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Genetic Diversity Within and Among Sinai Populations of Three *Ballota* Species (Lamiaceae)

M. S. Zaghloul, J. L. Hamrick, A. A. Moustafa, W. M. Kamel, and R. El-Ghareeb

Botany Department, Faculty of Science, Suez Canal University, Ismailia, Egypt (Zaghloul, Moustafa, and Kamel); Departments of Plant Biology and Genetics, University of Georgia, Athens, GA 30602 (Hamrick); and Botany Department, Faculty of Science, Alexandria University, Alexandria, Egypt (El-Ghareeb).

Address correspondence to M. S. Zaghloul at the address above, or e-mail: saadzaghloul@hotmail.com or zaghloul_mohamed@yahoo.com.

Abstract

Ballota undulata, Ballota kaiseri, and *Ballota saxatilis* are very rare (and endemic—*B. kaiseri*), threatened species growing in St. Catherine Protectorate, southern Sinai, Egypt. They are subjected to a number of threats that have caused populations to decline in both number and size. For the long-term survival of these species, an appropriate conservation strategy for the maintenance of their genetic variation should be developed. This study measures genetic diversity within and among populations of these *Ballota* species and determines the conservation implications of the results. The genetic analyses demonstrated that the three *Ballota* species maintain relatively high levels of genetic diversity (H_e = 0.195–0.317) and that most of the their genetic diversity was found within populations (G_{ST} = 0.045–0.099). Indirect estimates of historical gene flow for *B. undulata* and *B. saxatilis* were relatively high (Nm(W) = 5.25 and 3.37, respectively) but suggest that there is somewhat less gene movement among *B. kaiseri* populations (Nm(W) = 2.29). The levels of genetic diversity maintained within populations of the three *Ballota* species indicate that an appropriate sampling design for ex situ safeguarding should capture the majority of the genetic diversity found within these taxa.

A long-term objective of most conservation efforts is to maintain the evolutionary viability of taxa, maximizing their chances for persistence in the face of changing environmental conditions. Genetic considerations may also have short-term importance for the development of strategies for the successful management of rare plants. Thus, plant conservation biologists face a series of decisions when developing plans for the preservation of a species ex situ or for its preservation or reestablishment in the wild. It is important to determine to what extent local populations are genetically differentiated, whether such differences have adaptive value, and whether mixing gene pools from different populations will affect successful establishment and long-term survival.

The level and distribution of genetic diversity within any plant species is influenced by interactions among at least three factors. The first factor encompasses abiotic conditions including climate, habitat conditions, and soil factors. Biotic interactions such as competition, symbiosis, parasitism, and predation form the second factor. The third and last factor includes species characteristics such as population size, mating system, gene flow, and dispersal. All the three factors influence the genetic composition of populations in both a directed (e.g., selection) or a stochastic fashion (Frankel et al. 1995).

Although a rare species may occur within reserves, its populations may not be viable or may represent only a small proportion of the total genetic variation within the species (Cropper 1993). Genetic analyses can help conservation managers to choose protected areas by providing genetic information on rare plant populations. Genetic studies can identify priority populations to conserve, alter the boundaries of reserves, and change reserve design (Hopper and Coates 1990). A major conclusion that has emerged from the past three decades of plant population genetic research is that species differ greatly in their levels and distribution of genetic variation (Hamrick and Godt 1989). An understanding of the variation in genetic composition and population genetic structure is basic to planning the conservation of plant biodiversity. One immediate implication is that optimal sampling strategies to conserve genetic diversity will differ among species.

Descriptions of the level and distribution of genetic variation maintained within a species permit comparisons of the

species of conservation interest with other species having similar life history characteristics and similar geographic ranges. Comparisons with congeneric species sharing a common evolutionary past and having similar mating systems and seed-dispersal mechanisms are perhaps the most informative (e.g., Godt et al. 1995; Karron 1987) but are not always feasible.

Studies of geographically restricted species are of interest to evolutionary biologists because rapid evolutionary change often takes place in isolated populations (Stebbins 1979; Wright 1931). Information on the ecology and the genetics of these taxa is also important to those responsible for the management of rare species, a problem that is gaining increasing attention (e.g., Frankel and Soulé 1981; Ledig 1986; Soulé 1980). Drury (1974) noted that although many researchers predicted low levels of genetic polymorphism in plant species with limited ranges and small numbers of individuals, few had measured genetic variation in such taxa. During the past three decades, many researchers used allozyme analyses to investigate the genetic composition and structure of plant populations (e.g., Brown 1979; Gottlieb 1981; Hamrick and Godt 1989) and there have been several studies involving rare, endemic species with small geographic ranges (e.g., Godt and Hamrick 1998; Karron 1987; Moran and Hopper 1983).

Ballota undulata, Ballota kaiseri, and *Ballota saxatilis* are rare (and endemic—*B. kaiseri*) species growing in southern Sinai, Egypt, that are subjected to threats that have caused declines in population number and size. The loss of genetic variation through stochastic factors and the deleterious effects of inbreeding in small populations are potential threats that may compromise attempts to maintain the long-term viability of populations of these rare species. Our goal in this study is to measure genetic variation within and among *Ballota* populations and to determine implications for the conservation of these species.

