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Comparison of population genetic diversity between a rare, narrowly distributed species and a common, widespread species of *Alnus* (Betulaceae)[1]

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Comparisons of population genetic diversity between related rare and widespread species provide valuable insights to the consequences of rarity and are critical for conservation planning. Population genetic diversity of *A. maritima*, a rare species, was compared with its common, widespread congener *A. serrulata* to evaluate the impacts of small population size and high isolation on genetic diversity in *A. maritima* and to provide population genetic data to be used in conservation planning for *A. maritima*. Genetic data were also used to evaluate whether the disjunct distribution of *A. maritima* was due to range reduction or anthropogenic dispersal. Genetic diversity was lower in *A. maritima* (H_e = 0.217) than in *A. serrulata* (H_e = 0.268), and there is also higher inbreeding within *A. maritima* populations (f = 0.483) than *A. serrulata* populations (f = 0.269). The partitioning of genetic variation was also higher among *A. maritima* populations (Θ = 0.278), but not significantly different from that of *A. serrulata* (Θ = 0.197). Significant genetic differences among *A. maritima* populations support using local populations as seed sources for regional conservation efforts. The results also indicate that the highly disjunct distribution of *A. maritima* is due to natural range reduction in the past and not anthropogenic establishment of Oklahoma and Georgia populations.

Key words: allozyme; *Alnus*; Betulaceae; Delmarva peninsula; Georgia; Oklahoma; population genetics; rare plants.

Geographic distribution is an important factor shaping the genetic characteristics of plant populations. Rare or endemic plant species with limited geographic distributions tend to have lower levels of genetic variation than species with more widespread distributions due to the effects of either directional selection promoting adaptation to local environments or random processes such as inbreeding, genetic bottlenecks, or drift acting in small populations. Isolation may further reduce levels of genetic diversity within plant populations and enhance differences among them due to restricted gene flow (Hamrick et al., 1992). In several important reviews of the allozyme literature, mean values for statistics to describe population genetic diversity for plants with different life history traits (Hamrick et al., 1979; Hamrick and Godt, 1990, 1996) have been established. These data have served as benchmarks in numerous studies that compared population genetic characteristics of related rare and widespread species (Karron et al., 1988; Sherman-Broyles et al., 1992; Linhart and Premoli, 1993; Purdy and Bayer, 1995; Avis and Hamrick, 1996; Gitzendanner and Soltis, 2000; Cole, 2003). Comparisons of related species to investigate the impacts of rarity and small population size are particularly valuable because they can factor out shared traits such as breeding system or life history, which are important characteristics that shape the levels and structuring of population genetic variation (Karron et al., 1988; Hamrick and Godt, 1996; Gitzendanner and Soltis, 2000; Cole, 2003). Further, comparisons of related taxa allow specific insights into the impacts of isolation and restricted distribution on population genetic attributes of rare plant species that conservation biologists can use in developing effective management strategies (Hamrick, 1983; Swensen et al., 1995; Avise and Hamrick, 1996; Rieseberg and Swensen, 1996; Gemmill et al., 1998; Chung, 1999; Hedge and Ellstrand, 1999; Francisco-Ortega et al., 2000, Oliva-Tejera et al., 2006; Mateu-Andrés and Segarra-Moragues, 2004).

Alnus maritima (Marsh.) Muhl. ex Nutt (seaside alder) has the most highly disjunct distribution of any tree species in North America (Little, 1975). It occurs naturally in three highly separated locations (Fig. 1): the Delmarva Peninsula of Delaware and Maryland (*A. maritima* subsp. *maritima* Schrader & Graves), south central Oklahoma (*A. maritima* subsp. *oklahomensis* Schrader & Graves), and northwest Georgia (*A. maritima* subsp. *georgiensis* Schrader & Graves). The existence of Delmarva and Oklahoma populations has been known for over 100 years, but the Georgia population was not discovered until 1997 (Furlow, 1979; Ranger, 1997; Schrader and Graves, 2002, 2004; USDA and NRCS, 2002).

A majority of *A. maritima* populations are small, typically containing fewer than 20 individuals. However, larger populations containing 70–100 individuals do exist along the Blue River in Oklahoma, at Drummond Swamp in Georgia, and in the Prime Hook National Wildlife Refuge in Delaware. *Alnus maritima* is a globally rare species. Due to the small number and size of existing populations, the National Biological Information Infrastructure and the Association for Biodiversity Information have given *A. maritima* a global conserva tion status ranking of G3, marking it as a globally rare species facing a moderate vulnerability of extinction because of its restricted range and relatively few populations (NatureServe, 2007). In Delaware and Maryland, *A. maritima* is given an S3 ranking (Delaware Natural Heritage Program, 2003; Maryland Department of Natural Resources, 2004), indicating that it is rare and

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Fig. 1. Distribution of *Alnus maritima* and *A. serrulata* in the United States.

on the Watch List of monitored species in those states. In Oklahoma, *A. maritima* is an S2 species; it is rare and potentially vulnerable to extirpation in the state (Oklahoma Biological Survey, 2003). The Georgia Department of Natural Resources (2006) has given *A. maritima* an S1 ranking because it is highly rare and critically imperiled in the state.

Stibolt (1981) suggested that the disjunct distribution of *A. maritima* is the result of indigenous peoples of North America either intentionally or unintentionally transporting seeds from Delmarva to Oklahoma. However, no ethnobotanical data support this speculation beyond records of the Delaware Tribe traveling through Oklahoma before the discovery of the Oklahoma populations in 1872 (Stibolt, 1981). Furlow (1979) proposed that ancestors of *A. maritima* dispersed into North America across the Bering land bridge and spread across North America. Range reduction from environmental changes following the Pleistocene glacial period resulted in the highly disjunct distribution of *A. maritima*. On the basis of biogeographic and molecular analyses (Chen et al., 1999; Chen and Li, 2004; Schrader and Graves, 2000, 2002) the existing *A. maritima* populations have been concluded to be relicts of a more extensive distribution in the past.

In contrast, *Alnus serrulata* (Aiton) Willd. (hazel alder) grows throughout the eastern third of North America. It is a common riparian and wetland species that grows in moist soils along the banks of streams, rivers, ponds, and lakes (Fig. 1). Ancestors of *A. serrulata* are also thought to have dispersed to North America from Asia. However, this species either did not experience extreme range reduction or was able to repopulate areas following the glacial periods (Furlow, 1979; Chen et al., 1999). Populations of *A. serrulata* are sympatric with *A. maritima* except in Oklahoma, where the distribution of *A. maritima* lies west of the *A. serrulata* range.

