Genetic Variation and Evidence of Hybridization in the Genus *Rhus* (Anacardiaceae)

J. M. Burke and J. L. Hamrick

Rhus michauxii, **a rare plant species endemic to the southeastern United States, was previously known only from central North Carolina and one site in Georgia. An additional site, which is now believed to represent the largest known concentration of *R. michauxii,* was recently discovered at Ft. Pickett near Blackstone, Virginia. Morphological characteristics in several of the Ft. Pickett *Rhus* populations appear to be intermediate between *R. michauxii* and the widespread *R. glabra,* a closely related congener that co-occurs at Ft. Pickett. Although morphological evidence of hybridization between *R. michauxii* and *R. glabra* in North Carolina has been provided previously, genetic marker data are lacking. In the present study we examined levels of allozyme variation at 11 polymorphic loci within and among seven populations of *R. michauxii,* one population of *R. glabra,* and four putative hybrid populations at Ft. Pickett. Overall, *R. michauxii* had typical levels of within-population genetic variation when compared to other species with similar life-history characteristics. In contrast, the proportion of genetic variation among populations (G_{ST}) was considerably lower than expected. Finally, *R. michauxii* and *R. glabra* appear to have a fixed allelic difference at the *Idh2* locus. This enabled us to confirm hybridization in all four of the putative hybrid populations and one of the *R. michauxii* populations.**

Species with restricted geographic distributions and relatively small population sizes are of particular concern to conservation biologists. Not only are these species at risk of extinction because of a variety of ecological factors (e.g., habitat destruction and environmental changes), but they are also susceptible to the loss of genetic variation due to inbreeding and genetic drift. Reviews of both the animal and plant allozyme literature have revealed a tendency for species with limited geographic ranges to have relatively low levels of within-population genetic variation and higher levels of among-population variation in comparison to more widespread species (Hamrick and Godt 1989; Hamrick et al. 1992; Nevo 1985). Furthermore, recent work has suggested that inbreeding, as evidenced by decreased heterozygosity, can significantly increase the extinction risk of small, isolated populations (Saccheri et al. 1998).

An additional risk that rare and endangered taxa may face is hybridization with a more common taxon. The risks associated with natural hybridization are twofold (Levin et al. 1996). First, if F_1 or later generation hybrids exhibit reduced viability and/or fertility the rare taxon may suffer from outbreeding depression, thereby reducing the potential for individuals to replace themselves (Price and Waser 1979; Templeton 1986). In contrast, if hybrids are viable and fertile, the rare taxon may face genetic assimilation, or swamping, by the more common taxon (Leary et al. 1993; O'Brien et al. 1990; Rieseberg et al. 1989; Wayne and Jenks 1991). In either case, natural hybridization may increase the likelihood of extinction of the rare taxon. Recent theoretical work has suggested that hybridization may be the most rapidly acting genetic threat to rare species, with extinction occurring in as few as five generations (Wolf et al. 2001).

Rhus michauxii Sargent (Anacardiaceae), or false poison sumac, is a rare "endangered endemic" (Sutter et al. 1983) which, until recently, was known only from central North Carolina and one site in Georgia (Figure 1; U.S. Fish and Wildlife Service 1993). A previous survey of allozyme diversity in the North Carolina populations of *R. michauxii* revealed that, as expected, this rare endemic exhibited relatively low levels of genetic diversity compared to its more widespread congeners

From the Departments of Genetics (Burke and Hamrick) and Botany (Hamrick), University of Georgia, Athens, GA 30602. J. M. Burke is currently at the Department of Biology, Indiana University, 142 Jordan Hall, Bloomington, IN 47405. The authors thank L. Boyte, M. Bucher, V. Emrick, R. Sims, and T. Southall for assisting with the field work, and M. Burke and E. Stacy for assistance in the lab. This work was supported by a grant from the U.S. Fish and Wildlife Service. J.M.B. was supported by NSF/USDA/DOE Plant Molecular Evolution Training grant BIR-9220329. Address correspondence to J. M. Burke at the address above or e-mail: jmburke@indiana.edu.