Materials and Methods

Study Species

The genus *Ballota* (Lamiaceae) consists of perennial undershrubs that are obtusely tetragonal. Three *Ballota* species: *B. undulata* (rare), *B. saxatilis* (endangered), and *B. kaiseri* (extremely endangered and endemic) grow in the Sinai. Populations of these three species are endangered by human impact through severe overgrazing by goats and sheep. *Ballota* are grazed in the spring when they have new soft, green branches and before flowering. The effect of grazing is aggravated by the prevailing aridity in the area, where annual rainfall averages less than 50 mm/year (Zaghloul 1997).

B. kaiseri is a rare, endemic species restricted to crevices in outcrops of smooth-faced granite, to elongated gaps, and to narrow ravines in such rocky terrain. *B. saxatilis* has a discontinuous distribution and may be a Mediterranean relict. *B. undulata* is also Mediterranean but has a more continuous distribution in a wider range of relatively moist microhabitats (Danin 1986). Their distribution is affected positively by elevation. Whereas *B. undulata* prefers low-pH soils, *B. kaiseri* and *B. saxatilis* share soil microhabitats with high clay and silt and organic matter. These habitats have low sand content, low pH, and relatively high soil moisture (Zaghloul 2003). The distribution of the *Ballota* species is determined by the integrated influence of the environmental factors rather than by independent effects (Gorham 1954; Waring and Major 1964).

Population Samples and Seed Collection

Twenty-nine *Ballota* populations were sampled from 17 sites (Table 1; Figure 1) within the St. Catherine Protectorate in late summer of 2000. Four populations were represented by more than 25 seed families, twelve by 12–20, and thirteen by 1–5 (Table 1). The number of individuals sampled depended on actual population size as well as seed availability. Sites supporting more than one *Ballota* species were treated as separate populations for the genetic analyses.

When the wadi's drainage lines were traced, the distance between sampled populations ranged from 500 m (between El-Sarw Garden and Abu Hemat Gorge) to 9250 m (between W. El-Asbaei'a and W. El-Tala'a), whereas the direct geographic distance ranged from 425 m (between El-Sarw Garden and Abu Hemat Gorge) to 6050 m (between W. El-Asbaei'a and W. El-Tala'a). The mean tracing distance was 3570 m between *B. undulata* populations, 2684 m between *B. kaiseri* populations, and 1250 m between *B. saxatilis* populations. The mean direct distance was 2519 m between *B. undulata* populations, 1782 m between *B. kaiseri* populations, and 1000 m between *B. saxatilis* populations.

Seed Germination

Open-pollinated seed were germinated on cotton pads in petri dishes in growth chambers under a regime of 15°C/5°C day and night temperature and 12 h/12 h light for 25–30 days. After germination in the growth chamber, seedlings were transferred to the greenhouse for 5 days to adapt to the new environmental conditions and then were transplanted to Fafard mix no. 3B. Seedlings were allowed to grow for about 30 days (i.e., to 5–10 cm height).

Starch Gel Electrophoresis

Whole *Ballota* seedling tissues were ground manually with sea sand using a small precooled mortar and pestle. Preliminary trials determined that the extraction buffer of Wendel and Parks (1982) produced superior resolution. After homogenization, crude extracts were absorbed onto 4- × 6-mm sample wicks made from No. 3 Whatman filter paper. Wicks were stored in 96-well microtest plates at −70°C until needed for electrophoresis.

Twenty-four isozymes were tested in the preliminary survey to identify potentially reliable loci. Adequate resolution was obtained for 12 enzyme systems: aconitase (Aco; EC 4.2.1.3), isocitrate dehydrogenase (Idh; EC 1.1.1.42), fluorescent esterase (Fe; EC 3.1.1.6), triose phosphate isomerase (Tpi; EC 5.3.1.1), menadione reductase (Mnr; EC 1.6.99.2),

Table 1. Summary of population locations sampled for three *Ballota* species from the Sinai