Alnus maritima and *A. serrulata* both have multistemmed growth forms, with individuals growing as clearly delimited clumps. *Alnus maritima* occurs in shallow, open, moving water along streams and rivers or in swamp or marsh areas. High shade intolerance in *A. maritima* restricts plants to full sun areas (Schrader et al., 2006), whereas greater shade tolerance allows *A. serrulata* to grow in both shade and full sun. In sympatric locations, *A. maritima* occupies full sun sites, while *A. serrulata* grows in more shaded, drier areas.

Alnus maritima is the only North American member of the small subgenus *Clethropsis*, and *A. serrulata* is a member of the larger subgenus *Alnus*. The primary difference between subgenera is the timing of flowering. As in other members of subgenus *Alnus*, the catkins of *A. serrulata* are initiated in the summer and become dormant during the winter. The catkins open for pollination in the following spring, and seeds mature and are released in late summer or autumn. In contrast, *A. maritima* forms catkins in the spring, opens for pollination in summer or autumn, and releases seeds in late autumn or early winter (Chen and Li, 2004). Although *A. maritima* seeds germinate readily in greenhouse or laboratory conditions, there is no evidence of successful seedling recruitment in natural populations. Establishment from seed is characteristically low in *Alnus* spp., with most individuals reproducing asexually via suckers that arise from the roots (Huenneke, 1987).

The focus of the current study is to compare the levels and structuring of population genetic diversity between *A. maritima,* a rare species with a highly disjunct distribution, and *A. serrulata*, a common, widespread congener. The species are sympatric in the majority of locations where *A. maritima* occurs. Unlike others who similarly compared rare species to common species that could be sampled over a much larger geographic scale (Cole, 2003), we sampled populations of both

species at an identical geographic scale and, in many instances, in the same location, to more accurately determine population genetic consequences of isolation and rarity in *A. maritima.* These data will also allow further evaluation of whether the disjunct distribution of *A. maritima* is the result of natural or anthropogenic forces. If it is the result of range reduction, then the levels and structuring of genetic variation are expected to be consistent with patterns observed in other rare species in general and *Alnus* species in particular. If the *A. maritima* distribution is the result of human dispersal, then the Oklahoma and Georgia populations should have significantly lower genetic variation than Delmarva populations and contain a subset of the genetic variation present in Delmarva populations.

MATERIALS AND METHODS

Sampled populations—Leaf tissue was collected from plants of *A. maritima* in Delmarva, Oklahoma, and in Georgia (Table 1). Leaf tissue was collected from only one branch per clump to avoid repeated sampling from a single plant. Delmarva samples were collected at streamside locations in Dorchester County, Maryland (DELM1 and DELM2) and along the Marshyhope and Nanticoke Rivers, in Sussex County, Delaware (DELM3 and DELM4). In all Delmarva locations except DELM2, *A. maritima* was growing in areas with dense vegetation, and clumps were becoming overtopped by other vegetation, indicating these were late successional habitats. Plants in DELM2 were established in an open marshy area of the Nanticoke River. *Alnus maritima* samples in Oklahoma were collected from the banks of the Blue River in the Blue River Wildlife Management Area (OKM1) and along the banks of Pennington Creek (OKM2) in Johnston County. Georgia samples of *A. maritima* were collected at the lone Georgia population at Drummond Swamp in Bartow County (GAM1). Clumps were established in open areas of the swamp with full sun exposure. *Alnus serrulata* samples were collected at the same four Delmarva locations where *A. maritima* samples were collected (DELS1, DELS2, DELS3, DELS4). In Oklahoma, *A. serrulata* samples (OKS1, OKS2) were collected at sites east of OKM1 and OKM2. In Georgia, *A. serrulata* samples were collected in more shaded areas at Drummond Swamp (GAS1) and along the Yellow River in Gwinnett County east of Atlanta (GAS2). Samples were stored on ice and returned to the laboratory for genetic analysis.

Allozyme analyses—Leaf tissue from each sampled plant was quick frozen with liquid nitrogen and crushed with a mortar and pestle. Proteins were extracted using a phosphate polyvinyl pyrolidone buffer (Mitton et al., 1979). Tissue homogenates were stored in 1.5-ml microcentrifuge tubes at –80°C. Prior to electrophoresis, homogenates were thawed, and protein extracts from tissue homogenates were absorbed onto 4 × 6 mm filter paper wicks.

Electrophoretic analyses were conducted on 12% horizontal, hydrolyzed potato starch gels. Over 30 allozyme systems were initially surveyed for each species using different buffer–stain combinations. Eight putative allozyme loci were resolved for *A. maritima* using modifications of Soltis et al. (1983) buffer systems 6, 8, and 11 and Conkle et al. (1982) buffer system B. System 6 (gel buffer: 0.015 M Tris [Sigma 7–9, Sigma-Aldrich, St. Louis, Missouri, USA] 0.004 M citric acid, pH 7.8; electrode buffer: 0.3 M boric acid, 0.1 M NaOH, pH 8.6) resolved one locus each for diaphorase (*Dia*) and menadione nitrate reductase (*Mnr*). System 8 (gel buffer: 0.004 M LiOH, 0.025 M boric acid, 0.042 M Tris [Sigma 7–9] (Sigma-Aldrich, St. Louis, Missouri, USA), 0.007 M citric acid, pH 7.6; electrode buffer: 0.039 M LiOH, 0.263 M boric acid, pH 8) resolved amylase (*Amy*) and fluorescent esterase (*Fe*). System 11 (gel buffer: 0.045 M Tris [Sigma 7–9] 0.007 M citric acid, 0.004 M LiOH, 0.019 M boric acid, pH 8.3; electrode buffer: 0.038 M LiOH, 0.188 M boric acid, pH 8.3) resolved single loci for fructose 1,6-diphosphatase (*Fdp*) and shikimate dehydrogenase (*Skdh*). System B (gel buffer: 00.1 M Tris (Sigma 7–9), 0.016 M citric acid monohydrate, pH 8.45; electrode buffer: 0.27 M boric acid, 0.05 M NaOH, pH 8.0) resolved loci for beta-galactosidase (*Bgal*) and glutamic oxalic transaminase (*Got*).

Fourteen putative allozyme loci were resolved in *A. serrulata* using the same four buffer systems. System 6 resolved two loci for fluorescent esterase (*Fe-1, Fe-2*) and one locus for phosphoglucoisomerase (*Pgi*). System 8 resolved single loci for diaphorase (*Dia*) and menadione nitrate reductase (*Mnr*). System 11 resolved single loci for malate dehydrogenase (*Mdh*), 6-phosphogluconate dehydrogenase (*6-Pgdh*), and shikimate dehydrogenase (*Skdh*), and two loci for fructose 1,6-diphosphatase (*Fdp*-1, *Fdp*-2) and phosphoglucomutase (*Pgm1, Pgm2*). Single loci were resolved for beta-galactosidase (*Bgal*) and glutamic oxalic transaminase (*Got*) on System B.