 93:37–41

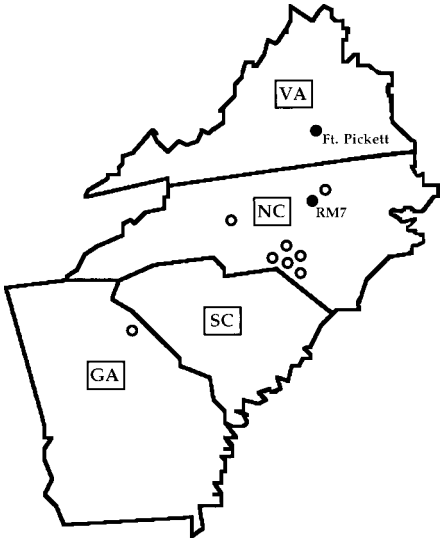

Figure 1. Approximate locations of extant populations of *Rhus michauxii* in the southeastern United States (U.S. Fish and Wildlife Service 1993). Open circles correspond to previously identified locales that were not included in our study. Closed circles correspond to population RM7 and the Ft. Pickett, Virginia, populations included in our study.

Table 1. Summary of locations, sample sizes, and genetic variation for the six *R. michauxii,* one *R. glabra,* and five hybrid populations

Population	Species	Location	N	P	A_p	A_e	H_e (SE)	H_o (SE)
RM1	*R. michauxii*	Ft. Pickett, VA	48	0.35	2.00	1.11	0.07 (0.03)	0.09 (0.01)
RM2	*R. michauxii*	Ft. Pickett, VA	48	0.41	2.00	1.07	0.06 (0.02)	0.06 (0.01)
RM3	*R. michauxii*	Ft. Pickett, VA	48	0.47	2.00	1.07	0.06 (0.02)	0.06 (0.01)
RM4	*R. michauxii*	Ft. Pickett, VA	48	0.53	2.00	1.19	0.12 (0.04)	0.12 (0.01)
RM5	*R. michauxii*	Ft. Pickett, VA	48	0.47	2.00	1.16	0.12 (0.03)	0.11 (0.01)
RM7	*R. michauxii*	Wake County, NC	30	0.44	2.00	1.10	0.08 (0.03)	0.08 (0.01)
Mean			**45**	**0.45**	**2.00**	**1.12**	**0.08 (0.01)**	**0.09 (0.01)**
Pooled			**270**	**0.59**	**2.00**	**1.12**	**0.08**	**—**
RG	*R. glabra*	Ft. Pickett, VA	48	0.50	2.00	1.22	0.13 (0.04)	0.08 (0.01)
HYB1	Hybrid	Ft. Pickett, VA	48	0.50	2.00	1.13	0.09 (0.04)	0.11 (0.01)
HYB2	Hybrid	Ft. Pickett, VA	48	0.53	2.11	1.13	0.11 (0.03)	0.12 (0.01)
HYB3	Hybrid	Ft. Pickett, VA	48	0.41	2.00	1.14	0.08 (0.04)	0.13 (0.01)
HYB4	Hybrid	Ft. Pickett, VA	48	0.56	2.00	1.22	0.12 (0.05)	0.17 (0.02)
RM6	Hybrid[a]	Ft. Pickett, VA	48	0.56	2.00	1.22	0.14 (0.04)	0.21 (0.02)
Mean			**48**	**0.51**	**2.02**	**1.17**	**0.11 (0.02)**	**0.15 (0.01)**
Pooled			**240**	**0.65**	**2.09**	**1.17**	**0.11**	**—**

[a] Population RM6 was initially identified as *R. michauxii* in the field, but was later classified as hybrid on the basis of the genetic analysis.

(Sherman-Broyles et al. 1992). In the early 1990s an additional site located at Ft. Pickett near Blackstone, Virginia, was discovered. Although this site is now believed to represent the largest known concentration of *R. michauxii,* the morphological characteristics of several populations of *Rhus* at Ft. Pickett appear to be intermediate between *R. michauxii* and *R. glabra* L. (smooth sumac), a closely related congener that co-occurs at Ft. Pickett. In contrast to the geographically restricted *R. michauxii, R. glabra* is the most widespread woody plant species in North America, with a range spanning all 48 contiguous states, southern Canada, and northern Mexico (Little 1980). The morphological intermediacy of these Ft. Pickett populations suggests that they are the product of interspecific hybridization. The latter third of the *R. glabra* flowering season overlaps the first third of the *R. michauxii* flowering season, and 20% of the hybrid seeds produced from experimental crosses between these two species were viable (Hardin and Phillips 1985). Furthermore, Hardin and Phillips (1985) have provided morphological evidence that *R. michauxii* and *R. glabra* hybridize in nature. Because their analysis was limited to morphology, however, molecular marker data confirming hybridization are lacking.