Species	Population	Abbreviation	Number of families collected	Number of sampled families
B. undulata	W. El-Arbeie'en (the mouth)	UArbMo	42	39
	W. El-Arbaie'en (Abu Hemat Gorge)	UArbHe	17	12
	W. El-Arbaie'en (Ramadan Garden)	UArbRa	13	11
	W. El-Arbaie'en (El-Dier Garden)	UArbDi	41	31
	W. El-Arbaie'en (El-Sarw Garden)	UArbSa	25	23
	W. El-Ahmar	UAhmar	17	16
	W. El-Shraiq (mouth of W. El-Fara'a)	UFaraa	38	33
	Shaq Mousa	UShaMo	13	12
	W. El-Garagnia	UGarag	2	2
	Abu Geifa	UAbuGe	4	2
	W. El-Tofahah	UTofah	17	10
	W. El-Dier	UDier	15	13
	W. El-Asbaei'a	UAsbai	16	12
	W. Toboq	UToboq	13	11
	Mt. Mousa (Stairway)	UMousa	16	16
	W. El-Tala'a (Hussien El-Hashash Garden)	UTalHu	14	11
Subtotal	16		303	254
B. kaiseri	W. El-Arbaie'en (El-Sarw Garden)	KArbSa	1	1
	W. El-Arbaie'en (Abu Hemat George)	KArbHe	21	16
	W. El-Arbaie'en (Ramadan Garden)	KArbRa	4	4
	W. El-Arbaie'en (El-Deir Garden)	KArbDi	8	3
	W. El-Shraiq (mouth of W. El-Fara'a)	KFaraa	1	1
	Kahf El-Ghoula	KKaGh	3	2
	W. El-Dier	KDier	2	2
Subtotal	7		40	29
B. saxatilis	W. El-Arbaie'en (Abu Hemat George)	SArbHe	19	17
	W. El-Arbaie'en (Ramadan Garden)	SArbRa	1	1
	W. El-Arbaie'en (El-Deir Garden)	SArbDi	3	3
	W. El-Arbaie'en (El-Sarw Garden)	SArbSa	2	1
	Kahf El-Ghoula	SKaGh	2	2
Subtotal	5		27	24
Ballota sp.	W. El-Arbaie'en (El-Deir Garden)	CArbDi	1	1
Total	29 populations		371	308

aspartate aminotransferase (Aat; EC 2.6.1.1), diaphorase (Dia; EC 1.8.1.4), acid phosphatase (Acp; EC 3.13.2), phosphoglucoisomerase (Pgi; EC 5.3.1.8), phosphoglucomutase (Pgm; EC 5.4.2.2), malate dehydrogenase (Mdh; EC 1.1.1.37), and 6-phosphogluconate dehydrogenase (6-Pgd; EC 1.1.1.44). Five gel and electrode buffer systems resolved these enzymes: system 4 (Soltis et al. 1983), Aco; system 11 (Soltis et al. 1983), Idh; system 6 (Soltis et al. 1983), Acp, Pgi, and Pgm; system 8- (a modification of system 8 of Soltis et al. 1983), Fe, Tpi, Mnr, Aat, and Dia; and a morphiline citrate (Clayton and Tretiak 1972) system, Mdh and 6-Pgd. Generally, stain recipes are from Soltis et al. (1983), with the exception of Dia and Mnr, which came from Cheliak and Pitel (1984).

Genetic Diversity and Structure

Genetic diversity within this *Ballota* complex was determined by using a computer program (LYNSPROG) developed by M. D. Loveless and A. Schnabel. The following standard genetic diversity statistics were obtained: proportion of polymorphic loci (P), number of alleles per polymorphic locus (AP), the effective number of alleles per locus (A_e), observed heterozygosity (H_o), and expected heterozygosity (H_e). These parameters were calculated at both the species and within-population levels. Gene diversity statistics (H_T, H_S, and G_{ST}) of Nei (1973) were also calculated for each polymorphic locus. Heterogeneity in allele frequencies among populations was tested for significance using a chi-square test (Workman and Niswander 1970). Wright's (1965) *F*-statistics were calculated to determine deviations from Hardy-Weinberg expectations and were tested by chi-square (Li and Horvitz 1955). Rousset (1997) suggested plotting $F_{ST}/(1 - F_{ST})$ versus spatial distance, where the F_{ST}'s are pairwise values. Pairwise F_{ST} were calculated using FSTAT, version 1.2 computer program (Goudet 1995). We examined the correlation between the $F_{ST}/(1 - F_{ST})$ and geographic distance (direct and drainage tracing) with Mantel's test using Mantel Nonparametric Test Calculator, version 2 (Liedloff 1999).

Although we are aware of the problems of estimating gene flow (Nm) from genetic structure parameters (Whitlock and McCauley 1999), we felt that it would be informative to compare estimates among the three species. Historic levels of gene flow were estimated using measures of genetic

