

Spatial Genetic Structure of the Southeastern North American Endemic, *Ceratiola ericoides* (Empetraceae)

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Abstract

Understanding the spatial distribution of genetic diversity (i.e., spatial genetic structure [SGS]) within plant populations can elucidate mechanisms of seed dispersal and patterns of recruitment that may play an important role in shaping the demography and spatial distribution of individuals in subsequent generations. Here we investigate the SGS of allozyme diversity in 2 populations of the southeastern North American endemic shrub, *Ceratiola ericoides*. The data suggest that the 2 populations have similar patterns of SGS at distances of 0–45 m that likely reflect the isolation by distance (IBD) model of seed dispersal. However, at distances ≥ 50 m, the pattern of SGS differs substantially between the 2 populations. Whereas one population continues to reflect the classical IBD pattern, the second population shows a marked increase in autocorrelation coefficient (r) values at 50–75 m. Furthermore, r values at these distances are as much as 33% higher than at 0–5 m where the highest r value would be predicted by IBD. A likely explanation is the differing frequencies of 2 fruit morphologies in these populations and the greater role that birds play in seed dispersal in the second population.

Patterns of genetic variation within natural plant populations (i.e., spatial genetic structure [SGS]) provide interesting and sometimes unexpected insights into mechanisms of seed dispersal as well as patterns of recruitment within populations. SGS is defined as the nonrandom distribution of genetically similar individuals within populations (McCauley 1997). In plants, SGS is influenced by clonal reproduction, mating systems, patterns of seed dispersal and establishment, population density of established plants (Hamrick and Nason 1996), and microhabitat selection. Patterns of seed dispersal and establishment tend to have the greatest impact on the spatial distribution of genetic diversity within plant populations (Nason and Hamrick 1997) and can influence the demography and spatial distribution of individuals of subsequent generations.

The typical model of seed dispersal applied to SGS in populations of perennial plant species is isolation by distance (IBD). This model generally assumes that fruits or seeds have leptokurtic or normal dispersal kernels with more seeds being dispersed near the maternal parent and fewer seeds being dispersed to greater distances. These dispersal patterns are expected to produce seed or seedling shadows centered on maternal individuals. Actual dispersal distances and the densities of maternal individuals will

determine the degree of overlap of the various seed shadows and levels of genetic relatedness between pairs of individuals.

These IBD models have 2 implicit assumptions that may be commonly violated in natural plant populations. First, certain dispersal agents may not disperse seeds or fruits in a leptokurtic or normal pattern. Rather, many animal dispersal agents disperse seeds in a clustered or patchy fashion (e.g., Jones et al. 2006; Jordano et al. 2007). For example, many tropical fruit-eating bats take fruits from trees and fly to feeding roosts where they deposit seeds in a highly patchy fashion often at some distance from the maternal individual. The genetic relatedness observed in seedlings germinating below these roosts will depend on the particular foraging behavior of the bats using that roost but can result in high levels of genetic relatedness (Aldrich and Hamrick 1998).

The second assumption is that seedling establishment patterns are dependent on seed dispersal patterns. This, of course, is not always the case. Janzen (1970) and Connell (1971, 1978) pointed out that seedling survival is often highest in areas removed from conspecific adults. Also, many species have specific requirements for germination and establishment (e.g., bare mineral soil, tree fall gaps) that may be patchily distributed at some distance from maternal individuals.

Finally, seedlings may only be capable of surviving in habitats in which they are protected from biotic or abiotic mortality. The location of germination and establishment “safe sites” relative to the location of maternal plants and the dispersal ability of the species will determine the levels of genetic relatedness observed. If a safe site is located near a single maternal individual, then the resulting juvenile cohort may be dominated by this maternal individual and the level of genetic relatedness will be high. In contrast, if seedlings establish at some distance from every adult (i.e., Janzen–Connell model or the colonization of an open habitat), successful seedlings may be derived from the tails of the dispersal kernels of several adults and levels of genetic relatedness may be low.

