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MORPHOLOGICAL AND GENETIC VARIATION AMONG POPULATIONS OF THE RARE KACHINA DAISY (*ERIGERON KACHINENSIS*) FROM SOUTHEASTERN UTAH

Loreen Allphin¹ and Michael D. Windham²

ABSTRACT.—*Erigeron kachinensis*, the Kachina daisy, is a rare species restricted to canyons in southeastern Utah. The species is known to exhibit low fecundity due to low percent fertilization of ovules and high percent abortion of fertilized ovules. Previous reproductive studies suggest that low fecundity is a consequence of small population size and inbreeding depression. This study examines genetic diversity within and among populations of *E. kachinensis* in Natural Bridges National Monument using enzyme electrophoresis. Field populations are found to have significantly different morphologies. However, morphological differences were less pronounced among populations grown in the greenhouse. The Kachina daisy exhibits levels of genetic variability in its populations similar to that of other outcrossed species. Genetic diversity statistics demonstrate that only 22.8% of genetic variation is distributed among populations. Genetic distance could not be correlated with geographic distance. Most of the populations showed significant deviation of fixation indices from zero for multiple loci. Observation of genotype frequencies demonstrates that populations are fixing on different genotypes and may be experiencing initial stages of genetic drift. Mean observed heterozygosity was 0.166 and was found to increase with increasing size and/or age in populations.

Key words: *Erigeron*, *heterozygosity*, *genetic variability*, *gene flow*, *fleabane*.

Ecological factors and life history traits may affect the amount and distribution of genetic diversity within and among natural populations (Hamrick et al. 1979, Nevo et al. 1984, Hamrick and Godt 1990). The pool of genetic diversity in wild populations is critical for a species' survival if environmental pressures exceed its limits of developmental plasticity (Frankel 1983). Moreover, genetic variability is associated with increased fitness in populations of many plant and animal species (Hamrick et al. 1979, Wills 1981, Danzmann et al. 1986, Ledig 1986). For example, increased levels of heterozygosity have been shown to increase longevity in long-lived perennial plant species (Schaal and Levin 1976, Hamrick et al. 1979). In addition, greater levels of genetic variation may buffer genotypes against environmental challenges. Conversely, the loss of genetic variability might render a population more vulnerable to extinction in cases of habitat perturbation, reproductive bottlenecks, etc. (Wright 1933, O'Brien et al. 1985, Lacy 1987, Simberloff 1988, Barrett and Kohn 1991).

According to Wright's (1943, 1946) isolation-by-distance models, mating is dependent on the distance between individuals and their ability

to disperse propagules. Based on these models, inbreeding may occur if isolation increases and prevents gene flow between small patch populations. Rare and/or endangered plant species often occur in small, disjunct populations with reduced genetic diversity due to increased habitat fragmentation and inbreeding (Stebbins 1980, Simberloff 1988, Barrett and Kohn 1991, Godt et al. 1996, Sun 1996).

Erigeron kachinensis Welsh and Moore (Asteraceae), the Kachina daisy, is a rare endemic of the Colorado Plateau regions of southeastern Utah and Colorado. The species exists in alcove seeps that arise along walls of deep canyons in the Cedar Mesa Sandstone. As water percolates through sandstone, it will eventually reach an impermeable layer and exude out of the canyon wall forming seeps. Plants adhere to the surface of the rock in such seeps, forming what are known as hanging gardens. The Kachina daisy is limited in distribution to these hanging gardens, which are often rare or sporadic in distribution (May 1995). Because this species is restricted to seeps and alcoves that are small in size, typically along a single seepline within a canyon, there is concern for its preservation.

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Several factors contribute to rarity within *E. kachinensis* and elucidate its need for preservation. The Kachina daisy is an obligately outcrossing species with a homomorphic, sporophytic type self-incompatibility system (Allphin et al. 2002). The primary pollinators of the species are small generalist flies. The species exhibits low reproductive success due to low levels of successful fertilization events and a high percentage abortion of developing fruits (Allphin and Harper 1997). Its low reproductive success has been attributed at least in part to genetic causes, specifically inbreeding and small population size (Allphin et al. 2002). Reproductive success in the species appears to be limited by both the species' inability to find compatible mates due to its incompatibility system, and recessive lethals exposed during sexual recombination in its small populations (Allphin et al. 2002).

Increased tourism in the canyon country of southeastern Utah poses another serious threat to the species. Hanging garden plant communities in the canyons of the Colorado Plateau are fragile (Fowler et al. 1995, May 1995). Shady alcoves that are habitat for the Kachina daisy are also favorite areas for hikers because they provide refuge from the hot summer sun. Many of these alcoves contain ancient Anasazi Indian ruins that also attract tourists. Populations of the Kachina daisy are also potentially threatened by flash floods, alcove roof rock spall, and other natural catastrophes. For example, if drought caused seep lines occupied by this species to dry up, many populations would be eliminated.

The Kachina daisy was proposed as "endangered" by the U.S. Fish and Wildlife Service (USFWS) in 1976 (U.S. Department of Interior 1975, 1976). Later proposals downgraded the original recommendation to "threatened" status (U.S. Department of Interior 1988). The Kachina daisy was more recently listed by the USFWS as a category 2 species, a species for which more information is needed before assigning final designation. However, all category 2 species are now officially deleted from federal lists (U.S. Department of Interior 1996). The Bureau of Land Management in Utah currently lists the Kachina daisy as a sensitive species.