The goals of our study were to (1) examine allozyme variation within and among the Ft. Pickett populations of *R. michauxii* in relation to the North Carolina populations previously analyzed by Sherman-Broyles et al. (1992), and (2) characterize putative hybrid populations to determine if there is genetic evidence of hybridization between *R. michauxii* and *R. glabra.*

Materials and Methods

Sample Collection and Electrophoresis

Leaves were collected from individuals in six populations of *R. michauxii,* one population of *R. glabra,* and four populations containing putative hybrids between *R. michauxii* and *R. glabra* at Ft. Pickett, Virginia (Table 1 and Figure 1). All collection sites at Ft. Pickett were located in an area of approximately 2 km × 8 km. One additional population of *R. michauxii* from Wake County, North Carolina, was sampled to provide continuity with the previous research on this species (i.e., Sherman-Broyles et al. 1992). Wherever possible, leaf samples were collected from 48 individuals per population. Populations RG and RM7 consisted of 43 and 30 individuals, respectively. Because these species exhibit clonal growth, samples were taken from widely spaced (≥2 m) individuals to avoid repeated sampling of the same genet. All samples were placed in plastic bags and stored on ice to prevent protein denaturation in the field.

Leaf tissue from each individual was crushed under liquid nitrogen with a mortar and pestle. Proteins were then extracted and stabilized by the addition of a phosphate polyvinylpyrrolidone buffer (Mitton et al. 1979). The crude enzyme extract was absorbed onto filter paper wicks cut from Whatman 3MM chromatography paper, placed in 96-well microtest plates, and stored at −70°C. Samples were electrophoresed on 10% starch gels using five different electrode buffer systems; 17 loci were resolved using 13 histochemical stains (Soltis et al. 1983; Table 2). To facilitate consistent and accurate scoring, standard samples of *R. michauxii* and *R. glabra* were run on each gel.

Genetic Data Analyses

Statistics of genetic diversity were calculated at both the population and species levels. The five measures of within-population genetic diversity analyzed were percent polymorphic loci (P; a locus was considered polymorphic if it had more than one allele), mean number of alleles per locus (A), mean number of alleles per polymorphic locus (A_p), effective number of alleles per locus ($A_e = 1/\Sigma p_i^2$, where p_i is the frequency of the ith allele at a locus, averaged over loci) and expected heterozygosity ($H_e = 1 - \Sigma p_i^2$; also referred to as genetic diversity). Species-level parameters were estimated from pooled datasets. Deviations from Hardy–Weinberg equilibrium were calculated for each polymorphic locus in each population using Wright's fixation index ($F = 1 - H_o/H_e$; Wright 1922) and tested using chi-squared tests. Significance values were adjusted for multiple comparisons using the sequential Bonferroni procedure of Holm (1979).

Among-population genetic variation was estimated in two ways. First, total genetic diversity at polymorphic loci (H_T) was partitioned into within- (H_S) and among-population (D_{ST}) components (such that $H_T = H_S + D_{ST}$). The proportion of genetic vari-

Table 2. Electrode and gel buffer systems used to resolve the 17 loci

System	Electrode buffer	Gel buffer	Enzyme systems
4	0.263 M boric acid	0.008 M Tris	*Mdh1, 2*
	0.388 M LiOH	0.003 M citric acid	*Skdh*
	pH 8.0	pH 7.5	*6-Pgd*
6	0.3 M boric acid	0.015 M Tris	*Acp*
	0.1 M NaOH	0.004 citric acid	*Ce1, 2*
	pH 8.6		*Pgm2*
7	0.188 M boric acid	0.045 M Tris	*Aat*
	0.038 M LiOH	0.019 M boric acid	*Dia*
	pH 8.3	0.007 M citric acid	
		0.004 M LiOH	
		pH 8.3	
8	0.263 M boric acid	0.033 M Tris	*Lap2*
	0.038 M LiOH	0.029 M boric acid	*Fe1*
		0.006 M citric acid	*Tpi2*
		0.004 M LiOH	
		pH 7.6	
11	0.4 M citric acid (trisodium)	0.009 M L-histidine HCl	*Pgi1, 2*
	pH 7.0	pH 7.0	*Idh1, 2*

Buffer systems are as described by Soltis et al. (1983) with the exception of systems 6 and 8, which have both been modified.

