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Genetic diversity in *Astragalus tennesseensis* and the federal endangered *Dalea foliosa* (Fabaceae)[1]

Adrienne L. Edwards[2,3]
Illinois Natural History Survey, Champaign, IL 61820

Bethany Wiltshire, and Daniel L. Nickrent
Department of Plant Biology, Southern Illinois University, Carbondale, IL 62901-6509

EDWARDS, A. L. (Illinois Natural History Survey, Champaign, Illinois, 61820), B. WILTSHIRE AND D. L. NICKRENT (Department of Plant Biology, Southern Illinois University, Carbondale, Illinois, 62901-6509). Genetic diversity in *Astragalus tennesseensis* and the federal endangered *Dalea foliosa* (Fabaceae). J. Torrey Bot. Soc. 131: 279–291. 2004.—The Tennessee milk vetch, *Astragalus tennesseensis* Gray, and the leafy prairie clover, *Dalea foliosa* (Gray) Barneby, are cedar glade endemics with disjunct populations in formerly glaciated Illinois dolomite prairies. We explored the effects of geographical isolation on the levels and distribution of genetic variation in these rare species. Isozyme analyses were conducted using leaf tissue from 19 populations (429 individuals) of *Astragalus tennesseensis* and 10 populations (240 individuals) of *Dalea foliosa* throughout their geographical ranges in Illinois, Tennessee, and Alabama. Across all *A. tennesseensis* populations sampled, 13 out of 15 loci (87%) were polymorphic, (14–57% within populations), averaging 2.6 alleles per polymorphic locus. In *D. foliosa*, 11 out of 20 (55%) loci were polymorphic, (0–30% within populations), averaging 2.0 alleles per polymorphic locus. Across loci, levels of observed and expected heterozygosity in *A. tennesseensis* (H_O = 0.103 and H_E = 0.121) were more than twice that found in *D. foliosa* (H_O = 0.037 and H_E = 0.043). For both species, levels of heterozygosity were highest in Tennessee and lowest in Illinois, where levels half that found in Tennessee. Differentiation among populations within geographical regions (G_{STC} = 0.161 and 0.145 for *A. tenneseensis* and *D. foliosa*, respectively) was lower than when not clustered by regions (G_{ST} = 0.217 and 0.441). In *A. tennesseensis*, the majority of species-level genetic diversity was contained within populations (H_S = 0.141 as compared to total diversity H_T = 0.179), but in *D. foliosa*, the opposite was true (H_S = 0.060 as compared to H_T = 0.186). Given the high degree of differentiation among geographical regions, particularly in formerly glaciated Illinois, conservation strategies for these species should include consideration of their geographical affinities.

Key words: *Astragalus*, conservation biology, *Dalea*, population genetic structure, rare plants.

The evolutionary forces of mutation, natural selection, migration, and genetic drift, superimposed on the ecological requirements of a species, determine levels and patterns of genetic variation (Wright 1940, Kareiva 1990, Nei 1973). Large populations should have higher levels of genetic variation within populations than small populations (Kimura and Crow 1964), and isolated populations should exhibit greater genetic differentiation between populations than relatively contiguous ones (Endler 1977). Essentially, isolated populations of small size are more susceptible to the effects of random events (Hamilton 1982, Beardmore 1983, Barrett and Kohn 1991), which is of particular concern for rare species. Primarily outcrossing species that are restricted to rare, isolated habitats particularly more likely to suffer from lowered levels of heterozygosity and the negative effects of inbreeding in small populations (Wright 1977, Schoen and Brown 1991). Organisms with limited dispersal potential that also are limited to particular habitats are often threatened due to habitat destruction and landscape fragmentation. Naturally isolated habitats provide the opportunity to examine the accumulated effects of isolation and fragmentation on the levels and distribution of genetic variation (Hamrick and Godt 1996, Edwards and Sharitz 2000).

Calcareous cedar glades are an example of naturally isolated habitats that have been reduced in extent due to anthropogenic influences across landscapes. Shallow soils and rock outcrops of limestone or dolomite characterize these glades; dry conditions prevail during the growing season, although soils can be saturated or flooded in winter and spring (Neslon 1987). Dominated by herbaceous vegetation, cedar

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[3] E-mail: aedwards@inhs.uicu.edu

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glades range in size from ~0.2 to several hundred ha. Disturbances thought to minimize woody plant encroachment include drought, frost heaving, grazing, weathering, and fire (Quarterman 1950, Nelson 1987). Calcareous glade systems are scattered geographically from southern Missouri, Illinois, and Indiana, to northeast Texas, and east to southwestern Virginia and northwestern Georgia (Kurz 1981, Redfearn 1983, Bacone *et al.* 1984, LeGrand 1988, Baskin and Baskin 1989, Bartgis 1993, Heikens and Robertson 1995). The centers of greatest concentration are in the Ozark Plateau Region of Missouri (Steyermark 1940, Nelson and Ladd 1983) and the Central Basin of Tennessee (Quarterman 1950). At least 44 plant species are endemic or nearly endemic to calcareous glades (Baskin and Baskin 1989).

Of the 29 Tennessee cedar glade endemic plants described by Baskin and Baskin (1989), only two species had populations that were disjunct to areas glaciated during the Pleistocene, in what is now Illinois: *Astragalus tennesseensis* and *Dalea foliosa*. Based on morphological affinities with western congeners, Baskin and Baskin (1986) suggested that these two legumes were derived from species in the Southwest or the Ozarks, and that the disjunct Illinois populations represent relicts of a migration north along the glades of western Kentucky, southern Illinois and the dry hill prairies along the Mississippi and Illinois Rivers in the mid-Holocene. Since no collections have been made of either species between Nashville and central Illinois (Kurz and Bowles 1981), it is assumed that the Illinois populations have been isolated from the extensive southern populations for at least several hundred years. Because of their extreme isolation from the centers of distribution in central Tennessee, the potential exists for the disjunct Illinois populations to be genetically distinct. Today *A. tennesseensis* and *D. foliosa* are restricted in Illinois to specific sites along the Des Plaines and Illinois Rivers, respectively. Alabama populations of both species are remnants of the southern extensions of the Tennessee glade system and are now effectively isolated from the nearest Tennessee populations. Populations in Alabama and are declining