Although the species was originally known only from hanging garden communities in Natural Bridges National Monument, San Juan County, Utah, 3 potential races or clusters of populations have been recognized for this

species: hanging garden, high elevation, and Colorado (Welsh et al. 1993, Allphin and Harper 1994). New collections of the species at high elevations and Colorado have significantly increased the number of known populations of the Kachina daisy and have raised the question of its necessity for protection. However, a previous genetic evaluation of the group suggests that the high-elevation and Colorado races likely represent different taxa from *E. kachinensis* and thus will not be discussed further in this manuscript (Allphin et al. 1996). Nevertheless, the typical Kachina daisy, restricted to seeps and alcoves, is extremely rare and known only from a small number of populations and drainages in the Colorado Plateau region of Utah (Allphin 1992).

The maintenance of genetic diversity in populations of plant species such as the Kachina daisy is an important aspect of many conservation programs (Marshall and Brown 1975, Frankel and Soulé 1981, Dole and Sun 1992, Rieseberg and Swensen 1996). Since the Kachina daisy occurs in small, isolated populations exhibiting reduced fitness likely due to inbreeding (Allphin et al. 2002), it is important to understand how genetic diversity is distributed within and among its natural populations.

Therefore, the research presented in this manuscript will examine the amount and distribution of genetic diversity within and among populations of *E. kachinensis* and assess the amount of gene flow among its relatively isolated populations. Significant morphological and pathogenic differences have been observed among populations of this species (Allphin and Harper 1997). Therefore, we will also determine if genetic differentiation among populations, rather than phenotypic plasticity across varying environments, might account for observed morphological and pathogenic differences.

MATERIALS AND METHODS

Study Site

Natural populations of *E. kachinensis* sampled for this study occur in 6 alcove seeps located in Natural Bridges National Monument, San Juan County, Utah (Allphin and Harper 1994, 1997). The alcoves all occur at the same elevation (1768 m), traverse Cedar Mesa Sandstone, and are shaded for much of the day by high canyon walls. However, the study alcoves differ with respect to the amount and timing of

TABLE 1. Population names, abbreviations, and sizes; various population characteristics; and number of individuals sampled by population and size-class for *E. kachinensis*.

Population	Population number	Abbreviation	Aspect ^(c) ^a	Density ^a (ind./m ²)	Estimated population size	Number of individuals sampled ^b	Number of individuals sampled/size-class ^c
North-facing A	1	NFA	302	15.1	200–300	25	5–8
North-facing B	2	NFB	328	12.7	100–200	25	5–8
South-facing A	3	SFA	160	6.1	50–100	25	5–8
South-facing B	4	SFB	197	15.7	50–100	25	5–7
West-facing A	5	WFA	252	7.9	100–150	25	5–7
West-facing B	6	WFB	225	27.4	50–100	25	5–7

^aData taken from Allphin and Harper (1994, 1997).

^bRepresents the number of individuals sampled in natural populations and used to compute diversity statistics. This number does not include the seedlings that were sampled for life stage comparisons.

^cRepresents the range in number of individuals sampled per size-class (1–4) in natural populations.

direct sunlight received and soil moisture. Two of the alcoves face north, 2 face south, and 2 face west (Table 1). Each alcove, within the pairs that share the same aspect, differs with respect to the amount of soil moisture, ranging from 5.8% to 25.6% (Allphin and Harper 1994, 1997; Table 1, 9).

Morphological and Pathogenic Comparisons

To determine if observed morphological differences among populations are due to genetic differences or environmental plasticity, we assessed morphological differences across 6 study populations from both field- and greenhouse-grown individuals. For assessment of morphological differences among the 6 field populations, we randomly selected 100 previously tagged individuals from each of 6 study alcoves (Allphin and Harper 1994, 1997). The following morphological measurements were taken from previous field studies for comparison across the alcoves: leaf length (average of 3 longest leaves), clump diameter, and number of flower heads (characteristics which showed significant differences among populations; Allphin and Harper 1994, 1997). Statistical comparisons among populations were performed using a 1-way analysis of variance and Tukey multiple means comparison (Computing Resource Center 1992).

The same morphological characteristics, in addition to leaf width and head diameter (these characteristics were not measured in field study but were observed to vary in the greenhouse), were also measured across greenhouse-grown progeny from each of the 6 study populations. To facilitate greenhouse comparisons,

we randomly collected seed from all 6 natural populations, germinated it in the greenhouse, and allowed it to grow to maturity. Note that all greenhouse individuals received optimal resources during the experimental period. Greenhouse-grown individuals allowed for morphological comparisons under a common-garden situation. Because morphological differences in field populations may be attributed to abiotic variability in habitat, greenhouse comparisons allowed us to determine whether or not observed morphological differences in field populations were environmentally or genetically controlled. Statistical comparisons among populations were performed for these morphological characteristics using 1-way analysis of variance and Tukey multiple means comparison (SAS Institute, Inc. 1994).

The incidence of pathogens, fungal infections, and herbivory was recorded for all 100 individuals in each of the 6 study populations in an earlier study of *E. kachinensis* (Allphin and Harper 1997). The data compared the percent of infected individuals in each population across the 6 study alcoves. For this study we reexamine these data for evidence of differential resistance to these stressors among study populations and determine if differential resistance is related to genetic differentiation among populations.