Table 3. Summary of *Idh2* allele frequencies in the six *R. michauxii,* one *R. glabra,* and five hybrid populations

Population	Allele 3	Allele 4	Allele 5
RM1	0.98	0	0.02
RM2	1.00	0	0
RM3	1.00	0	0
RM4	0.96	0	0.04
RM5	0.83	0	0.17
RM7	1.00	0	0
RG	0	1.00	0
HYB1	0.99	0.01	0
HYB2	0.86	0.14	0
HYB3	0.55	0.45	0
HYB4	0.50	0.50	0
RM6	0.50	0.50	0

ation among populations was then calculated as $G_{ST} = D_{ST}/H_T$ (Nei 1973, 1977). Values of G_{ST} were tested for significance by $\chi^2 = 2NG_{ST}(a - 1)$ with df $= (a - 1)(n - 1)$, where N is the total number of individuals analyzed, a is the number of alleles at the locus of interest, and n is the number of populations (Workman and Niswander 1970). Significance values were adjusted for multiple comparisons using the sequential Bonferroni procedure of Holm (1979). Second, Nei's genetic identities and distances were calculated for all possible pairwise comparisons of populations (Nei 1972).

Results

Idh2 as a Diagnostic Locus

Rhus michauxii and *R. glabra* from Ft. Pickett appear to have a fixed allelic difference at *Idh2* (Table 3). All individuals identified as *R. glabra* on the basis of morphology (population RG) were homozygous for the "4" allele, whereas individuals in six of the seven populations identified as *R. michauxii* carried only the "3" or "5" alleles ("5" occurred at a frequency ≤0.17 whenever present). The one exception to this was population RM6. Although this population was initially identified as *R. michauxii,* every individual sampled was heterozygous for the "3" and "4" alleles. If it is assumed that, at *Idh2,* the "4" allele is specific to *R. glabra,* and that the "3" and "5" alleles are specific to *R. michauxii,* all F_1 hybrid offspring, as well as a portion of later generation hybrids, should be heterozygous for the marker alleles. In fact, all putative hybrid populations, like RM6, contained individuals heterozygous for the "3" and "4" alleles, although in very different frequencies. It therefore seems likely that RM6 is actually a hybrid population that was mistakenly identified as *R. michauxii* in the field. Furthermore, although these species exhibit clonal growth, inspection of individual genotypes at just three loci (*Aat, Dia,* and *Mdh2*) reveals a minimum of seven distinct multilocus genotypes. Therefore, although population RM6 is fixed heterozygous at *Idh2,* the other loci are segregating, indicating that we have not sampled from a single, large clone. These data suggest that population RM6 may largely (or completely) consist of F_1 hybrid individuals. For this reason, population RM6 is considered a hybrid population for the remainder of the analyses.

Genetic Diversity

The putative hybrid populations have somewhat more genetic variation than *R. michauxii,* and considerably more variation than the single *R. glabra* population studied here (Table 1). The percent polymorphic loci (P) pooled across all populations was 0.65 for the hybrids (A_p = 2.09), compared to 0.59 for *R. michauxii* (A_p = 2.00) and 0.50 for *R. glabra* (A_p = 2.00). At the individual population level, P varied from 0.41 to 0.56 (mean = 0.51, A_p = 2.02) in the hybrids, and from 0.35 to 0.53 (mean = 0.45, A_p = 2.00) in *R. michauxii.* Because only one population of *R. glabra* was sampled, pooled and individual population-level values are identical. Genetic diversity within populations (H_e) ranged from 0.08 to 0.14 (mean = 0.11) in the hybrid populations, from 0.06 to 0.12 (mean = 0.08) in *R. michauxii,* and was 0.13 in *R. glabra.* Observed heterozygosity (H_o) ranged from 0.11 to 0.21 (mean = 0.15) in the hybrid populations, from 0.06 to 0.11 (mean = 0.09) in *R. michauxii,* and was 0.08 in *R. glabra.*

Single-locus genotypic frequencies generally conformed to Hardy–Weinberg expectations. After controlling for multiple comparisons, 12 of the 199 chi-squared tests showed significant deviations. These included seven cases of significant heterozygote excess (*Aat* in RM1; *Fe1* in HYB1, HYB2, HYB4, and RM6; and *Idh2* in HYB4 and RM6) and five cases of significant heterozygote deficit (*Dia* in RG, *Fe1* in RM5, and *Mdh2* in HYB1, RM2, and RM4).

