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Genetic Insights into the Biogeography of the Southeastern North American Endemic, *Ceratiola ericoides* (Empetraceae)

Dorset W. Trapnell, John Paul Schmidt, Pedro F. Quintana-Ascencio, and Jim L. Hamrick

From the Department of Plant Biology, University of Georgia, Athens, GA 30602 (Trapnell); Institute of Ecology, University of Georgia, Athens, GA 30602 (Schmidt); Department of Biology, University of Central Florida, Orlando, FL 32816 (Quintana-Ascencio); and Departments of Plant Biology and Genetics, University of Georgia, Athens, GA 30602 (Hamrick).

Address correspondence to D. W. Trapnell at address above, or e-mail: dorset@plantbio.uga.edu.

Abstract

The southeastern United States harbors an unusually large number of endemic plant taxa, which may reflect the refugial nature of the region during Pleistocene glacial maxima. Understanding the genetic diversity and structure of extant plant taxa can provide insights into the biogeographical processes that shaped them genetically. Here, we investigate the levels and partitioning of allozyme diversity in the southeastern North American endemic, *Ceratiola ericoides*, which displayed greater genetic variation and structure than other endemics. Central Florida populations represent a center of genetic diversity, whereas South Carolina and Georgia Fall Line sandhill populations have a subset of the Central Florida genetic diversity and may be relicts of a once continuous distribution. This much broader, continuous distribution throughout the southeastern United States occurred during glacial maxima when the scrub habitat, dominated by *C. ericoides*, expanded considerably owing to drier climatic conditions. Georgia Coastal Plain populations appear to have been independently founded more recently by propagules from Central Florida and the Fall Line sandhills because they have an even more limited subset of genetic diversity and greater genetic heterogeneity among populations. Since their establishment, coastal plain populations appear to have had little, if any, gene exchange among each other or with the relatively proximate Fall Line sandhill populations. These data underscore the importance of understanding the genetic composition and historical biogeography of species before intelligent management or restoration decisions can be made regarding their preservation.

The southeastern United States has a disproportionately large number of endemic plant species (Delcourt HR and Delcourt PA 1991); there are approximately 385 plant species endemic to Florida, which is exceeded only by California and Hawaii (Gentry 1986). This is rather remarkable, considering Florida's relatively small geographic area and lack of substantial topographic variation. The ancient xeric oak scrub habitat of northern and central peninsular Florida is particularly recognized for its high degree of endemism (Abrahamson et al. 1984; Christman and Judd 1990; Webb 1990). These taxa may represent a footprint of the refugial nature of the region for both plants and animals during Pleistocene glacial maxima (e.g., Avise et al. 1979; Sewell et al. 1996; Maskas and Cruzan 2000; Soltis et al. 2006). As glacial ice sheets advanced southward, conspecifics that became physically separated in different refugia may have evolved independently during relatively long glacial periods, eventually giving rise to reproductively isolated species. Holocene warming, retreat of glacial ice sheets, and the northward shifting of ecotones permitted many refugial species to greatly expand their ranges. Although this pattern was typical, not all species' ranges contracted during the glacial maxima. One such species, whose range is thought to have expanded during the last glacial epoch as evidenced by palynological records, is the sandhill scrub shrub *Ceratiola ericoides* Michaux (Watts 1975). In this species, we would expect to see different patterns of genetic variation and structure from that of typical refugial species.

Genetic examination of extant plant species together with knowledge of their geologic histories may make it possible to develop insights into the biogeographical processes that shaped their genetic composition. It is particularly important for the preservation of rare endemic

species to identify centers of genetic diversity and to understand the processes that influenced their genetic composition (Godt and Hamrick 2001). Our objectives are to investigate the levels and distribution of genetic diversity in *C. ericoides*, an endemic southeastern North American shrub, which is listed as threatened in Georgia (http://georgiawildlife.dnr.state.ga.us). This species grows in xeric, patchily distributed sandy substrates. Populations of species that favor this soil type are often isolated (Quintana-Ascencio and Menges 1996). However, Florida has several large, continuous regions of ancient sandhill habitat. MacDonald and Hamrick (1996) showed that *C. ericoides* occupying different sandhill ridges are genetically divergent and that, within each ridge, distant populations along the north–south axis are more genetically similar than more closely occurring populations along an east–west axis cutting across ridges. These authors suggested that populations have a north–south biogeographic relatedness as a result of the narrow but continuous coastal dunes that run from the Atlantic coast of Florida into Georgia. In Georgia and South Carolina, *C. ericoides* occurs in smaller, more widely separated populations.

