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Allozyme Diversity in *Macbridea alba* (Lamiaceae), an Endemic Florida Mint

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Abstract

Macbridea alba is a herbaceous perennial mint endemic to the panhandle region of Florida. We used starch gel electrophoresis to describe allozyme diversity and genetic structure in this federally threatened plant. Ten populations were analyzed, with an average sample size of 47 plants (range 41–48 plants) per population. Of the 22 loci analyzed, 11 (50%) were polymorphic, with an average of 36.2% of the loci polymorphic within populations. Gene diversity measures for the species ($H_{cs} = 0.121$) and for the populations ($H_{cp} = 0.099$) were slightly higher than means found for other surveyed endemic plants. Compared to nine previously analyzed woody mints, however, *M. alba* is genetically depauperate. Little genetic structure was evident within the species, with 92% of the total genetic variation found within populations. Genetic identities between population pairs were high (mean $I = 0.98$). The perennial life habit of *M. alba* and long-distance pollination by bumblebees may contribute to the maintenance of genetic diversity within this threatened species.

Of the 50 states in the United States, Florida, which harbors more than 3800 plant species, ranks third in fern and seed plant species richness (Wunderlin and Hansen 2000). About 221 plant species are endemic to this peninsular state. Approximately 500 Florida plants are listed as threatened or endangered by the state; more than 50 of these are on the Federal Endangered Species List (http://floridaconservation.org). Many plant species endemic to Florida are at increasing risk of extinction because of rapid human population growth and the ever-increasing pressure such growth brings. As plant habitats become developments, as wetlands, uplands, and savannahs are altered or diminished in size, and as natural processes are altered or suppressed, many native Florida plant populations are declining in size. Associated with decreasing population sizes are increased extinction risks from stochastic factors (e.g., drought), environmental factors (e.g., decreased pollinator service), and genetic factors (e.g., increased inbreeding and decreased genetic diversity). Relatively little is known of the genetic diversity and structure of endangered and threatened plant species within this biotically diverse state. Knowledge of genetic structure and diversity is critical for the formulation of scientifically based, species-specific conservation and restoration plans for these rare and threatened species.

Among the species at risk in Florida is the herbaceous perennial *Macbridea alba* Chapman, a member of the mint (Lamiaceae) family. Listed as a federally threatened species, *M. alba* is endemic to four counties of the Florida panhandle (Bay, Gulf, Franklin, and Liberty). In 1989 the Florida Natural Areas Inventory documented 63 "occurrences" of this species, more than two-thirds of which were within the Apalachicola National Forest (U.S. Fish and Wildlife Service 1994).

Macbridea alba, like many herbaceous Florida endemics, is a fire-adapted species. Within its restricted range, *M. alba* is found in a variety of fire-maintained habitats, including wet to mesic pine flatwoods, wet savannas, and seepage slopes (Walker J, personal observation). Although the species persists for several years in areas where fire has been excluded, flowering and seed set decline with time since fire (Madsen 1999; Walker and White 1994).

The specific epithet ("alba") of *M. alba* refers to the brilliant white flowers of this diminutive (3–4 dm) mint. The superficial resemblance of the small clusters of buds and flowers within *M. alba*'s white inflorescence to eggs and bird's heads has led to the quaint common name given to the species: "white birds-in-a-nest."

Macbridea alba reproduces sexually via hermaphroditic flowers that require insect vectors for pollination (Madsen 1999). The production of short rhizomes permits limited vegetative spread. Bumblebees are the primary pollinators (Madsen 1999). The species usually flowers during the late spring and summer months (May–July), although phenology can be fire related, with flowering occurring subsequent to

burns (Walker J, personal observation). Each flower can produce four nutlets, which are primarily gravity dispersed. The species is self-compatible (Walker and Madsen 1997), but selfed seeds exhibit inbreeding depression (Walker J, unpublished data). Quantitative estimates of *M. alba*'s mating system (i.e., percent outcrossing) are not available.

The objective of this study was to describe genetic diversity and structure within *M. alba*. Its genetic diversity and structure are contrasted with other mint species, with endemic plants and with plant species having similar life-history characteristics.

Materials and Methods

Macbridea alba leaves were collected from 10 populations, 9 of which were in Apalachicola National Forest. Field searches (in 2000) based on herbarium records suggested that many "populations" or "occurrences" of this species off the Apalachicola National Forest may have been extirpated. It is also possible that these populations were dormant during 2000; given unfavorable environmental conditions, *M. alba*, a perennial, can persist underground until conditions improve (Walker J, unpublished data). Two populations (one roadside population off Forest land, and one on Forest land) were collected during the spring of 2000; the remaining populations (all from within the Forest) were sampled in spring 2001. All populations within the Apalachicola National Forest were in the southwest corner of the Forest. Distances between populations within the Forest ranged from about 1 km to 18 km (mean 6.9 km; SD = 5.0). The sole population located outside the Forest was about 23–28 km from those within.