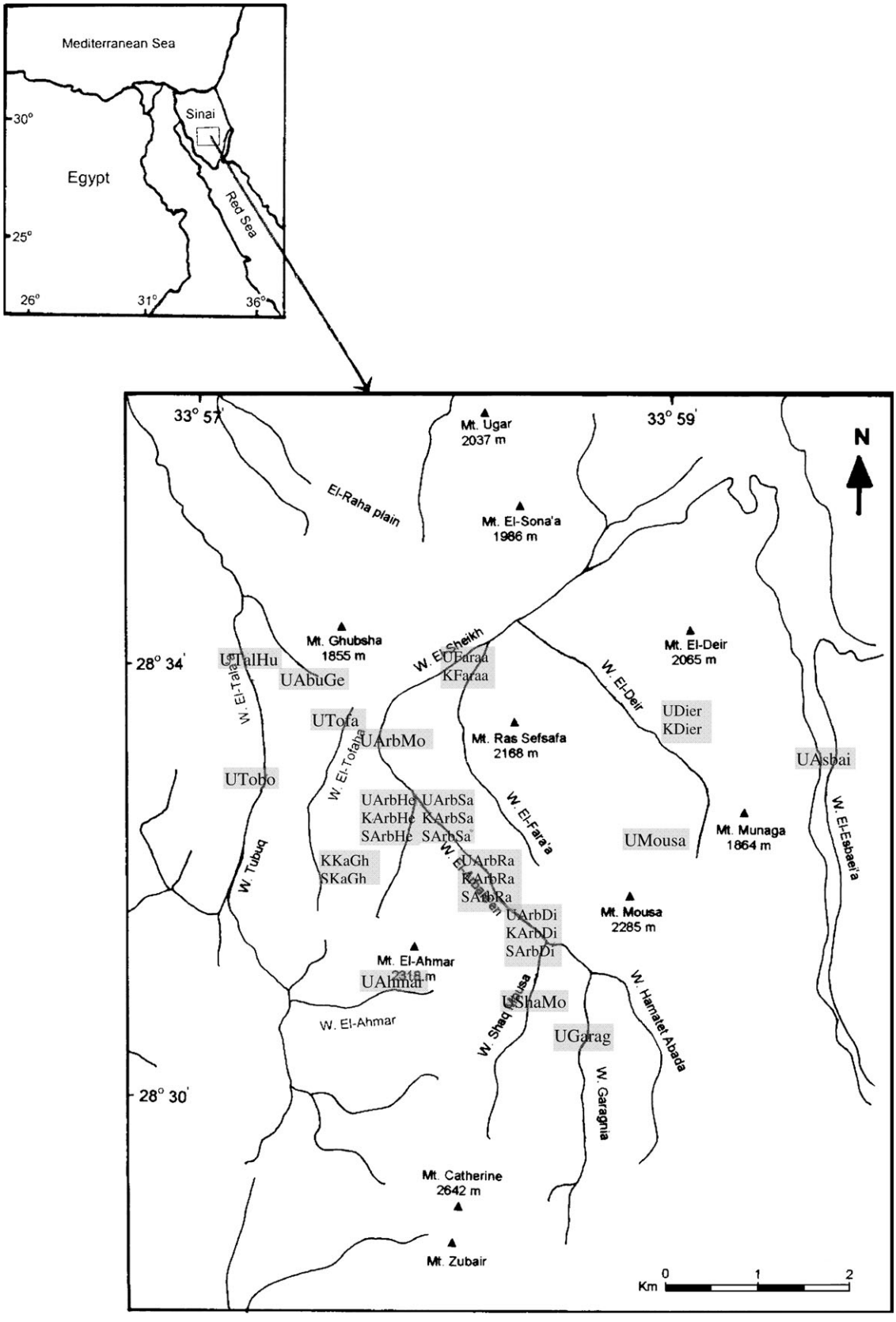

Figure 1. Locations of sampled *Ballota* populations. Sites beginning with a U indicate *B. undulata*, a K indicate *B. kaiseri*, and S indicate *B. saxatilis*.