The Tennessee glade endemics identified by Baskin and Baskin (1989) tend to be poor competitors, with insect-pollinated flowers, and gravity-dispersed seeds that are capable of forming at least short-lived seed banks. *Astragalus tennesseensis* and *D. foliosa* share these traits, in addition to being short-lived perennials that do not spread clonally (Baskin and Baskin 1989, Schwegman 1998, Molano-Flores 2004). Both species appear to require very specific microhabitat conditions. The microhabitat in which *A. tennesseensis* occurs is along the glade margins, in areas often partially shaded by Eastern red cedar (*Juniperus virginiana*). This species is widespread throughout the Tennessee glade system, yet entirely restricted to the cedar glade habitat. *Dalea foliosa* apparently requires a microhabitat defined by a balance of sun exposure and moisture along the edge of the glade (DeMauro and Bowles 1993). It occupies slight slopes that allow drainage and prefers the open sun of the glades, but also grows in the partial shade provided by cedars or other woody species that line the glade. Illinois populations of these species occur in full sun on dry-mesic dolomite prairies.

The purpose of this study was to use isozymes as genetic markers to examine genetic diversity within and among populations of *Dalea foliosa* and *Astragalus tennesseensis* in Illinois, Tennessee, and Alabama. We were interested in the levels of genetic diversity in these rare species, and the distribution of diversity within and among populations, and among disjunct geographical areas. More specifically, we were interested in determining the extent to which the disjunct populations in Alabama and previously glaciated Illinois had diverged genetically from the center of the geographical range in Tennessee. We hypothesized that both species would show low levels of diversity, that the disjunct populations of these species would be genetically depauperate and with few unique alleles, and that genetic variation would be distributed mostly among rather than within populations.

Materials and Methods. SPECIES BIOLOGY. Tennessee milk vetch, *Astragalus tennesseensis* Gray is a short-lived perennial (living up to 5 years), with an age of first reproduction of 2–5 years (Baskin and Baskin 1989). The plant grows to 10 cm, and new shoots arise from the ground-level stem in early spring. Creamy white to yellow flowers that appear in May are primarily bumblebee pollinated (Baskin and Baskin 1989), and ripe fruits fall from the plant in July and August before seed dehiscence. Seeds possess a thick seed coat, requiring scarification to germinate, and can remain viable in the soil for more than 10 years (Baskin and Baskin 1989).

Astragalus tennesseensis is considered to

have Western affinities. In his revision of the genus, Barneby (1964) created Section *Tennesseensis* for *A. tennesseensis* based on its unique morphology relative to other North American *Astragalus* species and its unusual characteristics of pilose pubescence covering the entire plant and honeycomb-like fruit venation. Barneby (1964) hypothesized that species of northeastern Mexico, *A. sanguineus* Rydb., and the western plains, *A. plattensis* Nutt., were the closest relatives of *A. tennesseensis*.

Leafy prairie clover, *D. foliosa* (Gray) Barneby (synonym *Petalostemon foliosum* Gray) is a short-lived perennial (living up to 7 years), with an age of first reproduction of 2–3 years (Baskin and Baskin 1989). Plants grow to 80 cm; new shoots emerge from the basal overwintering root crown each spring. Flowering late July through August, purple flowers are clustered in compressed terminal racemes and are primarily bumblebee pollinated (Baskin and Baskin 1989). Fruits reach maturity in mid-September and fall from erect stems throughout winter; seeds are capable of persisting in seed banks at least 8 years (Baskin and Baskin 1998a). Seeds germinate in May, but seedlings are not very drought-resistant; insufficient moisture and frost heaving are the primary sources of seedling mortality (Baskin and Baskin 1973).

Leafy prairie clover is morphologically distinct from eastern *Dalea* species. Its leaflet number is greater than other eastern members of the genus, averaging 20–27 (Fernald 1950). Barneby (1977) described *D. foliosa* as a western species, and Wemple (1970) concluded that another quite rare species restricted to limestone cliffs in western Texas, *D. sabinale* (Watson) Shinners, is its closest relative. These taxonomic affinities suggest a western origin of this species, despite having a center of distribution in Tennessee (Baskin and Baskin 1986).

Astragalus tennesseensis is rare, although sometimes locally abundant, throughout its historical range (Fig. 1). More than 120 populations of *A. tennesseensis* have been documented in Tennessee, where it is thought to exist on all glade systems in sometimes large populations (Baskin *et al.* 1972; Tennessee Department of Ecological Services, pers. comm.). Seven extant populations of *Astragalus* are known in Alabama glades, averaging < 50 individuals each (Webb et al. 1992). In Illinois, *A. tennesseensis* exists in only one natural population, and one artificial population derived from that seed bank; it originally occurred in dolomite dry gravel prairies along rivers in northern and central Illinois but was almost extirpated from the state due to overgrazing and gravel mining (McFall 1991, Herkert and Ebinger 2002). The species is state-endangered in Illinois, state-threatened in Alabama, and of special concern in Tennessee.

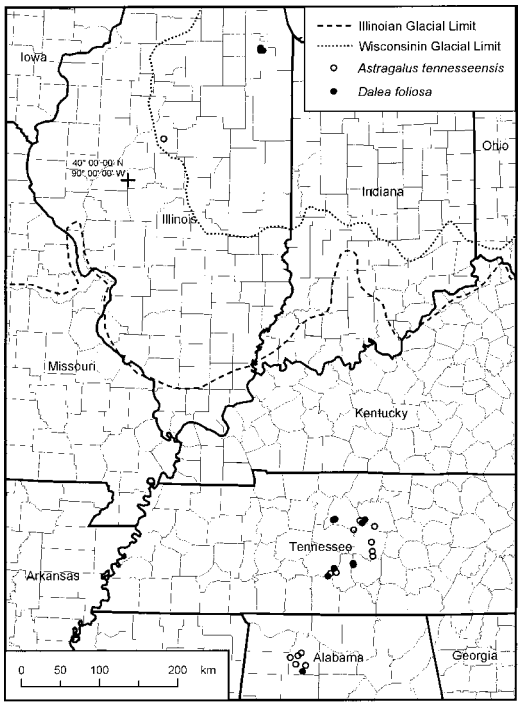

FIG. 1. Locations of 18 sampled populations of *Astragalus tennesseensis* (open circles) and nine populations (filled circles) of *Dalea foliosa* from throughout their respective geographical ranges.