Due to an extended (3-year) drought in the southeastern United States, many plants were in poor condition during both sampling years. Leaves were smaller than usual, and many had extensive herbivore damage. Sample sizes per population ranged from 41 to 48 (mean 46.7; SD = 2.6). Population sizes were not estimated. This savannah species can be fairly cryptic in the grass, even when in flower. Because juveniles are difficult to identify and individual plants can be dormant for at least a year, population size estimates require long-term, detailed demographic studies. Currently one of the authors (J.W.) is conducting such studies at several sites; these should provide data on plant longevity, population sizes, and fluctuations.

Several leaves from each sampled plant were bagged and placed on ice in the field and transported to the University of Georgia. Within 60 hours of sample collection, enzymes were extracted from plant samples by finely cutting several leaves from each sample and crushing them with a mortar and pestle to which an extraction buffer (Wendel and Parks 1982) had been added. Preliminary electrophoretic trial runs on greenhouse-grown material indicated that this extraction buffer provided superior allozyme resolution to one designed for pines (Mitton et al. 1979). Enzyme extracts were absorbed onto chromatography paper wicks that were stored in microtest plates at −70°C until used in genetic analyses.

Allozyme diversity was determined via standard starch gel electrophoresis. The following enzymes were assayed, with the loci resolved indicated in parentheses: amino acid transferase (*Aat-2*), aconitase (*Acon-1* and *Acon-2*), colorimetric esterase (*Ce-1, Ce-2,* and *Ce-3*), cathodal peroxidase (*Cper*), diaphorase (*Dia-1* and *Dia-2*), isocitrate dehydrogenase (*Idh-1* and *Idh-2*), malate dehydrogenase (*Mdh-1, Mdh-2,* and *Mdh-3*), malic enzyme (*Me-1*), phosphoglucoisomerase (*Pgi-1*), phosphoglucomutase (*Pgm-2*), peroxidase (*Per*), 6-phosphogluconate dehydrogenase (*6-Pgdh-1*), shikimate dehydrogenase (*Skdh-1*), and UTP-glucose-1-phosphate (*Ugpp-1* and *Ugpp-2*). Stain recipes were modified from Soltis et al. (1983), except for diaphorase (Cheliak and Pitel 1984) and UTP-glucose-1-phosphate (Manchenko 1994). The numbered gel and electrode buffer systems used are described in Table 1 of Soltis et al. (1983). Buffer system 4 was used to resolve *Acon-1, Acon-2, Idh-1, Idh-2, 6-Pgdh, Skdh-1, Ugpp-1,* and *Ugpp-2*; buffer system 6 resolved *Ce-1, Ce-2, Ce-3, Cper, Me-1,* and *Per*; *Pgm-2* was resolved on buffer system 11. A modified buffer system 8 was used for *Dia-1, Dia-2,* and *Pgi-1*. A continuous morpholine citrate buffer system (Conkle et al. 1982) was used to resolve *Aat-2, Mdh-1, Mdh-2,* and *Mdh-3*.

Genetic diversity measures were calculated for each population (Hedrick 1985) and for the species (Hamrick and Godt 1989) using a statistical program developed by M. D. Loveless and A. Schnabel. These measures included the percentage of polymorphic loci (P), the mean number of alleles per locus (A) and per polymorphic locus (AP), the effective number of alleles (A_e), and observed (H_o) and expected (H_e) heterozygosity. Species values (Hamrick and Godt 1989) are subscripted with an "s" and population means are subscripted with a "p." Deviations from Hardy-Weinberg expectations were estimated for each polymorphic locus with Wright's fixation index (F; Wright 1922). Indices were tested for significance using chi-square tests (Li and Horvitz 1953).

To describe genetic structure, the proportion of total genetic diversity (G_{ST}) found among populations was calculated for each polymorphic locus (Nei 1973, 1977). Chi-square tests were employed to test the significance of these values at each locus (Workman and Niswander 1970). G_{ST} values were averaged across polymorphic loci to estimate the proportion of total genetic diversity found among populations. Nei's genetic identity (Nei 1972) was also calculated for each pair of populations.

