Genetic Diversity and Population Size in Four Rare Southern Appalachian Plant Species

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Abstract: *Allozyme diversity was examined in four rare, high-montane plant species from the Appalachian Mountains of southeastern North America. These species may represent relictual members or descendants of an alpine community that was more widespread during the late Pleistocene. We sampled five populations of* Geum radiatum *(Rosaceae),* Carex misera *(Cyperaceae),* Trichophorum cespitosum *(Cyperaceae), and the four known populations of* Calamagrostis cainii *(Poaceae). Genetic diversity was low for all species but was typical of that found for plant species with limited ranges. Low genetic diversity may reflect historical events associated with changes in the species' biogeography. As the Pleistocene climate warmed, suitable habitat decreased in areal extent and became fragmented, probably resulting in smaller, more-isolated populations. In recent times these species, which co-occur in fragile rock outcrop habitats, have been adversely affected by human activities. Genetic analyses revealed reduced diversity in populations of decreasing size for three species. Estimates of gene flow were low (*Nm < 1.0*) in all four species. Positive associations between genetic diversity and population size, evidence of recent population declines, and the low estimates of gene flow suggest that genetic drift may play a prominent role in shaping the present-day genetic composition of these species. Furthermore, these data suggest that the genetically depauperate populations are unlikely to regain genetic variation without human intervention.*

Diversidad Genética y Tamaño Poblacional de Cuatro Especies de Plantas Raras en el sur de los Apalaches.

Resumen: *Se examinó la diversidad de alozimas en cuatro especies raras de plantas de las Montañas Apalaches en el sureste de Norte América. Estas especies pueden ser relictos o descendientes de una comunidad alpina de amplia distribución en el Pleistoceno tardío. Se muestrearon cinco poblaciones de Geum radiatum (Rosaceae), Carex misera (Cyperaceae), Trichophorum cespitosum (Cyperaceae) y las cuatro poblaciones conocidas de Calamagrostis cainii (Poaceae). La diversidad genética de todas las especies fue baja, pero correspondió a la que presentan especies de plantas con rangos limitados. La baja diversidad genética puede reflejar eventos históricos asociados con cambios en la biogeografía de las especies. Al calentarse el clima del Pleistoceno el hábitat adecuado disminuyó en extensión y se fragmentó, lo cual produjo poblaciones pequeñas y aisladas. En tiempos recientes, estas especies, que coexisten en hábitats rocosos frágiles, han sido afectadas adversamente por actividades humanas. Análisis genéticos indicaron diversidad reducida en poblaciones de tamaño decreciente en tres de las especies. La estimación del flujo de genes fue baja (Nm < 1.0) en las cuatro especies. La asociación positiva entre diversidad genética y tamaño poblacional, evidencia de declinaciones poblaciones.Recientes y la baja estimación del clujo de genes, sugiere que la deriva genética puede jugar un papel prominente en el moldeado de la composición genética actual de estas especies. Mas aún, estos datos sugieren que es improbable que las poblaciones genéticamente empobrecidas recuperen su variación genética sin la intervención humana.*

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Introduction

The rapid pace of environmental change has led to increasing concern for the viability of many plant and animal species. Although the short-term persistence of many species will be determined by their ability to recover numerically from population declines and extinctions (Lande 1988; Schemske et al. 1994), a species' long-term viability is ultimately linked to the genetic variation it maintains (Vida 1994; Vrijenhoek 1994). Species that lack genetic diversity overall or those lacking variation for specific traits (e.g., disease resistance) may lack the ability to adapt to new and changing environmental conditions and are thus more prone to extinction.

The maintenance of genetic diversity is often linked to population size. Population genetic theory predicts the loss of genetic diversity in populations that remain small for several generations (genetic drift), in populations initiated from a small number of colonists (founder effect), and in populations that suffer rapid declines in size (population bottlenecks), particularly if recovery is slow or if size fluctuations are frequent (Barrett & Kohn 1991). Many rare and endangered plant species have recently experienced declines in population size and numbers. Indeed, documentation of such declines is among the criteria for obtaining protected status in the United States (Smith 1980). Although the genetic consequences of decreasing population size are predicted by theory, empirical data documenting these effects are scant.

We describe the genetic structure of four rare plant species that co-occur on high-montane rock outcrops in the southern Appalachian Mountains of the southeastern United States. The outcrop flora of this region harbors a number of rare species that have been adversely affected by anthropogenic disturbances, particularly direct trampling and soil disturbance. We had three major goals:(1) to determine whether these species had levels of genetic variation comparable to other endemic plant species; (2) to examine patterns of genetic variation to determine the relationship of genetic diversity to population size; and (3) to aid management of these species by identifying genetically diverse (or unique) and genetically depauperate populations. Genetically diverse or unique populations may deserve highest priority for protection, whereas genetically depauperate populations may be prime candidates for management.