Table 2. Genetic diversity statistics for 29 populations of *Ballota* species

Population	*N*	P	AP	A_e	H_o (SD)	H_e (SD)
UArbMo	108	81.0	2.65	1.25	0.150 (0.031)	0.159 (0.037)
UArbHe	30	90.5	2.63	1.39	0.224 (0.073)	0.248 (0.036)
UArbRa	31	76.2	2.44	1.26	0.139 (0.059)	0.169 (0.034)
UArbDi	84	95.2	2.60	1.32	0.152 (0.038)	0.197 (0.038)
UArbSa	65	90.5	2.47	1.35	0.187 (0.046)	0.225 (0.035)
UAhmar	48	76.2	2.38	1.22	0.121 (0.044)	0.148 (0.035)
UFaraa	94	85.7	2.72	1.31	0.158 (0.036)	0.192 (0.039)
UShaMo	31	71.4	2.53	1.30	0.149 (0.059)	0.182 (0.039)
UGarag	6	47.6	2.00	1.20	0.139 (0.116)	0.126 (0.038)
UAbuGe	3	52.4	2.09	1.40	0.238 (0.157)	0.214 (0.049)
UTofah	30	61.9	2.38	1.22	0.106 (0.053)	0.144 (0.034)
UDier	35	66.7	2.64	1.27	0.144 (0.055)	0.166 (0.040)
UAsbai	33	76.2	2.31	1.26	0.134 (0.054)	0.168 (0.037)
UToboq	33	85.7	2.44	1.30	0.161 (0.062)	0.191 (0.037)
UMousa	48	81.0	2.53	1.31	0.147 (0.046)	0.181 (0.042)
UTalHu	24	81.0	2.41	1.31	0.168 (0.070)	0.194 (0.038)
Submean (SD)		76.2 (2.21)	2.45 (0.20)	1.29 (0.06)	0.157 (0.017)	0.182 (0.010)
B. undulata pooled	703	95.2	3.50	1.30		0.195 (0.035)
KArbSa	3	23.8	2.00	1.14	0.127 (0.119)	0.085 (0.035)
KArbHe	41	85.7	2.61	1.59	0.239 (0.063)	0.309 (0.046)
KArbRa	9	71.4	2.27	1.37	0.216 (0.120)	0.230 (0.039)
KArbDi	9	71.4	2.13	1.46	0.185 (0.113)	0.256 (0.046)
KFaraa	3	33.3	2.14	1.31	0.270 (0.103)	0.156 (0.051)
KKaGh	2	28.6	2.00	1.23	0.119 (0.134)	0.125 (0.045)
KDier	2	47.6	2.00	1.34	0.300 (0.209)	0.194 (0.047)
Submean (SD)		51.7	2.16 (0.22)	1.35 (0.15)	0.208 (0.049)	0.194 (0.017)
B. kaiseri pooled	69	95.2	2.75	1.53		0.297 (0.042)
SArbHe	45	90.5	2.74	1.63	0.238 (0.060)	0.324 (0.047)
SArbRa	1	9.5	2.00	1.10	0.095 (0.000)	0.048 (0.033)
SArbDi	9	66.7	2.14	1.42	0.267 (0.118)	0.238 (0.047)
SArbSa	2	52.4	2.18	1.56	0.439 (0.156)	0.256 (0.056)
SKaGh	5	19.0	2.00	1.15	0.069 (0.085)	0.077 (0.042)
Submean (SD)		47.6	2.2 (0.30)	1.37 (0.24)	0.222 (0.044)	0.188 (0.020)
B. saxatilis pooled	62	90.5	2.89	1.59		0.317 (0.045)
CArbDi	3	38.1	2.00	1.21	0.150 (0.149)	0.131 (0.042)
Mean (SD)		64.0	2.33 (0.26)	1.32 (0.13)	0.180 (0.018)	0.184 (0.008)
Overall genus	837	95.2	3.70	1.35		0.227

N, the number of seedlings assayed; P, % polymorphic loci; AP, mean number of alleles per polymorphic locus; A_e, effective number of alleles per locus; H_o, observed heterozygosity; H_e, unbiased heterozygosity expected under Hardy-Weinberg assumptions. Populations where first letter is a U indicates *B. undulata;* K, *B. kaiseri;* S, *B. saxatilis;* and C, confused.

structure (F_{ST}). This approach estimates the number of migrants per generation (Nm). Slatkin's equation (Barton and Slatkin 1986; Slatkin 1985) estimates Nm as a function of the average frequency of alleles found exclusively in one population (private alleles). The method assumes genetic and demographic equilibrium and neutral migration.

Genetic Identity and Distance

Nei's (1972) measures of genetic distance (D) and identity (I) were estimated for intraspecific pairwise comparisons between populations. Allele frequency data were used to calculate Nei's (1972) genetic distance between population pairs using LYNSPROG. The UPGMA dendrogram was produced by POPGENE (version 1.3, Yeh et al. 1999), using Nei's (1978) unbiased genetic distances to alleviate bias due to small sample sizes.

Results

Loci and Alleles Scored

Enzyme electrophoresis resulted in clear staining for 12 enzyme systems encoded by 21 putative loci. All enzymes migrated anodally. Several additional enzymes were initially surveyed but were abandoned due to poor resolution. The *Pgm-1* locus was monomorphic in all populations of the three *Ballota* species. The other 20 loci were polymorphic in at least one population. A locus was considered to be polymorphic if two or more alleles were detected, regardless of their frequencies. A total of 75 alleles were observed across all three species ($\bar{x} = 3.57$ alleles per locus) (Table 2). At only one locus (*Mnr-1*) was the frequency of the common allele more than 90% in all populations of the three *Ballota* species. The common allele at *Idh-2* and *Mdh-2* was >0.90 in the *B. saxatilis* populations. At *Tpi-2* the common allele was monomorphic

Table 3. Nei's genetic diversity statistics and estimates of gene flow in the three *Ballota* taxa[a]

Species	H_T	H_S	G_{ST}	F_{IS}	Nm(W)	Nm(S)
B. undulata	0.205	0.196	0.045	0.130	5.25	5.16 (12)
B. kaiseri	0.312	0.282	0.099	0.102	2.29	0.51 (13)
B. saxatilis	0.350	0.321	0.069	0.156	3.37	1.87 (17)
Endemics (52)[b]	0.263	0.163	0.248		1.96	1.58

[a] Total genetic diversity (H_T), genetic diversity found within populations (H_S), the proportion of total genetic diversity found among populations (G_{ST}), the deviation of genotype frequencies from Hardy-Weinberg expectations (F_{IS}) were calculated for the polymorphic loci. Nm indicates the number of migrants per generation using Wright's equation (Nm(W)) and Barton and Slatkin's (Nm(S)) equation (the number of private alleles are in parentheses).

