ALLOZYME DIFFERENTIATION AMONG *BEALIA MEXICANA, MUHLENBERGIA ARGENTEA,* AND *M. LUCIDA* (POACEAE: ERAGROSTIDEAE)

PAUL M. PETERSON
Department of Botany, National Museum of Natural History, Smithsonian Institution, Washington, DC 20560

MELVIN R. DUVALL
Department of Botany and Plant Sciences, University of California, Riverside, CA 92521

ALAN H. CHRISTENSEN
Department of Biology, George Mason University, Fairfax, VA 22030

ABSTRACT

Allozyme data were used to evaluate the genetic variation, relationships, and population genetic structure of three endemic grasses: *Bealia mexicana, Muhlenbergia argentea,* and *M. lucida.* Electrophoretic examination of 20 putative enzyme loci in 14 populations revealed that all three species have high genetic variability (*H* ranging from 0.19 to 0.26; *F* ranging from 0.073 to −1.000) indicative of mixed mating and outcrossing plants, this variation being sequestered within populations in *B. mexicana* and *M. lucida* and among populations in *M. argentea.* The highest allozymic similarity occurred between *B. mexicana* and *M. argentea* (I = 0.83) rather than between *M. lucida* and *M.argentea* (I = 0.59). At present, populations of the geographically restricted *B. mexicana* and *M. argentea* are not threatened due to lack of genetic variation and their survival depends on the maintenance of critical habitat.

RESUMEN

Mediante el análisis de alozimas se evaluó la variación genética, relaciones y estructura genético de tres gramíneas endémicas: *Bealia mexicana, Muhlenbergia argentea* y *M. lucida.* El examen electroforético de 20 loci putativo enzimaticos en 14 poblaciones reveló que las tres especies tienen una alta variabilidad genética (*H* varía de 0.19 a 0.26; *F* varía de 0.073 a −1.000), indicadora de aparejamiento variado y polinización cruzada, estando esta variación dividida dentro de las poblaciones en *B. mexicana* and *M. lucida* y entre poblaciones en *M. argentea.* Se encontró mayor similitud alozimica entre *B. mexicana* y *M. argentea* (I = 0.83) que entre *M. lucida* y *M. argentea* (I = 0.59). Actualmente poblaciones de las geograficamente restringidas *B. mexicana* y *M. argentea* no se encuentran en amenaza debido a falta de variación genética y su supervivencia depende de que se mantenga su hábitat crítico.

Muhlenbergia Schreb. is a large, primarily New World genus of perhaps 160 species, most of which occur in the arid lands of the southwestern United States and north-central Mexico. The genus, as now interpreted, is morphologically very diverse and probably represents a taxonomic dumping ground (Correll and Johnston 1970;

MADROÑO, Vol. 40, No. 3, pp. 148–160, 1993

Peterson and Annable 1991; Peterson et al. 1989). The revision of the entire genus is currently underway and will undoubtedly result in additional re-alignment of some species as has already been accomplished for some taxa (Peterson 1989; Peterson and Annable 1990, 1991, 1992; Peterson unpublished data).

Bealia mexicana Scribn. in Beal, *Muhlenbergia argentea* Vasey, and *M. lucida* Swallen are morphologically very similar and have been suggested by various authors as being closely related (Hitchcock 1935; Swallen 1936; Peterson 1989). They all possess an unusual feature, the occurrence of a deeply bilobed lemma with a crisped-curled to flexuous awn borne between the two lobes (Peterson 1989). However, the lobe apices in *B. mexicana* and *M. argentea* are obtuse to rounded and in *M. lucida* the apices are acute. *Muhlenbergia lucida* also has tightly involute leaf blades whereas *B. mexicana* and *M. argentea* have flat to very loosely involute leaf blades.

Muhlenbergia argentea and *M. lucida* are caespitose perennials from the Sierra Madre Occidental of western Chihuahua, Mexico. *Muhlenbergia argentea* is restricted in range and habitat and occurs on reddish rhyolitic lava flows in seasonally wet rocky crevices usually associated with a cliff face, at elevations between 1780–2100 m. It is known from only three localities, one just above the Cascada de Basaseachic and the other two northeast of La Bufa on the cliffs above the Rio Batópilas. *Muhlenbergia lucida* is more wide ranging in the Sierra, where it occurs on gray to reddish or white volcanic pumice, lapilli tuff and altered rhyolite lava flows in dry rocky sites among boulders at elevations between 2000–2600 m.