The Study Species

The four plant species studied (*Calamagrostis cainii* Hitchcock, *Carex misera* Buckley, *Geum radiatum* Gray, and *Trichophorum cespitosum* (L.) Hartm.) grow on rocky promontories, steep rock faces, and narrow ledges at high elevations in the southern Appalachians. The attractiveness of scenic vistas at these high elevation sites, coupled with the typically thin, fragile soils of the rock outcrop habitat make these plants particularly vulnerable to destruction by hikers, climbers, and sightseers (Johnson 1995). Collectors have also been attracted to the most showy of these species, *G. radiatum* (U.S. Fish and Wildlife Service 1993).

Geum radiatum (Rosaceae), commonly known as spreading avens, is a federally endangered perennial herb that produces basal rosettes. Eleven populations of *G. radiatum* occur in Tennessee and North Carolina. Five additional populations are known to have been extirpated. Four of the extant populations have undergone significant declines (ranging from 67 to 96%) in the last decade, whereas four have experienced less drastic declines and three have remained relatively stable (U.S. Fish and Wildlife Service 1993). *Geum radiatum* reproduces vegetatively by rhizomes and sexually via achenes arising from perfect, yellow flowers found in few-flowered, indefinite cymes. Seedling establishment is rare (Johnson 1995). Various insects, including dipterans and hymenopterans, have been observed visiting flowers (Brackley & Burger 1980; Johnson 1995).

About 25 populations of *Carex misera* (Cyperaceae) are found in the southern Appalachians. Commonly termed "wretched sedge," *C. misera* is a monoecious, wind-pollinated perennial herb that has acquired state-imposed protected status in Georgia, Tennessee, and North Carolina (Massey et al. 1983). The cosmopolitan genus *Carex* has about 1000 species (Mabberley 1987) and is one of 11 genera containing the highest number of taxa of conservation concern (32) in the U.S. (Falk 1992).

Calamagrostis cainii (Poaceae) is a rhizomatous perennial grass endemic to the southern Appalachians. It is considered distinct from other eastern North American species of *Calamagrostis* and has morphological affinities to three *Calamagrostis* taxa (a circumpolar congener and two California endemics) from which it has presumably been isolated for some time (Wiser 1991). *Calamagrostis cainii* is known from four populations and is a candidate for federal protection.

In contrast to the three endemic species studied, *Trichophorum cespitosum* (formerly *Scirpus cespitosus* Bigelow; Cyperaceae) has an extensive range, and is found most commonly in the alpine zone of the northern Appalachians and in the tundra and peatlands of the northern U.S. and Canada. The 25 southern Appalachian populations of *T. cespitosum* (all located in North Carolina) are distantly disjunct from the rest of the species' range. Thus, in the southern portion of its range *T. cespitosum* may have genetic characteristics similar to endemic species. *Trichophorum cespitosum*, a bulrush, is a perennial rhizomatous sedge that forms dense tussocks.

Primary seed dispersal of the four study species is likely through gravity and wind, with secondary dis-

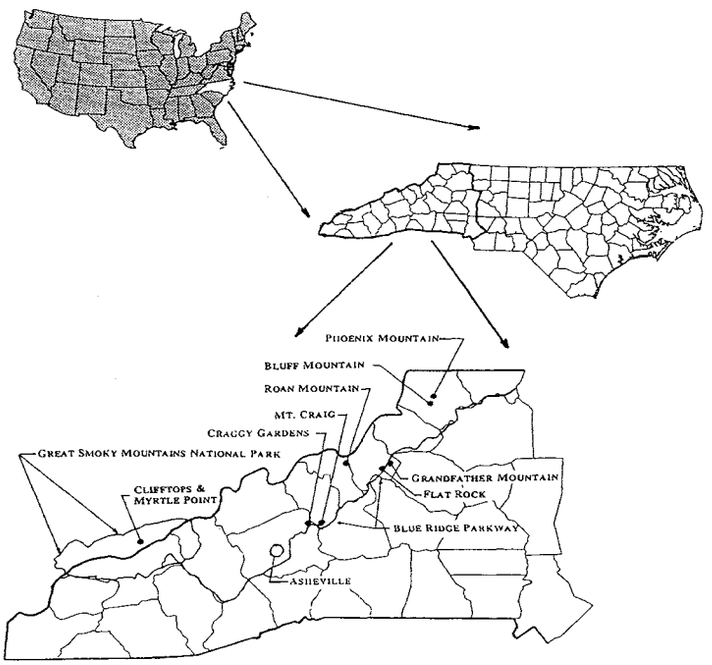

Figure 1. Locations of collection sites in the southern Appalachians of North Carolina and Tennessee. Sites were selected to sample rare plant populations across a wide range of environmental conditions and to maximize the number of species sampled at each site. When a given site did not include all four rare species, a nearby site containing the missing species was also selected.

persal facilitated by water movement through crevices and channels between rock-outcrop habitat patches.