[b] Data from Hamrick and Godt (1989) and Hamrick et al. (1991).

in all populations of *B. saxatilis*, in all but two populations (UArbDi and UTalHu) of *B. undulata*, where its frequency was 0.979 and 0.988, and in all except one population (KArbDi) of *B. kaiseri*, where the frequency of the common allele was just 0.556.

The common allele was the same in every population for eight polymorphic loci. In the other 12 polymorphic loci, one of the low-frequency alleles became the more common allele or was fixed in one or more populations. Although this switch happened in both *B. undulata* and *B. kaiseri* populations, it was most obvious in the *B. saxatilis* populations. Seventeen rare alleles (23.3%) were observed in one to three populations.

Genetic Diversity Within Populations

The total number of alleles in each population for the 21 loci ranged from 31 to 53 with a mean of 44.6 (71 total) for *B. undulata* populations, from 26 to 50 with a mean of 34.4 (56 total) for *B. kaiseri* populations, and from 23 to 54 with a mean of 34.6 (57 total) for *B. saxatilis*. The overall population mean number of alleles for the genus was 39.9 (75 total).

Genetic diversity in *Ballota* species was quantified using standard measures of genetic diversity (Table 2). These values varied among populations, with P ranging from 47.6% to 95.2% ($\bar{x}$ = 76.2% and 95.2% for the species) in *B. undulata*, from 23.8% to 85.7% ($\bar{x}$ = 51.7% and 95.2% for the species) in *B. kaiseri*, and from 9.5% to 90.5% ($\bar{x}$ = 47.6% and 90.5% for the species) in *B. saxatilis*. The AP ranged from 2.00 to 2.65 with a mean of 2.45 for the *B. undulata* populations and 3.50 for the species, from 2.00 to 2.61 with a mean of 2.16 for the *B. kaiseri* populations and 2.75 for the species, and from 2.00 to 2.74 with a mean of 2.21 for the *B. saxatilis* populations and 2.89 for the species. The mean effective number of alleles per locus within each population ranged from 1.22 to 1.40 ($\bar{x}$ = 1.29) for *B. undulata*, from 1.23 to 1.59 ($\bar{x}$ = 1.35) for *B. kaiseri*, and from 1.10 to 1.63 ($\bar{x}$ = 1.37) for *B. saxatilis*. The mean was 1.32 for the genus. H_o ranged from 0.106 to 0.238 ($\bar{x}$ = 0.157) in *B. undulata*, from 0.119 to 0.300 ($\bar{x}$ = 0.208) in *B. kaiseri*, and from 0.069 to 0.439 ($\bar{x}$ = 0.222) in *B. saxatilis*. H_e ranged from 0.126 to 0.248 ($\bar{x}$ = 0.182) in *B. undulata*, from 0.085 to 0.256 ($\bar{x}$ = 0.194) in *B. kaiseri*, and from 0.048 to 0.324 ($\bar{x}$ = 0.188) in *B. saxatilis*. Expected heterozygosity at the species level was 0.195 for *B. undulata*, 0.297 for *B. kaiseri*, and 0.317 for *B. saxatilis*. In most populations, observed heterozygosity was less than Hardy-Weinberg expectations except in three populations in both *B. kaiseri* (KArbSa, KFaraa, and KDier) and *B. saxatilis* (SArbRa, SArbDi, and SArbSa). These results may be due to small sample size in populations of these two species.

Deviations from Hardy-Weinberg expectations were measured by Wright's F_{IS}. Values of F_{IS} were 0.130 in *B. undulata*, 0.102 in *B. kaiseri*, and 0.156 in *B. saxatilis*, suggesting that most populations approach Hardy-Weinberg expectations (Table 3).

Genetic Structure Among Populations

Heterogeneity chi-square analyses indicated very highly significant ($P \leq .001$) allele frequency differences among *B. undulata* populations for 18 of the 20 polymorphic loci. Allele frequency differences at *Tpi-2* and *Mnr-1*, two loci with low H_T values, were insignificant ($P = .554$ and $P = .135$, respectively). Allele frequency differences among *B. kaiseri* populations were significant ($P < .05$) at nine loci, whereas seven loci were significant ($P < .05$) among the *B. saxatilis* populations.

Although populations differed significantly in allele frequencies, the proportion of total genetic variation found among populations (G_{ST}) was relatively low for *B. kaiseri* (0.099), *B. saxatilis* (0.069), and *B. undulata* (0.045). Mantel's test showed that there is a very highly significant ($P \leq .005$) positive association between $F_{ST}/(1 - F_{ST})$ and geographic distance ($r^2 = .473$ and $r^2 = .476$ for direct and drainage-tracing measures, respectively) among populations of *B. undulate* and *B. kaiseri* ($r^2 = .534$ and $r^2 = .501$) and a positive but nonsignificant association ($r^2 = .177$ and $r^2 = .278$) among *B. saxatilis* populations.