Dalea foliosa is known from only ~25 populations throughout its historical range (Fig. 1). On cedar glades, there are approximately 16 populations in Tennessee, and five in Alabama (DeMauro and Bowles 1993). In Illinois there are three natural populations, one relocated population, and one derived population, all on dry dolomite gravel prairie. Roughly half of all *Dalea* populations consist of < 25 individuals, and most of the populations are not protected (DeMauro and Bowles 1993). The U.S. Fish and Wildlife Service declared *D. foliosa* a federal endangered species under the 1973 Endangered Species Act (U.S. Fish and Wildlife Service 1991). State conservation agencies have reintroduced both species to sites where the species were historically documented within these states and in sites where they were extirpated in Indiana (Bowles 1988, DeMauro and Bowles 1993).

Table 1. Population identification codes, locations, numbers of individuals sampled (N), approximate number of individuals present at the time of sample collection (size), and the landowner status for 19 *Astragalus tennesseensis* and 10 *Dalea foliosa* populations.

Code	Location	N	Size	Status
Astragalus tennesseensis				
Flat Rock	Lawrence Co., AL	29	50–150	private
Landers Glade	Lawrence Co., AL	16	<50	private
Needmore	Lawrence Co., AL	10	<50	private
Witt Glade	Lawrence Co., AL	31	50–150	private
Woodruff Glade	Lawrence Co., AL	22	<50	private
Manito Prairie	Tazewell Co., IL	27	100	Illinois Nature Preserve
Morton Arboretum	Will Co., IL	15	<50	Morton Arboretum (restoration)
Burnt Hill	Bedford Co., TN	13	>200	private
Cedar Grove	Davidson Co., TN	21	500–1000	private
Mt. View Road	Davidson Co., TN	22	150	private
Sneed Road	Davidson Co., TN	12	>500	The Nature Conservancy
Kennie Nelson	Marshall Co., TN	30	200–300	private
Blue Spring	Maury Co., TN	27	100	Tennessee Valley Authority
Bell Glade	Rutherford Co., TN	30	1000	private
Couchville Glade	Rutherford Co., TN	20	>500	The Nature Conservancy
Hall Farm	Rutherford Co., TN	29	300–400	private
Williams Glade	Rutherford Co., TN	26	1000	private
Gwynn Road	Wilson Co., TN	26	500	private
Lane Farm	Wilson Co., TN	23	200	private
Dalea foliosa				
Landersville Glade	Lawrence Co., AL	33	340	Tennessee Valley Authority
Lockport Prairie	Will Co., IL	43	>2300	County Forest Preserve
Midewin	Will Co., IL	39	100	National Tallgrass Prairie
Romeoville	Will Co., IL	11	300	County Forest Preserve
Burnt Hill	Bedford Co., TN	15	890	private
Sneed Road	Davidson Co., TN	25	500–1000	The Nature Conservancy
Columbia Dam	Maury Co., TN	30	1130	Tennessee Valley Authority
Sowell Mill Pike	Maury Co., TN	21	145	private
Leeman Glade	Rutherford CO., TN	19	195	private
Richmond Shop	Wilson Co., TN	4	16	Tennessee Dept. of Conservation

POPULATION SAMPLING AND ELECTROPHORETIC PROCEDURES. Four hundred twenty nine individuals from 19 populations of *Astragalus tennesseensis* were sampled (Fig. 1; Table 1). We selected the five largest Alabama populations, one native and one derived population in Illinois, and twelve Tennessee populations representing pairs from each of the geographical clusters of the species in that state. In Illinois, the derived population (ILMA) was established from seed collected from the single remaining native population (ILMP). The Tennessee populations of the milk vetch were large populations chosen from element occurrence records from the Ecological Services Division natural history database maintained by the Tennessee Department of Conservation. All populations were sampled in 1992.

Samples were collected from 239 individuals of *Dalea foliosa* across 10 populations (Fig. 1; Table 1). Populations were chosen based on size and geographical distribution, and included the three native populations in Illinois, the Romeoville Prairie, Lockport Prairie, and Midewin Tall Grass Prairie. All populations were sampled in 1992, with the exception of the recently discovered Midewin population in Illinois (ILMD), which was sampled in 2001. Of the two extant Alabama populations, the larger Lawrence County population was sampled. Tennessee populations were chosen from element occurrence records from the Ecological Services Division natural history database (Tennessee Department of Conservation). The six populations sampled in the state included the four largest populations as well as two small populations, Sowell Mill Pike and Richmond Shop Barren, representing northern and southern distribution clusters.

For both species, we sampled individual plants haphazardly within populations. Both mature flowering and vegetative plants were sampled, but seedlings were considered too small from which to obtain sufficient tissue for isozyme extraction. When possible, at least 20 in-

dividuals were sampled. When fewer than twenty individuals were found, every mature individual was sampled. Samples for both species consisted of five leaves or 3 g per individual. Apical leaves were used whenever possible. Each plant sample was individually numbered and labeled by population name, sealed in a plastic bag, and stored on ice for transport to the lab. Samples were kept refrigerated for less than two weeks. If not extracted within this time period, leaflets were removed from the leaf and instantaneously frozen in the bag with liquid nitrogen, then maintained at −75°C until extraction.