Enzyme stain recipes followed Soltis et al. (1983), except for *Dia* and *Mnr* (Cheliak and Pitel, 1984). *Amy* was scored from white, destained bands, a sign that the enzyme degraded starch, on gels stained with a triose phosphate isomerase (*Tpi*) overlay. Different loci and alleles within loci were numbered sequentially, giving the most anodally migrating locus/allele the lowest number.

Genetic diversity and structure—Standard parameters of species- and population-level genetic diversity were calculated from allozyme data using LYNSPROG, a statistical program developed by M. D. Loveless (Department of Biology, College of Wooster, Wooster, Ohio, USA) and A. F. Schnabel (Department of Biology, University of Indiana, South Bend, Indiana, USA). Genetic diversity was measured by percentage of polymorphic loci (P_p), average number of alleles per locus (A), effective number of alleles per locus (A_e), expected heterozygosity (H_e), and observed heterozygosity (H_o). Expected heterozygosity was calculated at the population and species levels. Wright's (1922) inbreeding coefficient (F) was calculated for each polymorphic locus in each population to measure deviations of genotype frequencies at individual loci from Hardy–Weinberg expectations. Positive F-values indicate excess homozygosity, while negative values indicate excess heterozygosity. Statistical significance of F-values was evaluated by a χ^2 test: $\chi^2 = F^2N(a-1)$, df = $a(a-1)/2$, where N is the total sample size, and a is the number of alleles at the locus (Li and Horvitz, 1953).

The distribution of genetic diversity for polymorphic loci was evaluated through Weir and Cockerham's (1984) parameters F, f, and Θ. Values were calculated using the program GDA version 1d16c (Lewis and Zaykin, 2001) according to the methods of Weir (1996). The parameters F, f, and Θ are conceptually similar to Wright's (1965) F-statistics F_{IT}, F_{IS}, and $F_{ST,}$ respectively. F and f indicate levels of inbreeding over all populations and within populations, respectively, and Θ measures genetic differentiation among populations. Deviations of F and f from zero were evaluated by the χ^2 test described earlier for F. Statistical significance of Θ was likewise tested by a χ^2 test, $X^2 = 2N\Theta(a-1)$, df = $(a-1)(n-1)$ where N is the total sample size, a is the number of alleles, and n is the number of populations (Workman and Niswander, 1970).

Means and standard errors of genetic diversity and structure values were calculated via Tukey's jackknife (Sokal and Rohlf, 1981). Genetic diversity values were jackknifed across populations, and genetic structure values were jackknifed across loci. Means of genetic diversity and structure statistics were directly compared between species via t tests.

Gene flow was indirectly estimated using Takahata's (1984) modification of Wright's equation $Nm = [(1 - G_{ST})/4G_{ST}]\alpha$, where $\alpha = [n/(n-1)]^2$, n is the number of populations, and Nm is the number of migrants per generation (Crow and Aoki, 1984). For our calculations, Θ-values were substituted for G_{ST}. Genetic identity (I) was calculated for all pairwise combinations of populations to measure their genetic similarity (Nei, 1972; 1977). Genetic identities were then used to conduct cluster analyses via the UPGMA procedure (Rohlf, 1988).

TABLE 1. Locations and sample size (N) of seven *Alnus maritima* (M) and eight *A. serrulata* (S) study populations in Delmarva (DEL), Oklahoma (OK), and Georgia (GA).

Population(N)	County, state	Latitude	Longitude
Delmarva			
DELM1(11)/DELS1(11)	Dorchester, MD	N 38°32′	W 75°43′
DELM2(19)/DELS2(6)	Dorchester, MD	N 38°34′	W 75°47′
DELM3(4)/DELS3(6)	Sussex, DE	N 38°31′	W 75°28′
DELM4(8)/DELS4(11)	Sussex, DE	N 38°37′	W 75°49′
Oklahoma			
OKM1(47)	Johnston, OK	N 34°19′	W 96°35′
OKM2(21)	Johnston, OK	N 34°21′	W 96°41′
OKS1(22)	McCurtain, OK	N 34°08′	W 94°42′
OKS2(9)	Choctaw, OK	N 34°08′	W 95°32′
Georgia			
GAM1(73)/GAS1(9)	Bartow, GA	N 34°07′	W 84°57′
GAS2(32)	Gwinnett, GA	N 33°47′	W 84°08′

Note: DE, Delaware; GE, Georgia; MD, Maryland; OK, Oklahoma

RESULTS

A.maritima genetic diversity—Five of the eight loci (62.5%) resolved for *A. maritima* were polymorphic in at least one population (*Amy*, *Dia*, *Fdp*, *Mnr*, and *Skdh*). Allelic diversity was estimated at $A = 1.55$ (SE = 0.011), and $A_e = 1.30$ (SE = 0.02) (Table 2). *Amy* and *Fdp* were polymorphic in all three regions. *Dia* was polymorphic in Georgia and Delmarva, and *Mnr* was polymorphic in Delmarva and one Oklahoma population. *Skdh* was polymorphic in all populations except DELM4. Of the polymorphic loci, only *Dia* had the same allele present at the highest frequency across all populations; the most common allele varied among populations for all other polymorphic loci. No private alleles were identified in any *A. maritima* population. In the examination of multilocus genotypes, we found a high degree of genotypic similarity among individuals within populations, but multilocus genotypes differed in a majority of instances, which indicated that clumps are unique plants and not separate ramets of a single, large clonal genet.

Species-level genetic diversity was estimated at 0.217 (SE = 0.030). Mean expected heterozygosity within populations (H_e) ranged from 0.099 (OKM2) to 0.246 (DELM2). Observed heterozygosity (H_o) ranged from a low of 0.064 (OKM1) to a high of 0.188 (DELM3). Mean observed heterozygosity across all *A. maritima* populations ($H_o = 0.111$, SE = 0.009) was lower than expected ($H_e = 0.180$ SE = 0.008) (Table 2). In the χ^2 analysis of Wright's *F* for each polymorphic locus within populations, *F* was significantly greater than 0.0 in 16 of 35 instances, and no *F*-values were significantly less than 0.0. In DELM1, only one locus (*Mnr*) had a significant fixation index, whereas all polymorphic loci in DELM2 had significant *F*-values. In the other *A. maritima* populations, *F*-values were significant at either two or three polymorphic loci.