Estimates of historical values of gene flow were high (Table 3). *B. undulata* had the highest values (Nm(W) = 5.25), followed by *B. saxatilis* (Nm(W) = 3.37) and *B. kaiseri* (Nm(W) = 2.29). These values were considerably higher than the mean for endemics (Nm(W) = 1.96; Hamrick et al. 1991). A high number of alleles (12 in *B. undulata*, 13 in *B. kaiseri*, and 17 in *B. saxatilis*) were found exclusively in single populations (i.e., private alleles). Estimates of gene flow (Nm(S) = 5.16, 0.51, and 1.87) by Barton and Slatkin's (1986) method were still high in *B. undulata* and *B. saxatilis* relative to the average for endemics (Table 3).

The average genetic identity of pairwise comparisons averaged 0.977 for *B. undulata* populations, 0.903 for *B. kaiseri*

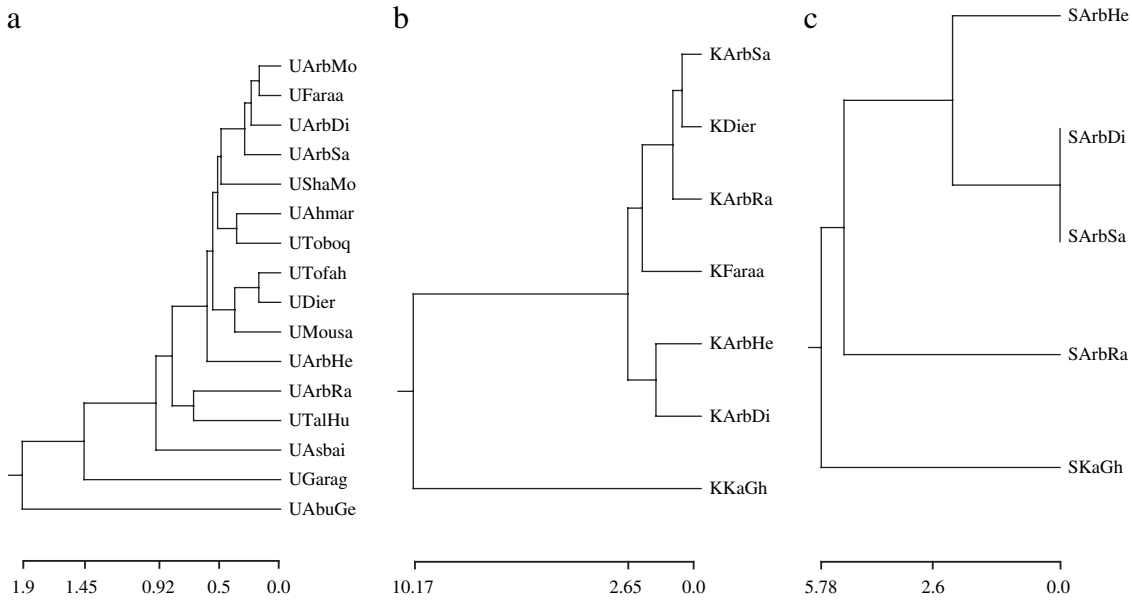

Figure 2. Dendrograms of each of the three *Ballota* species using Nei's unbiased genetic distance: (a) *B. undulata,* (b) *B. kaiseri,* (c) *B. saxatilis.*

populations, and 0.879 for *B. saxatilis* populations. Genetic identity values for KKaGh and SKaGh were relatively low with all the other populations (mean of 0.790 and 0.788, respectively). UPGMA phenograms were constructed to examine genetic relationships among populations of the three *Ballota* species (Figure 2). Within *B. kaiseri* (Figure 2b), population KKaGh was genetically distinct from all other populations. The situation was the same for *B. saxatilis* (Figure 2c) at the same site (SKaGh) followed by population SArbRa. Within *B. undulata*, population UAbuGe was most distinct followed by population UGarag (Figure 2a). Population UTofah was grouped with population UDier although they occur in distinctive geographic locations.

Discussion

The long-term management and conservation of rare and threatened plant species requires an understanding of their genetic structure and population biology. Conservation genetic studies suggest that genetic diversity significantly influences the long-term viability and persistence of local populations (Frankham 1996; Vrijenhoek 1994). An understanding of the geographic patterns of genetic diversity is important to conservation biology and allows systematic rather than opportunistic decision making concerning the selection of populations and areas for in situ protection (Pressey et al. 1993). Therefore, awareness of genetic variation patterns may be vital to defining criteria and prioritizing sites for conservation and the wise use of genetic resources (Brooks et al. 1992). Ex situ conservation efforts, as well as in situ restoration efforts, require the identification of genetically diverse populations because such populations may be the best source of propagules for conservation efforts. Conversely, genetically depauperate populations may be prime candidates for population size enhancement and the infusion of additional genetic variation (Godt et al. 1995).