Methods

Five populations of *G. radiatum*, *C. misera*, and *T. cespitosum* and the four known populations of *C. cainii* were sampled (Fig. 1; Table 1). Population size was estimated and designated as follows: small ($n < 100$), medium ($100 \leq n \leq 1000$), or large ($n > 1000$). Genetic analyses (by M. J. W. Godt & J. Hamrick) were "blind" to population size (estimated by B. Johnson). *Calamogrostis cainii* population MPT was not collected by the authors and thus was excluded from the size classification. Forty-eight individual plants were sampled for each species at each location, except for *G. radiatum* at Phoenix (60 individuals sampled) and Roan Mountain (72 individuals), *T. cespitosum* at Roan Mountain (23 individuals), and *C. cainii* at Craggy Gardens (41 individuals). Leaf samples were transported on ice to the laboratory, where they were crushed under liquid nitrogen using a mortar and pestle. A crushing buffer (Mitton et al. 1979) was added to the resultant leaf powder to solubilize and stabilize the enzymes. Enzyme extracts were absorbed onto chromatography paper wicks that were stored in microtiter plates at −70°C. Ultra-cold storage of the enzyme extracts permitted all populations of a species to be analyzed simultaneously. Starch-gel electrophoresis of enzymes was used to estimate genetic diversity.

A subset of the following enzymes were stained for each of the four species: acid phosphatase (ACPH), adenylate kinase (AK), alcohol dehydrogenase (ADH), aldolase (ALD), colorimetric esterase (CE), diaphorase (DIA), fluorescent esterase (FE), fructose-1,6-diphosphatase (F-1,6) β-galactosidase (β-GAL), glutamate dehydrogenase (GDH), glutamate oxaloacetate transaminase (GOT), isocitrate dehydrogenase (IDH), leucine aminopeptidase (LAP), malate dehydrogenase (MDH), mannose phosphate isomerase (MPI), menadione reductase (MNR), 6-phosphogluconate dehydrogenase (6-PGDH), phosphoglucoisomerase (PGI), phosphoglucomutase (PGM), shikimate dehydrogenase (SKDH), and triose phosphate isomerase (TPI). The loci resolved for each species and the buffers used are given in Table 2. Stain recipes for β-GAL, DIA, and MNR are given in Cheliak and Pitel (1984). All other recipes were taken from Soltis et al. (1983).

Table 1. Collection localities and sample sizes for the four study species.

*Location**	*Abbreviation*	Calamagrostis cainii	Carex misera	Geum radiatum	Trichophorum cespitosum
Clifftops	CT	48	48	48	48
Myrtle Point	MP	48	—	—	—
Craggy Gardens	CG	41	48	48	48
Mount Craig	MC	48	—	—	—
Roan Mountain	RM	—	48	72	23
Flat Rock	FR	—	—	—	48
Grandfather Mountain	GF	—	48	48	48
Bluff Mountain	BF	—	—	—	—
Phoenix Mountain	PM	—	48	60	—

**Clifftops, Mount LeConte, Great Smoky Mountain National Park, Sevier County, Tennessee (TN); Myrtle Point, Mount LeConte, Great Smoky Mountain National Park, Sevier County, TN; Craggy Gardens, Blue Ridge Parkway, Buncombe County, North Carolina (NC); Mount Craig, Mount Mitchell State Park, Yancey County, NC; Roan Mountain, U.S. Forest Service, Mitchell County, NC; Flat Rock, Blue Ridge Parkway, Avery County, NC; Grandfather Mountain, private, Avery and Wautuga Counties, NC; Bluff Mountain, The Nature Conservancy, Wilkes County, NC; Phoenix Mountain, private, Ashe County, NC.*

Table 2. The allozyme loci resolved for each of the four species studied and the buffer system used to resolve them.*