Bealia Scribn. in Hack. is a monotypic genus known from only a few localities in the Sierra Madre Occidental in Chihuahua and Durango, Mexico. Until only recently, *Bealia mexicana* was placed in *Muhlenbergia* (*M. biloba* Hitchc.), but with new cytological and existing morphological evidence this species has been reinstated in its own genus (Peterson 1989). *Bealia mexicana* can be differentiated from *Muhlenbergia* by possessing deeply bilobed lemmas (1–1.4 mm long) with rounded to obtuse lobes, crisped-curled to flexuous awns born between the lobes, minutely glandular pedicels, loosely pilose to villous, single-nerved glumes, and by its annual life form. In *Muhlenbergia* the lemma is usually not deeply bilobed, although the apex can sometimes be emarginate to shallowly lobed with acuminate to aristate teeth less than 1 mm long (*M. argentea* is the exception), the awns are usually straight to flexuous (*M. argentea, M. crispiseta* Hitchc. and *M. flaviseta* Scribn. have crisped-curled awns), the pedicels are eglandular, and both perennials and annuals are common. *Bealia mexicana* is presently known from only four localities in the Sierra and is restricted to whitish, shallow and sandy, volcanic, lapilli tuff soils on flat escarpments or ledges at elevations between 2000–2300 m.

In *Muhlenbergia* and other Eragrostideae the base chromosome number is generally 10 and only *Bealia, Blepharoneuron* Nash, *Chaboissaea* Fourn., *Crypsis* Ait., *Dasyochloa* Willd. ex Rydb., *Erioneuron* Nash, and *Munroa* Torr. have a base number of 8 (Gould 1958; Hammel and Reeder 1979; Peterson 1988, 1989; Peterson and Annable 1990, 1992; Reeder 1967, 1968, 1971, 1977; Reeder and Reeder 1988; Tateoka 1961). Cladistic analysis of chloroplast DNA restriction fragment variation suggests a close relationship among *Bealia, Blepharoneuron, Chaboissaea,* and *Muhlenbergia,* excluding *Dasyochloa, Erioneuron,* and *Munroa* (Duvall et al. in review). *Crypsis,* although morphologically similar to *Muhlenbergia,* is probably more closely allied with *Sporobolus* where it shares 1-nerved lemmas, caryopses with free pericarps, and hairy ligules.

The present study was initiated to estimate the genetic differentiation within and among populations of *B. mexicana, M. argentea,* and *M. lucida.* We also wanted to determine whether allozymic data could clarify relationships and allow the evaluation of the population genetic structure between the annual and perennial life form in highly endemic species. There is very little information describing the genetic diversity of geographically restricted, potentially endangered grasses. This study is the first population-based analysis of soluble enzymes in *Muhlenbergia* and *Bealia,* a related genus.

Material and Methods

One hundred individuals from four populations of *B. mexicana,* 78 individuals from three populations of *M. argentea,* and 178 individuals from seven populations of *M. lucida* were examined for electrophoretic variation (Table 1). Fresh leaf blades or entire plants, if a small annual, were collected in the field, placed in a 3.6 ml Nunc cryotube, and frozen on site in liquid nitrogen.

Floral buds were field collected and fixed in ethanol–acetic acid (3:1, V:V) prior to storage under refrigeration in 70% ethanol. Meiotic chromosome counts were obtained from aceto-carmine squashes on pollen mother cells. Representative cells were recorded with sketches and photographed using Kodak Technical Pan film. Chromosome number determinations were based on observations of 10 or more cells at diakinesis.

Sample preparation and electrophoresis of enzymes followed the general methodology of Morden et al. (1987). Approximately 300 mg of mature tissue from each plant was homogenized in up to 25 drops of grinding buffer (Morden et al. 1987) together with about 50 mg of sea sand to enhance disruption of cells. Extracts were absorbed into 2 × 11 mm Whatman filter paper wicks and stored at −80°C. Electrophoresis was conducted in the four gel/buffer systems of Morden et al. (1987): L, M, N, and T, however, starch content

TABLE 1. FIELD COLLECTIONS OF *BEALIA MEXICANA, MUHLENBERGIA ARGENTEA, M. LUCIDA, CHABOISSAEA DECUMBENS, C. LIGULATA,* AND *C. SUBBIFLORA* ANALYZED BY ENZYME ELECTROPHORESIS. Chromosome numbers are given in parenthesis. Voucher specimens are deposited at US.