Results

Fifty percent of the 22 loci scored for *M. alba* were polymorphic. An additional locus (*Aat-1*) was also highly polymorphic (as indicated in electrophoretic trials on greenhouse-grown material), but it could not be reliably scored on the field samples due to low enzymatic activity in the drought-stressed samples. When this locus is included,

Table 1. Genetic diversity statistics[a] for *Machridea alba*

Population	*P*	*AP*	*A*	No. of alleles	A_e	H_o (SD)	H_e (SD)
1	33.3	2.29	1.43	30	1.13	0.077 (0.036)	0.078 (0.039)
2	36.4	2.63	1.59	35	1.22	0.107 (0.044)	0.123 (0.043)
3	40.9	2.78	1.73	38	1.18	0.105 (0.038)	0.110 (0.037)
4	33.3	2.29	1.43	30	1.15	0.079 (0.030)	0.087 (0.036)
5	31.8	2.29	1.41	29	1.17	0.091 (0.033)	0.094 (0.038)
6	50.0	2.27	1.64	36	1.19	0.105 (0.038)	0.112 (0.038)
7	40.9	2.33	1.55	34	1.21	0.098 (0.035)	0.110 (0.044)
8	31.8	2.29	1.41	31	1.14	0.089 (0.036)	0.083 (0.039)
9	31.8	2.29	1.41	31	1.16	0.077 (0.033)	0.095 (0.036)
10	31.8	2.29	1.41	31	1.16	0.094 (0.036)	0.097 (0.038)
Mean	36.2	2.37	1.50	32.5	1.17	0.092	0.099
SD	3.2	0.18	0.12	3.0	0.03	0.011	0.012
Species total	55.0	2.91	1.95	43	1.21	—	0.121

[a] *P* is the percentage of polymorphic loci, *AP* is the mean number of alleles per polymorphic locus, *A* is the mean number of alleles per locus, A_e is the effective number of alleles per locus, H_e is gene diversity (expected heterozygosity), and H_o is observed heterozygosity. SD is standard deviation.

the percentage of polymorphic loci for the species (P_s) is 55%. Within populations the percentage of polymorphic loci ranged from 32% to 50%, with an average (P_p) of 36.2% (Table 1). The average number of alleles per polymorphic locus (AP_s) was 2.91 for the species; the population mean (AP_p) was 2.37. The highest number of alleles per locus was five (for *Dia-1*); 5 of the 11 polymorphic loci were diallelic (Table 2). The effective number of alleles per locus (A_{es}) was 1.21 for the species and averaged (A_{ep}) 1.17 within populations (Table 1). Despite a fairly high proportion of polymorphic loci, gene diversity was relatively low (H_{es} = 0.121 for the species; the population mean was H_{ep} = 0.099). Among populations, gene diversity ranged from 0.078 to 0.123 (Table 1). Five alleles were detected only in single populations. Two of these alleles were in populations 2 and 6, and three were in population 3. Overall these alleles were in low frequencies, ranging from 0.01 to 0.15, with an overall mean frequency of 0.056.

Total gene diversity (H_T) at polymorphic loci was 0.241, with most (92%) of the variation being found within (rather than among) populations, as indicated by the G_{ST} value (0.08; Table 2). Despite the low G_{ST} value, statistically significant ($P < .05$) differences were found among populations for 9 of the 11 polymorphic loci (Table 2). Six of the 11 polymorphic loci (with $H_T > 0.200$) were major contributors to overall gene diversity. Nei's genetic identity values (I) ranged from 0.93 to 0.99 with a mean of 0.98 (SD = 0.02), indicating that allele frequencies were fairly similar among populations.

Seventy-eight fixation indices were tested for deviations from Hardy-Weinberg expectations, with 14 significant ($P < .05$) results. Twelve of the significant fixation indices were positive, indicating heterozygote deficits, and two were negative, indicating an excess of heterozygotes. Despite these statistically significant results, the overall F_{IS} value was only slightly above zero (0.055), indicating that populations were very close to Hardy-Weinberg equilibrium.

Discussion

Machridea alba is a narrowly distributed endemic species; it is restricted to a small area of the Florida panhandle and has probably never been widespread. Reviews of the allozyme literature indicate that geographically restricted species tend to maintain less genetic diversity within their populations than more widespread species (Hamrick and Godt 1989). This generalization seems to hold across phylogenetically diverse species groups (Hamrick and Godt 1989), as well as among comparisons between rare and widespread congeners (Gitzendanner and Soltis 2000). The lower genetic diversity maintained by endemic species may reflect their evolutionary origins, or it may be the result of small population sizes within their recent past. Without historical and phylogenetic data, it is difficult to distinguish between these alternatives. In any case, low genetic diversity may limit a species' ability to adapt to changing environmental conditions (Ellstrand and Elam 1993).