Species	*Buffer system*	*Loci resolved*
Calamagrostis cainii	2	*Idh-1*; *6Pgdh-1*
	4	*Got-2*; *Skdh*
	6	*Adh-1* and *-2*; *Acph-3*; *Dia-1* and *-2*; *Gdh-1*; *Lap-1* *Mdh-1*; *Mnr-2* and *-3*; *Pgi-1* and *-2*; *Pgm-1*; *Tpi-1* and *-2*
	8	*Fe-1*, *-2* and *-3*; *Got-1*
	11	*Ak-1*; *Mpi-1*
Carex misera	2	*Ald-1*; *Pgi-1* and *-2*; *Pgm-1* and *-2*
	4	*Mdh-1*; *Mpi*
	6	*Adh-1*; *Acph-1*
	7	*Dia-1*; *Mnr-1* and *-2*
	8	*β-Gal-1*; *Fe-1*, *-2*, *-3*, and *-4*; *Got-1* and *-2*; *Tpi-1* and *-2*
	11	*F-1,6-4*; *Idh-2*; *6Pgdh-1* and *-2*; *Pgm-1* and *-2*; *Skdh-1* and *-2*
Geum radiatum	2	*6Pgdh-1*, *-2* and *-3*; *Idh-1*
	6	*Acph-1*, *Dia-1*, *-2*, *-3* and *-4*; *Mnr-2*; *Pgm-1*, *-2* and *-3*; *Tpi-1*, *-2* and *-3*
	7	*Got-2*
	8	*Fe-1* and *-2*; *Lap-1* and *-2*
	11	*Pgi-1*, *-2* and *-3*; *Skdh-2*
Trichophorum cespitosum	2	*Idh-1* and *-2*
	6	*Acph-1* and *-2*; *Adh-1*; *Ce-1*, *-2* and *-3*; *Mnr-1*, *-2*, *-3* and *-4*; *Pgi-1* and *-2*; *Tpi-1* and *-2*
	7	*Ak-3*; *Got-1* and *-2*
	8	*β-Gal-1*; *Fe-1*, *-2*, *-3* and *-4*; *Lap-1* and *-2*;
	11	*F-1, 6-1*; *Mdh-1*, *-2* and *-3*; *6Pgdh-1*; *Pgm-1*, *-2* and *-3*; *Skdh-2*

**Abbreviations for the loci are given in the materials and methods section of the text. The buffers are numbered as in Soltis et al. (1983). Buffer system eight was modified slightly.*

Genetic diversity statistics were calculated for the species (as described in Hamrick & Godt 1989) and for each population (as described in Hedrick 1985). These statistics included the percent polymorphic loci (P), the mean number of alleles per locus (A) and per polymorphic locus (AP), the effective number of alleles per locus (A_e), observed heterozygosity (H_o) and expected heterozygosity or gene diversity (H_e). Genetic parameters subscripted with an s (e.g., H_{es}) indicate species values, whereas parameters subscripted with a p (e.g., H_{ep}) indicate population values.

Deviations from Hardy-Weinberg expectations were examined for each polymorphic locus within every population by calculating Wright's fixation index (Wright 1922). Fixation indices were tested for significance (F should equal zero under panmixia in the absence of selection) by Chi-square tests (Li & Horvitz 1953).

Population divergence was estimated in two ways. First, Nei's gene diversity statistics (Nei 1973, 1977) were used to estimate the proportion of genetic diversity found among populations (G_{ST}) for each polymorphic locus. These values were averaged across loci to obtain an overall estimate of population divergence. Each G_{ST} value was tested for significance by the Chi-square test, $\chi^2 = 2TG_{ST}(k - 1)$ with $(k - 1)(s - 1)$ degrees of freedom, where T is the total sample size, k is the number of alleles at the locus, and s is the number of populations (Workman & Niswander 1970). Genetic identity and distance measures were also calculated for each pair of conspecific populations (Nei 1972). Associations between genetic distance and linear geographic distance were examined using a correlation analysis in SAS (SAS Institute 1988).

Table 3. Species-wide genetic diversity[a] (and the number of loci analyzed) for each of the study species compared to endemics and short-lived herbaceous species.

Species	L	P_s	AP_s	A_s	A_{cs}	H_{cs}
Calamagrostis cainii	25	56.0	2.36	1.76	1.14	0.089
Carex misera	28	67.9	2.42	1.96	1.21	0.125
Geum radiatum	25	28.0	2.57	1.44	1.20	0.098
Trichophorum cespitosum	35	40.0	2.07	1.43	1.14	0.081
Endemics (159)[b]	18	43.8	2.99	1.88	1.16	0.110
Short-lived herbs (236)[b]	16	45.0	2.73	1.78	1.18	0.136

[a] L is the number of loci; P_s the percent polymorphic loci, AP_s the mean number of alleles per polymorphic locus, A_s the mean number of alleles per locus, A_{es} the effective number of alleles, and H_{es} gene diversity or expected heterozygosity.

[b] Unpublished data; updated from Hamrick and Godt 1989. Numbers in parentheses are the number of taxa reviewed.

Table 4. Mean population genetic diversity[a] for each of the study species as compared to endemic and short-lived herbaceous species.