Enzyme Electrophoresis

The level of genetic diversity was assessed within and among the 6 study populations from Natural Bridges National Monument using enzyme electrophoresis (Soltis et al. 1983, Odrzykoski and Gottlieb 1984, Allphin et al. 1998). Approximately 4–5 young leaves were

collected from 25 individuals in each population (Table 1). The approximate proportion of the total population size that this sample represents can be estimated from Table 1. Samples from each population consisted of 6 or 7 individuals in each of 4 size-classes based upon number of rosettes: (1) a single rosette, (2) two rosettes, (3) three rosettes, and (4) \geq four rosettes per plant (Table 1). Seedling leaf samples were obtained from (8–10) seedlings grown from germinated seed that had been randomly collected from each study population and sown in the greenhouse. Because the number of rosettes in these populations has been shown to increase with age in this species (Allphin and Harper 1997), the separation of samples with respect to size allowed us to indirectly evaluate whether genetic variation, heterozygosity, was correlated with increased age in this species (Schaal and Levin 1976, Hamrick et al. 1979).

All leaf tissue samples were ground in a phosphate-PVP grinding buffer (Soltis et al. 1983) using a mortar and pestle. Ground material was absorbed into wicks made of Whatman 3 MM filter paper and stored in an ultra cold freezer (-70°) prior to electrophoresis. Electrophoresis was performed using a variety of gel and electrode conditions (Soltis et al. 1983, Odrzykoski and Gottlieb 1984; Table 2). Samples were analyzed using 12% starch gels that were sliced and stained for allozyme markers following standard protocols (Soltis et al. 1983). Twenty enzymes were surveyed for variability. Allozyme markers from 12 polymorphic enzyme loci that provided consistent, interpretable results were analyzed (Table 2). The presence or absence of each allele detected was recorded for all populations.

Genetic data collected from the 12 variable loci were used to compute a variety of genetic diversity statistics for each study population following Hamrick et al. (1979), Hedrick (1985), and Hamrick and Godt (1990) using LYNSTRONG, a genetic statistics program written by M.D. Loveless (College of Wooster, Wooster, OH). These genetic diversity statistics include mean observed heterozygosity (H_o , a direct estimate), expected heterozygosity (H_e , based on Hardy-Weinberg equilibrium model), polymorphic index (P , mean proportion of polymorphic loci), number of alleles per locus (A), mean number of effective alleles per locus (A_e , number of equally frequent alle-

les in an ideal population that would produce the same homozygosity as the actual population; Hartl 1988), and Nei's genetic distance (D ; Nei 1972). Correlation was performed in the form of linear regression to determine whether there was a significant positive relationship between geographic distance and genetic distance (SAS Institute, Inc. 1994). Mean observed heterozygosity (H_o) was also computed by size-class (life stage) for the Kachina daisy. Because of the small sample sizes for each life stage in each population, populations were pooled. Therefore, mean H_o values for this analysis represent a mean across all individuals of a particular life stage across all populations. Significant differences in these values were assessed using a 1-way analysis of variance (Computing Resource Center 1992).

A fixation index (F) was computed for each locus and population using methods of Wright (1965). Chi-square analysis of Wright's fixation indices for study populations was used to test significance of deviations from Hardy-Weinberg (H-W) expectations (0). Fixation indices and deviations from H-W expectations were computed following Hamrick et al. (1979), Hedrick (1985), and Hamrick and Godt (1990) using LYNSTRONG.

Distribution of genetic variation among populations was estimated using Nei's (1973) genetic diversity statistics employing LYNSTRONG. These statistics include total genetic diversity (H_T), intrapopulation genetic diversity (H_S), diversity among populations (D_{ST}), the proportion of genetic variation distributed among populations (G_{ST}), and inter- to intrapopulation gene diversity (R_{ST}). The average number of diploid migrants exchanged among local populations per generation, N_m (Wright 1951, Slatkin and Barton 1989), was also estimated ($N_m = (1/G_{ST} - 1)$).

Ruzicka's (1958) index of similarity was computed for all pairwise comparisons of populations based on allele frequency data for all loci. Average linkage clustering was performed on this similarity matrix using the unweighted pair-group method (UPGMA; Krebs 1989). Cluster analysis permitted the construction of a dendrogram, or cluster diagram, illustrating genetic similarities among populations. This was compared to a 2nd dendrogram based upon various abiotic/site characteristics. In order to generate the 2nd dendrogram, a similarity matrix was computed based upon Ruzicka's index of

TABLE 2. Enzymes used in population genetic analyses of *E. kachinensis*.

Enzyme	Acronym	Gel/electrode buffers ^a	No. loci scored
Esterase	EST	8	1
Isocitrate dehydrogenase	IDH	11 & M	1
Leucine aminopeptidase	LAP	8	1
Malate dehydrogenase	MDH	M	1
NADH-diaphorase	NADH	8	1
Phosphoglucisomerase	PGI	6 & 8	2
Phosphoglucmutase	PGM	6	2
6-Phosphogluconate dehydrogenase	6-PGD	11 & M	1
Shikimate dehydrogenase	SKDH	11 & M	1
Triosephosphate dehydrogenase	TPI	6 & 8	1
TOTAL NUMBER OF LOCI			12

^aSystems 6, 8, 11 after Soltis et al. (1983); system M a 7.5 pH version of the morpholine citrate system after Odrzykoski and Gottlieb (1984).

similarity (Ruzicka 1958) for various abiotic/site characteristics for each population of *E. kachinensis* as reported in an earlier study (Allphin and Harper 1994). Average linkage clustering using UPGMA was also performed on this similarity matrix (Krebs 1989) and presented in the form of a dendrogram to demonstrate habitat similarities among study populations.