The two factors thought to be responsible for the depletion of genetic variation are: (1) change in allelic frequencies due to genetic drift, which may lead to fixation (Carson 1983; Nei et al. 1975), and (2) strongly directional selection toward genetic uniformity in a limited array of environments (Van Valen 1965). Loss of genetic variation due to these factors is more likely to occur in geographically restricted species, with few individuals, than in widespread species, with many individuals (Babbel and Selander 1974; Frankel and Soulé 1981).

Comparisons of genetic diversity within a rare species with that of a closely related species with similar life history characteristics but a different geographic distribution can aid interpretations of the effects of rarity on genetic diversity and structure (e.g., Karron 1987; Karron et al. 1988; Lynch and Vaillancourt 1995). Our results show that all the three *Ballota* species maintain relatively high levels of genetic diversity (Table 2). Typically, species with restricted distributions maintain less genetic diversity than more widespread species (Hamrick and Godt 1989), but with these three *Ballota* species, the two more restricted and endangered species (*B. saxatilis* and *B. kaiseri*) maintain more genetic diversity (H_e) than the more widespread *B. undulata*. Because *B. undulata* has a high proportion of polymorphic loci and more alleles per polymorphic locus, its lower H_e value is due to it having more skewed allele frequencies than the more restricted species. Similar results have been found for several rare plant

species and their more widespread congeners (e.g., Godt and Hamrick 1998; Lewis and Crawford 1995; Shapcott and Playford 1996), emphasizing the importance of empirical data for understanding the genetic diversity and structure of specific species. Factors such as hybridization, recent speciation from a widespread species, multiple origins, comparatively recent bottlenecks, and the maintenance of genetic diversity within refuge populations have all been suggested as causes of relatively high genetic diversity in rare plant species (Lewis and Crawford 1995; Purdy and Bayer 1996; Smith and Pham 1996).

For the three *Ballota* species, most of the total genetic diversity was found within populations. Furthermore, genetic identities between populations were high within each taxon and are similar to values for conspecific populations (Crawford 1983). Differences in polymorphism among restricted species may be related to the extent to which their populations occur in heterogeneous habitats (Babbel and Selander 1974; Van Valen 1965). In general, the rare plant literature supports the view that genetic variation among populations is significant reproductively, ecologically, and evolutionarily and should be maintained (Hamrick et al. 1991; Holsinger and Gottlieb 1991). Small differences among populations may represent incipient ecotypic differentiation or even the beginning of the speciation process if populations are isolated from one another (Falk and Olwell 1992). Huenneke (1991) noted the ecological significance of genetic variation within and among populations for microhabitat differentiation, resistance to pathogens and herbivores, and overall ecological amplitude. Menges (1992) has described the correlation between low genetic variability in populations and increased vulnerability to genetic, environmental, catastrophic, and demographic stochastic events.

High levels of interpopulation divergence are typical of selfing species (Hamrick and Godt 1989) and of consistently small, discrete populations that have experienced little genetic interchange (Wright 1931). The floral architecture of *Ballota* promotes outcrossing, which is consistent with the lack of seed production from greenhouse-grown plants. Fixation indices for the three *Ballota* species also suggest that populations of these taxa are outcrossed. A small heterozygote deficit was found for the *B. undulata* populations, indicating that some selfing or biparental inbreeding may occur or that plants are intermating and dispersing over a smaller scale than that sampled (i.e., a Wahlund effect). The results of a mating system analyses for these *Ballota* species (Zaghloul 2003) indicated that *B. undulata*, *B. kaiseri*, and *B. saxatilis* are largely outcrossed (79%–100%).

When populations remain small for any extended period, sampling effects may become cumulative. This gives rise to random changes in gene frequency due to the sampling of gametes from generation to generation. In large populations, on average, only small, random changes in gene frequencies occur as a result of drift; however, where population sizes are small (e.g., <100), gene frequencies can undergo relatively large fluctuations between generations, resulting in a loss of low-frequency alleles. Therefore, large populations should maintain more genetic variability than small populations (Barrett and Kohn 1991). Although most of the sampled *Ballota* populations are currently small and appear to be relatively isolated, indirect estimates of historical gene flow for *B. undulata* and *B. saxatilis* (Table 3) were relatively high ($Nm(W) = 5.25$ and 3.37, respectively). Both estimates of gene flow suggest that there has been somewhat less gene movement among *B. kaiseri* populations compared with *B. undulata* and *B. saxatilis* populations. A migration rate of $Nm > 1.0$ is theoretically necessary to counter genetic divergence of populations due to genetic drift (Wright 1931). The high allelic diversity and heterozygosity within these *Ballota* populations and the low G_{ST} estimates suggest that historically populations of these species were more nearly continuous and that gene exchange among these populations was relatively common. Apparently, the loss of genetic variation due to genetic drift has not yet had a major influence on these *Ballota* populations. Because *Ballota* are perennials and may be long-lived, recent genetic isolation and reduction of population sizes due to increased human activities (e.g., grazing) may not have significantly affected genetic diversity. Hence, the conservation of genetic diversity naturally occurring in these species should still be possible by a combination in situ and ex situ conservation efforts.

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