Species	L	P_p	AP_p	A_p	A_{ep}	H_{ep}	*Range in* I	*Mean* I (SD)
Calamagrostis cainii	25	33.9	2.13	1.39	1.09	0.056	0.94–0.99	0.97 (0.02)
Carex misera	28	34.8	2.26	1.44	1.14	0.082	0.89–0.99	0.95 (0.03)
Geum radiatum	25	23.2	2.22	1.29	1.14	0.074	0.93–0.99	0.96 (0.02)
Trichophorum cespitosum	35	17.7	2.06	1.19	1.06	0.039	0.88–0.96	0.94 (0.03)
Endemics (159)[b]	18	29.2	2.58	1.43	1.10	0.076	—	—
Short-lived herbaceous (236)[b]	16	29.2	2.39	1.41	1.13	0.103	—	—

[a] *L is the number of loci; P_p the percent polymorphic loci; AP_p the mean number of alleles per polymorphic locus; A_p the mean number of alleles per locus; A_{ep} the effective number of alleles; H_{ep} the gene diversity or expected heterozygosity; and I the genetic identity.*
[b] *Unpublished data; updated from Hamrick and Godt (1989). Numbers in parentheses are the number of taxa reviewed.*

Gene flow was estimated from population genetic structure by the equation

$$G_{ST} = [1/(4Nm\alpha + 1)],$$

where $\alpha = (n/n - 1)^2$ and n is the number of populations, G_{ST} is the multiallelic equivalent of F_{ST}, and Nm is the number of migrants per generation (Wright 1931; Crow & Aoki 1984). A second indirect estimate of gene flow was made based on the mean frequency of alleles found exclusively in single populations ("private alleles" [Slatkin 1985; Barton & Slatkin 1986]).

Results

Genetic variation was low in these four species, as is typical of endemic plant species (Tables 3 & 4). Relative to the mean value for endemic plants, all four species displayed lower numbers of alleles per polymorphic locus. In addition, three species (*C. cainii*, *G. radiatum* and *T. cespitosum*) had less genetic diversity at the species level (H_{es}) than expected for endemic plants. Within populations, however, genetic diversity (H_{ep}) was comparable in *G. radiatum* and *C. misera* to the mean value

Table 5. Genetic diversity statistics[a] and population sizes for the four species studied.

Population/ location[b]	*Estimated size*[c]	P	A	A_e	AP	H_o	H_e	I
Calamagrostis cainii								
CT	L	44.0	1.56	1.21	2.27	0.107	0.108	0.97
MCG	M	39.1	1.39	1.08	2.00	0.072	0.056	0.97
CG	S	16.0	1.16	1.04	2.00	0.016	0.023	0.97
MPT	—	36.4	1.45	1.04	2.25	0.031	0.037	0.95
Carex misera								
GF	L	35.7	1.39	1.17	2.10	0.089	0.103	0.96
BM	L	35.7	1.43	1.16	2.20	0.082	0.091	0.95
RM	L	17.9	1.25	1.09	2.40	0.065	0.049	0.93
CT	L	46.2	1.65	1.13	2.42	0.090	0.084	0.93
CG	L	38.5	1.46	1.12	2.20	0.084	0.082	0.97
Geum radiatum								
PX	L	28.0	1.32	1.17	2.14	0.056	0.091	0.97
RM	L	28.0	1.40	1.15	2.43	0.050	0.086	0.96
GF	L	24.0	1.32	1.13	2.33	0.049	0.066	0.95
CT	M	20.0	1.24	1.12	2.20	0.054	0.064	0.97
CG	M	16.0	1.16	1.11	2.00	0.050	0.061	0.95
Trichophorum cespitosum								
GF	L	31.4	1.34	1.08	2.09	0.042	0.060	0.95
FR	M	14.3	1.17	1.10	2.20	0.035	0.056	0.94
CG	M	11.4	1.11	1.06	2.00	0.021	0.034	0.95
CT	M	17.1	1.17	1.05	2.00	0.024	0.027	0.93
RM	S	14.3	1.14	1.03	2.00	0.011	0.020	0.92

[a] *P is the percent polymorphic loci; A the mean number of alleles per locus; A_e the effective number of alleles; AP the mean number of alleles per polymorphic locus; H_o the observed heterozygosity; H_e the gene diversity or expected heterozygosity; and I the mean identity with all population pairs.*
[b] *See Table 1 for definitions of location abbreviations.*
[c] *S, small; M, medium; and L, large (see text).*

Table 6. Nei's genetic diversity statistics (1973, 1977) and indirect measures of gene flow.*

Species	H_T	H_S	G_{ST}	Nm(S)	Nm(W)
Calamagrostis cainii	0.159	0.117	0.130	0.29 (10)	0.94
Geum radiatum	0.185	0.119	0.191	32.29 (1)	0.68
Carex misera	0.349	0.266	0.161	0.26 (10)	0.83
Trichophorum cespitosus	0.202	0.105	0.283	0.76 (5)	0.41
Endemics	0.274	0.191	0.226	1.58	1.96

**Total genetic diversity averaged across all polymorphic loci is H_T, genetic diversity found within populations is H_S, and the proportion of total genetic diversity found among populations is G_{ST}. Nm indicates the number of migrants per generation and has been calculated using Wright's equation as modified by Crow and Aoki (Nm(W); 1984) and using Barton and Slatkin's equation (Nm(S); 1986), with the number of "private" alleles noted in parentheses. The genetic diversity data for endemics is from a review by Hamrick and Godt (1989); the gene flow data for endemics is taken from Hamrick et al. (1995).*

found for endemics, whereas *C. cainii* and *T. cespitosum* had lower values.