Enzymes were extracted by grinding two grams of leaf tissue with a spinning ground glass homogenizer in 1.5 ml Tris-HCl extraction buffer, pH 7.5 (Soltis et al. 1983). Five percent polyvinylpyrrolidone (MW 40,000) and 0.1% 2-mercaptoethanol were added to the buffer prior to use. Samples were kept on ice throughout the extraction process. Sample extracts were poured into labeled microcentrifuge tubes and stored at −75°C. About 0.1 ml of raw extract was used for horizontal electrophoresis, adsorbed onto 6 x 19 mm Whatman #3 filter paper wicks and loaded into 13% starch gels (Starch Art, Smithville, TX).

Three gel/electrode buffer systems were used to resolve nine enzyme systems in each species. For *Dalea*, alcohol dehydrogenase (ADH), and phosphoglucoisomerase (PGI) were resolved on the pH 8.1 citrate-borate buffer of Ridgeway et al. (1970). Esterases (EST), glutamate oxaloacetate transaminase (GOT), menadione reductase (MDR), 6-phosphogluconate dehydrogenase (6-PGD), and triose phosphate isomerase (TPI) were resolved on a Tris-EDTA borate, pH 8.0 gel of Selander et al. (1971). A tris-citrate pH 7.2 (Soltis et al. 1983) buffer was used for isocitrate dehydrogenase (IDH) and malate dehydrogenase (MDH). Enzyme systems showing activity but not sufficiently resolved for use were: aconitase (ACO), aldolase (ALD), glyceraldehyde-3-phosphate dehydrogenase (G-3-PDH), glucose-6-phosphate dehydrogenase (G-6-PDH), glutathione reductase (GSR), and leucine amino peptidase (LAP). For *Astragalus*, ADH, GOT, MDR, and TPI were resolved on the Ridgeway et al. (1970) buffer. ALD, G-3-PDH, and LAP were resolved on the Tris-EDTA borate gel, and MDH and 6-PGD were obtained using the tris-citrate gel. Active enzyme systems not consistently resolved for this species were: ACO, adenylate kinase (AK), diaphorase (DIA), EST, G-6-PDH, GSR, IDH, and PGI. Enzyme staining protocols were essentially as described in Weeden and Wendel (1989).

Gel banding patterns were recorded photographically and the electromorphs (isozyme bands) were measured from the prints. Banding patterns were analyzed with the knowledge of enzyme subunit composition and number of loci per enzyme system commonly seen in other plants (Gottlieb 1981, Weeden and Wendel 1989). Allelic isozymes were measured and recorded as relative mobilities using the most common allele as the standard (relative mobility of 100). The standards used were Lockport Prairie individual 19 for *Dalea foliosa* and Sneed Road population individual 9 for *Astragalus tennesseensis*. When more than one locus appeared for an enzyme system, the most anodal one was designated locus 1. All samples were run in 1992 with the exception of samples from ILMD, which were run in 2001.

STATISTICAL ANALYSES. Allele frequencies and isozyme variability were calculated by population, by geographical region, and by species. Homogeneity of allele frequencies was tested using chi-square (Workman and Niswander 1970). Measures of genetic diversity were summarized as the proportion of polymorphic loci (%P), mean number of alleles per polymorphic locus (AP), mean number of alleles per locus (A), mean expected heterozygosity (H_E), and mean observed heterozygosity (H_O). Departures of Wright's (1922) fixation index from Hardy-Weinberg equilibrium within populations for each polymorphic locus were checked using chi-square goodness-of-fit tests. F_{IT} and F_{IS} for polymorphic loci were calculated according to Nei (1973), with G_{ST} (equivalent to F_{ST}) averaged over polymorphic loci according to Hamrick and Godt (1989; see Culley et al. 2002). Departures from zero of these calculated F-statistics were also tested using chi-square tables (Li and Horovitz 1953). We used LYNSPROG, (Fortran77 program developed by M. D. Loveless (College of Wooster, Wooster, Ohio, USA) and A. F. Schnabel (University of Indiana, South Bend, Indiana, USA), to summarize allele frequencies and to obtain diversity estimates averaged across polymorphic loci. We used GDA (http://lewis.eeb.uconn.edu/lewishome/software.html, developed by P. Lewis and D. Zaykin) to obtain summaries of genetic diversity, and F-statistics with their associated bootstrapped confidence intervals calculated according to Weir (1996). In addition, we used BIOSYS-1 (Swofford and Se-

Table 2. Summary of within-population variability for 19 *Astragalus tennesseenis* populations calculated using GDA: percentage of polymorphic loci (% P), mean number of alleles per polymorphic locus (AP), mean number of alleles per locus (A), mean observed heterozygosity (H_O), mean expected heterozygosity (H_E), calculated by population, geographical region, and species.