Total genetic diversity at the polymorphic loci (H_T) averaged 0.170 and 0.176 for *R. michauxii* and the hybrids, respectively (Table 4). The proportion of genetic diversity among populations (G_{ST}) ranged from 0.012 (*Skdh*) to 0.495 (*Fe1*) in *R. michauxii,* with a mean of 0.094, and from 0.018 (*Mdh2*) to 0.229 (*Idh2*) in the hybrids, with a mean of 0.087. Six of the 10 G_{ST} values were significant in *R. michauxii,* whereas 10 of the 11 G_{ST} values were significant in the hybrids. Genetic identities among the hybrid populations ranged from 0.97 to 0.99 (mean = 0.98) while genetic identities among the *R. michauxii* populations ranged from 0.93 to 1.00 (mean = 0.98). Genetic identities among all populations (hybrids, *R. michauxii,* and *R. glabra*) ranged from 0.85 to 1.00. The hybrids and *R. michauxii* had the highest mean identity (0.97), followed by the hybrids and *R. glabra* (0.89). *R. michauxii* and *R. glabra* were the most divergent groups (0.87). This latter value was nearly identical to the value provided by Sherman-Broyles et al. (1992) for this comparison.

Discussion

Overall, *R. michauxii* exhibits somewhat more within-population genetic variation

Table 4. Nei's (1973) statistics of genetic diversity for all polymorphic loci in the six *R. michauxii* and five hybrid populations

	R. michauxii				Hybrids			
Locus	H_T	H_S	D_{ST}	G_{ST}	H_T	H_S	D_{ST}	G_{ST}
Aat	0.324	0.282	0.042	0.131*	0.204	0.174	0.029	0.143*
Acp	0.231	0.216	0.016	0.067*	0.066	0.062	0.004	0.066*
Dia	0.205	0.198	0.006	0.031*	0.135	0.130	0.005	0.034*
Fe1	0.453	0.229	0.224	0.495*	0.492	0.458	0.033	0.068*
Idh2	0.080	0.072	0.008	0.102*	0.397	0.306	0.091	0.229*
Mdh2	0.101	0.098	0.002	0.023	0.097	0.095	0.002	0.018
Pgi2	—	—	—	—	0.066	0.063	0.003	0.051*
Pgm2	0.103	0.100	0.002	0.024	0.131	0.124	0.007	0.051*
Skdh	0.011	0.011	0.001	0.012	0.085	0.078	0.006	0.076*
Tpi2	0.177	0.172	0.005	0.031*	0.133	0.123	0.010	0.076*
6-Pgd	0.019	0.019	0.001	0.027	0.129	0.111	0.018	0.141*
Mean	**0.170**	**0.140**	**0.031**	**0.094**	**0.176**	**0.157**	**0.019**	**0.087**

* $P \leq 0.05$, adjusted using the sequential Bonferroni procedure of Holm (1979).

at Ft. Pickett than in the previously studied North Carolina populations (Table 1; Sherman-Broyles et al. 1992). Although the Ft. Pickett populations had slightly fewer alleles per polymorphic locus (A_p = 2.00 versus A_p = 2.08), values for both percent polymorphic loci (P = 0.45 versus P = 0.18) and genetic diversity (H_e = 0.08 versus H_e = 0.05) were higher at Ft. Pickett than in the populations studied by Sherman-Broyles et al. (1992). The higher levels of genetic diversity at Ft. Pickett may be a function of population size. In contrast to Ft. Pickett, which represents the largest known concentration of *R. michauxii,* the North Carolina populations are relatively small and isolated, and often have skewed sex ratios, suggesting that genetic drift may have played a greater role in North Carolina than at Ft. Pickett. However, the levels of genetic variation reported in our study are fairly typical when compared to other species with similar life-history characteristics. Genetic diversity in *R. michauxii* at Ft. Pickett (H_e = 0.08) is nearly identical to the average reported for woody species with an endemic distribution (0.078) as well as animal-pollinated woody species with a mixed mating system (0.075; Hamrick et al. 1992).