To gain further insight into the biogeographic history of *C. ericoides*, we sampled 3 regions within its range that represent different sandy habitats that arose by distinct geologic processes. The sites were the ancient and endemic-rich Lake Wales Dune Ridge of Central Florida, riverine sandhills associated with the Georgia Coastal Plain, and Fall Line sandhills of northeast Georgia and southwest South Carolina. Specifically, we addressed 3 questions: 1) How genetically diverse is this southeastern endemic scrub species? 2) How is its genetic variation partitioned among populations and among geographic regions in light of climatic changes during the last glacial maxima and the biogeographic histories of the geographic regions in which it occurs? Greater genetic differentiation among populations (Rousset 1997) and lower genetic diversity within populations are expected as the distance between populations increases in the northern range of sandhill/scrub species. 3) Does population size affect genetic diversity within populations? Has recent range retraction and increased disturbance led to the loss of genetic variation? We predicted that, relative to refugial species, *C. ericoides* would have less genetic structure and more genetic diversity, assuming that genetic drift has not had a significant impact on current populations. Furthermore, we would expect to find less genetic diversity in smaller populations or ones that may have experienced recent population bottlenecks.

Materials and Methods

Study Species

Ceratiola ericoides Michaux, Sandhill or Florida Rosemary, is a monotypic genus in the Empetraceae. It is a large, long-lived, evergreen, dioecious shrub (up to 2.5 m) with needle-like leaves. Flowers bloom from September to October, have wind-borne pollen, and produce small fruits (~3 mm diameter) that ripen from January to April, just as migratory birds travel northwards. An individual can produce hundreds of yellowish or olive berries. Although many fruits simply fall to the ground, others are consumed by harvester ants (Johnson 1982), rodents, and several bird species (Van Dersal 1938) including rufous-sided towhees (*Pipilo erythrophthalmus*) and scrub jays (*Aphelocoma coerulescens*; Gibson and Menges 1994). Seeds from the ingested fruit survive passage through the intestinal tracts of these birds (Johnson 1982). Persistent seed banks have been documented (Menges ES, unpublished data), and seedling recruitment is associated with disturbances, such as fire that kills *C. ericoides* adults and reduces shrub cover (Johnson 1982; Gibson and Menges 1994).

Ceratiola ericoides occurs on dry, sandy soils and grows in sand pine and oak scrubs on coastal and inland river dunes, sand ridges, and sandhills in the southeastern United States from Mississippi to South Carolina. It grows in well-drained, sterile marine sands found in dune deposits (Laessle 1958) associated with Miocene, Pliocene, and Pleistocene shorelines and offshore bars (Christman and Judd 1990) as well as estuaries and riverbeds. In Florida, *C. ericoides* is a key scrub species that is widely distributed and locally abundant within a narrow range of scrub habitats that form on the central ridges and along either coast, often forming pure stands of uneven-aged individuals (Johnson 1982). It occurs sporadically along the Gulf Coast of Mississippi, Alabama, and Florida as well as on riverine and Fall Line sandhills in Georgia and South Carolina. In Georgia (where it was listed as threatened in May 2007; http://georgiawildlife.dnr.state.ga.us) and South Carolina, even-aged populations occupy isolated patches that are separated by stands of turkey oak (*Quercus laevis*) and other sandhill tree species with many suitable, uncolonized patches scattered throughout.

Sampling, Enzyme Extraction, and Electrophoresis

Leaf tissue was collected from branch tips of approximately 48 individuals from each of 12 natural populations (Table 1). The approximate number of flowering individuals per population was recorded. Because we were interested in the effects of different biogeographic histories, populations were selected from 3 regions of varying geologic age and geographic position. Four populations were sampled from the 25-mya Lake Wales Dune Ridge and its vicinity in Central Florida, 4 from similarly aged but higher latitude Fall Line sandhill populations (2 each from eastern Georgia and western South Carolina; Figure 1) and 4 from the approximately 10 000-year-old riverine sandhill habitats on the Georgia Coastal Plain. Leaf tissue samples were chilled and, within 48 h of collection, crushed in chilled mortars with a pestle, liquid nitrogen, and a pinch of sea sand to disrupt cellular compartmentalization. Enzymes were extracted with a polyvinylpyrrolidone phosphate extraction buffer (Mitton et al. 1979). The resulting slurry containing crude protein extract was absorbed onto 4 × 6 mm wicks punched from Whatman 3 mm chromatography paper. Wicks were stored in microtest plates at −70 °C until used for electrophoresis. Wicks were placed in horizontal starch gels (10%) and electrophoresis was performed. Fifteen