RESULTS

Significant differences for morphological characters among populations from field and greenhouse individuals are given in Table 3. Most morphological differentiation among populations is apparently due to environmental factors. From the field data, alcoves 2 and 5 had the smallest leaves and smallest clump diameters (Table 3; Allphin and Harper 1997). Alcoves 4 and 6 exhibited the largest leaves and clump diameters (Table 3; Allphin and Harper 1997). These data are consistent with environmental data. Alcoves 2 and 5 are the driest sites and alcoves 4 and 6 the wettest (Allphin and Harper 1994; Tables 1, 9). Greater morphological differences were observed for field populations than among cultivated individuals (Table 3). Actual morphological differences due to genetic factors were more rare, yet more pronounced, for greenhouse-grown individuals. Population (alcove) 1 exhibits significantly smaller leaves, leaf widths, clump diameters, and head diameters than other populations grown in common-garden conditions (Table 3).

Levels of genetic variability for allozyme loci are summarized in Table 4. Mean observed heterozygosity varies among the 6 populations

(alcoves). Populations 4 (south-facing B) and 6 (west-facing B) exhibited the highest mean observed heterozygosity and polymorphic indices ($H_o = 0.201, 0.195, P = 0.187, 0.231$; Table 3). Conversely, population 5 showed the lowest level of genetic variability and lowest polymorphic index ($H_o = 0.120, P = 0.155$). Mean heterozygosity across all populations was moderate ($H_o = 0.166$; Table 4). Expected heterozygosity was different from observed heterozygosity in most populations (Table 4).

Increased number of rosettes for individuals in populations of the Kachina daisy is consistent with increasing age in field populations (Allphin and Harper 1997). Mean observed heterozygosity (computed as a mean value across all loci for individuals in the same age/size class in the population) increases with increasing size-class (based on number of rosettes) in the Kachina daisy (Fig. 1). Seedlings (from year sampled) exhibit relatively high diversity ($H_o = 0.266$). Diversity is significantly lower for size-class 1 than the seedling class and continues to increase across size-classes. The youngest size-class, SC1, is significantly less heterozygous than size-classes 3 and 4 at $P \leq 0.05$.

Genetic diversity statistics for all populations are summarized in Table 5. Total gene diversity is fairly high ($H_t = 0.311$). Most of this diversity is distributed within populations ($H_s = 0.249$). G_{ST} was estimated as 0.228, and thus 22.8% of genetic variation is distributed among populations (Table 5). This results in a relatively high estimate of N_m , the number of migrants exchanged between local populations per generation. Gene flow (N_m) was estimated to be 0.847. This is interpreted as an exchange of between 8 and 9 migrants every 10 generations

TABLE 3. Mean measurements for several morphological characteristics of 6 study populations of the Kachina daisy in Natural Bridges National Monument. Cultivated populations were sown from seed of the 6 field populations and grown under greenhouse conditions at the University of Utah. Means for any population which do not share the same letter are significantly different at $P \leq 0.05$. Columns with lines indicate that data are unavailable. Morphological data from field populations were taken from Allphin and Harper (1997).

Population (alcove)	Leaf length (cm)	Leaf width (cm)	Clump diam. (cm)	No. flower heads	Head diam. (cm)
Field					
1	2.7 b (0.07)	—	4.8 b (0.13)	1.3 a (0.08)	—
2	1.9 a (0.06)	—	3.6 a (0.10)	0.9 b (0.10)	—
3	2.5 b (0.06)	—	6.2 c (0.18)	1.9 c (0.19)	—
4	3.6 c (0.08)	—	7.3 d (0.18)	2.3 c (0.20)	—
5	2.1 a (0.06)	— (0.12)	4.4 b (0.10)	0.6 b	—
6	3.4 c (0.08)	—	6.6 c (0.18)	1.4 a (0.14)	—
Cultivated					
1	3.7 a (1.03)	0.7 a (0.19)	6.9 a (2.33)	67.9 a (32.69)	1.0 a (0.18)
2	5.8 b (1.77)	1.3 b (0.15)	18.0 b (5.66)	80.0 a (28.28)	1.3 b (0.21)
3	5.4 b (1.27)	1.2 b (0.25)	16.9 b (2.79)	39.0 b (15.25)	1.3 b (.180)
4	5.2 b (1.52)	1.2 b (0.32)	13.6 bc (8.42)	34.0 b (17.75)	1.1 a (0.08)
5	4.9 b (0.82)	1.2 b (0.36)	9.8 c (6.89)	63.62 a (41.81)	1.3 b (0.19)
6	5.4 b (1.49)	1.4 b (0.43)	11.9 bc (6.30)	47.0 ab (21.49)	1.4 b (0.14)

TABLE 4. Level of intrapopulation allozyme variation as measured across 25 individuals in each of 6 study populations of the Kachina daisy in Natural Bridges National Monument. Values are given as means across 12 enzyme loci for each population.

Population (alcove)	Number of unique genotypes ^a	Mean observed heterozygosity (H_o)	Mean expected heterozygosity (H_e)	Polymorphic index (PI)	Mean no. alleles (A)	Mean no. effective alleles (A_e)
NFA (1)	1	0.150	0.157	0.157	1.58	1.29
NFB (2)	1	0.177	0.179	0.179	1.58	1.30
SFA (3)	1	0.154	0.181	0.181	1.67	1.32
SFB (4)	2	0.201	0.187	0.187	1.42	1.34
WFA (5)	2	0.120	0.155	0.155	1.67	1.26
WFB (6)	2	0.195	0.230	0.231	1.83	1.47
ALL POPULATIONS		0.166	0.182	0.182	1.63	1.33

^aLines illustrate that the unique genotype(s) is (are) shared by both populations in the species pair but no other populations.