Allozyme diversity in *M. alba* is slightly higher than mean values found for endemic plant species. For example, species' genetic diversity (H_{es}) is 0.123 for *M. alba* and 0.096 for 81 endemics (Hamrick and Godt 1989), while mean

Table 2. Nei's (1973, 1977) genetic diversity statistics[a] (based on polymorphic loci) for *Machridea alba*

Locus	No. of alleles	H_T	H_S	G_{ST}
Acon-2	2	0.478	0.452	0.055
Ce-1	2	0.002	0.002	0.009
Ce-3	3	0.334	0.201	0.399
Ce-4	2	0.002	0.002	0.009
Cper	3	0.352	0.336	0.046
Dia-1	5	0.246	0.236	0.039
Pgm-2	2	0.493	0.438	0.112
Per	3	0.080	0.075	0.061
6-Pgdh-1	2	0.020	0.020	0.017
Ugpp-1	4	0.133	0.123	0.070
Ugpp-2	4	0.517	0.473	0.084
Mean	2.9	0.242	0.214	0.082

G_{ST} values are statistically significant ($P < .05$) except for *Ce-1* and *6-Pgdh-1*.

[a] Total genetic diversity is H_T, genetic diversity found within populations is H_S, and the proportion of total genetic diversity found among populations is G_{ST}.

Table 3. Comparisons of genetic diversity in *Macbridea alba* and other southeastern mint (Lamiaceae) species, short-lived perennials, and endemic plants

Species	No. of Population	No. of loci	P_s	AP_s	H_{es}	P_p	AP_p	H_{ep}	G_{ST}
Conradina brevifolia Shinners[a]	3	12	83.3	3.50	0.349	73.2	3.04	0.323	0.099
Conradina canescens (T & G) Gray[a]	3	12	91.7	4.27	0.365	71.4	2.98	0.291	0.197
Conradina etonia Kral & McCartney[a]	1	12	—	—	—	41.7	3.00	0.231	—
Conradina grandiflora (Small)[a]	2	12	75.0	3.56	0.364	59.7	3.44	0.294	0.172
Conradina verticillata Jennison[a]	2	12	58.3	2.71	0.227	50.0	2.64	0.219	0.123
Dicerandra christmanii Huck & Judd[b]	1	17	—	—	—	58.8	2.70	0.210	—
Dicerandra cornutissima Huck[b]	1	17	—	—	—	43.8	2.30	0.102	—
Dicerandra frutescens Shinners[b]	2	17	—	—	—	64.7	2.90	0.246	—
Dicerandra immaculata Lakela[b]	1	17	—	—	—	43.8	2.30	0.102	—
Mean	1.7	14.2	77.1	3.51	0.326	56.3	2.81	0.224	0.148
SD	0.8	2.6	14.3	0.64	0.067	12.1	0.37	0.079	0.045
***Macbridea alba* Chapman**	**10**	**22**	**50.0**	**2.91**	**0.121**	**36.2**	**2.37**	**0.099**	**0.082**
Short-lived herbaceous perennials[c]	—	—	41.3	—	0.116	28.0	—	0.096	0.233
Endemic plants[c]	—	—	40.0	—	0.096	26.3	—	0.063	0.248

See Table 1 for a definition of the genetic parameters.

[a] Crook (1998).

[b] Macdonald and Hamrick (1996).

[c] Hamrick and Godt (1989).

population gene diversity (H_{cp}) is 0.099 for *M. alba* and 0.063 for 100 endemics (Hamrick and Godt 1989). The percentage of polymorphic loci is also somewhat higher for *M. alba* than means found for endemic plants, for the species overall, and within populations [P_s = 40.0% and P_p = 26.3 % for endemics (N = 81 and 100, respectively; Hamrick and Godt 1989), whereas P_s = 55% and P_p = 36.2 % for *M. alba*].

The comparison of genetic diversity within rare species and their widespread congeners may provide insight into potential causes of diminished genetic diversity, because congeners share recent phylogenetic histories, and they often share life-history characters that influence genetic diversity (Gitzendanner and Soltis 2000; Hamrick and Godt 1989; Karron 1987). For example, if genetic diversity is low within a common widespread species, as well as within its rare congener, it is quite possible that the diminished genetic diversity is associated with the phylogenetic history of the genus. Rare species that share common alleles with widespread congeners, but lack alleles found in low or intermediate frequencies in their widespread relatives may be derivative species that originated with low genetic diversity (Loveless and Hamrick 1988; Pleasants and Wendel 1989). Alternatively, low genetic diversity in a rare (particularly an endangered) species, compared to its widespread congener, may indicate the random loss of variation due to continuously small population sizes or transient population crashes. None of these scenarios can be determined with assurance, however, without historical data. Such data are generally unavailable for plants, although there are exceptions (e.g., Landergott et al. 2001).