The proportion of genetic diversity among populations of *R. michauxii* and the hybrids was very similar (0.094 and 0.087, respectively), indicating relatively low levels of among-population differentiation (Table 4). These G_{ST} values are much lower than the values reported previously for *R. michauxii* (G_{ST} = 0.335; Sherman-Broyles et al. 1992). In addition, the averages reported for woody endemics (G_{ST} = 0.141) and animal-pollinated woody species with mixed mating systems (G_{ST} = 0.122) are both somewhat higher than those reported here (Hamrick et al. 1992). A probable explanation for the low level of differentiation at Ft. Pickett is that the populations sampled are geographically close to one another (<8 km) and may experience higher levels of gene flow than the more isolated North Carolina populations.

The genetic data presented here support Hardin and Philips' (1985) contention that hybridization can and does occur between *R. michauxii* and *R. glabra* in nature. It is difficult, however, to determine the overall frequency of hybridization from these data. Populations HYB3, HYB4, and RM6 consist almost exclusively of individuals that are heterozygous at *Idh2* for the alleles that are characteristic of the two species, indicating that these populations may largely consist of F_1 hybrid individuals. This result suggests that hybridization has played a major role in the production of these three populations. In contrast, populations HYB1 and HYB2 have the *R. michauxii* allele in high frequency (0.990 and 0.865, respectively), with relatively low levels of the *R. glabra* allele in the heterozygous state. There are two possible explanations for this finding. First, hybridization may have occurred infrequently at these sites, producing populations consisting of a majority of pure *R. michauxii* individuals with a few first-generation (i.e., F_1) hybrids mixed in. Conversely, hybridization may have occurred relatively frequently at these sites, producing populations of advanced-generation hybrids that have backcrossed extensively to *R. michauxii.*

The hybrid populations are nearly identical to *R. michauxii* (Nei's I = 0.97). When compared to *R. glabra,* however, the hybrids have a slightly higher identity than does *R. michauxii* (0.89 versus 0.87). This difference disappears when *Idh2* is removed from the analysis, indicating that the similarity of the hybrids with *R. glabra* is mainly due to alleles at this locus. Unfortunately these findings do not help to distinguish between relatively rare hybridization in a population consisting mainly of pure *R. michauxii* and frequent hybridization accompanied by extensive backcrossing to *R. michauxii.* The complete absence of *R. glabra* alleles at *Idh2* in six of the seven populations initially identified as *R. michauxii* argues against the possibility of widespread introgression between these two species. It appears that hybridization between the two species, while not unusual, is generally local in nature. It is important to note that populations HYB1, HYB2, HYB3, and HYB4 were identified in the field as hybrids because, although they were morphologically similar to *R. michauxii,* some of their traits (e.g., rachis coloration, degree of pubescence, etc.) appeared to be intermediate between those of *R. michauxii* and *R. glabra.* It is therefore possible that these "*michauxii*-like" hybrids represent only a portion of those hybrids actually present. There may be additional hybrid populations consisting of "*glabra*-like" hybrids that were missed due to our sampling strategy.

Conclusion

Rhus michauxii, like other rare endemics, may be susceptible to extinction due to a combination of factors. Although Ft. Pickett represents the largest known concentration of *R. michauxii,* populations at this site are geographically quite close to one another and highly susceptible to localized environmental changes. In addition, recent work has suggested that inbreeding can significantly increase extinction risk in local populations (Saccheri et al. 1998). While species with restricted ranges typically have low levels of genetic diversity, our results reveal that there is little evidence of inbreeding at Ft. Pickett. Finally, our results indicate that *R. michauxii* hybridizes with the more common *R. glabra* at this location. Due to the apparently localized nature of hybridization at Ft. Pickett, however, hybridization does not appear to be an immediate threat to the existence of *R. michauxii* at this location. The effect of natural hybridization on rare and endangered species ultimately depends on factors such as the initial frequency of the rare taxon, the rate of hybrid production, and the relative fitness of the resulting hybrid individuals (Wolf et al. 2001). Future studies aimed at quantifying these parameters will provide critical data for predicting the long-term effect

of hybridization on the survival of *R. michauxii*.