For *C. cainii*, *G. radiatum*, and *T. cespitosum*, genetic diversity (H_e) was positively associated with population size (Table 5). The range in genetic diversity was large; genetic diversity varied 1.5 times between the smallest (CG) and largest (PX) *G. radiatum* populations, whereas the smallest populations of *C. cainii* and *T. cespitosum* maintained about one-third the genetic diversity displayed in their largest populations. Sampled populations of *C. misera* were all classed as large. It is noteworthy that this species maintained the highest levels of genetic diversity of the four analyzed.

The four species exhibited moderate amounts of population differentiation. The proportion of total genetic variation found among populations (G_{ST}; Table 6) ranged from 13.0% (for *C. cainii*) to 28.3% (for *T. cespitosum*). Significant correlations (i.e., $p \leq 0.05$) between genetic distance and geographic distance were not found.

Although historical patterns of population divergence can contribute to allele frequency differences between populations, *Nm* values are typically interpreted to reflect recent gene-flow patterns. Estimated migration rates (i.e., *Nm* values) are low for the four species (Table 6), except for the rate calculated on the basis of rare alleles for *G. radiatum*. This estimate for *G. radiatum* was based on a single, rare heterozygote and thus does not provide as robust an estimate of gene flow as that based on the G_{ST} value (Slatkin & Barton 1989). All six polymorphic loci in *G. radiatum* exhibited significant heterogeneity in allele frequencies ($p < 0.05$) among populations, whereas 16 of 19 loci differed across *C. misera* populations, 12 of 14 across *T. cespitosum* populations, and 8 of 14 for *C. cainii*.

Mean genetic identity values for each of the species was ≥ 0.94 (Table 4). *Carex misera* exhibited the largest range in genetic identity values ($I = 0.89$ to 0.99), whereas *T. cespitosum* had populations that were least similar to one another ($I = 0.88$ to 0.96). Genetic identity values were high for all population pairs of *C. cainii* and *G. radiatum*.

Significant deviations from Hardy-Weinberg expectations were found for each of the four species. In general, a deficit of heterozygotes was apparent at several loci. For *C. cainii*, 10 of 11 significant fixation indices (25 total tests) were positive; for *C. misera* 13 of 19 significant fixation indices (46 total tests) were positive; for *G. radiatum* 15 of 20 significant fixation indices (20 total tests) were positive; and for *T. cespitosum* all 12 significant fixation indices (25 total tests) were positive.

Discussion

The genetic diversity maintained within a species is a function of historical events and recent evolutionary processes. Because very little is usually known of a species' evolutionary and ecological history, explanations for levels of genetic diversity maintained within a species or population rely primarily on inference. Insights into historical events that may have affected the level of variation within a species can sometimes be gained through consideration of its biogeography (Schwaegerle & Schaal 1979; Godt et al. 1995) or via comparison with congeners (Karron 1987; Karron et al. 1988; Loveless & Hamrick 1988; Pleasants & Wendel, 1989; Cole & Biesboer 1992). Thus, the low "species-wide" genetic diversity found for these four species may reflect historical events associated with long-term climatic changes and shifts in vegetation patterns in the southeastern United States. On the other hand, recent ecological and demographic characteristics of the species can provide explanations for the levels and patterns of genetic diversity found within and among extant populations (Van Treuren et al. 1991).

Biogeography of the Study Species

The flora of high-elevation rock outcrops in the southern Appalachians includes numerous rare plant species. Some of these species are members of the northern alpine and tundra floras (e.g., *T. cespitosum*) that are represented by disjunct southern Appalachian populations. Other species, such as *G. radiatum*, *C. cainii*, and *C. misera*, are southern Appalachian endemics. All four species are probably remnants or descendants of a more widespread, late Pleistocene alpine flora (Wiser 1994). Large areas of alpine meadow and exposed rock outcrops without woody species would have offered extensive suitable habitat for these species or their progenitors during the late Pleistocene. Contemporary distribution patterns of several species provide support for this view.

For example, the rush *Juncus trifidus* occupies the most restricted range of microhabitats of six rare southern Appalachian outcrop species analyzed by Johnson (1995), but in more northern areas it occupies a variety of alpine habitats and is a dominant species in some. *Geum peckii*, a northern relative of *G. radiatum*, not only occupies rock outcrops in the White Mountains of New Hampshire, but it also occurs in wet meadows, on streamsides, and on nonforested, moist scree slopes (Bliss 1963; Crow 1982; Wiser 1993). In the northern Appalachian alpine zone, *T. cespitosum* occurs on streamsides and in bogs, as well as on rock outcrops (Bliss 1963; Wiser 1993). In even more northerly areas, *T. cespitosum* is found in peatlands, bogs, and tundra (Scoggan 1978).