B. mexicana. MEXICO. **Chihuahua:** Parque Natural Cumbres de Majalca, Colonia Cumbres de Majalca, *Peterson, Annable & Herrera 7946*; Parque Natural Cumbres de Majalca, W of Cumbres de Majalca, *Peterson, Annable & Herrera 7981* (n=16); S of Villa Matamoros, *Peterson & King 8264* (n=16). **Durango:** SW of El Ojito, *Peterson, Annable & Herrera 8090.*

M. argentea. MEXICO. **Chihuahua:** Parque Natural Barranca del Cobre, NE of La Bufa, *Peterson, Annable & Herrera 8044, 8066* (n=10); Cascada de Basaseachic, *Peterson & King 8248.*

M. lucida. MEXICO. **Chihuahua:** Parque Natural Cumbres de Majalca, Colonia Cumbres de Majalca, *Peterson, Annable & Herrera 7973*; Parque Natural Cumbres de Majalca, W of Cumbres de Majalca, *Peterson, Annable & Herrera 7978*; Parque Natural Barranca del Cobre, S of Creel, *Peterson, Annable & Herrera 8029*; Parque Natural Barranca del Cobre, NE of Batópilas, *Peterson, Annable & Herrera 8039*; E of Guachochi, *Peterson, Annable & Herrera 8083*; W of La Junta, *Peterson & King 8202*; Parque Natural Cumbres de Majalca, Cascada de Basaseachic, *Peterson & King 8249.*

C. decumbens. MEXICO. **Chihuahua:** W of Cuauhtemoc, *Peterson, Annable & Herrera 7983.*

C. ligulata. MEXICO. **Chihuahua:** N of Cuauhtemoc, *Peterson & Annable 8111.*

C. subbiflora. MEXICO. **Durango:** N of Durango, *Peterson & King 8266.*

was modified to optimize gel handling characteristics and improve resolution. All gels consisted of a 2.6:1, Electrostarch : Sigma Starch ratio with a total starch content of 13.5%, 11.5%, 11.5%, and 13.0% for the L, M, N, and T systems, respectively. For each population, samples from all individuals were included together on the same gel. Selected individuals from different populations were then analyzed together for purposes of interspecific and interpopulational caparisons. Gels were sliced and stained for the following 14 enzymes: aspartate aminotransferase (AAT), aconitase (ACO), adenylate kinase (ADK), aminopeptidase (AMP), fructokinase (FRK), glutamate dehydrogenase (GDH), glutamate-pyruvate transaminase (GPT), isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), 6-phosphogluconate dehydrogenase (PGD), phosphoglucose isomerase (PGI), phosphoglucomutase (PGM), shikimate dehydrogenase (SAD), and triose phosphate isomerase (TPI). Only the plastid form of IDH was surveyed. Loci were designated sequentially with the most anodally-migrating isozyme designated 1, the next 2, and so on. Alleles were designated sequentially with the most anodally migrating allele given an *a,* the next *b,* and so on.

Values for Nei's (1972) genetic identity (I) and distance measures were computed for pairwise comparisons using BIOSYS-1 (Swofford and Selander 1989). Standard measures of genetic variation (Table 3) were computed for *B. mexicana, M. argentea,* and *M. lucida,*

including mean number of alleles per locus (A), proportion of polymorphic loci (P), mean heterozygosity (H), and mean fixation (F) index which measures the deviation of genotypic proportions from Hardy-Weinberg expectations (Jain and Workman 1967; Swofford and Selander 1989). The distribution of genetic variation within the three taxa was determined using F-statistics (Table 4) where F_{IS} is the fixation index within populations, F_{IT} is the overall fixation index, and F_{ST} measures the amount of differentiation among populations (Wright 1965, 1969). The patristic distance matrix was calculated using the Prevosti distance index (Wright 1978) and after optimization of branch lengths a corresponding Wagner tree (Fig. 1) was produced using *Chaboissaea decumbens* (Swallen) J. & C. Reeder, *C. ligulata* Fourn., and *C. subbiflora* (Hitchc.) J. & C. Reeder as outgroups (Swofford and Selander 1989).

Results

New chromosome counts (Table 1) were made in three populations: two of *B. mexicana* ($n=16$) and one of *M. argentea* ($n=10$). A previous reported chromosome count for *B. mexicana* from the same locality as population *8090* indicated a diploid at $n=8$ (Peterson 1988). The earlier count is probably in error since no irregularities in the meiotic chromosome pairing, such as univalents or trivalents, were noted in the new preparations of *B. mexicana.* Therefore, all populations analyzed in this study were interpreted to be diploid. However, *B. mexicana* could still be an autopolyploid or an allopolyploid with some duplicated loci expressed and some silenced.