Genetic diversity in Alnus serrulata—Of the 14 loci resolved for *A. serrulata*, 11 (78.6%) were polymorphic in at least one population (*Dia*, *Fdp1*, *Fe1*, *Fe2*, *Got*, *Mdh*, *Mnr*, *Pgi*, *Pgm1*, *Pgm2*, and *Skdh*). Three alleles were scored for *Fe1*, *Pgi*, *Pgm2*, and *Mnr*, and two alleles were scored at other polymorphic loci. Mean allelic diversity was estimated across populations at $A = 1.71$ (SE = 0.02) and $A_e = 1.39$ (SE = 0.01) (Table 2). The proportion of polymorphic loci was lowest in DELS2 and highest in OKS1 and GAS1. Loci *Fdp1*, *Fe1*, and *Got* were polymorphic in all populations. *Skdh* was polymorphic in Georgia and Oklahoma populations, *Fe2* was polymorphic in only the two Oklahoma populations, and *Dia* was polymorphic in DES1 and DES2. There were no consistent patterns of loci being fixed or polymorphic among populations or regions. For example, *Mnr* was polymorphic in only GAS1, DELS1, and DELS3, while *Pgm1* was polymorphic in all populations except DELS2 and GAS2. Private alleles were identified for two loci: allele *Pgm2–1* in OKS1 and allele *Mnr-3* in GAS1.

Species-level genetic diversity for *A. serrulata* was estimated at 0.268 (SE = 0.019). Observed heterozygosity was lowest in DELS4 ($H_o = 0.144$) and highest in DELS3 ($H_o = 0.217$). Expected heterozygosity was lowest in DELS2 ($H_e = 0.154$) and highest in OKS1 ($H_e = 0.268$). Mean observed heterozygosity ($H_o = 0.172$ SE = 0.004) was lower than expected ($H_e = 0.203$ SE = 0.006), and H_o was lower than H_e in all *A. serrulata* populations except DELS2 and DELS3 (Table 2). In χ^2 tests of individual loci within populations, *F* was significantly different from 0.0 in only 13 of 88 instances with 12 fixation index values significantly greater than and one significantly less than 0.0. No heterozygotes were detected for *Mdh* in GAS1, *Pgi* in DELS2, and *Pgm2* in OKS1, DELS1, DELS3, and DELS4 although multiple alleles were present.

Genetic structure of Alnus maritima—The mean values of *F* (0.636, SE = 0.032) and *f* (0.483, SE = 0.042), and the individual values of *F* and *f* for the loci in *A. maritima* were all significantly greater than 0.0 indicating a high level of inbreeding within populations and for the species (Table 3). The mean

TABLE 2. Estimates of genetic diversity for seven *A. maritima* populations based on eight allozyme loci and for eight *A. serrulata* populations based on 14 allozyme loci. Means and (standard error) for population-level diversity statistics were calculated across populations using Tukey's jackknife.

Population	*N*	P_p	*A*	A_e	H_o	H_e
A. maritima						
OKM1	47	50.0	1.50	1.28	0.064	0.157
OKM2	21	50.0	1.50	1.13	0.065	0.099
GAM1	73	50.0	1.50	1.23	0.078	0.141
DELM1	11	62.5	1.63	1.33	0.159	0.198
DELM2	19	62.5	1.63	1.44	0.066	0.246
DELM3	4	62.5	1.63	1.37	0.188	0.219
DELM4	8	50.0	1.50	1.33	0.156	0.197
Mean	—	55.4 (6.59)	1.55 (0.01)	1.30 (0.02)	0.111 (0.009)	0.180 (0.008)
Species		62.5	1.63			0.217 (0.030)
A. serrulata						
OKS1	22	64.3	1.86	1.52	0.211	0.268
OKS2	9	50.0	1.64	1.33	0.151	0.176
GAS1	9	64.3	1.79	1.47	0.167	0.258
GAS2	32	42.9	1.73	1.36	0.165	0.195
DELS1	11	57.1	1.79	1.42	0.184	0.211
DELS2	6	35.7	1.43	1.30	0.167	0.154
DELS3	6	50.0	1.71	1.42	0.217	0.204
DELS4	11	57.1	1.79	1.34	0.144	0.189
Mean	—	52.7 (4.63)	1.71 (0.02)	1.39 (0.01)	0.172 (0.004)	0.203 (0.006)
Species		72.7	2.36			0.268 (0.019)

Note: N, sample size; P_p, percentage of polymorphic loci; *A* average number of alleles per locus; A_e, effective number of alleles per locus; H_e, expected heterozygosity, H_o, observed heterozygosity.

TABLE 3. Summary of genetic structure parameters calculated for polymorphic loci in *A. maritima* and *A. serrulata.* Means and (standard errors) for parameters were calculated across loci using Tukey's jackknife.

Locus	*N*	*F*	*f*	Θ
A. maritima				
Amy	7	0.530*	0.211*	0.404*
Dia	7	0.525*	0.464*	0.114*
F16	7	0.601*	0.526*	0.160*
Mnr	7	0.828*	0.552*	0.615*
Skdh	7	0.696*	0.663*	0.097*
Mean	7	0.636* (0.032)	0.483* (0.042)	0.278* (0.056)
A. serrulata				
Dia	8	0.395*	0.362*	0.051
F161	8	0.165	0.190	–0.031
Fe1	8	0.003	–0.063	0.061
Fe2	8	0.205*	0.125	0.091*
Got	8	0.414*	0.324*	0.133*
Mdh	8	0.217*	0.080	0.149*
Mnr	8	0.326*	0.297*	0.042
Pgi	8	0.472*	0.282*	0.263*
Pgm1	8	0.944*	0.799*	0.722*
Pgm2	8	0.339*	0.179	0.195*
Skdh	8	0.460*	0.388*	0.119*
Mean	8	0.358* (0.024)	0.269* (0.022)	0.197* (0.021)

Notes: N = sample size; * = values differ significantly from 0.0 ($P < 0.05$).

Θ-value (0.278, SE = 0.056) and values for individual loci were likewise significant; indicating that approximately 28% of the total genetic diversity is due to significant heterogeneity of allele frequencies among all *A. maritima* populations.

Despite differences among populations and regions, mean genetic identity ($I = 0.839$) was high among populations. The dendrogram of the UPGMA analysis (Fig. 2) indicates that populations within regions are genetically more similar to one another than to populations in other regions. Furthermore, *A. maritima* populations within the Oklahoma and Delamarva regions had similar levels of genetic identity. The lone Georgia population clustered more closely to the Oklahoma populations than to the Delmarva populations. Estimated gene flow for *A. maritima* was low with Nm = 0.885.

Genetic structure in Alnus serrulata—As with *A. maritima*, mean values for *F* (0.358, SE = 0.024) and *f* (0.269, SE = 0.022) were significantly greater than 0.0 (Table 3), indicating significant inbreeding in *A. serrulata*. The *F*-value was significantly greater than zero for all loci except *Fdp1* and *Fe1*, and *f* was significantly greater than zero for seven loci. The mean Θ (0.197, SE = 0.021) was significant, indicating that approximately 20% of the total genetic diversity is due to genetic differentiation among populations (Table 3). Genetic identity was high among *A. serrulata* populations ($I = 0.908$), and populations within regions clustered together in UPGMA analyses (Fig. 3). Unlike *A. maritima*, Georgia *A. serrulata* populations were more similar to Delmarva populations than to Oklahoma populations. Gene flow in *A. serrulata* was estimated at Nm = 1.33.

