. E. SOULÉ. 1981. Conservation and evolution. Cambridge University Press, Cambridge.

GOTTLIEB, L. D. 1974. Gene duplication and fixed heterozygosity for alcohol dehydrogenase in the diploid *Clarkia franciscana. Proceedings of the National Academy of Sciences, USA* 71: 1816–1818.

———. 1977. Electrophoretic evidence and plant systematics. *Annals of the Missouri Botanical Garden* 64: 161–180.

———. 1981. Gene number in species of Asteraceae that have different chromosome numbers. *Proceedings of the National Academy of Sciences, USA* 78: 3726–3729.

HAMRICK, J. L. 1989. Isozymes and the analysis of genetic structure in plant populations. *In* D. E. Soltis and P. S. Soltis [eds.], Isozymes in plant biology, 87–105. Dioscorides Press, Portland, OR.

HARPER, R. M. 1944. Notes on *Plantago* with special reference to *P. cordata. Castanea* 26: 119–130.

KARRON, J. D. 1987. A comparison of levels of genetic polymorphism and self-compatibility in geographically restricted and widespread plant congeners. *Evolutionary Ecology* 1: 47–58.

———. 1991. Patterns of genetic variation and breeding systems in rare plant species. *In* D. A. Falk and K. E. Holsinger [eds.], Conservation of rare plants: biology and genetics, 87–98. Oxford University Press, Oxford.

KEPHART, S. R. 1990. Starch gel electrophoresis of plant isozymes: a comparative analysis of techniques. *American Journal of Botany* 77: 693–712.

KURZ, D. R., AND M. L. BOWLES. 1981. A status report on *Plantago cordata* in Illinois. Natural Land Institute, Rockford, IL.

LACY, R. C. 1988. A report on population genetics in conservation. *Conservation Biology* 2: 245–247.

LEDIG, F. T., AND M. T. CONKLE. 1983. Gene diversity and genetic structure in a narrow endemic, Torrey pine (*Pinus torreyana* Parry ex. Carr). *Evolution* 37: 79–85.

LES, D. H., J. A. REINARTZ, AND E. J. ESSELMAN. 1991. Genetic consequences of rarity in *Aster furcatus* (Asteraceae), a threatened, self-incompatible plant. *Evolution* 45: 1641–1650.

LESICA, P., R. F. LEARY, F. W. ALLENDORF, AND D. E. BILDERBACK. 1988. Lack of genic diversity within and among populations of an endangered plant, *Howellia aquatilis. Conservation Biology* 2: 275–282.

LEWIS, P., AND R. WHITKUS. 1989. GENESTAT-PC (Version 2.1). Department of Botany, The Ohio State University, Columbus, OH.

LEWONTIN, R. C. 1974. The genetic basis of evolutionary change. Columbia University Press, New York, NY.

LOVELESS, M. D., AND J. L. HAMRICK. 1984. Ecological determinants

of genetic structure in plant populations. *Annual Review of Ecology and Systematics* 15: 65–95.

MEAGHER, T. R., J. ANTONOVICS, AND R. PRIMACK. 1978. Experimental ecological genetics in *Plantago* III. Genetic variation and demography in relation to survival of *Plantago cordata,* a rare species. *Biological Conservation* 14: 243–258.

MORAN, G. F., AND S. D. HOPPER. 1983. Genetic diversity and the insular population structure of the rare granite rock species, *Eucalyptus caesia* Benth. *Australian Journal of Botany* 31: 161–172.

MORGAN, S. 1980. Status report on *Plantago cordata* Lam. Missouri Department of Conservation, Jefferson City, MO.

NAMKOONG, G. 1983. Preserving natural diversity. *In* C. M. Schonewald-Cox, S. M. Chambers, B. MacBryde, and L. Thomas [eds.], Genetics and conservation, 317–334. Benjamin/Cummings, Menlo Park, CA.

NEI, M. T. 1972. Genetic distances between populations. *American Naturalist* 106: 283–292.

———. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences, USA* 70: 3321–3323.

———, AND R. K. CHESSER. 1983. Estimation of fixation indices and gene diversities. *Annals of Human Genetics* 47: 253–259.

———, T. MARUYAMA, AND R. CHAKRABORTY. 1975. The bottleneck effect and genetic variability in populations. *Evolution* 29: 1–10.

OHNO, S. 1970. Evolution by gene duplication. Springer-Verlag, New York, NY.

PRIMACK, R. B. 1979. Reproductive effort in annual and perennial species of *Plantago* (Plantaginaceae). *The American Naturalist* 114: 51–61.

———. 1980. Phenotypic variation of rare and widespread species of *Plantago. Rhodora* 82: 87–95.

RABINOWITZ, D. 1981. Seven forms of rarity. *In* H. Synge [ed.], The biological aspects of rare plant conservation, 205–217. John Wiley and Sons, New York, NY.

STEBBINS, G. L. 1942. The genetic approach to problems of rare and endemic species. *Madroño* 6: 241–258.

STROMBERG, J., AND F. STEARNS. 1989. *Plantago cordata* in Wisconsin. *The Michigan Botanist* 28: 3–16.

TESSENE, M. F. 1968. Preliminary reports on the flora of Wisconsin. No. 59. Plantaginaceae-plantain family. *Transactions of the Wisconsin Academy of Science, Arts and Letters* 56: 281–313.

———. 1969. Systematic and ecological studies on *Plantago cordata. The Michigan Botanist* 8: 72–104.

WALLER, D. M., D. M. O'MALLEY, AND S. C. GAWLER. 1987. Genetic variation in *Pedicularis furbishiae* (Scrophulariaceae). *Conservation Biology* 1: 335–340.

WILKINSON, L. 1988. SYSTAT: the system for statistics. SYSTAT, Inc., Evanston, IL.