Twelve enzymes with 20 putative loci were consistently scorable by starch gel electrophoresis: AAT-1, AAT-2, AAT-3, ACO-1, ACO-2, AMP-2, GDH, GPT-1, GPT-2, IDH-1, MDH-1, MDH-2, MDH-3, PGD-1, PGD-2, PGI-1, PGI-2, PGM-1, SAD, TPI-1. Several enzymes or putative loci, viz., ADK, FRK, AMP-1, and TPI-2, were not scored because of faint or inconsistent staining. Phosphoglucomutase, PGM-2, was absent or did not stain in all populations of *Bealia* and was not included in the analysis. All plants examined possessed enzyme bands of identical mobility for GPT-2 whereas GPT-1 and PGI-1 were monomorphic for different alleles in *B. mexicana, M. argentea,* and *M. lucida* and were not included in the genetic analysis except when generating the Wagner tree. Only segregating loci were used to determine the genetic variation in populations, taxa, and F-statistics (Tables 3, 4). The following nonsegregating loci that were fixed for a pair of different alleles were removed from the analysis: *B. mexicana* (AAT-2, MDH-1, MDH-2, MDH-3, PGD-1), *M. argentea* (AMP, MDH-1, MDH-2, PGD-1), and *M. lucida* (AAT-2, MDH-1, MDH-2, PGD-1, PGI-2).

Intraspecific allele frequencies at 17 variable loci are given in Table 2. Population allele frequencies, fixation indices per polymorphic locus, and interpopulational genetic identity values can be obtained from PMP upon request. Observed alleles per polymorphic locus per taxon ranged from two in AAT-3, ACO-1, ACO-2, and GDH to five in MDH-2. The greatest number of alleles per locus observed in any population was four (*M. lucida 8029* for MDH-2). Seven, five, and two unique alleles were detected in *B. mexicana, M. argentea,* and *M. lucida,* respectively (Table 2). Three of these unique alleles were present in all individuals surveyed of *B. mexicana* (AMP-2c, MDH-1b, and PGI-a) and one unique allele was present in all individuals of *M. lucida* (PGD-2a).

The mean number of alleles per locus (A) within populations ranged from 1.1 to 1.8 and the mean proportion of polymorphic loci (P) within populations ranged from 0.14 to 0.60 (Table 3). The mean heterozygosity direct count estimate (H) within populations ranged from 0.143 to 0.297, indicating a moderately high level of heterozygosity at most polymorphic loci and in most populations. The mean fixation index (F) within populations or inbreeding coefficient was 0.073 and −0.010 in two different populations of *M. argentea* indicating no significant deviation from Hardy-Weinberg expectations. All other populations ranged from −0.186 to −1.000, suggesting a high excess of heterozygotes relative to Hardy-Weinberg expectations.

Partitioning of genetic diversity within *B. mexicana, M. argentea,* and *M. lucida* was determined using F-statistics where the fixation index within populations (F_{IS}) ranged from −0.079 to −0.867 (Table 4). The amount of genetic diversity among populations within each species (F_{ST}) ranged from 0.142 to 0.192. In *M. argentea* the genetic diversity was partitioned slightly higher among populations (F_{ST} = 0.158) versus within (F_{IS} = −0.079). In contrast, in *B. mexicana* and *M. lucida,* the primary component of F_{IT} was F_{IS} (−0.540 and −0.867, respectively) with the F_{ST} values being very small (0.192 and 0.142). This indicates greater heterogeneity within populations of *B. mexicana* and *M. lucida* than among them. The overall fixation index (F_{IT}) ranges from −0.079, i.e., approaching Hardy-Weinberg equilibrium, to −0.602, indicating a large excess of heterozygotes relative to Hardy-Weinberg expectations.

Genetic identities among populations of each species demonstrate a high level of similarity, ranging from 0.95 to 0.98 whereas identities between species are 0.57 between *B. mexicana* and *M. lucida,* 0.59 between *M. argentea* and *M. lucida,* and 0.83 between *B. mexicana* and *M. argentea* (Table 5).

A Wagner tree (Fig. 1) summarizes the interpopulational relationship based on gentic distance values. Three species belonging to the genus *Chaboissaea* (*C. decumbens, C. ligulata,* and *C. subbiflora*)

TABLE 2. INTRASPECIFIC ALLELE FREQUENCY DATA FOR 17 POLYMORPHIC LOCI AMONG *BEALIA MEXICANA*, *MUHLENBERGIA ARGENTEA*, AND *M. LUCIDA*.