Code	% P	AP	A	H_O	H_E	F_{IS}
Astragalus tennesseensis						
Flat Rock	33.3	2.2	1.4	0.094	0.094	0.002
Landers Glade	26.7	2.5	1.4	0.083	0.107	0.229
Needmore	26.7	2.0	1.3	0.088	0.081	−0.093
Witt Glade	40.0	3.0	1.8	0.096	0.114	0.162
Woodruff	53.3	2.0	1.5	0.082	0.088	0.067
Alabama Means	38.6	2.3	1.5	0.095	0.104	0.085
Manito Prairie	26.7	2.0	1.3	0.054	0.054	0.002
Morton Arboretum	13.3	2.5	1.2	0.024	0.033	0.286
Illinois Means	21.4	2.3	1.3	0.042	0.044	0.145
Burnt Hill	26.7	2.8	1.5	0.067	0.106	0.376
Cedar Grove	40.0	2.5	1.6	0.124	0.118	−0.043
Mt. View Road	40.0	2.7	1.7	0.100	0.136	0.268
Sneed Road	40.0	2.5	1.6	0.138	0.132	−0.041
Kennie Nelson	46.7	3.1	2.0	0.112	0.143	0.215
Blue Spring	33.0	2.6	1.5	0.086	0.101	0.150
Bell Glade	53.3	2.5	1.8	0.111	0.129	0.147
Couchville Glade	26.7	3.0	1.5	0.077	0.103	0.325
Hall Farm	26.7	3.0	1.5	0.094	0.096	0.018
Williams Glade	53.3	2.6	1.9	0.147	0.153	0.030
Gwynn Road	46.7	2.6	1.7	0.112	0.141	0.212
Lane Farm	26.7	3.3	1.6	0.110	0.125	0.129
Tennessee Means	41.0	2.7	1.7	0.120	0.141	0.145
Species Means	38.4	2.6	1.6	0.103	0.121	0.130
Dalea foliosa						
Landersville, AL	5.0	2.0	1.1	0.002	0.002	0.000
Lockport	5.0	2.0	1.1	0.011	0.025	0.573
Midewin	0.0	—	1.0	0.000	0.000	0.000
Romeoville	10.0	2.0	1.1	0.014	0.013	−0.034
Illinois Means	5.0	2.0	1.1	0.007	0.020	0.374
Burnt Hill	30.0	2.2	1.4	0.067	0.089	0.263
Sneed Road	25.0	2.2	1.3	0.080	0.081	0.011
Columbia Dam	30.0	2.0	1.3	0.057	0.066	0.135
Sowell Mill Pike	10.0	2.0	1.1	0.021	0.019	−0.139
Leeman Glade	10.0	2.0	1.1	0.023	0.035	0.354
Richmond Shop	20.0	2.0	1.2	0.100	0.096	−0.043
Tennessee Means	20.8	2.1	1.2	0.058	0.064	0.108
Species Means	14.5	2.0	1.2	0.037	0.043	0.113

lander 1981) to analyze and graphically depict the phenetic relationships among populations, employing Cavalli-Sforza and Edwards (1967) arc chord distance matrices and resulting phenograms.

Results. LEVELS AND DISTRIBUTION OF GENETIC VARIATION. At the species level, these two rare legume species had low to quite low levels of polymorphism (Table 2). Across all *A. tennesseensis* populations sampled, 13 of the 15 loci scored (87%) were polymorphic; *g3pdh–1* and *tpi-1* were monomorphic. Within populations, the percentage of polymorphic loci ranged from 13.3–53.3%. Two loci that were fixed in the Tennessee populations (*mdr-1* and *tpi-3*) were variable for other alleles in at least one other geographical region. Alabama had three monomorphic loci (*adh-1, g3pdh–1,* and *tpi-3*), but Illinois had 10 monomorphic loci and only four polymorphic loci (*adh-1, ald-1, got-2,* and *mdr-2*). Most of these polymorphisms were due to rare alleles. Across all *D. foliosa* populations, only 11 of the 20 loci scored (55%) were polymorphic; *adh-1, adh-2, est-1, mdr-2, mdr-3, mdh-4, 6pgd–1, tpi-1,* and *tpi-2* were monomorphic. Percentages of polymorphic loci within populations ranged from 0.0–30.0%. All but one (*got-3*) of the polymorphic loci in *D. foliosa* were found in the Tennessee populations. However, *D. foliosa* in Alabama had only a single polymorphic locus (*got-3*), and in Illinois had

only two polymorphic loci (*got-2* and *pgi-2*). Allele frequency data are available from the authors by request.

There were 2.6 alleles per polymorphic locus in *A. tennesseensis*, and an average of 1.6 across all loci (Table 2). Levels of polymorphism were slightly lower for Alabama and Illinois, and slightly higher in Tennessee. Forty-three alleles were resolved across the 15 loci. Within geographical regions, the 12 Tennessee populations contained 39 alleles, the five Alabama populations contained 32 alleles, and the two Illinois populations had only 19 alleles. Alleles that were unique to the Tennessee region included *got-2e, mdh-4d, 6pgd–1e, 6pgd–1f, 6pgd–2b, tpi-1b,* all at frequencies $<$ 20%. Two alleles were unique in Alabama, *mdr-1c* and *6pgd–2e,* at frequencies $<$ 0.5%. Alleles that were unique in Illinois included one at low frequency (*ald-b* at 8.6%), and two alleles that were fixed in the two populations but absent (*tpi-3c*) or present at frequencies $<$ 2% (*lap-b*) in the other two regions. In addition, Illinois populations were fixed for alleles at *mdh-1* and *6pgd–1* that were at frequencies of $<$ 60% elsewhere. Despite the common origin of the two Illinois populations, the Manito Prairie source population had two alleles not found in the restoration population (*adh-b* and *got-2c*), and the Morton Arboretum restoration had one allele not sampled in the source population (*ald-d*), all at frequencies $<$ 3%.

Allelic diversity in *D. foliosa* was lower overall, with 2.0 alleles per polymorphic locus and an average of 1.2 across all loci (Table 2). Levels of polymorphism differed little within regions. Thirty-five alleles were resolved across the 20 loci in *D. foliosa*. The six Tennessee populations had 33 alleles, and the one Alabama and three Illinois populations had 21 and 22 total alleles, respectively. Tennessee had a majority of the unique alleles. Four of these alleles were quite common in the region: *idh-1b* at 30%, *mdh-2b* at 55%, *mdh-3b* at 52%, and *pgi-2b* at 41%. Unique alleles that occurred at frequencies $<$ 6% included *mdr-1b, mdh-5b, mdh-5c, idh-1c,* and *mdr-1c*. A single unique allele was found in Alabama (*got-3b* at $<$ 2%) and in Illinois (*pgi-2d* at $<$ 0.5%). Of two alleles in got-2, the fast allele occurred at frequencies of 73% and 56% in Illinois and Tennessee, but the slow allele was fixed in the single Alabama population surveyed.

None of the fixation indices for polymorphic loci in either species were significantly negative. An excess of homozygotes was indicated for a few polymorphic loci: 11 out of 101 (10.9%) fixation indices in *A. tenneseensis*, and three out of 27 (11.1%) fixation indices in *D. foliosa* were significantly greater than zero. Allele frequencies were heterogeneous among populations in *A. tennesseensis* at all loci except *g3pdh–1* and *mdr-1*. Allele frequencies were heterogeneous among populations in *D. foliosa* at *got-2, idh-1, mdr-1, mdh-1, mdh-2, mdh-3, mdh-5, pgi-2,* and *6pgd–2*.