(Slatkin and Barton 1989). However, this appears to be an overestimate of gene flow when genetic distances are compared with geographic distance (Table 6). Genetic distance is not significantly correlated with geographic distance ($r^2 = 0.0592$). Distant populations are just as likely to have alleles in common as pop-

ulations in close proximity. Gene flow (Nm) values lower than 1 can lead to population differentiation (Wright 1951).

Although the genetic diversity statistics demonstrate that most variability is distributed within populations in the Kachina daisy, genotypic frequencies among populations for 2 enzyme

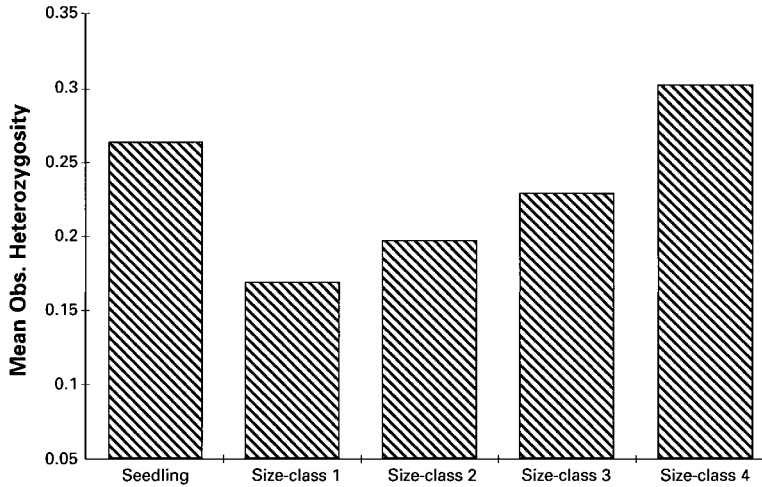


Fig. 1. Mean observed heterozygosity across seedlings and 4 other size/age classes (as described in Methods) in *E. kachinensis*. Seedlings were sown from seed collected in all natural populations of *E. kachinensis* and grown in the greenhouse. Mean observed heterozygosity for the seedling class is significantly higher than for size-class 1. Size-class 1 exhibits significantly lower mean observed heterozygosity than size-classes 3 and 4 at $P = 0.05$.

loci in particular suggest that populations are fixing on different genotypes. Table 7 summarizes genotype frequencies for 6-PGD-2 and EST-1. At EST-1 higher genotype frequencies are observed for AA and AB genotypes for populations 1, 2, and 6, and yet populations 3 and 4 generally lack allele A. At 6-PGD-2 populations 1 and 2 (north-facing populations) exhibit higher frequencies for genotypes AB and BB, while populations 3 and 4 (south-facing) are missing allele B entirely. Moreover, 2 of the populations (3, 4) have unique genotypes not found in other populations. Two of the population pairs (both north-facing or both west-facing) had unique genotypes not found in other sampled populations. Furthermore, at PGI-2 population 3 (SFA) carries an allele that cannot be found in any other population (Fig. 2, Table 4).

Fixation index (F) was determined for all loci in each population of *E. kachinensis*. Chi-square analysis demonstrates significant deviation from Hardy-Weinberg expectations for some of the populations and loci (Table 8). West-facing B, one of the smallest and most genetically variable of the study populations, showed significant deviation from H-W expectations at 4 loci. All populations showed significant deviation from H-W expectations for 1 or more loci (Table 8). Since 6 populations and 12

TABLE 5. Genetic diversity statistics from 6 populations of *E. kachinensis* in Natural Bridges National Monument, San Juan County, Utah. Data given are mean values for 12 enzyme loci.

Total gene diversity (H_T)	0.311
Within populations (H_S)	0.249
Among populations (D_{ST})	0.066
Coefficient of gene differentiation ($G_{ST} = D_{ST} / H_T$)	0.228
Inter- to intrapopulation gene diversity (R_{ST})	0.441

loci were sampled, there are 72 determinations for F deviations from H-W equilibrium. For *E. kachinensis*, 7 of the 72 determinations ($\sim 10\%$) deviated significantly ($P = 0.05$).

The dendrogram based on allele frequency data demonstrates genetic similarities among populations (Fig. 3A). Populations 1 and 2 are the most similar (86.2%). These populations are only approximately 100 m from each other, and gene flow likely occurs. Populations 3 and 4 are also very similar (83.7%). These 2 populations are approximately 250 m apart; thus, gene flow is also likely between these populations. However, population 6 (WFB) clusters with populations 1 and 2, but it is geographically the most distant of the populations (over 2 miles). West-facing A and B (5 and 6) are located only 500 m from one another but are genetically dissimilar (Fig. 3A).

TABLE 6. Geographic distance and genetic distances between sampled populations of *E. kachinensis*. Geographic distances (m) are given above the diagonal, and Nei's (1972) genetic distances (D) are given below the diagonal.

Population	NF-A 1	NF-B 2	SF-A 3	SF-B 4	WF-A 5	WF-B 6
1	—	100	1610	1860	3980	3480
2	0.0192	—	1519	1769	3880	3380
3	0.0989	0.1448	—	250	2360	1860
4	0.1122	0.1446	0.284	—	2110	1610
5	0.1126	0.1307	0.0516	0.0457	—	500
6	0.0376	0.0355	0.0376	0.0553	0.0799	—

TABLE 7. Genotype frequencies at 2 of the enzyme loci (6-PGD-2 and EST) for study populations of *E. kachinensis* in Natural Bridges National Monument.