Here we examine SGS within populations of *Ceratiola ericoides* Michaux, a southeastern endemic shrub, which has a patchy distribution throughout much of its range and is threatened in Georgia (as of January 2008 in <http://georgiawildlife.dnr.state.ga.us>). This species grows in xeric, patchily distributed sandy substrates. We sampled 2 populations of *C. ericoides* from habitats that arose by distinct historical geologic processes. One population was located in the riverine sandhills of the Georgia Coastal Plain and the other was in the Fall Line sandhills of northeast Georgia. Our prediction is that both populations will reflect the IBD model whereby a large proportion of seeds are deposited within close proximity to the maternal plant with fewer and fewer seeds dispersed as the distance from the maternal individual increases.

Materials and Methods

Study Species

Ceratiola ericoides Michaux, Sandhill or Florida Rosemary, belongs to 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 northward. An individual can produce hundreds of yellowish or olive fruit, similar to a drupe but containing 2 nutlets. Female shrubs produce 2 fruit morphs; more commonly observed dry, hard fruits and less numerous fleshy fruits. Both fruit types are typically found in varying proportions on all female shrubs (Schmidt JP, personal observation). Seed production does not begin until a plant is 10–16 years of age (Gibson and Menges 1994; Patrick et al. 1995). Although many fruits simply fall to the ground, fruits are also consumed by harvester ants (Johnson 1982), rodents, and several bird species (Van Dersal 1938) including rufous-sided towhees (*Pipilo erythrophthalmus*) and scrub jays (*Apbe-locoma 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 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 (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 January 2008 (<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

Leaves were collected from branch tips of 146 and 243 individuals from each of 2 populations. The smaller population, CBK, is located in the Georgia Coastal Plain (32.3°N and 82.2°W) and was comprised of approximately 250 plants of which 86 were adult females, 76 were adult males, and 88 were juveniles. The second population, FTG, consisting of approximately 500 individuals (77 adult females, 73 adult males, and 350 juveniles), occurs on the Georgia Fall Line sandhills (33.3°N and 82.3°W). Juveniles were identified by their lack of flowers and/or fruit, evidence of which persists on adult shrubs year round. The coordinates of each plant from which a sample was collected were recorded with a Trimble GeoExplorer 3 global positioning system (GPS) unit and associated Trimble Pathfinder software was used to perform differential correction from base station files. 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 from the tissue 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 enzyme stains in 4 buffer systems resolved 9 putative polymorphic (i.e., more than one allele per locus) and 10 monomorphic loci. Enzymes stained and polymorphic loci utilized in the study (in parentheses) for each of the 4 buffer systems were: 1) system 4, UTP-glucose-1-phosphate (UGPP2); 2) system 6, phosphoglucomutase (PGM2) and triosephosphate isomerase (TPI2); 3) system 8-, aspartate aminotransferase (AAT2), fluorescent esterase (FE), and menadione reductase (MNR); and 4) system 11, isocitrate dehydrogenase (IDH), malate dehydrogenase (MDH), and phosphoglucoisomerase (PGI2). All buffer and stain recipes were adapted from Soltis et al. (1983) except AAT 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 Mendelian inheritance patterns expected for each enzyme system (Weeden and Wendel 1989).

Spatial Patterns of Individuals' Distribution

We statistically analyzed mapped spatial point patterns with SPPA program (Haase 1995) using Ripley's K -function (Ripley 1976, 1981; Diggle 1983; Upton and Fingleton 1985; Haase 1995), which is a second-order analysis of point patterns in 2-dimensional space. Simulation of data permitted 95% confidence intervals (CIs) to be generated to test for significance.

Spatial Genetic Structure Analyses

A pairwise comparison of genetic similarity of individuals with respect to spatial distance separating those individuals within populations was performed to assess the presence/absence of SGS using GenAlEx 6 (Peakall and Smouse 2006). Genetic distances based on multiallelic codominant multilocus arrays as described by Smouse and Peakall (1999) were plotted against geographic distances separating pairs of individuals. By analyzing the full dataset, this approach reduces stochastic noise and strengthens the spatial signal. The autocorrelation coefficient (r), similar to Moran's I (Moran 1950), ranges from -1 to 1 . Significance of the spatial autocorrelation values was tested by constructing a classic 2-tailed 95% CI around the null hypothesis of no SGS (i.e., $r = 0$) by performing 999 random permutations of genotypes among geographic locations. Spatial autocorrelation was calculated at 5-m distance intervals up to 380 m in population CBK and 365 m in population FTG. SGS was further quantified using the S_p statistic described by Vekemans and Hardy (2004) and calculated using SPAGeDi version 1.2 (Hardy and Vekemans 2002) such that $S_p = -b/(1 - F_1)$ where b = slope of the regression of f_{ij} (coefficient of coancestry) on ln (distance) separating possible pairs of individuals and F_1 is the mean f_{ij} between pairs of individuals in the first distance class (i.e., nearest neighbors; 0–5 m in this study). This statistic transforms values of different relatedness statistics into a common statistic that allows examination of genetic structure at any spatial scale and permits comparisons with other species.