Measures of genetic diversity at the species level and within populations were moderate to low compared to other geographically restricted species (Table 2). Averaged across loci, levels of observed and expected heterozygosity in *A. tennesseensis* (H_O = 0.103 and H_E = 0.121) were more than twice that found in *D. foliosa* (H_O = 0.037 and H_E = 0.043). Within geographical regions for both species, levels of heterozygosity were highest in Tennessee. For both species, the levels of heterozygosity in Illinois were less than half that found for populations in Tennessee. Average observed heterozygosities were most often lower than or equivalent to expected values.

Population Differentiation. F-statistics calculated according to Weir (1984) resulted in high values of population differentiation ($>$ 0.01); as a consequence, we calculated F- and diversity statistics strictly according to Nei (1973) and Hamrick and Godt (1989), as recommended by Chakraborty and Leimar (1987). The majority of the polymorphic isozyme loci in both species were significantly variable across populations (12 out of 13 in *A. tennesseensis*, and 8 out of 11 in *D. foliosus*), contributing to population divergence. Estimates of mean population differentiation differed dramatically between the two species, (G_{ST}; Table 3). In *A. tennesseensis*, the differentiation among geographical regions (G_{STC} = 0.161) was lower than when regional differences were not taken into account (G_{ST} = 0.217). At the species level, the majority of genetic diversity was contained within populations (H_S = 0.141) compared to the total diversity across polymorphic loci (H_T = 0.179). Within geographical regions, individual populations contained an even greater percentage of regional diversity, but the average population in one region had less in common with the average population in another region. Populations within Alabama were most distinct from each other (G_{ST} = 0.092), followed by Tennessee popula-

Table 3. Multilocus estimates of hierarchical F-statistics and diversity statistics calculated across polymorphic loci according Hamrick and Godt (1989). No regional cluster could be assessed for *D. foliosa* in Alabama because only one population was sampled. The Illinois population containing no polymorphisms is not included in the summary estimates for *D. foliosa*. G_{ST} = proportion of total diversity among populations, G_{STC} = proportion of total diversity among geographical regions.

Species Cluster	F_{IS}	F_{IT}	H_T	H_S	G_{ST}	G_{STC}
Astragalus tenneseensis						
Alabama	0.038	0.115	0.162	0.132	0.092	
Illinois	0.019	0.047	0.177	0.165	0.029	
Tennessee	0.053	0.106	0.185	0.168	0.059	
All populations	0.052	0.260	0.179	0.141	0.217	0.161
Dalea foliosa						
Alabama	0.001					
Illinois	0.232	0.287	0.258	0.221	0.091	
Tennessee	0.038	0.451	0.259	0.096	0.460	
All populations	0.047	0.438	0.186	0.060	0.441	0.145

tions (G_{ST} = 0.059), and were least distinct between the single natural Illinois population and the one derived from it (G_{ST} = 0.029).

In *D. foliosa*, differences among geographical regions were even more striking (Table 3). Differentiation among populations within geographical regions (G_{STC} = 0.145) was substantially lower than when populations were not clustered by regions (G_{ST} = 0.441). At the species level, the majority of genetic variation was contained among populations relative to the total (H_T = 0.186), rather than within populations (H_S = 0.060). In Tennessee, the center of geographical concentration, populations were surprisingly distinct from one another (G_{ST} = 0.460), and even more of the regional diversity (H_T = 0.259) was contained among populations rather than within (H_S = 0.096). In contrast, the extremely low levels of diversity in Illinois contributed to lower differentiation among populations in that region (G_{ST} = 0.091), resulting in the majority of regional diversity (H_T = 0.258) represented within populations (H_S = 0.221). Regional differences for both species are reflected by consistently lower F_{IS} estimates compared to F_{IT}.

Genetic matrices based on chord distance (Cavalli-Sforza and Edwards 1967) for the two species illustrates the distribution of genetic variation across regions (Figs. 2, 3). Pair-wise distances between populations in *A. tennesseensis* ranged from 0.066 to 0.449. Distances between the Illinois population and the other two regions were all > 0.38, whereas, distances between Alabama and Tennessee were all < 0.25. Distances within regions were also all < 0.23. Pair-wise chord distances in *D. foliosa* ranged from 0.053

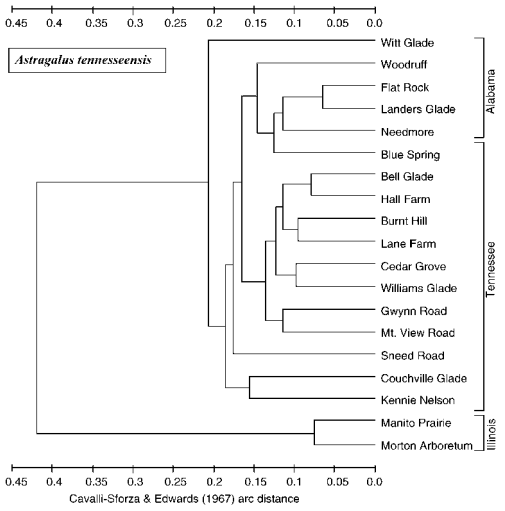

FIG. 2. Phenetic relationships among 19 populations of *Astragalus tennesseensis*, employing Cavalli-Sforza and Edwards (1967) arc chord distance matrices.