Locus	Allele	*B. mexicana*	*M. argentea*	*M. lucida*
AAT-1	a	0.000	0.013	0.000
	b	1.000	0.948	0.000
	c	0.000	0.039	1.000
AAT-2	a	0.475	0.012	0.000
	b	0.490	0.000	0.500
	c	0.035	0.988	0.500
AAT-3	a	1.000	0.936	1.000
	b	0.000	0.064	0.000
ACO-1	a	0.900	1.000	0.112
	b	0.100	0.000	0.888
ACO-2	a	0.725	0.585	1.000
	b	0.275	0.415	0.000
AMP-2	a	0.420	0.500	0.214
	b	0.035	0.000	0.000
	c	0.545	0.000	0.000
	d	0.000	0.500	0.786
GDH	a	1.000	0.469	0.003
	b	0.000	0.531	0.997
IDH-1	a	1.000	0.906	0.000
	b	0.000	0.094	0.914
	c	0.000	0.000	0.086
MDH-1	a	0.000	0.500	0.500
	b	0.500	0.000	0.000
	c	0.500	0.500	0.500
MDH-2	a	0.060	0.168	0.000
	b	0.440	0.332	0.000
	c	0.000	0.332	0.500
	d	0.500	0.000	0.448
	e	0.000	0.168	0.052
MDH-3	a	0.000	0.006	0.004
	b	0.500	0.367	0.537
	c	0.500	0.627	0.459
PGD-1	a	0.495	0.500	0.500
	b	0.500	0.500	0.500
	c	0.005	0.000	0.000
PGD-2	a	0.000	0.000	1.000
	b	0.975	0.967	0.000
	c	0.025	0.033	0.000
PGI-2	a	0.510	0.000	0.000
	b	0.490	0.777	0.448
	c	0.000	0.153	0.494
	d	0.000	0.070	0.058
PGM-1	a	0.295	0.000	0.503
	b	0.495	0.506	0.497
	c	0.210	0.449	0.000
	d	0.000	0.045	0.000

TABLE 2. CONTINUED

Locus	Allele	*B. mexicana*	*M. argentea*	*M. lucida*
SAD	a	0.000	0.147	0.000
	b	0.765	0.853	1.000
	c	0.235	0.000	0.000
TPI-1	a	0.010	0.000	1.000
	b	0.960	1.000	0.000
	c	0.030	0.000	0.000

were designated as outgroups based on their morphological similarity to the study species and similar base chromosome number (x=8) with *B. mexicana*. All populations of each respective species form a clade; populations of *M. argentea* and *B. mexicana* showed the least genetic distance between species (Fig. 1).

DISCUSSION

Populations of *B. mexicana, M. argentea,* and *M. lucida* possess moderately high levels of genetic variation comparable to that found in outcrossing and mixed mating plant species (Hamrick et al. 1979). This is reflected in the mean heterozygosity levels per species of 19

TABLE 3. GENETIC VARIATION IN POPULATIONS AND TAXA OF *BEALIA MEXICANA, MUHLENBERGIA ARGENTEA,* AND *M. LUCIDA*: SAMPLE SIZE (n); MEAN NUMBER OF ALLELES PER LOCUS (A); MEAN PROPORTION OF POLYMORPHIC LOCI (P); 95% CRITERION, MEAN HETEROZYGOSITY (H), DIRECT COUNT ESTIMATE; AND MEAN FIXATION INDEX (F).

Taxon & coll. no.	n	A	P	H	F
Within populations					
B. mexicana 7946	25	1.6	0.43	0.294	−0.408
B. mexicana 7981	25	1.5	0.43	0.297	−0.474
B. mexicana 8090	25	1.6	0.50	0.234	−0.189
B. mexicana 8264	25	1.3	0.29	0.206	−0.686
M. argentea 8044	25	1.8	0.60	0.205	−0.010
M. argentea 8066	27	1.7	0.60	0.185	−0.142
M. argentea 8248	26	1.5	0.40	0.172	0.073
M. lucida 7973	26	1.1	0.14	0.143	−1.000
M. lucida 7978	26	1.1	0.14	0.143	−1.000
M. lucida 8029	25	1.3	0.29	0.191	−0.520
M. lucida 8039	26	1.4	0.36	0.277	−0.736
M. lucida 8083	26	1.2	0.21	0.157	−0.702
M. lucida 8202	24	1.2	0.21	0.164	−0.652
M. lucida 8249	25	1.3	0.29	0.286	−1.000
Within taxa					
B. mexicana		1.5	0.41	0.26	−0.49
M. argentea		1.7	0.53	0.19	−0.03
M. lucida		1.2	0.24	0.19	−0.80

TABLE 4. SUMMARY OF F-STATISTICS AND UNIQUE ALLELES (PARENTHESIS INDICATES PRESENCE IN ALL INDIVIDUALS) WITHIN POPULATIONS OF *BEALIA MEXICANA*, *MUHLENBERGIA ARGENTEA*, AND *M. LUCIDA*.