Results

Populations CBK and FTG were polymorphic for 4 and 8 of the 9 polymorphic loci, respectively. Population FTG had a somewhat higher number of alleles per polymorphic locus than did CBK (2.25 vs. 2.00, respectively) and much higher genetic diversity (H_e of 0.059 vs. 0.015). However, both populations possessed less genetic variation than levels observed in the population mean for the species (31% of the loci polymorphic, an average of 2.26 alleles per polymorphic

Table 1. Autocorrelation coefficients (r) and number of pairs of individuals in each distance class for *Ceratiola ericoides* populations CBK and FTG

Distance class	CBK		FTG	
	r values	Number of pairs/distance class	r values	Number of pairs/distance class
5	0.111*	253	0.128*	1173
10	0.028	332	0.078*	959
15	0.018	255	0.092*	1045
20	0.026	313	0.046*	1095
25	0.021	277	0.089*	1530
30	0.007	242	0.075*	1108
35	0.023	232	0.073*	1476
40	0.013	301	0.095*	808
45	-0.020	273	0.026	579
50	0.015	276	0.192*	446
55	0.002	264	0.173*	424
60	-0.059	302	0.094*	975
65	-0.050	246	0.138*	649
70	-0.042	288	0.114*	541
75	0.001	293	0.129*	445
80	0.063	311	0.021	366

* Indicates r values that deviate significantly from random.

locus [AP], and a mean genetic diversity [H_e] of 0.079; Trapnell et al. 2007).

The Ripley's K -function analysis showed that both populations had significant spatial clustering (i.e., structure) of individuals at all distances. Ripley's statistic, transformed into $L(r)$, ranged from 22.32 at 5 m to 43.34 at 40 m in CBK and 26.97 at 5 m to 55.61 at 40 m in FTG. Significant genetic structure was observed in both populations with GenAlEx although at different scales (Table 1; Figure 1). Population CBK displayed significant structure only at a distance of ≤ 5 m ($r = 0.111$), whereas FTG had r values significantly different from random at every distance up to 75 m (except 45 m) as well as at 105 and 115 m. Significant r values varied from 0.046 to 0.192 with the highest autocorrelation coefficients for individuals separated by 50 m ($r = 0.192$), 55 m ($r = 0.173$), and 65 m ($r = 0.138$). Analyses using SPAGeDi yielded patterns of SGS that were similar to those generated by GenAlEx. The S_p statistics produced from the SPAGeDi analyses were quite different between the 2 populations. Population CBK had a value of 0.0038, whereas FTG had a substantially higher S_p value (0.0411).

Discussion

Both populations demonstrated significant SGS, however, both GenAlEx and SPAGeDi analyses indicate that the patterns of genetic variation observed in the 2 populations differ. The GenAlEx analyses demonstrated that both populations have significant SGS at distances ≤ 5 m suggesting localized seed dispersal resulting from gravity