Population	AA	AB	BB
EST			
N-facing A (1)	0.167	0.667	0.167
N-facing B (2)	0.480	0.480	0.040
S-facing A (3)	0	0.093	0.917
S-facing B (4)	0	0	1.00
W-facing A (5)	0	0.417	0.583
W-facing B (6)	0.292	0.583	0.125
6-PGD-2			
N-facing A (1)	0.160	0.480	0.360
N-facing B (2)	0.158	0.210	0.632
S-facing A (3)	1.00	0	0
S-facing B (4)	1.00	0	0
W-facing A (5)	0.750	0.250	0
W-facing B (6)	0.375	0.250	0.375

When this dendrogram is compared with another based on abiotic/site characteristics of the 6 study alcoves (Allphin and Harper 1994), a different clustering pattern results (Fig. 3B). Populations are only ~63% similar at best. Abiotic/site characteristics differ the most among populations that are most similar genetically (populations 1, 2). Environmental variability has been shown to select for allozyme markers (Milton 1994), but these data show that allozyme variability is independent of environment.

DISCUSSION

The results presented in this manuscript have many evolutionary and conservation implications. Population size is often correlated with an increase in genetic diversity (heterozygosity) in natural populations (Billington 1991, Stangel et al. 1992). However, in the Kachina daisy, 2 of the smallest populations (SFB and WFB) have the highest heterozygosity and polymorphic indices. These data are consistent with findings in other rare species (Sherwin et al.

1991, Allphin et al. 1998, Maki and Morita 1998). In addition, genetic similarities among populations were found to be independent of ecological (abiotic) similarities among populations.

In the Kachina daisy, most genetic variability appears to be distributed within rather than among populations. This would not appear to support the idea of historic, long-term population isolation and genetic drift. The distribution of genetic diversity may be affected by spatial and historical factors (Kimura and Maruyama 1971, Slatkin 1987, Sheely and Meagher 1996). Because the historical range and past population sizes of the Kachina daisy are unknown, interpretation of the data presented in this manuscript is difficult. However, most populations have fixation indices that are significantly different from zero (no fixation) for at least 1 or more loci. These data suggest that most populations of the Kachina daisy do not randomly cross. Maki (1999) found similar findings in a threatened insular endemic in the genus *Aster*.

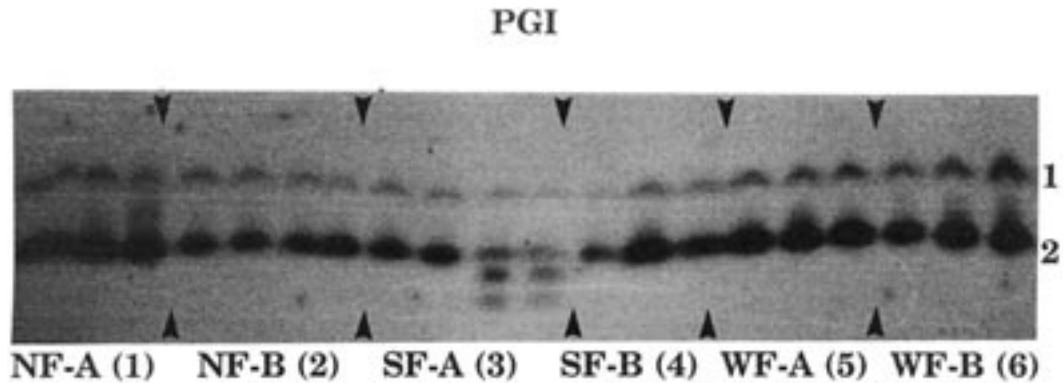


Fig. 2. Photograph of an allozyme showing variability at the PGI-2 locus. Notice only a single population (alcove 3) has allele 2.

TABLE 8. Fixation index (*F*) values for selected enzyme loci of each population generated using LYNSPROG. Population *F* values exhibiting significant chi-square deviations from zero are followed by a symbol. Chi-square values for fixation index values were generated using LYNSPROG, following the methods of Hedrick (1985). ***P* = 0.01; * *P* = 0.05; †*P* = 0.10.

Population	6PGD-1	MDH-2	IDH-2	NADH-1	SKDH-1
NFA (1)	0.0200	-0.1190	0.2833	0.7873**	0.0000
NFB (2)	0.4714*	-0.3056†	0.0882	0.0000	0.0559
SFA (3)	0.0000	-0.2162	0.4778*	-0.0387	0.5000†
SFB (4)	0.0000	0.1048	0.2656	-0.3824†	0.0143
WFA (5)	-0.1190	-0.1463	0.1188	0.6543**	0.0571
WFB (6)	0.5104*	-0.4242*	0.3354†	0.5131**	0.2781

High fixation indices could result from inbreeding, selection pressures, random genetic drift, founder events, etc. We suggest that populations of the Kachina daisy do not show signs of historic, long-term isolation and drift because habitat fragmentation and reduction in population size are of fairly recent origin. However, other additional lines of evidence suggest that populations of the Kachina daisy might be in the early stages of genetic differentiation and genetic drift. For example, populations of the Kachina daisy appear to have differential ability to resist pathogens and predators (Allphin and Harper 1997). In addition, populations differentially fix alternate genotypes at some loci (Table 7).