Taxon	F_{IS}	F_{IT}	F_{ST}	Unique alleles
B. mexicana	−0.540	−0.244	0.192	7 (3)
M. argentea	−0.079	0.091	0.158	4
M. lucida	−0.867	−0.602	0.142	2 (1)

to 26% and the predominantly negative mean fixation indices per species (0.073 to −1.000) indicating a heterozygote excess (Table 3). Although all three species are limited to a particular edaphic habitat, *M. argentea* which is known only from two localities and has the most restricted range, exhibits the lowest mean fixation index of −0.03, showing no deviation from Hardy-Weinberg expectations. Likewise, the more widespread perennial, *M. lucida* exhibits the highest excess of heterozygotes (F = −0.80) relative to Hardy-Weinberg expectations. The somewhat restricted annual, *B. mexicana,* is intermediate with a mean fixation index of −0.49 but higher direct count, mean heterzygosity (H = 26%). These results do not lend support to the theoretical prediction that species with limited ranges will exhibit lower levels of genetic polymorphisms (Drury 1974; Hamrick et al. 1979; Karron 1987; Karron et al. 1988), although these values could be attributable to the decreasing number of population samples in each respective species. Even though these three species are currently known from very few populations, their genetic diversity is unusually high, and again this could be attributable to the paucity of sampling. However, a similar situation was also found in the highly restricted *Helianthus praecox* Engelm. & Gray ssp. *hirtus* Heiser where the genetic diversity at isozyme loci was similar to the widespread *H. praecox* ssp. *runyonii* Heiser (Rieseberg and Doyle 1989).

Bealia mexicana and *M. lucida* show little genetic differentiation among populations (F_{ST}) but exhibit high values of variability within

TABLE 5. MEAN GENETIC IDENTITY VALUES (NEI 1972) AND RANGES FOR PAIRWISE COMPARISONS OF POPULATIONS OF *BEALIA MEXICANA*, *MUHLENBERGIA ARGENTEA*, AND *M. LUCIDA*.

Taxa	*B. mexicana*	*M. argentea*	*M. lucida*
B. mexicana	0.96 (0.95–0.99)	0.83 (0.79–0.86)	0.57 (0.52–0.66)
M. argentea		0.95 (0.94–0.96)	0.59 (0.52–0.68)
M. lucida			0.98 (0.96–1.00)

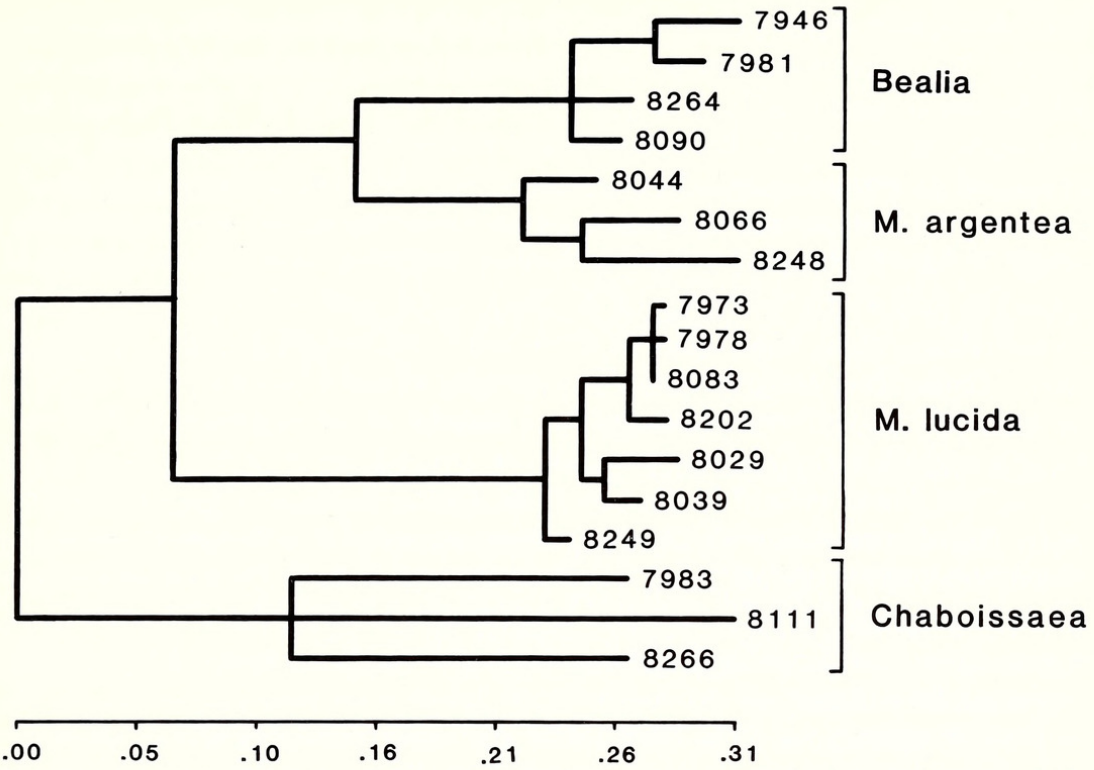

FIG. 1. Wagner tree showing the genetic distance among *Bealia mexicana, Muhlenbergia argentea,* and *M. lucida. Chaboissaea decumbens, C. ligulata,* and *C. subbiflora* were designated as outgroups. Correlation coefficient = 0.99; length = 1.5; numbers refer to population collections given in Table 1; scale indicates distance from root.