References

Hamrick JL and Godt MJW, 1989. Allozyme diversity in plant species. In: Plant population genetics, breeding and genetic resources (Brown AHD, Clegg MT, Kahler AL, and Weir BS, eds). Sunderland, MA: Sinauer Associates; 43–63.

Hamrick JL, Godt MJW, and Sherman-Broyles SL, 1992. Factors influencing levels of genetic diversity in woody plant species. New For 6:95–124.

Hardin JW and Philips LL, 1985. Hybridization in eastern North American *Rhus* (Anacardiaceae). ASB Bull 32: 99–106.

Holm S, 1979. A simple sequentially rejective multiple tests procedure. Scand J Stat 6:65–70.

Leary RF, Allendorf FW, and Forbes SH, 1993. Conservation genetics of bull trout in the Columbia and Klamath drainages. Conserv Biol 7:856–865.

Levin DA, Francisco-Ortega J, and Jansen RK, 1996. Hybridization and the extinction of rare plant species. Conserv Biol 10:10–16.

Little EL, 1980. The Audubon Society field guide to North American trees. New York: Alfred A. Knopf.

Mitton JB, Linhart YB, Hamrick JL, and Beckman JS, 1979. Observations of the genetic structure and mating system of ponderosa pine in the Colorado Front Range. Theor Appl Genet 51:5–13.

Nei M, 1972. Genetic distance between populations. Am Nat 106:283–292.

Nei M, 1973. Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70:3321–3323.

Nei M, 1977. F-statistics and analysis of gene diversity in subdivided populations. Ann Hum Genet 41:225–233.

Nevo E, 1985. Ecological and populational correlates of allozyme polymorphisms in mammals. Acta Zool Fenn 170:25–29.

O'Brien SJ, Roelke ME, Yuhki N, Richards KW, Johnson WE, Franklin WL, Anderson AE, Bass OL Jr, Belden RC, and Martenson JS, 1990. Genetic introgression in the Florida panther *Felis concolor* Coryi. Nat Geog Res 6: 485–494.

Price MV and Waser NM, 1979. Pollen dispersal and optimal outcrossing in *Delphinium nelsonii.* Nature 227: 294–297.

Rieseberg LH, Zona S, Aberbom L, and Martin T, 1989. Hybridization in the island endemic, Catalina mahogany. Conserv Biol 3:52–58.

Saccheri I, Kuussaari M, Kankare M, Vikman P, Fortelius W, and Hanski I. 1998. Inbreeding and extinction in a butterfly metapopulation. Nature 392:491–494.

Sherman-Broyles SL, Gibson JP, Hamrick JL, Bucher MA, and Gibson MJ, 1992. Comparisons of allozyme diversity among rare and widespread *Rhus* species. Syst Bot 17:551–559.

Soltis DE, Haufler CH, Darrow DC, and Gastony GJ, 1983. Starch gel electrophoresis of ferns: a compilation of crushing buffers, gel and electrode buffers, and staining schedules. Am Fern J 73:373–380.

Sutter RD, Mansberg L, and Moore J, 1983. Endangered, threatened, and rare plant species of North Carolina: a revised list. ASB Bull 30:153–163.

Templeton AR, 1986. Coadaptation and outbreeding depression. In: Conservation biology: the science of scarcity and diversity (Soulé ME, ed). Sunderland, MA: Sinauer Associates; 105–115.

U.S. Fish and Wildlife Service. 1993. Michaux's sumac recovery plan. Atlanta: U.S. Fish and Wildlife Service.

Wayne RK and Jenks SM, 1991. Mitochondrial DNA analysis implying extensive hybridization in the endangered red wolf *Canus rufus.* Nature 351:564–568.

Wolf DE, Takebayashi N, and Rieseberg LH, 2001. Predicting the risk of extinction through hybridization. Conserv Biol 15:1039–1053.

Workman PL and Niswander JD. 1970. Population studies on southwestern Indian tribes II. Local differentiation in the Papago. Am J Hum Genet 22:24–49.

Wright S, 1922. Coefficients of inbreeding and relationship. Am Nat 56:330–338.

Received October 18, 2000
Accepted September 5, 2001

Corresponding Editor: Kendall R. Lamkey