Comparison of Alnus maritima and A. serrulata—Although higher for *A. serrulata*, mean species-level diversity values did not differ significantly between species. However, genetic diversities at the population level as measured by H_o, H_e, *A*, A_e, and P_p were all significantly higher in *A. serrulata* than *A. maritima.* Furthermore, *F* and *f* were significantly higher in *A. maritima* than *A. serrulata*, indicating different levels of inbreeding overall and within populations between species. Despite differences in the levels of genetic diversity and inbreeding between species, Θ-values were not significantly different. Thus, both species partition genetic diversity among populations in much the same way.

DISCUSSION

Rare, endemic, and otherwise geographically restricted species tend to have lower levels of genetic diversity than widespread species (Hamrick and Godt, 1990; Sherman-Broyles et al., 1992; Linhart and Premoli, 1993; Avis and Hamrick, 1996; Gitzendanner and Soltis, 2000; Cole, 2003). Consistent with this trend, there is lower genetic diversity in *A. maritima* than its widespread congener *A. serrulata* (Tables 2, 3). Lower genetic diversity and higher inbreeding in *A. maritima* are likely due to populations being smaller and more highly isolated from one another than are *A. serrulata* populations.

The significant Θ-values for both *A. maritima* and *A. serrulata* reflect significant genetic structure among populations. Significant *f*-values measured in both species indicate high coancestry of alleles within individuals in populations caused by a high occurrence of consanguineous matings despite a predominantly outcrossing mating system (P. Gibson, unpublished data). Significant

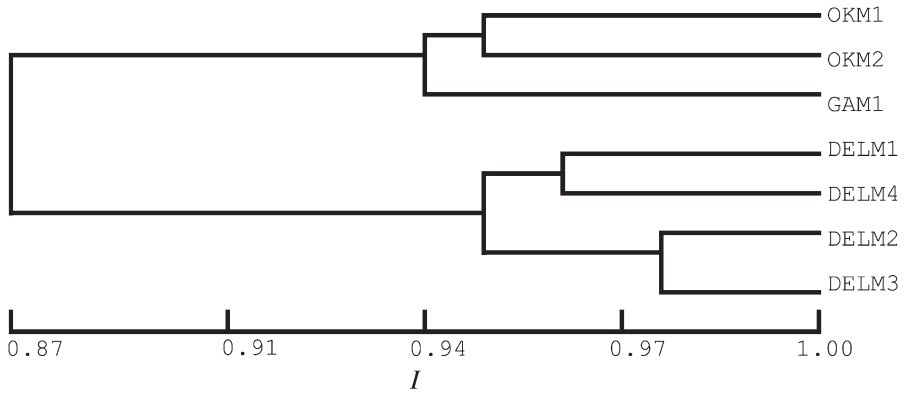

Fig. 2. UPGMA cluster dendrogram of seven *Alnus maritima* populations based upon Nei's genetic identity (*I*).

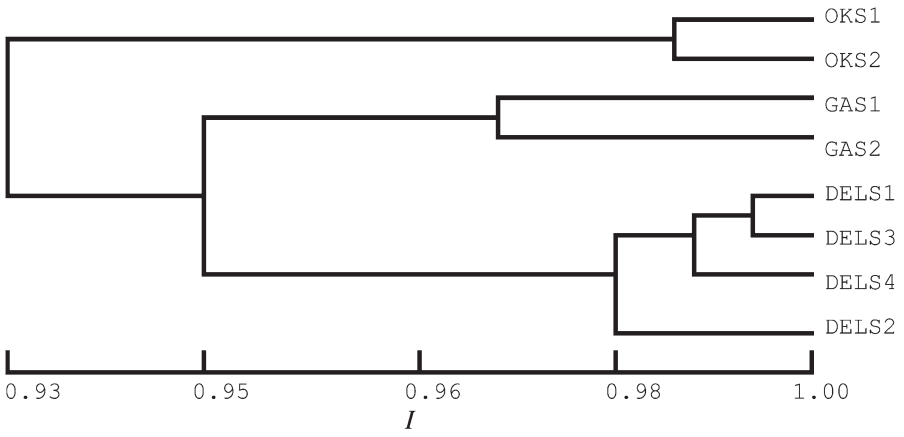

Fig. 3. UPGMA cluster dendrogram of eight *Alnus serrulata* populations based upon Nei's genetic identity (*I*).

F-values likewise indicate a high level of inbreeding overall in both species. The significant heterozygote deficiency at the species-level for both *A. maritima* and *A. serrulata* is due to a Wahlund effect caused by high levels of inbreeding within populations and significant genetic differentiation among populations.

The percentage of polymorphic loci and number of alleles per locus in both species is similar to mean values reported by Hamrick and Godt (1990) and Hamrick et al. (1992) for long-lived woody perennials at the species ($A = 2.22$, $P_p = 65.0$) and population ($A = 1.76$, $P_p = 49.3$) levels. However, H_e values in both study species are slightly higher than typical at the species ($H_e = 0.177$) and population levels ($H_e = 0.148$). In terms of geographic distribution, values of genetic diversity for *A. maritima* tended to be higher than those reported for rare species ($A = 1.82$, $P_p = 42.5$, $H_e = 0.078$), but more similar to those for species with narrow distributions ($A = 2.08$, $P_p = 61.0$, $H_e = 0.165$). Likewise, genetic diversity in *A. serrulata* was similar to that reported for widespread species ($A = 2.11$, $P_p = 67.8$, $H_e = 0.257$). Our results are also generally consistent with the different genetic diversity statistics reported Cole (2003) for rare and common species at the population (rare: $A = 1.42$, $P_p = 27.6$, $H_e = 0.113$, $H_o = 0.100$; common: $A = 1.72$, $P_p = 44.1$, $H_e = 0.150$, $H_o = 0.139$) and species levels (rare: $A = 1.74$, $P_p = 40.7$, $H_e = 0.142$; common: $A = 2.34$, $P_p = 58.8$, $H_e = 0.199$). Our estimates of inbreeding, however, were higher than Cole (2003) reported for rare ($F_{IS} = 0.175$) and common ($F_{IS} = 0.184$) species, but measures of genetic structure (rare spp. $F_{ST} = 0.212$; common spp. $F_{ST} = 0.198$) were similar.