populations (Table 4). Again, the highest levels of genetic heterogeneity reside within populations of the more widespread *M. lucida* ($F_{IS} = -0.867$), followed by the somewhat restricted *B. mexicana* ($F_{IS} = -0.540$). Although many species of *Muhlenbergia* and other eragrostoid species are often weedy, these two species occupy very restricted habitats that are relatively undisturbed by man. The genetic diversity of *B. mexicana* and *M. lucida* seems to support Loveless and Hamrick's (1984) hypothesis that stable, long-lived population structure of a non-weedy plant species reduces differentiation among populations and increases variability within populations.

In contrast to the uniform levels of electrophoretic variation observed within populations of *B. mexicana* and *M. lucida,* the distribution of genetic variation within *M. argentea* differs markedly. A major portion of the genetic variation in *M. argentea* resides among populations rather than within. This could be a consequence of a small sample size, genetic drift, and/or founder effects (Loveless and Hamrick 1984).

The interspecific mean genetic identity values (Table 5) within each of the three species is similar to values reported among populations (I = 0.95) of other plant species and the mean identities among the three taxa are more similar to genetic identities reported

for congeneric plant populations (I = 0.67) (Gottlieb 1981; Crawford 1983). The results indicate that for genes coding for soluble enzymes, *M. lucida* is genetically distinct from *B. mexicana* (I = 0.57) and *M. argentea* (I = 0.59). By possessing a deeply bilobed lemma with rounded to obtuse lobes *B. mexicana* and *M. argentea* appear morphologically most similar and indeed their pairwise mean identity value is the highest among the three species at 0.83. Although the base chromosome number of *B. mexicana* and *M. argentea* is not concordant, their high genetic similarity indicates a close relationship. These data suggest that *M. argentea* should be placed in *Bealia* or that the recent reinstatement of *Bealia* is unwarranted. Results from a cpDNA restriction site survey among the Eragrostideae genera of the New World indicates that *Bealia* is more closely aligned with *Blepharoneuron* by sharing three parallel site losses than with *Muhlenbergia* (Duvall et al. in review). More data from a thorough sampling within *Muhlenbergia,* specifically from cpDNA restriction site analysis, will perhaps shed some light on this question (Peterson unpublished data).

The Wagner tree (Fig. 1) allows the interpretation of interpopulational relationships and aligns all populations within a species in a separate clade. Due to the very infrequent occurrence of natural populations of these three species, population samples were not always widely spaced geographically. Sympatric populations of *B. mexicana* (*7946, 7981*) and *M. lucida* (*7973, 7978*) occurred within 3.2 km of each other and indeed, within each species pair, show the least genetic distance from one another. However, two populations of *M. argentea* (*8044, 8066*), separated by only 2.3 km, are genetically distant. The other population of *M. argentea* (*8248*) is more than 115 km distant but is genetically more similar with *8066.*

In summary, *B. mexicana* and *M. lucida* have high levels of within population genetic variation often associated with highly outcrossing plants and *M. argentea* has slightly higher genetic variation among populations. Even though *M. argentea* and *B. mexicana* are species with very restricted ranges and different life forms, the genetic diversity of their soluble enzymes is high. At present the only threat to the survival of *B. mexicana* and *M. argentea* is loss of habitat due to human interference. Since two populations of *B. mexicana* and all three known populations of *M. argentea* occur in National Parks, their continued survival at this time is not in jeopardy. Populations of *Bealia mexicana* and *M. argentea* show a higher level of allozymic similarity than either do with *M. lucida.* Other studies have indicated that *Muhlenbergia* is morphologically very diverse and is perhaps polyphyletic (Correll and Johnston 1970; Peterson et al. 1989; Peterson and Annable 1990, 1991, 1992). A complete re-evaluation of all the species within *Muhlenbergia* analyzing mor-

phological and cpDNA variation is in progress which will shed further light on the proper alignment of *M. argentea.*

ACKNOWLEDGMENTS

This study was supported by grants from the Smithsonian Institution Research Opportunities and Scholarly Studies Funds. Special thanks are given to Carol R. Annable, Yolanda Herrera, and Robert M. King for help collecting the specimens in the field, Mark T. Strong and CRA for laboratory assistance, Socorro González E. for preparing the Spanish abstract, and Emmet Judziewicz and Cliff Morden for reviewing the manuscript.