Macbridea alba has a single congener, *Macbridea caroliniana*, which is restricted to a few counties in North and South Carolina. Although not federally listed as a threatened species, *M. caroliniana* may be as rare (or perhaps rarer) than *M. alba* (Godt MJ, personal communication with southeastern botanists). Genetic diversity comparisons between these congeners are precluded due to a lack of genetic data for *M. caroliniana*.

Allozyme diversity has been described for at least nine mints other than *M. alba* (Crook 1998; Macdonald and Hamrick 1996), and they provide comparative data (Table 3). All nine mint species are found within the southeastern United States, and four (the *Dicerandra* species) are rare Florida endemics. Genetic diversity within most of these mint species is substantially higher than that found for *M. alba*. For example, overall mean population genetic diversity (H_{cp}) is 0.224 for the nine mints (SD = 0.079; range 0.102–0.323), whereas H_{cp} = 0.099 for *M. alba*, a twofold difference. This difference is especially striking since few populations (populations one to three) were analyzed for the *Conradina* and *Dicerandra* species. However, two endemic Florida mints (*Dicerandra cornutissima* and *Dicerandra immaculata*) have genetic diversity values (H_{cp} = 0.102) similar to *M. alba*. In contrast to *M. alba*, the *Conradina* and *Dicerandra* species are "woody mints." Woody species tend to have much higher genetic diversity than herbs (Hamrick and Godt 1996; Hamrick et al. 1992). This may be evolutionarily associated with their longer life spans, during which they are likely to be exposed to more rapidly evolving diseases and pests (Hamrick 1978). Woodiness may account for the overall difference in genetic variation between other southeastern mint species and *M. alba*.

Overall, Wright's F statistics indicated that *M. alba*'s populations were close to Hardy-Weinberg equilibrium, suggesting that the populations were randomly mating. However, estimates of a species' mating system based on the genetic analyses of adult plants may be misleading, especially if inbreeding depression has been documented within early life stages. Genetic analysis of progeny from a number of maternal families would be necessary to directly estimate

outcrossing rates and biparental inbreeding (mating among related individuals) for a complete picture of *M. alba*'s current mating system.

Little genetic divergence was evident among the 10 sampled *M. alba* populations. The mean genetic identity for *M. alba* ($I = 0.98$) was higher than the mean value ($I = 0.95$) Gottlieb (1977) found in a review of conspecific populations. Furthermore, the range of genetic identities between population pairs of *M. alba* was small ($I = 0.93$–0.99). Genetic identities are influenced both by polymorphic loci and the number of monomorphic loci; G_{ST} values, based on polymorphic loci, provide an additional perspective on population divergence. We found that 92% of the genetic variation at polymorphic loci was found within *M. alba* populations ($G_{ST} = 0.08$), again indicating very little population divergence. Curiously, more genetic structure was evident for four other genetically analyzed southeastern mint species (Table 3).

Macbridea alba also exhibited less genetic structure compared to means found for short-lived herbaceous perennials ($G_{ST} = 0.233$, $N = 119$) and endemic species ($G_{ST} = 0.248$, $N = 52$) (Hamrick and Godt 1989). The low level of population divergence found for *M. alba* may be associated with the restricted range of the species, coupled with strong-flying pollinators that may move pollen among populations.

Low levels of population divergence suggest that genetic drift is not currently of great concern for this species. Population management for this threatened species should include careful management of fire regimes and the maintenance of large populations. Fire management is an important consideration for gene flow among populations, since timing of flowering can be offset by fires. If nearby populations are burned at different times, interpopulation gene flow may be precluded due to differing phenologies of plants at the sites. Reestablishment of populations at protected sites within the historic range of the species should be considered to help ensure the long-term viability of the species. Currently most populations are apparently found only in the Apalachicola National Forest. The establishment of some "safe sites" off the forest may be beneficial because localized species are vulnerable to even geographically restricted catastrophic events, diseases, and pests. Furthermore, because of conflicting goals and mandates, land management policies of the U.S. Forest Service may not always be beneficial for the long-term viability of all endangered species within a region. Propagules used to restore populations should be taken from ecologically similar sites. However, more than one population could be used as a source of propagules; this should allow recombination and natural selection to sort out favorable genotypes in restored populations.

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