The levels and structuring of genetic variation in both *A. maritima* and *A. serrulata* are consistent with what has been observed in other species with similar life history traits. Although the small, highly isolated populations of *A. maritima* would be expected to have extremely low levels of genetic diversity, perennial growth has likely promoted the maintenance of genetic variation within populations as has been reported for other long-lived, woody, perennial taxa (Hamrick and Godt, 1990, 1996). Further, populations of clonal shrubs may additionally be buffered against loss of genetic variation even more so than trees because their multistemmed growth form can allow persistence of an individual genet following death or damage to a ramet even in the absence of establishment from seed. Clumps in Oklahoma have been observed to resprout vigorously following flooding, which allows plants to persist in disturbed riparian communities.

Although genetic diversity is lower within *A. maritima* populations, the overall pattern of population genetic diversity and structure is not markedly different from that of other North American alders. Population genetic analyses have been conducted for two other common, widespread North American alder species: *A. crispa* (Bousquet et al., 1987) and *A. rugosa* (Bousquet et al., 1988). Measures of genetic diversity in *A. crispa* ($A = 2.39$, $P_p = 56.0$, $H_e = 0.136$) and *A. rugosa* ($A = 2.1$, $P_p = 60.0$, $H_e = 0.165$) are similar to values for *A. maritima* and *A. serrulata*, but there was considerably less genetic differentiation among population ($F_{ST} = 0.05$ in both species). This difference is probably due to the studies of *A. crispa* and *A. rugosa* being conducted among neighboring populations that were primarily along the same waterway. Low differentiation among populations of these alder species was concluded to be due in part to high gene flow among networks of populations (Bousquet et al., 1987, 1988). Populations of *A. maritima* in the different regions of our study are separated by over 800 miles (1300 km) and clearly isolated from one another. Thus, it is not at all unexpected that they would differ highly from one another. However, within regions, it is possible that there could potentially be gene flow among *A. maritima* populations, except in Georgia where there is only one population. If a greater number of *A. serrulata* populations located between those that were sampled were to be included in this study, Θ-estimates for this species would likely decrease due to gene flow among populations. Cursory analysis of only populations within the same region for both species produced Θ-values ranging from 0.01 to 0.05. Therefore, within different regions, there is similar genetic differentiation among populations to what has been documented in other North American *Alnus* species. There has likely been extensive gene flow among populations within regions that has maintained high effective population sizes despite the small size of individual populations. Additional mating system and seedling establishment studies are required to directly measure levels of gene flow and more fully understand the impacts of the reproductive system on genetic dynamics of *Alnus* in general and *A. maritima* in particular.

Schrader and Graves (2002, 2004) concluded that morphological and molecular differences in inter sequence simple repeats (ISSR) among *A. maritima* growing in Delmarva, Oklahoma, and Georgia were sufficient to warrant subspecies designation for plants in the three areas (subspecies *maritima*, *oklahomensis*, and *georgiensis*). Their analyses indicated that the

Delmarva and Georgia populations were more similar to one another than to the Oklahoma populations and that the Oklahoma populations had diverged first from the *A. maritima* lineage that eventually established the Georgia and Delmarva populations. In contrast, our results based on allozyme data indicate that, overall, the Oklahoma and Georgia populations are more similar to one another than they are to Delmarva *A. maritima*. Oklahoma *A. maritima* populations also have allele frequency differences from other populations, such as the fixation of *Dia-2*. Our results cannot determine whether fixation is due to drift, genetic bottlenecks, intense selection, or other factors acting on this locus.

The results of this study further support the conclusion that the distribution of *A. maritima* in North America is due to natural range reduction and not human-mediated establishment of the Oklahoma and Georgia populations. If populations of *A. maritima* in Oklahoma and Georgia were established with plants from the Delmarva locations, in the UPGMA analyses of *I*, those populations would probably cluster within the Delmarva group and not have the geographic pattern we found. Instead, our results show a geographically based component in analyses of *I* such that populations within regions cluster together. However, these results should be approached with caution as the high similarity between OKM1, OKM2, and GAM1 is strongly influenced by the *Dia* and *Mnr* loci, which are either fixed for the same allele or have the same allele in extremely high frequency. If Oklahoma and Georgia *A. maritima* populations were established by indigenous peoples who transported plants from Delmarva to Oklahoma and Georgia, allelic diversity in these areas would be expected to have much less variation as a result of the several genetic bottlenecks that would have occurred from founder events. However, this is not the case because genetic variation is similar among populations in all three regions. The high levels of genetic diversity in Oklahoma and Georgia populations indicate that humans were not likely to have established populations in these areas unless there was intentional transport of very large numbers of seeds to establish *A. maritima* populations in these areas. There is no ethnobotanical record of such cultural importance, and intentional propagation for this species by any group of indigenous peoples makes this scenario highly unlikely. Thus, our genetic data further support the conclusion of a natural range reduction in *A. maritima.*

While the range of *A. maritima* has likely contracted in North America, it retains genetic variation within populations much like more common alder species and slightly more variation than is typical for rare species. Given the genetic heterogeneity among populations, it will be important to protect and maintain many different populations of this species to preserve variation in the *A. maritima* genome. Conservation efforts should focus on local seed sources for any reestablishment efforts to maintain integrity of the subspecies and preserve locally adapted genotypes. Efforts to maintain genetic variation within populations are particularly important given the absence of seed-established individuals in any population of *A. maritima*. Individual plants produce copious quantities of viable seeds that germinate readily in a greenhouse environment (P. Gibson and S. Rice, personal observations). However, no evidence of new individuals establishing from seed has been found in surveys of our field sites. In a preliminary mating system analysis of the Georgia *A. maritima* population, we estimated high outcrossing in progeny arrays from several trees (P. Gibson, unpublished data). High outcrossing should promote maintenance of genetic variation within populations if those seed successfully establish new individuals. It is interesting, however, that significant inbreeding is detected in populations despite a predominantly outcrossing mating system. Further analyses using more sensitive and variable markers should be conducted to estimate more accurately the levels of selfing, consanguineous mating, and outcrossing in populations of different sizes. The mating system dynamics of *A. maritima* is a topic that will need to be addressed in future studies of seaside alder. Without the establishment of new individuals from seed, genetic diversity will likely continue to decay within existing populations as individuals die of natural causes, are replaced by other species via succession, or are killed due to anthropogenic causes. It will be necessary to measure genetic characteristics of the seed pool to develop effective conservation strategies that will promote establishment of new individuals and maintenance of population genetic diversity. Any future conservation efforts for *A. maritima*, therefore, must also consider ways to promote recruitment of new individuals. Mating system studies combined with analyses of seed germination and viability can determine whether inbreeding depression may be a factor limiting the recruitment and establishment of new individuals.