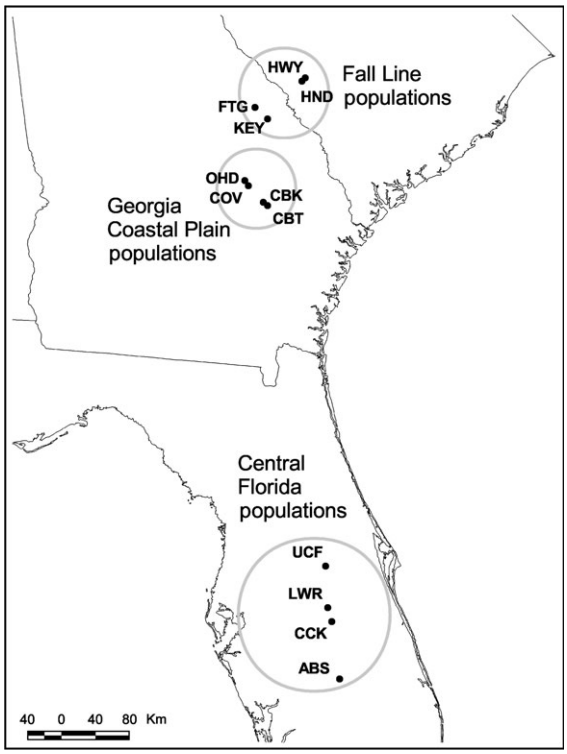

Figure 1. Location of *Ceratiola ericoides* study populations: HWY (33.6°N, 81.7°W), HND (33.6°N, 81.7°W), FTG (33.3°N, 82.3°W), KEY (33.2°N, 82.2°W), OHD (32.5°N, 82.5°W), COV (32.5°N, 82.4°W), CBK (32.3°N, 82.2°W), CBT (32.3°N, 82.2°W), UCF (28.4°N, 81.4°W), LWR (28.0°N, 81.4°W), CCK (27.9°N, 81.4°W), and ABS (27.2°N, 81.3°W).

enzyme stains in 4 buffer systems resolved 14 putative polymorphic (i.e., 2 or more alleles observed) and 5 monomorphic loci. Enzymes stained and loci identified (in parentheses) for each of the 4 buffer systems were as follows: 1) system 4, aconitase (*ACO1* and *ACO2*) and UTP-glucose-1-phosphate (*UGPP1* and *UGPP2*); 2) system 6, alcohol dehydrogenase (*ADH*), leucineamino peptidase (*LAP*), phosphoglucomutase (*PGM1* and *PGM2*), and triosephosphate isomerase (*TPI1* and *TPI2*); 3) system 8-, aspartate aminotransferase (*AAT2*), diaphorase (*DIA*), fluorescent esterase (*FE*), malic enzyme (*ME*), and menadione reductase (*MNR*); 4) system 11, isocitrate dehydrogenase (*IDH*), malate dehydrogenase (*MDH*), phosphoglucoisomerase (*PGI2*), and 6-phosphogluconate dehydrogenase (*6-PGD*). All buffer and stain recipes were adapted from Soltis et al. (1983) except AAT, DIA, and MNR (Cheliak and Pitel 1984) and UGPP (Manchenko 1994). Buffer system 8- is a modification of buffer system 8 as described by Soltis et al. (1983). Banding patterns were consistent with those expected for each enzyme system (Weeden and Wendel 1989).