LITERATURE CITED

CORRELL, D. S. and M. C. JOHNSTON. 1970. Manual of the vascular plants of Texas. Texas Research Foundation, Renner.

CRAWFORD, D. J. 1983. Phylogenetic and systematic inferences from electrophoretic studies. Pp. 257–287 *in* S. D. Tanksley and T. J. Orton (eds.), Isozymes in plant genetics and breeding. Elsevier Science Publishers, Amsterdam.

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

DUVALL, M. R., P. M. PETERSON, and A. H. CHRISTENSEN. In review. Alliances of *Muhlenbergia* within New World Eragrostideae are identified by phylogenetic analysis of mapped restriction sites from plastid DNA's. American Journal of Botany.

GOTTLIEB, L. D. 1981. Electrophoretic evidence and plant populations. Progress in Phytochemistry 7:1–46.

GOULD, F. W. 1958. Chromosome numbers in southwestern grasses. American Journal of Botany 45:757–767.

HAMMEL, B. E. and J. R. REEDER. 1979. The genus *Crypsis* (Gramineae) in the United States. Systematic Botany 4:267–280.

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

HITCHCOCK, A. S. 1935. *Muhlenbergia.* (Poales) Poaceae (pars). North American Flora 17:431–476.

JAIN, S. K. and P. L. WORKMAN. 1967. Generalized F-statistics and the theory of inbreeding and selection. Nature 214:674–678.

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

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

LOVELESS, M. D. and J. L. HAMRICK. 1984. Ecological determinants of genetic structure in plant populations. Annual Review of Ecology and Systematics 15: 65–95.

MORDEN, C. W., J. DOEBLEY, and K. F. SCHERTZ. 1987. A manual of techniques for starch gel electrophoresis of *Sorghum* isozymes. Texas Agricultural Experiment Station, MP-1635, College Station.

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

PETERSON, P. M. 1988. Chromosome numbers in the annual *Muhlenbergia* (Poaceae). Madroño 35:320–324.

———. 1989. Re-evaluation of *Bealia mexicana* (Poaceae: Eragrostideae). Madroño 36:260–265.

——— and C. R. ANNABLE. 1990. A revision of *Blepharoneuron* (Poaceae: Eragrostideae). Systematic Botany 15:515–525.
——— and ———. 1991. Systematics of the annual species of *Muhlenbergia* (Poaceae-Eragrostideae). Systematic Botany Monographs 31:1–109.
——— and ———. 1992. A revision of *Chaboissaea* (Poaceae: Eragrostideae). Madroño 38:8–30.
———, ———, and V. R. FRANCESCHI. 1989. Comparative leaf anatomy of the annual *Muhlenbergia* (Poaceae). Nordic Journal of Botany 8:575–583.
REEDER, J. R. 1967. Notes on Mexican grasses VI. Miscellaneous chromosome numbers—2. Bulletin of the Torrey Botanical Club 94:1–17.
———. 1968. Notes on Mexican grasses VIII. Miscellaneous chromosome numbers. Bulletin of the Torrey Botanical Club 95:69–86.
———. 1971. Notes on Mexican grasses IX. Miscellaneous chromosome numbers—3. Brittonia 23:105–117.
———. 1977. Chromosome numbers in western grasses. American Journal of Botany 64:102–110.
——— and C. G. REEDER. 1988. Aneuploidy in the *Muhlenbergia subbiflora* complex (Gramineae). Phytologia 65:155–157.
RIESEBERG, L. H. and M. F. DOYLE. 1989. Allozyme variation in *Helianthus praecox* ssp *hirtus*, a rare sunflower from southern Texas. Aliso 12:379–386.
SWALLEN, J. R. 1936. Three new grasses from Mexico and Chile. Journal of the Washington Academy of Sciences 26:207–209.
SWOFFORD, D. L. and R. B. SELANDER. 1989. *Biosys-1*, a computer program for the analysis of allelic variation in population genetics and biochemical systematics. Release 1.7. University of Illinois, Urbana.
TATEOKA, T. 1961. A biosystematic study of *Tridens* (Gramineae). American Journal of Botany 48:565–573.
WRIGHT, S. 1965. The interpretation of population structure by F-statistics with special regard to systems of mating. Evolution 19:395–420.
———. 1969. Evolution and genetics of populations, Vol. 2. The theory of gene frequencies. University of Chicago Press, Chicago.
———. 1978. Evolution and genetics of populations, Vol. 4. Variability within and among natural populations. University of Chicago Press, Chicago.

(Received 26 Aug 1992; revision accepted 12 Oct 1992.)