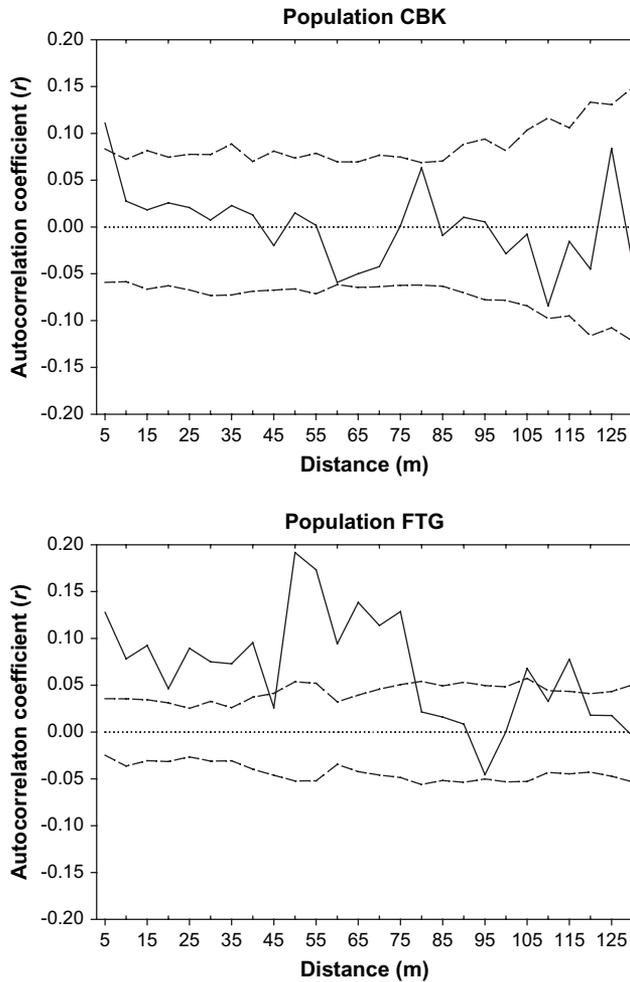


Figure 1. GenAlEx spatial autocorrelagrams for *Ceratiola ericoides* populations CBK and FTG. Note that the figures are truncated at 125 m although the actual pairwise distances extended to 380 m in CBK and 365 m in FTG.

deposition. Beyond 5 m, CBK has no significant SGS, whereas FTG has significant structuring at all distance classes up to 75 m (except at 45 m) as well as at 105 and 115 m. Furthermore, FTG has substantially higher autocorrelation coefficients (r) at every distance. However, despite these differences, the overall pattern from 0 to 45 m is similar in the 2 populations, with generally declining r values as the distance between pairs of individuals increases (Figure 1). The significance of SGS in FTG but not CBK reflects, in part, the larger sample size of FTG and thus its more compact CI (Figure 1). However, at 50 m and beyond, the pattern in the 2 populations diverges markedly with FTG having substantially higher r values at 50–75 m than at the smallest distance class of 5 m (Figure 1). Although the pattern of pollen movement is important in determining the degree of relatedness between propagules and therefore the magnitude of the autocorrelation coefficients, pollen movement does not affect the spatial scale of intrapopulation genetic structure (Dyer 2007). Rather, SGS is shaped

largely by the pattern of seed dispersal and establishment as well as the degree of overlap of seed shadows (i.e., maternal density). Unlike CBK where most seed dispersal is localized, FTG has a bimodal pattern of seed dispersal with some seeds falling to the ground below or near the maternal plants but with a larger proportion of seeds being dispersed 50–75 m. The SPAGeDi analyses revealed nearly identical patterns as GenAlEx in the 2 populations.

The 2 populations also produced very different S_p values. In CBK, S_p (0.0038) was lower than the mean of 0.0304 reported for 47 species examined by Vekemans and Hardy (2004) as well as the mean values found in plants with wind-dispersed pollen (mean $S_p = 0.0064$), gravity-dispersed seeds (mean $S_p = 0.0281$), animal-dispersed seeds (mean $S_p = 0.0088$), exclusively outcrossing species (mean $S_p = 0.0126$), and small trees (mean $S_p = 0.0259$). Only 3 of the 47 species surveyed by Vekemans and Hardy (2004) had lower S_p values. FTG, on the other hand, had substantially higher SGS with a S_p value of 0.0411 which is more than an order of magnitude greater than the S_p value for CBK and which exceeded the mean values for species sharing the same life-history traits as *C. ericoides* (i.e., wind-dispersed pollen, gravity-dispersed seeds, animal-dispersed seeds, outcrossing species, and small trees). In fact, the S_p value found in FTG exceeds the values found for 40 plant species summarized by Vekemans and Hardy (2004). Only 7 species surveyed by Vekemans and Hardy (2004) have S_p values greater than FTG. There are several factors that may contribute to these strongly contrasting patterns.