Genetic Analyses

Genetic diversity measures were estimated using a computer program, LYNSPROG, designed by M. D. Loveless and A. F. Schnabel. Measures of genetic diversity were percentage of polymorphic loci, *P*; mean number of alleles per polymorphic locus, *AP*; and genetic diversity, (i.e., expected heterozygosity H_e) (Nei 1973), the proportion of loci heterozygous per individual under Hardy–Weinberg expectations. Within population, values were calculated and then averaged across all populations. Heterogeneity in allele frequencies among populations was tested by the χ^2 method of Workman and Niswander (1970). Species values for these parameters were calculated by pooling data from all populations. The relationship between genetic diversity (*P*, *AP*, H_e, and observed heterozygosity [H_o]) and population size was examined with regression analyses. Jackknifing procedures (Weir 1996) were employed to determine significant differences in genetic diversity values between regions.

Observed heterozygosity (H_o) was compared with Hardy–Weinberg H_e for each polymorphic locus in each population by calculating Wright's fixation index (F_{IS}; i.e., inbreeding coefficient; Wright 1922, 1951). Deviations from Hardy–Weinberg expectations were tested for significance using χ^2 (Li and Horvitz 1953).

Partitioning of variation among populations was estimated according to Nei's (1973) measures (i.e., G_{ST}) of genetic diversity. Hierarchical genetic structure was determined at 3 spatial scales: among all populations, among regions (Central Florida, Fall Line sandhills, and Georgia Coastal Plain), and among populations within regions. Partitioning of genetic variation was also determined for pairs of regions to assess similarity. Analysis of molecular variance (AMOVA) was used to measure the extent and significance of partitioning variation at each level of the hierarchy (Peakall and Smouse 2001). Pairwise G_{ST} values were obtained for all possible pairs of populations using FSTAT (Goudet 2001). A Mantel test of correspondence between these pairwise G_{ST} values and geographic distances for each pair of populations was performed (Smouse et al. 1986) using NTSYS-PC (Rohlf 2000).

Nei's (1972) genetic distance statistics were calculated for each locus (monomorphic and polymorphic) and mean genetic distance values were calculated for each pair of populations. Neighbor-joining and unweighted pair group method with arithmetic mean (UPGMA) phenograms of genetic distances were generated using the analysis in phylogenetics and evolution (APE) library in R (R Development Core Team 2006) and Mega2 (Mukhopadhyay et al. 1999), respectively.

Results

Fourteen putative polymorphic and 5 monomorphic loci were resolved. Genetic diversity at the species level was high with 74% of the loci polymorphic (P_s), an average of 2.79 alleles per polymorphic locus (AP_s), and a mean genetic diversity (H_{es}) of 0.109 (Table 1). At the population level, *C. ericoides* displayed moderate levels of genetic diversity with mean values of P_p = 31%, AP_p = 2.26, and H_{ep} = 0.079 (Table 1). Tests for heterogeneity in allele frequencies among populations indicate that 13 of 14 polymorphic loci

Table 1. Summary of allozyme variation for 12 populations of *Ceratiola ericoides*

Sites	Population size	*N*	*P* (%)	*AP*	H_o (SD)	H_e (SD)
Central Florida						
ABS	550	48	61.1	2.64	0.166 (0.043)	0.158 (0.052)
CCK	500	48	68.4	2.31	0.146 (0.043)	0.151 (0.045)
LWR	700	48	57.9	2.64	0.172 (0.048)	0.196 (0.051)
UCF	30	26	33.3	2.67	0.102 (0.048)	0.109 (0.046)
Mean	445	43	55.2	2.56	0.147 (0.023)	0.154 (0.024)
Pooled	1780	170	73.7	2.79	-	0.182
Fall Line (GA/SC)						
KEY	90	48	15.8	2.33	0.060 (0.028)	0.059 (0.038)
FTG	500	48	21.1	2.25	0.073 (0.024)	0.059 (0.035)
HND	66	48	15.8	2.33	0.089 (0.028)	0.078 (0.046)
HWY	50	48	26.3	2.00	0.099 (0.023)	0.065 (0.035)
Mean	177	48	19.7	2.23	0.080 (0.013)	0.066 (0.019)
Pooled	706	192	31.6	2.17	-	0.078
Coastal Plain (GA)						
CBK	250	48	21.1	2.00	0.011 (0.015)	0.015 (0.007)
CBT	115	52	21.1	2.00	0.012 (0.014)	0.023 (0.015)
COV	60	48	10.5	2.00	0.029 (0.020)	0.024 (0.018)
OHD	500	48	21.1	2.00	0.011 (0.015)	0.011 (0.006)
Mean	231	49	18.4	2.00	0.016 (0.008)	0.018 (0.006)
Pooled	925	196	36.8	2.00	-	0.032
Population mean	284	47	31.1	2.26	0.081 (0.009)	0.079 (0.011)
Species level	3411	558	73.7	2.79	-	0.109