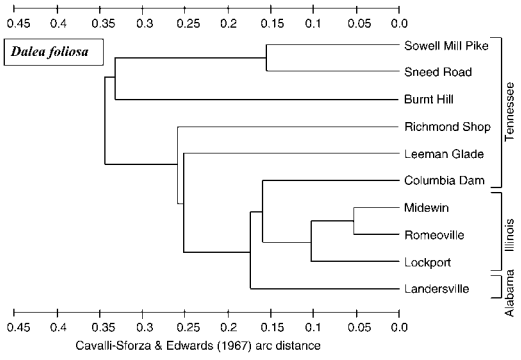

FIG. 3. Phenetic relationships among nine populations of *Dalea foliosa*, employing Cavalli-Sforza and Edwards (1967) arc chord distance matrices.

to 0.428, with distances between regions (0.11–0.40) and within regions (0.08–0.35) similarly variable.

Discussion. The two narrowly-distributed species we investigated share many life history characteristics, similar geographical distributions and total levels of diversity, but they differed in the distribution of genetic variation detected. Both species are nonclonal, short-lived perennials capable of developing persistent seed banks. *Astragalus tenneseensis* is common in the glades of Tennessee and Alabama, with a single natural population (and one restoration derived from it) in Illinois dolomite prairie. Mean locus polymorphism (27%− 53%) and expected heterozygosity (0.081–0.153) were similarly variable across populations and regions, with the exception of the native and restored Illinois populations (27% and 13% P, and 0.054 and 0.033 H_E, respectively). *Dalea foliosa*, once more common throughout its range, has been reduced to $<$ 25 small, isolated populations mostly in Tennessee glades, with a few populations in Alabama and Illinois. Little isozyme variation was detected in *D. foliosa*, with mean polymorphism ranging from 0%− 30% and expected heterozygosity of 0.000–0.096. Isozyme variability in this species also was unevenly distributed among populations, with the geographically peripheral populations in Alabama and Illinois having the lowest diversity. Overall, average polymorphism and expected heterozygosity within populations were higher in *A. tenneseensis* (except in Illinois), and lower in *D. foliosa*, than the averages for endemic plants (26.3 ± 2.1, and 0.063 ± 0.006, respectively; Hamrick and Godt 1989), despite higher values in Tennessee.

Regional differences in the distribution of diversity were reflected in F-statistics and phenograms for both species. In *A. tennesseensis*, the distribution of genetic variation between the central Tennessee populations and the peripheral Alabama populations were not uniform, and the Illinois populations were quite distinct from both regions (Table 3; Fig. 2). In general, the Alabama populations have differentiated from some of the Tennessee populations due to differences at only a couple loci. These populations show no severe reductions in heterozygosity due to isolation and genetic drift. A single Tennessee population grouped more closely with the Alabama populations because it had allele frequencies at a single locus that were more similar to the Alabama populations. The one native and one restored Illinois population contained fixed differences at two loci, and a shift in allele frequencies at two other loci that distinguish Illinois *A. tennesseensis* from the other regions. In contrast, the peripheral populations of *D. foliosa* in Alabama and Illinois contain only a subset of the variation found in the central Tennessee populations (Table 3; Fig. 3). Indeed, with the exception of a single variable locus in the Lockport population, and a single rare allele in the Romeoville population, the Illinois populations were fixed at all loci. A single locus (*got-2*) that was variable in some of the Tennessee populations for two alleles distinguished the Alabama population from the Illinois populations. Despite overall low levels of diversity in *D. foliosa*, a large proportion of the variation was found among populations.

Compared to either geographically restricted or widespread species of *Astragalus* that have been surveyed (Karron et al. 1988, Baskauf and Snapp 1998), *A. tenneseensis* averaged a greater proportion of polymorphic loci and a higher number of alleles per polymorphic locus. These comparisons are complicated, of course, by differences in sampling, and the number and choice of loci used in each study. In addition, diversity in extant vegetation may be lower than the total diversity maintained in long-lived seed banks (Templeton and Levin 1979). *Astragalus tenneseensis* seeds have been documented to persist in soil for more than 10 years (Baskin and Baskin 1989), and other species in the Fabaceae with similarly hard seed coats have been shown to remain viable for many decades (Baker 1989, Baskin and Baskin 1998b). Levels of genetic diversity in a another rare cedar glade endemic, *Astragalus bibullatus* Barneby and E. L. Bridges, were substantially higher in the persistent seed bank (Morris et al. 2002) than in extant populations (Baskauf and Snapp 1998), and within the range we observed in *A. tenneseensis*. Where *A. tenneseensis* is common in Alabama and Tennessee, it appears to be maintaining relatively high levels of diversity in extant populations. In Illinois, levels of diversity drop precipitously, most likely reflecting one or more genetic bottlenecks in areas that were once covered by the Wisconsin glaciation. However, fixed allelic differences between Illinois populations and elsewhere suggest isolation prior to the last glacial maximum, and derivation from glacial refugia distinct from the populations found in

present-day cedar glades in Alabama and Tennessee.

Populations of *Dalea foliosa* were quite genetically depauperate, especially in Alabama and Illinois. The only other species of *Dalea* that has been examined using isozymes is a relatively widespread congener, *D. purpurea*. Surprisingly, although *D. purpurea* has a broad geographical range throughout prairie communities (Gleason and Cronquist 1991), measures of genetic variation in both remnant and restored populations (Gustafson et al. 2002) were similar to those of *D. foliosa* in Tennessee. Indeed, both species have lower levels of diversity overall than is typically found in members of the Fabaceae (Hamrick and Godt 1997). Given the low measures of diversity for all the populations we surveyed, it is unlikely that seed banks, which can persist for at least 8 years (Baskin and Baskin 1998a), harbor much additional variation. The extremely low measures of diversity in the Alabama and Illinois populations of *D. foliosa* suggest that as this species migrated from the center of its distribution in Tennessee, already low levels of diversity were further reduced through the loss of alleles as populations were exposed to one or more genetic bottlenecks. Alternatively, the Illinois populations could be derived from extinct glacial refugia that were nonetheless already low in diversity.