Conservation efforts for *A. maritima* hinge on understanding the factors shaping its genetic diversity and structure. The preservation of local genetic diversity for all three subspecies requires the protection of as many adult clones as possible because of the lack of recruitment to populations from seeds. The lack of seedling establishment removes the benefits of sexual recombination, particularly the spread of beneficial alleles, which allow populations to respond to changing environmental conditions. For example, *A. maritima* has low tolerance for drought conditions, yet it is predicted that climate change could bring drier environmental conditions to Georgia and Oklahoma (Intergovernmental Panel on Climate Change, 2007). If populations are going to be able to respond to these changes, it is critical to preserve individuals that could potentially carry alleles that confer greater drought tolerance. Without these individuals and their genetic variation, changing climatic and other environmental conditions could be disastrous for the species. The evolutionary responses of annual species to climate change are predicted to be slower than the rate of climate change (Etterson and Shaw, 2001). The evolutionary response of perennial plants may be even slower due to the longer time to reach reproductive maturity, but their higher levels of genetic diversity may provide the additive genetic variation necessary to accurately respond to long-term changes. Theoretical and empirical conservation genetics studies can build upon the current understanding of the population genetic attributes of both common and rare woody species to evaluate and predict their evolutionary responses to changing environmental conditions.

LITERATURE CITED

AVISE, J. C., AND J. L. HAMRICK. 1996. Conservation genetics. Case histories from nature. Chapman & Hall, New York, New York, USA.

BOUSQUET, J., W. M. CHELIAK, AND M. LALONDE. 1987. Genetic differentiation among 22 populations of green alder (*Alnus crispa)* in central Quebec. *Canadian Journal of Forest Research* 17: 219–227.

BOUSQUET, J., W. M. CHELIAK, AND M. LALONDE. 1988. Allozyme variation within and among mature populations of speckled alder (*Alnus rugosa*) and relationships with green alder (*A. crispa*). *American Journal of Botany* 75: 1678–1686.

CHELIAK, W. M., AND J. A. PITEL. 1984. Techniques for starch gel electrophoresis of enzymes from forest tree species. Petawawa National Forestry Institute, Information Report P1-X-42. Canadian Forestry Service, Agriculture, Chalk River, Ontario, Canada.

CHEN, Z., AND J. LI. 2004. Phylogenetics and biogeography of *Alnus* (Betulaceae) inferred from sequences of nuclear ribosomal DNA ITS regions. *International Journal of Plant Sciences* 165: 325–335.

CHEN, Z., S. R. MANCHESTER, AND A.-Y. SUN. 1999. Phylogeny and evolution of the Betulaceae as inferred from DNA sequences, morphology, and paleobotany. *American Journal of Botany* 86: 1168–1181.

CHUNG, M. G. 1999. Allozyme diversity in the endangered shrub *Abeliophyllum distichum* (Oleaceae): A monotypic Korean genus. *International Journal of Plant Sciences* 160: 553–559.

COLE, C. T. 2003. Genetic variation in rare and common plants. *Annual Reviews of Ecology. Evolution and Systematics* 34: 213–237.

CONKLE, M. T., P. D. HODGSKISS, L. B. NUNNALLY, AND S. C. HUNTER. 1982. Starch gel electrophoresis of conifer seeds: A laboratory manual. General Technical Report PSW-64. U.S. Department of Agriculture Forest Service, Washington, D.C., USA.

CROW, J. F., AND K. AOKI. 1984. Group selection for a polygenic behavioral trait: Estimating the degree of population subdivision. *Proceedings of the National Academy of Sciences, USA* 81: 6073–6077.

DELAWARE NATURAL HERITAGE PROGRAM. 2003. Rare vascular plants of Delaware. Website http://www.dnrec.state.de.us/fw/rareplant03.pdf [accessed 25 August 2006].

ETTERSON, J. R., AND R. G. SHAW. 2001. Constraints to adaptive evolution in response to global warming. *Science* 294: 151–154.

FRANCISCO-ORTEGA, J., A. SANTOS-GUERRA, S.-C. KIM, AND D. J. CRAWFORD. 2000. Plant genetic diversity in the Canary Islands: A conservation perspective. *American Journal of Botany* 87: 909–919.

FURLOW, J. J. 1979. The systematics of the American species of *Alnus* (Betulaceae). *Rhodora* 81: 1–248.

GEMMILL, C. E. C., T. A. RANKER, D. RAGONE, S. P. PERLMAN, AND K. R. WOOD. 1998. Conservation genetics of the endangered endemic Hawaiian genus *Brighamia* (Campanulaceae). *American Journal of Botany* 85: 528–539.

GEORGIA DEPARTMENT OF NATURAL RESOURCES. 2006. Special concern plant species in Georgia. Website http://georgiawildlife.dnr.state.ga.us/content/specialconcernplants.asp [accessed 26 October 2006].

GITZENDANNER, M. A., AND P. S. SOLTIS. 2000. Patterns of genetic variation in rare and widespread plant congeners. *American Journal of Botany* 87: 783–792.

HAMRICK, J. L. 1983. The distribution of genetic variation within and among natural plant populations. *In* C. M. Schonewald-Cox, S. M. Chambers, B. MacBryde, and W. L. Thomas [eds.], Genetics and conservation, 335–348. Benjamin/Cummings, Menlo Park, California, USA.

HAMRICK, J. L., AND M. J. W. GODT. 1990. Allozyme diversity in plant species. *In* A. D. H. Brown, M. T. Clegg, A. L. Kahler, and B. S. Weir [eds.], Plant population genetics, breeding, and genetic resources 43–63. Sinauer, Sunderland, Massachusetts, USA.

HAMRICK, J. L., AND M. J. W. GODT. 1996. Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London, B, Biological Sciences* 351: 1291–1298.

HAMRICK, J. L., M. J. W. GODT, AND S. L. SHERMAN-BROYLES. 1992. Factors influencing levels of genetic diversity in woody plant species. *New Forests* 6: 95–124.

HAMRICK, J. L., Y. B. LINHART, AND J. B. MITTON. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation inplants. *Annual Review of Ecology and Systematics* 10: 173–200.

HEDGE, S. G., AND N. C. ELLSTRAND. 1999. Life history differences between rare and common British flowering plant species of California and the British Isles. *International Journal of Plant Sciences* 160: 1083–1091.

HUENNEKE, L. F. 1987. Demography of a clonal, shrub *Alnus incana* ssp. *rugosa* (Betulaceae). *American Midland Naturalist* 117: 43–55.

INTERGOVERNMENTAL PANEL ON CLIMATE CHANGE. 2007. Climate change and its impacts in the near and long term under different scenarios." Fourth Assessment Report, Topic 3. Website http://www.ipcc.ch/pdf/assessment-report/ar4/syr/ar4_syr_topic3.pdf [accessed 8 January 2008].