Variation is described by proportion of all loci that are polymorphic (% *P*), mean number of *AP*, mean H_o, and mean H_e. Population size represents the total number of individuals observed. *N* represents the number of samples assayed. SD are shown in parentheses. Gene frequency data are available from D.W.T. on request. SD, standard deviation.

were significant ($P < 0.05$). Private alleles (alleles found in a single population) occurred in 3 Central Florida populations (UCF and LWR each had 1 private allele, whereas CCK had 3). Three alleles were only found in Central Florida and Fall Line populations, and 3 alleles were only observed in Central Florida and Georgia Coastal Plain populations. Genetic diversity was highest in the Central Florida populations ($H_e = 0.154$), intermediate in the Fall Line populations (0.066), and lowest for Georgia Coastal Plain populations (0.018; Table 1). Jackknifing procedures indicated a significant difference in genetic diversity among the 3 regions ($P < 0.05$). There was a significant positive correlation between population size and *P* ($P < 0.05$), but positive correlations between population size and *AP*, H_e, and H_o were not significant. F_{IS} were significantly different ($P < 0.05$) from Hardy–Weinberg expected values in 13% (9 of the 70) of the χ^2 tests relative to the 5% expected by chance alone. The mean F_{IS} across all polymorphic loci was 0.116, suggesting a slight excess of homozygotes, perhaps due to spatial structuring within populations (Trapnell, Schmidt, Quintana-Ascencio, Hamrick in preparation).

Both measures of genetic structure showed similar results at each level of hierarchy, and AMOVA revealed that the partitioning of variation was significant in each case ($P < 0.05$; Table 2). At the broadest geographic level (i.e., all populations sampled), $G_{ST} = 0.295$ (Table 2). At the regional scale, G_{ST} was 0.202 (68% of total), whereas G_{ST} among populations within regions was 0.093 (32% of total). Among populations within regions, G_{ST} ranged from 0.059 (Central Florida) to 0.438 (Georgia Coastal Plain; Table 2). When partitioning of genetic diversity was examined for pairs of regions, the lowest G_{ST} (0.119) was found between Central Florida and the Fall Line, whereas the highest G_{ST} (0.257) was observed between Fall Line and Coastal Plain (Table 2). A 2-way Mantel test showed a negative, non-significant correlation ($r = -0.112$) between pairwise G_{ST} values and geographic distance for all possible pairs of

Table 2. Partitioning of nuclear genetic variation among *Ceratiola ericoides* populations and regions

	G_{ST}	AMOVA
Among all populations sampled	0.295	0.319*
Among Central Florida, Fall Line, and Georgia Coastal Plain regions	0.202	0.210*
Among populations within regions	0.093	0.109*
Between Central Florida and Fall Line regions	0.119	0.194*
Between Central Florida and Georgia Coastal Plain regions	0.144	0.185*
Between Fall Line and Georgia Coastal Plain regions	0.257	0.248*
Among Central Florida populations	0.059	0.057*
Among Fall Line populations	0.093	0.180*
Among Georgia Coastal Plain populations	0.438	0.502*

* $P < 0.05$.