For short-lived perennials whose populations fluctuate significantly between years, census population size may not be a good indicator of the genetic diversity contained within a population. As pointed out by Morris et al. (2002), seed banks of species with hard seed coats (common in members of Fabaceae) can be a richer source of population variation than extant plants. This may be true for at least some of the populations sampled in this study, but dispersal history and degree of isolation likely play a stronger role, as has been demonstrated for *Silene regia* (Menges and Dolan 1998). For *A. tennesseensis* and *D. foliosa*, both restricted to isolated suitable habitats, levels of allelic diversity and heterozygosity are more likely correlated with glaciation history and post-glacial dispersal than to extant population sizes or seed bank diversity. Dispersal and isolation have resulted in the loss and fixation of different alleles. In the case of *A. tennesseensis*, populations in Alabama and Illinois tend to be much smaller, but only the Illinois populations have significantly reduced levels of heterozygosity compared to the Tennessee populations (Figure 4A). For *D. foliosa*,

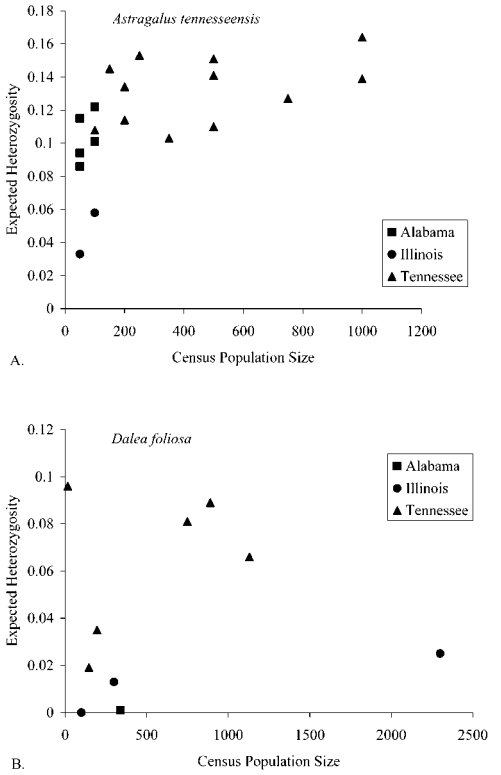

FIG. 4. Relationships between population census size and expected levels of heterozygosity for A. 19 populations of *Astragalus tennesseensis*, and B. nine populations of *Dalea foliosa*.

the population with the largest number of extant individuals was in Illinois, with levels of heterozygosity comparable only to the smallest Tennessee populations (Figure 4B). In contrast, one of the smallest Tennessee populations of *D. foliosa* had relatively high levels of diversity in the individuals sampled.

The appearance of unique alleles and enzymatic phenotypes in many of these populations demonstrates the amount of genetic variance held among populations. The isozyme variation of these species is fragmented along with the populations. Reproductive isolation is driving the high levels of genetic divergence among regions. Few of these populations are managed or protected, and when populations are lost, species-level diversity is imperiled as well. Based on regional and overall G_{ST} estimates, one could argue that by preserving a single population in each geographical region for *A. tennesseensis*, you could preserve the majority of the diversity present in the species; similarly, one population each in Alabama and Illinois combined with 4–5 from Tennessee might preserve

most of the species-level variation in *D. foliosa* (Table 3). However, due to environmental and demographic unpredictability at any single location, these numbers surely would be insufficient to hedge against extinction. Species restricted to isolated habitats are more vulnerable to environmental stochasticity that may be caused by, for example, fragmentation (e.g., increasing isolation, edge effects, and invasions by woody species) or even global warming (particularly if flood and drought cycles become more frequent, prolonged, and severe in these regions as some models predict [http://yosemite.epa.gov/oar/globalwarming.nsf/content/epa-regions.html]). Very few of the habitats containing these populations are protected; most are in private hands. The first step towards conserving these species is to protect more of the habitats to which they are confined.

Although some research has been done on seed bank longevity and seed germination requirements, there are no published studies documenting the breeding systems of these species. Indirect estimates of inbreeding ranged from effectively zero to about 38% in *A. tennesseensis*, suggesting that mating between close relatives may be occurring in at least some populations, although the effects are unknown. Indirect estimates of inbreeding in *D. foliosa* ranged from about zero to 57%, but these estimates are less robust due to overall low levels of genetic diversity. The low levels of isozyme diversity detected in *D. foliosa* may not have consequences for reproductive output. In the Illinois population for which no allozyme variation was detected (Midewin), viable seed production over a three-year period remained high, ranging between 70–80% (Molano-Flores 2004). Indeed, reproductive output as well as seedling survival for both species appears to be limited primarily by external factors, including deer and rabbit herbivory, drought, frost heaving, and trampling by off-road vehicles (Baskin and Baskin 1989, 1998a; Bowles 1988, Molano-Flores 2004). Clearly, their breeding systems and pollination biology should be examined further. The continued persistence of these short-lived perennials depends not just on protecting the habitats, but managing them to reduce sources of competition, herbivory, and disturbance that hinder reproductive output and seedling establishment.

We have demonstrated strong regional differences in the levels of genetic diversity and divergence in *A. tenneseensis* and *D. foliosa* which should be considered in conservation management. There is no conclusive evidence suggesting problems with inbreeding depression in either species, but the data are limited. It is unknown how other measures of diversity, including phenotypic plasticity, might vary among these long-isolated populations. For example, in the rare Mead's milkweed, an obligate outcrosser with high levels of population divergence (Tecic et al., 1998), isolated populations occur in nutrient-rich soils in the northern portion of the range and nutrient-poor soils in the southern portion of the range. When individuals from different regions were grown under common conditions, phenotypic differences in plants from different regions persisted (Bowles et al., 1998). Controlled pollinations and common garden experiments would enhance our understanding of the extent of inbreeding depression, and risks of small population sizes to reproductive success in *A. tenneseensis* and *D. foliosa*. In Illinois in particular, where both species are greatly reduced from their historical distributions, there is an active interest in reintroducing populations. Decisions to reintroduce populations to historical sites must take into consideration the source of the genetic material and potential for inbreeding in the founding populations. Introducing genetic material from one geographical region to another is not advised unless it can be shown that inbreeding depression is a threat to individual population viability.

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