The demographic composition of these 2 sites differs considerably, which likely reflects different fire regimes and the susceptibility of adults to fire-induced mortality. Evidence at CBK suggests that there have been small, highly localized fires at ~5-year intervals with the last major fire about 15 years before present (ybp). Population FTG has experienced more fire disturbance with burns occurring approximately every 3 years since 1970 (Schmidt JP, in review). Fire not only kills adults but also stimulates recruitment by removing competition and evidence suggests that smoke may trigger germination of *C. ericoides* seeds (Schmidt JP, in review). The seed bank is fairly substantial with seeds remaining viable for at least 7–11 years (Schmidt JP, unpublished data). FTG has a much higher density of adult individuals (mean of 2.6 m between adults vs. 4.7 m in CBK) and a higher proportion of juveniles. Adults are also smaller (mean diameter of 0.83 vs. 1.32 m in CBK). Thus, CBK has larger, older adult plants surrounded by rings of juveniles, whereas FTG has fewer, smaller adults and a dense carpet of juveniles. CBK also has more of an oak/pine canopy where birds can perch.

A second factor that may play a role in the contrasting dispersal patterns is differing proportions of the 2 fruit morphologies between the 2 sites. In CBK, only about 10% of the fruits are fleshy (Schmidt JP, personal observation); the more plentiful hard, dry fruits are presumably less attractive to birds, leaving the majority of the fruit to drop in the vicinity of the maternal plant. About 50% of the fruits in FTG are fleshy (Schmidt JP, personal observation) and thus

more likely to be consumed and dispersed by birds. So, whereas some fruits in FTG fall beneath the maternal plant, many are presumably consumed by birds and deposited elsewhere in the population beneath hospitable perching sites.

Differences in dominant fruiting types between the 2 populations should produce different patterns of SGS because FTG, with higher frequencies of fleshy fruits, is expected to experience greater seed dispersal. In one study, 50% of small passerine birds (e.g., warblers) dispersed seeds <51 m from the maternal tree, whereas 50% of medium-sized birds (e.g., crows and thrushes) dispersed seeds >110 m (Jordano et al. 2007). Furthermore, small birds dispersed seeds primarily beneath the canopies of fleshy fruited trees and shrubs, whereas medium-sized birds were more likely to disperse seeds to open areas or beneath pine trees (Jordano et al. 2007). CBK has a large number of safe perching sites (i.e., large *C. ericoides* adults, pines, and oaks) within the population, whereas FTG is more barren and hostile to bird dispersers because of the patchy distribution of adult plants as well as their smaller size and the near absence of an oak/pine canopy. If birds are moving only between fruiting individuals (i.e., females), the patchiness of perch sites is further increased. There is also an expectation that birds will seek the most productive fruiting plants that would further narrow selection to the largest maternal plants, of which there are relatively few in FTG presumably due to mortality from more frequent fires (Schmidt JP, in review). Because our sampling was not replicated, we cannot be certain that the observed pattern is not an artifact of some sort. However, if not artifactual, then the data suggest that the divergent patterns observed in CBK and FTG result from the foraging behavior of birds in populations with different demographic footprints. A bird will consume fruit from a single maternal plant before moving on to the next fruiting individual or to a perch site when satiated. Subsequent to a somewhat standard gut passage time, seeds belonging to a full- or half-sib cohort are deposited, presumably beneath another fruiting individual or nonfruiting perch site. It is, thus, possible that the foraging behavior of the frugivorous birds is responsible for the codispersal of seeds with a high degree of genetic relatedness and because of the demographic history of FTG, there are only a limited number of safe sites. Territoriality of the bird dispersers may further contribute to the observed pattern.

Results from these 2 *C. ericoides* populations demonstrate that populations within a single taxon can have very different SGS patterns. Population CBK has the more typical IBD pattern with the highest *r* value in the shortest distance class followed by a rapid decrease in the *r* values at greater distances. Population FTG, on the other hand, has a SGS pattern quite different from CBK and the IBD model. Several factors could have caused the observed differences but the most likely is the greater role that birds play in seed dispersal in FTG. The highly patchy deposition of seeds by birds could explain the observed SGS pattern. These results indicate that several factors (e.g., fire, population density, etc.) could impact the distribution of genetic

diversity within populations and illustrate the problem of attempting to interpret SGS data with a single dispersal model especially for species for which seed dispersal varies among its populations. Analyses of SGS can, therefore, provide important insights into differences in dispersal and recruitment patterns among populations of plant species.

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