Species of the southern Appalachian ice-age alpine community should have experienced range retractions as the climate warmed and vegetation zones shifted upward and northward. Their populations probably decreased in size and experienced increased isolation concomitantly with the decrease and fragmentation of the alpine habitat. The low genetic diversity found within these four species may reflect losses caused by genetic drift associated with range retraction and habitat fragmentation. The increasing isolation of these alpine communities may also have led to population divergence and perhaps speciation. *Geum radiatum*, for instance, may have shared a progenitor with its northern relative, *Geum peckii*, which it closely resembles (F. Brackley, personal communication).

Endemic species tend to have less genetic variation than more widespread species (Hamrick & Godt 1989). There is also some evidence suggesting that many narrowly distributed species maintain less genetic diversity than their more widespread relatives (Karron 1987; Karron et al. 1988), with one explanation being that the geographically restricted species acquired a subset of genetic variation from their more widespread progenitors (Loveless & Hamrick 1988; Cole & Biesboer 1992; Sherman-Broyles et al. 1992). This may have limited their adaptive abilities and, thus, restricted such species to tightly circumscribed habitats. Selection and drift within these habitats may have subsequently reduced the genetic variation available to the species. The four species examined in this study maintain levels of genetic diversity fairly typical of endemic species. Although our data do not permit evaluation of the historical level of genetic diversity maintained within these four species, it is tempting to speculate that the low level of variation found is associated with changes in their biogeography since the last ice age.

Genetic Diversity, Ecology, and Population Size

Allozyme diversity has been reported previously for *Carex misera*. Based on 27 loci examined in over 400 individuals from nine populations, Schell and Waterway (1992) reported considerably less variability than we report and higher population differentiation. The differences may be attributed in part to the loci examined. Species-estimates of genetic diversity should be higher, however, when more populations are examined. Differences in sampling strategies may also explain the discrepant results. We made a concerted effort to sample several habitat patches to maximize the range of microhabitats represented by the populations and to minimize sampling of multiple ramets of the same individual. More-localized sampling by Schell and Waterway (1992) may have led to lower estimates of polymorphism and higher estimates of among-population differentiation.

Associations between current population sizes and genetic diversity seen for *C. cainii*, *G. radiatum*, and *T. cespitosum*, coupled with documented declines of these species, suggest that genetic drift has played a role in the development of their present genetic structure. Population genetic theory dictates that the initial effect of small population size is the loss of rare alleles (Hedrick 1985), whereas significant reductions in heterozygosity should occur in populations that remain small for several generations (Barrett & Kohn 1991). Inbreeding in small populations also produces higher levels of homozygosity, and this may result in inbreeding depression (Barrett & Kohn 1991; Ellstrand & Elam 1993; Raijmann et al. 1994; Heschel & Paige 1995). The high number of significant positive inbreeding coefficients observed for these four species is consistent with the occurrence of inbreeding. But these observations can also arise through sampling across populations or plant patches that differ in gene frequency (the Wahlund effect). It is difficult to distinguish between these alternatives without a thorough examination of the mating system of each species. To our knowledge, the mating systems of these rare species have not been described.

In theory, decreased levels of genetic variation within populations can jeopardize their long-term persistence by reducing their ability to adapt to changing environmental conditions. Decreased genetic variation and increased homozygosity may affect population viability and growth rates in the short term also, by reducing individual fitness (Vrijenhoek 1994). But the immediate effects of diminishing genetic variation have been investigated for relatively few plant species. For several species, however, decreased fitness has been associated with small population size. For example, Polans and Allard (1989) documented the loss of genetic diversity and reduced fitness (as measured by quantitative characters) in small experimental *Lolium multiflorum* populations. In nature, small population size in *Ipomopsis aggregata* has been linked to increased susceptibility to environmental stress and decreased fitness (Heschel & Paige 1995). Extensive studies of *Salvia pratensis* and *Scabiosa columbaria*, two European species that have experienced

rapid population declines, have shown positive associations between population size and allozyme diversity (Van Treuren et al. 1991), as well as correlations between phenotypic variation (as measured in a common garden) and source population size (Ouborg et al. 1991). In addition, DeMauro (1993) has shown that a relictual population of the self-incompatible Lakeside daisy (*Hymenoxys acaulis* var. *glabra*) was effectively extinct due to a lack of variation at the self-incompatibility locus.