KARRON, J. D., Y. B. LINHART, C. A. CHAULK, AND C. A. ROBERTSON. 1988. Genetic structure of populations of geographically restricted and widespread species of *Astragalus* (Fabaceae). *American Journal of Botany* 75: 1114–1119.

LEWIS, P. O., AND D. ZAYKIN. 2001. Genetic Data Analysis: Computer program for the analysis of allelic data, version 1.0 (d16c). Free program distributed by the authors over the internet from Website http://lewis.eeb.uconn.edu/lewishome/software.html [accessed 5 February 2002].

LI, C. C., AND D. G. HORVITZ. 1953. Some methods of estimating the inbreeding coefficient. *American Journal of Human Genetics* 5: 107–117.

LINHART, Y. B., AND A. C. PREMOLI. 1993. Genetic variation in *Aletes acaulis* and its relative, the narrow endemic *A. humilis* (Apiaceae). *American Journal of Botany* 80: 598–605.

LITTLE, E. L. 1975. Our rare and endangered trees. *American Forests* 81: 16.

MARYLAND DEPARTMENT OF NATURAL RESOURCES. 2004. Current and historical rare, threatened and endangered species. Website http://www.dnr.state.md.us/wildlife/rte/rte04worc.pdf [accessed 25 August 2006].

MATEU-ANDRÉS, I., AND J. G. SEGARRA-MORAGUES. 2004. Reproductive system in the Iberian endangered endemic *Antirrhinum valentinum* f.q. (Antirrhineae, Scrophulariaceae): Consequences for species conservation. *International Journal of Plant Sciences* 165: 773–778.

MITTON, J. B., Y. B. LINHART, K. B. STURGEON, AND J. L. HAMRICK. 1979. Allozyme polymorphism detected in mature needle tissue of ponderosa pine. *Journal of Heredity* 70: 86–89.

NATURESERVE. 2007. NatureServe conservation status. Website http://www.natureserve.org/explorer/ranking.htm [accessed 25 August 2007].

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

NEI, M. 1977. *F*-statistics and analysis of gene diversity in subdivided populations. *Annals of Human Genetics* 41: 225–233.

OKLAHOMA BIOLOGICAL SURVEY. 2003. Working list of rare Oklahoma plants. Website http://www.biosurvey.ou.edu/download/heritage/plants0503.pdf [accessed 17 September 2006].

OLIVA-TEJERA, F., J. CAUJAPÉ, J. NAVARRO-DÉNIZ, A. REYES-BETANCORT, S. SCHOLZ, M. BACCARANI-ROSAS, AND N. CABRERA-GARCÍA. 2006. Patterns of genetic divergence of three Canarian endemic *Lotus* (Fabaceae): Implications for the conservation of the endangered *L. kunkelii. American Journal of Botany* 93: 1116–1124.

PURDY, B. G., AND R. J. BAYER. 1995. Allozyme variation in the Athabasca Sand Dune endemic *Salix salicicola*, and the closely related widespread species, *S. alaxensis. Systematic Botany* 20: 179–190.

RANGER, S. 1997. Four exciting fall '97 state records. *Botsoc News* 71: 1.

RIESEBERG, L. H., AND S. M. SWENSEN. 1996. Conservation genetics of endangered island plants. *In* J. C. Avise and J. L. Hamrick [eds.], Conservation genetics. Case histories from nature, 305–334. Chapman & Hall, New York, New York, USA.

ROHLF, F. J. 1988. Numerical taxonomy and multivariate analysis system. Exeter Publishers, Setauket, New York, USA.

SCHRADER, J. A., AND W. R. GRAVES. 2000. Timing of seed dispersal may limit the reproductive success of *Alnus maritima. Castanea* 65: 69–77.

SCHRADER, J. A., AND W. R. GRAVES. 2002. Intraspecific systematics of *Alnus maritima* (Betulaceae) from three widely disjunct provenances. *Castanea* 67: 380–401.

SCHRADER, J. A., AND W. R. GRAVES. 2004. Systematics of *Alnus maritima* (seaside alder) resolved by ISSR polymorphisms and morphological characters. *Journal of the American Society for Horticultural Science* 129: 231–236.

SCHRADER, J. A., W. R. GRAVES, S. A. RICE, AND J. P. GIBSON. 2006. Differences in shade tolerance help explain varying success of two sympatric *Alnus* species. *International Journal of Plant Sciences* 167: 979–989.

SHERMAN-BROYLES, S. L., J. P. GIBSON, J. L. HAMRICK, M. A. BUCHER, AND M. J. GIBSON. 1992. Comparison of allozyme diversity among rare and widespread *Rhus* species. *Systematic Botany* 17: 551–559.

SOKAL, R. R., AND F. J. ROHLF. 1981. Biometry, 2d. ed. Freeman, San Francisco, California, USA.

SOLTIS, D. E., C. H. HAUFLER, D. C. DARROW, AND G. J. GASTONY. 1983. Starch gel electrophoresis of ferns: A compilation of crushing buffers, gel and electrode buffers, and staining schedules. *American Fern Journal* 73: 9–27.

STIBOLT, V. M. 1981. The distribution of *Alnus maritima* Muhl. ex Nutt. (Betulaceae). *Castanea* 46: 195–200.

SWENSEN, S. M., G. J. ALLAN, M. HOWE, W. J. ELISENS, S. A. JUNAK, AND L. H. RIESEBERG. 1995. Genetic analysis of the endangered island endemic *Malacnothamnus* (Nutt.) Greene var. *nesioticus* (Rob.) Kern. *Conservation Biology* 9: 404–415.

TAKAHATA, N. 1983. Gene identity and genetic differentiation of populations in the finite island model. *Genetics* 104: 497–512.

USDA and NRCS [U.S. Department of Agriculture and Natural Resources Conservation Service]. 2002. The PLANTS database, version 3.5. National Plant Data Center, Baton Rouge, Louisiana, USA. Website http://plants.usda.gov [accessed 18 April 2006].

WEIR, B. S. 1996. Genetic data analysis. Sinauer, Sunderland, Massachusetts, USA.

WEIR, B. S., AND C. C. COCKERHAM. 1984. Estimating *F*-statistics for the analysis of population structure. *Evolution* 38: 1358–1370.

WORKMAN, P. L., AND J. D. NISWANDER. 1970. Population studies on southwestern Indian tribes. II. Local genetic differentiation in the Papago. *American Journal of Human Genetics* 22: 24–49.

WRIGHT, S. 1922. Coefficients of inbreeding and relationship. *American Naturalist* 56: 330–338.

WRIGHT, S. 1965. The interpretation of population genetic structure by *F*-statistics with special regard to systems of mating. *Evolution* 19: 395–420.