Habitat fragmentation might be a recent phenomenon in the Kachina daisy. Natural habitat fragmentation in this species occurs when seeps and hanging gardens become more isolated from one another, as seeplines in a canyon begin to dry. Drying along seeplines in

Natural Bridges National Monument appears to be a relatively recent event (Brough et al. 1987). We compared soil moisture data from 1991–92 (Allphin and Harper 1994) with soil moisture data obtained in 1997 at all study populations. These data demonstrate that all seeps with alcove overhangs (NFA and NFB) were drier in 1997 than in 1992 (Table 9). The populations without alcove overhangs (those populations receiving additional moisture from local precipitation) were the only ones that did not show a drier condition in 1997. Although we have only 2 data points and cannot conclusively say that the alcoves are drying, observations made in these alcoves over 10 years of monitoring suggest drying in these seeps. For example, we have observed an increase in rodent burrows at seeplines that were originally too wet to support habitation. Moreover, population size has gradually dwindled in the study populations over the past 10 years of study (Allphin and Harper 1994, 1997).

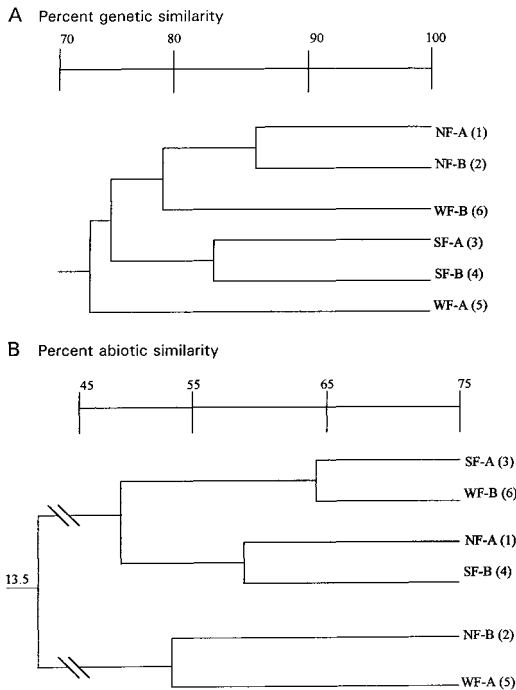


Fig. 3. A, Dendrogram illustrating similarities (percent) among sampled populations of *E. kachinensis* based upon allele frequencies for allozyme loci; B, dendrogram illustrating similarities (percent) among sampled populations of *E. kachinensis* based upon abiotic/site characteristics (Allphin and Harper 1994).

Since some populations still contain over 100 individuals, we may be only beginning to see the effects of isolation and small population size as a result of habitat fragmentation in these populations (Table 1). Although there was a moderately high estimate of gene flow between populations of the Kachina daisy based upon genetic distances, we could find no correlation between geographic and genetic distance in this species. This is not as expected under the isolation-by-distance model (Wright 1943, 1946). One might expect a lack of correlation between geographic and genetic distance if panmixia (or regular random mating) were occurring among populations (Wright 1946). This explanation seems unlikely for these populations of the Kachina daisy because of the long distances separating many of these populations, the bi-directionality of canyon systems, and the species' limited dispersal capabilities, due to its short-distance seed dispersal (primarily gravity) and small pollinators. A lack of corre-

TABLE 9. Mean soil moisture (% of dry weight) for 1990–1992 (mid-June) and 1997 (mid-June).

Population	Percent soil moisture (1990-1992) ^a	Percent soil moisture (1997)
1	12.3	15.0
2	5.8	8.3
3	18.4	18.4
4	12.2	6.1
5	12.7	8.7
6	25.6	22.8
Mean	14.5	13.2

^aData taken from Allphin and Harper (1994).

lation between genetic distance and geographic distance has been found for other rare species including the family Asteraceae (Allphin et al. 1998, Matthews and Howard 1999). Therefore, we suggest that N_m may not be reflective of actual gene flow events in the Kachina daisy, but possibly alleles shared in common through common ancestry. For example, if small population sizes were due to a recent habitat fragmentation, many alleles would still be shared among populations and not yet lost through genetic drift (Wright 1933, 1943, 1946).

Most morphological variation among natural populations of the Kachina daisy is apparently due to environmental factors. Morphological differences among populations reflecting genetic differences are evidenced by a few, very pronounced morphological differences between some of the populations of greenhouse-grown individuals (primarily alcove 1). Morphological characteristics appear to be very plastic in this species and not always indicative of genetic differences.

Additional patterns emerge from the genetic data that have biological and conservation implications. First, there is a positive relationship between heterozygosity and longevity in our data. If we ignore the seedling size-class, heterozygosity increases with increasing size-class in the Kachina daisy. Increased size of individuals in populations of the Kachina daisy has been shown to be consistent with increasing age in field populations (Allphin and Harper 1997). It is, therefore, probable that greater genetic variation increases the potential to achieve larger size (number of rosettes) or age, and is best interpreted as a correlation between heterozygosity and increasing longevity of

individuals, as has been demonstrated for trees and other long-lived perennials (Schaal and Levin 1976, Hamrick 1979, Hamrick et al. 1979). The Kachina daisy is a relatively long-lived species. Demographic monitoring (Allphin and Harper 1997 and unpublished data) has demonstrated no mortality in large individuals over 10 years of monitoring. This demographic research suggests that individuals may live as long as 20 years in the absence of environmental catastrophe. We suggest that the relationship between heterozygosity and longevity in the Kachina daisy might help to explain why this nonwoody perennial is so long-lived.