At the extreme, the lack of genetic variation (at one or more loci) can lead to the loss of entire species. For example, the virtual extinction of natural populations of the American chestnut (*Castanea dentata*) and stinking cedar (*Torreya taxifolia*), two North American trees, has been directly linked to their inability to adapt to new pathogens and/or changing environmental conditions (Elias 1987; Falk 1992). Thus, although few empirical studies have directly documented links between reduced genetic variation and the loss of individual fitness, decreased population vigor, and species extinction in the wild, these cases suggest that genetic variation can play an important role in the persistence of populations, even in the short term, and may ultimately determine the fate of some species.

The scope of our study precluded detailed investigations of the effects of lower genetic diversity on fitness of individuals and populations of the four study species. Observations suggest, however, that on average plants in the less genetically diverse populations may be less fit. For example, *G. radiatum* plants in the large Phoenix (PX) population were vegetatively more vigorous and exhibited substantially higher seed set than those within the small and genetically depauperate Craggy Gardens (CG) population (Johnson 1995). Factors in addition to genetics, such as habitat quality, pollinator efficiency, and competition, could have affected seed set. But a transplant experiment in which Phoenix plants were moved to Craggy Gardens suggested that the lower seed set of the native Craggy Gardens plants was not due to less favorable environmental conditions but was linked to decreased fitness that may be attributable to genetic drift and inbreeding (Johnson 1995). Furthermore, *Calamogrostis cainii* plants were vegetatively more vigorous and occupied a greater proportion of available habitat in the genetically diverse Clifftops (CT) population compared to the Craggy Gardens (CG) population (Johnson 1995). These observations suggest that low levels of genetic diversity at these presumably neutral allozyme loci may reflect losses of genetic diversity at loci that influence fitness.

For all four species, genetic identities were within the range commonly found for conspecific populations (Gottlieb 1977). When population identities were averaged across the sampled populations within species, all populations appeared to have a fairly high genetic identity with the others (Table 5). This is corroborated by the moderate G_{ST} values (0.13, 0.16, and 0.19) found for three of the species (*C. cainii*, *G. radiatum*, and *C. misera*). The high genetic identities and moderate level of population differentiation found in these three species suggest that their populations have not diverged substantially. The highest population differentiation ($G_{ST} = 0.283$) was found in the most widespread species, *T. cespitosum*. The genetic similarity of populations is an important consideration in the development of conservation and management strategies. Populations that share alleles are more likely to have experienced gene flow in the recent past than those that maintain different suites of alleles.

Estimated levels of gene flow in the four study species ($Nm(W) < 1.0$) provided further evidence that genetic drift may have played a prominent role in the development of their genetic structure (Wright 1931). Low rates of gene flow among populations of these four rare species, and their relative isolation, suggest that populations that have lost genetic variation because of genetic drift are unlikely to recover this variation without human intervention.

Conservation Considerations

Several management strategies could be used to ameliorate the effects of diminished genetic variation and reduced fitness within small populations of these southern Appalachian species. The most conservative strategy, and one that would avoid contamination of gene pools (and the potential breakup of adaptive genotypes), would be to increase population sizes using plants grown from seeds taken from the population to be restored. Although this is not likely to increase genetic diversity, further loss should be prevented. Because plant mortality tends to be concentrated in early life stages (Harper 1977), population size could be increased much more rapidly than would normally occur. Rapid population growth following bottlenecks minimizes loss of genetic variation (Nei et al. 1975). One caveat is that if substantial adaptive variation has been lost from the populations, few long-term benefits would be gained by this strategy, although such population size enhancement would, in the short term, buffer against extinction by demographic stochasticity. A second caveat is that a potentially important selective filter is bypassed by circumventing the seed-to-seedling stage of the life cycle.

An alternative strategy is the infusion of genetic diversity by the introduction of plants or propagules into genetically depauperate populations. Lacking knowledge of adaptive differences between populations of these species, the most conservative strategy would be to choose source populations that were genetically diverse and ecologically similar. Although we recognize that genetic supplementation via the introduction of plants

from other populations is controversial, we recommend that propagules from genetically diverse and ecologically similar populations be considered in restoration efforts undertaken with these and other species for which declines in population sizes, loss of genetic diversity, and reduced fitness have been documented. In the past, species management efforts implemented without regard for propagule source may have incurred unintended and potentially deleterious changes in population genetic structure and fitness (Millar & Libby 1989). However, reaction against such genetically naive management should not preclude efforts to reasonably assess and carefully manage genetic resources in native plant populations. Small, isolated populations that have experienced declines due to human disturbances and that show evidence of reduced vigor and inbreeding depression may benefit from the introduction of off-site germplasm (Van Treuren et al. 1993; Raijmann et al. 1994). Such introductions should provide demographic stability and may restore genetic diversity, providing the raw material for selection and adaptive population change. Indeed, such human-mediated introductions may be necessary if conservation biologists are concerned with restoring evolutionary processes (e.g., gene flow) that have been altered by human-induced habitat fragmentation and reductions in population numbers and sizes.

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