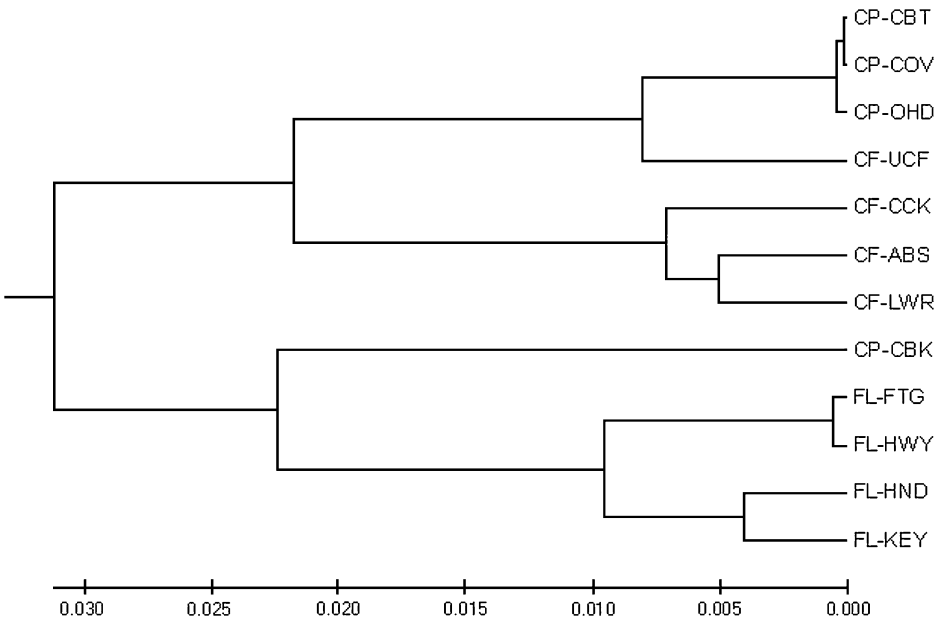

Figure 2. UPGMA phenogram of mean genetic distances (Nei 1972) among *Ceratiola ericoides* populations. CF, Central Florida; FL, Fall Line; CP, Coastal Plain of Georgia.

populations (pairs of populations were separated by 6–710 km). Similar tests performed for pairs of regions indicated a significant ($P < 0.05$) positive correlation in each case ($r = 0.800$ for Central Florida and Fall Line populations, $r = 0.417$ for Fall Line and Georgia Coastal Plain populations, and $r = 0.241$ for Central Florida and Georgia Coastal Plain populations).

Nei's genetic identity values between pairs of populations ranged from 0.9094 (populations CF-ABS and FL-HWY) to 0.9996 (populations CP-COV and CP-CBT). The UPGMA phenogram (Figure 2), based on genetic distance data, indicates that Georgia Coastal Plain populations are more similar to populations in Central Florida than either is to the Fall Line populations. The neighbor-joining analysis produced a similar phenogram.

Discussion

Relative to species with similar combinations of traits, *C. ericoides* maintains moderately high levels of genetic variation at the species level and moderate amounts of genetic structure among its populations (Hamrick and Godt 1989, 1996). Genetic diversity in *C. ericoides* ($H_{es} = 0.109$) is similar to that of other rare southeastern plant taxa ($H_{es} = 0.123$) and endemic species ($H_{es} = 0.096$; Godt and Hamrick 2001), which agrees with our prediction. Within its populations, *C. ericoides* has less diversity ($H_{ep} = 0.079$) than other rare southeastern plants ($H_{ep} = 0.100$) but more diversity than endemic plants ($H_{ep} = 0.063$; Godt and Hamrick 2001). *Ceratiola ericoides* partitions more of its genetic variation among populations ($G_{ST} = 0.187$ and 0.248 in rare southeastern plants and endemic taxa, respectively; Godt and Hamrick 2001). The relatively high G_{ST} (0.295) value is primarily due to variation among the 3 regions and high values among populations within the Georgia Coastal Plain. Population size was positively correlated with all measures of genetic diversity in accordance with our expectations but only significantly so with P (percentage of polymorphic loci). This suggests that larger populations had more rare alleles but that these alleles occurred at frequencies too low to significantly increase expected and H_{ep}.

Central Florida populations display the most diversity and the least genetic structure (both $P < 0.05$), have 15 alleles found only in this region and a total of 5 private alleles. No unique alleles were detected in the other 2 regions. The Lake Wales Ridge is probably the most ancient peninsular Florida refugium (MacNeil 1950) having remained above sea level more consistently during interglacial sea level fluctuations than other peninsular Florida ridges (Christman and Judd 1990). Georgia Coastal Plain populations had the lowest genetic diversity and the most genetic structure, a result consistent with recent, independent founding events. Central Florida and Fall Line regions are the most genetically similar, whereas the Fall Line and Coastal Plain populations are the most dissimilar despite their greater geographic proximity. It appears that Florida was the ancestral region for both northern clusters of populations but that there has been little subsequent gene flow between Fall Line and the Coastal Plain populations. The high level of isolation-by-distance ($r = 0.80$) between the Central Florida and Fall Line populations suggests that these regions were once continuous during much of their history so that gene flow/genetic drift equilibrium has resulted. The weaker Isolation by distance (IBD) correlation (24%) between the Central Florida and the riverine Coastal Plain populations may be indicative of recent long-distant

founder events, with little modern gene flow. This conclusion is supported by the UPGMA phenogram, which indicates that 3 of the Coastal Plain populations are more similar to populations in Central Florida. A notable exception is population CBK that clusters more closely with the Fall Line populations (Figure 2), a result that may indicate that CBK was established by a recent colonization event from a Fall Line population.