Another pattern emerges in the comparison of heterozygosity in seedlings with other size-classes of the Kachina daisy. Greenhouse-grown seedlings exhibited unusually high levels of heterozygosity compared to the size/age classes (1–3). This is greater genetic variability than would have been observed in seedlings in the natural populations, suggesting that the high level of heterozygosity in seedlings is an artifact of being grown in a greenhouse setting. For example, environmental constraints could have eliminated some genotypes from natural populations.

Additionally, there is evidence of low reproductive success due to high levels of embryo abortion in the Kachina daisy that can be explained by inbreeding and genetic load (Allphin and Harper 1997, Allphin et al. 2002). Genetic load, recessive deleterious or lethal genes present in a population, should be expected to increase in out-crossed species (Wiens 1984, Wiens et al. 1987, Charlesworth 1989a, 1989b). Such deleterious recessive genes appear in homozygous form as chromosomes are independently assorted in meiosis and produce lethal or sublethal combinations that result in the abortion of developing embryos or endosperm, or death of developing seedlings. Because the most reproductively active size-classes of the Kachina daisy are the largest size-classes (Allphin and Harper 1997), and this obligately outcrossing species exhibits relatively high levels of genetic variability for these size-classes, there is a greater potential for inbreeding due to genetic load because more recessive lethals can be maintained in the recessive state. As the populations of the Kachina daisy become isolated and individuals are forced to mate with close relatives, lethals

may be exposed in the homozygous state, causing the high level of embryo abortions observed in this species (Allphin and Harper 1997). Genetic data presented in this manuscript support the reproductive studies that suggest inbreeding (Allphin et al. 2002). In this study all populations showed significant deviation of fixation indices from zero for 1 or more loci (Table 8). These data suggest that populations of the Kachina daisy are not mating randomly and that inbreeding may be occurring in at least some of the natural populations.

To determine how genetic diversity in the Kachina daisy compares with other outcrossing, perennial endemic species, we compared our data with a synthesis paper by Hamrick and Godt (1990) summarizing levels of diversity across a wide sampling of plants. Mean observed heterozygosity in *E. kachinensis* is similar to that found in other outcrossing plant species and perennial dicots (Table 10; Hamrick and Godt 1990). However, genetic diversity in *E. kachinensis* is higher than the level of variation found in other narrow endemic species (Table 10; Hamrick and Godt 1990). Other species in Asteraceae show similar patterns (Maki and Morita 1998, Ayres and Ryan 1999). For example, *Aster spathulifolius*, a narrow endemic from Japan, also showed high genetic diversity for its endemism (Maki and Morita 1998).

Allozyme markers, as used to assess genetic diversity in the Kachina daisy, have been considered “neutral” genes reflecting evolutionary processes affecting the entire genome (Avice 1994). Mitton (1994), however, proposed that environmental factors may occasionally select for allozyme markers. He considers that allozymes are, therefore, not always indicative of random genetic change. However, our 2 dendrograms for similarity among populations of the Kachina daisy based upon genetic and environmental characteristics provide different topologies from one another (Fig. 3). This suggests that environmental conditions likely are not responsible for genetic differences across the study alcoves (populations) and that allozymes can be used as neutral markers in this species.

The patterns presented in this manuscript suggest some important conservation implications. Because populations show signs of inbreeding depression and are beginning to show signs of population differentiation and loss of

TABLE 10. Level of allozyme variation at species level for species in different categories. The data were extracted from Hamrick and Godt (1990). These data are compared with allozyme variation at the species level for *E. kachinensis*. Standard error values are given in parentheses below the means. Statistical differences among means for species or groups were determined using multiple means comparison (Snedecor and Cochran 1967). Means followed by the same letter in a column are not significantly different at $P = 0.05$.

Categories or species	Number taxa	Mean no. of pop.	Mean no. of loci	A	A _e	H _o
Dicots	329	11.9 (1.7)	16.8 (0.4)	1.79 a (0.04)	1.19 a (0.01)	0.136 a (0.007)
Short-lived perennials	152	8.8 (1.2)	17.2 (0.7)	1.70 a (0.06)	1.15 a (0.01)	0.116 a (0.009)
Outcrossing annuals	172	10.7 (2.1)	17.7 (0.7)	1.99 a (0.07)	1.24 b (0.02)	0.167 a (0.01)
Endemic	81	6.5 (0.9)	17.8 (0.6)	1.80 a (0.08)	1.15 a (0.04)	0.096 b (0.01)
<i>E. kachinensis</i>	1	6	12	1.63 a (0.06)	1.33 b (0.03)	0.166 a (0.01)

diversity, land managers may need to bring pollen or seed from other populations, or genetically more distant sources, to increase reproductive capacity in local populations of the Kachina daisy. However, we caution the movement of genes among populations if populations are beginning to become differentiated from one another.

Alcove seeps and hanging gardens that support Kachina daisy populations are fragile habitats. Backcountry recreation is increasing in the Colorado Plateau area of Utah. Trails into canyon bottoms run near populations of the Kachina daisy. One trail in Natural Bridges National Monument comes very close to alcove 6, which despite theoretic expectations was shown to have the highest genetic diversity and the smallest population size. Land managers should take special care to maintain this population. Trails may need to be rerouted to avoid human impact to Kachina daisy populations. With growing human impact to the canyon country, protection of all populations may prove necessary for preservation of genetic diversity within the Kachina daisy.

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