Pollen records indicate that scrub habitat expanded considerably during the drier glacial periods of the Pleistocene (Watts 1975), allowing species adapted to this habitat to flourish and expand their geographic ranges during glacial periods only to contract again during more mesic interglacial periods (Delcourt PA and Delcourt HR 1981). Thus, although *C. ericoides* is currently restricted to high, dry sand dunes, it was more continuously distributed over much of the southeast during the Pleistocene and early Holocene (Watts 1975). In Georgia and South Carolina, the Fall Line sandhills date from the Miocene (~25 mya) and thus populations of *C. ericoides* on these formations could have been continuous with the Central Florida populations as recently as 10 000 ybp. The riverine sandhills, smaller in extent and less continuous, formed when sand from exposed river bottoms was deposited by Coastal Plain rivers during the late Pleistocene and Holocene (~10 000 years ago to present; Ivester and Leigh 2003). Thus, populations of *C. ericoides* on these Coastal Plain sites have probably never been connected with populations in the other 2 regions. Our genetic evidence is consistent with this biogeographical scenario because Fall Line populations share rare alleles with the Florida populations, are more genetically similar to the Central Florida populations, and show IBD with the Central Florida populations.

An explanation for the lower levels of genetic diversity found in the Fall Line populations is less obvious, if our argument for their much greater age and more extensive past distribution is correct. The lower genetic diversity in the Fall Line sandhill populations could be the result of recent human-influenced fluctuations in their population sizes (i.e., genetic bottlenecks). Although the sandy substrates on which *C. ericoides* occurs are not likely to have been heavily disturbed by agriculture, they were certainly disturbed by logging of overstory long-leaf pine. The increased frequency and severity of fires owing to human activities may also have greatly reduced *C. ericoides* population sizes, because adults are killed by fire. During the last several hundred years, populations of *C. ericoides* may have experienced relatively frequent cycles of extinction and recolonization either by moderate distance seed dispersal from a few surviving individuals or by regeneration from a resident seed bank. The increased frequency of fires owing to human activity may have depleted the seed bank leading to restoration of the population by relatively few founding individuals. A scenario of repeated population bottlenecks would lead to the loss of low frequency alleles, lowering P, AP, and H_e relative to the more continuous (in time and space) Florida populations.

Because our work was conducted within the Florida refugial phylogeographic region, we are unable to compare our results with that of Avise and his colleagues who examined patterns across several southeastern phylogeographic regions (Avise 2000). However, the different phylogeographic patterns shown in the Fall Line and Coastal Plain populations agree with Soltis et al. (2006), who argue that phylogeographic patterns in the southeastern United States are more complex than originally thought. Our genetic data are consistent with what we know about the geologic differences and inferred paleoecology of the region, that is, older Fall Line populations were more widely distributed during the last glacial epoch when the climate was drier.

In conclusion, these data reveal the impact that Pleistocene glaciations and associated changes in climatic conditions as well as differing geologic histories had on the geographic distribution of this southeastern endemic over time. *Ceratiola ericoides* is unusual in that its range expanded during the last glacial maximum, unlike the pattern displayed by most other eastern taxa. Our results demonstrate the need for empirical data for the development of effective strategies to conserve genetic diversity in *C. ericoides* populations. A priori, we expected the Fall Line and Coastal Plain populations to be genetically more similar due to their geographic proximity as well as the absence of geographic or topographic barriers between the 2 regions. However our empirical data indicate that the Fall Line and Coastal Plain populations are more genetically similar to, but geographically distant from, the Florida populations than to each other. As a result, it would be a mistake to use individuals from the Fall Line populations to restore or augment the small Coastal Plain populations. Rather, our genetic analyses indicate that it would be more appropriate to use the more genetically similar Florida populations as sources of propagules for Coastal Plain sites.

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Corresponding Editor: David Wagner