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The Impact of Landscape Disturbance on Spatial Genetic Structure in the Guanacaste Tree, *Enterolobium cyclocarpum* (Fabaceae)

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Abstract

We examined spatial genetic structure (SGS) in *Enterolobium cyclocarpum* (the Guanacaste tree), a dominant tree of Central American dry forests in 4 sites in Guanacaste Province, Costa Rica. In disturbed dry forest sites (e.g., pastures), *E. cyclocarpum* is primarily dispersed by cattle and horses, whose movements are restricted by pasture boundaries. The study sites varied in tree densities and disturbance. Allozyme analyses of adult trees demonstrated significant levels of SGS in 3 of 4 sites. SGS was primarily due to clusters of young adults located along seasonal streams, rocky areas, and in abandoned pastures. SGS was highest in the first distance class in the least disturbed population, which also had the lowest density of large adults. Low, but significant SGS characterized the site with the highest number of large adults located in individual pastures. The semiurban site, had no clusters of young adults and, probably as a result, failed to exhibit SGS. Our results demonstrate that disturbance can strongly influence SGS patterns and are consistent with a landscape model in which the location of potential recruitment sites, restricted seed disperser movements, and the number and location of maternal individuals dictate the level and pattern of SGS.

Key words: *Enterolobium cyclocarpum*, *landscape genetics*, *landscape disturbance*, *seed dispersal*, *spatial genetic structure*, *tropical dry forest*

The distribution and maintenance of genetic diversity are recurring themes in evolutionary biology because genetic diversity is a prerequisite for adaptation to environmental variation over space and time (Frankel 1974). Typically, continuous natural populations of tree species exhibit extensive genetic diversity (Hamrick et al. 1992; Hamrick and Godt 1996) and that variation is often distributed nonrandomly in space (Wright 1951; Heywood 1991). For instance, in continuous populations with restricted seed dispersal, the nonrandom spatial distribution of related individuals can develop over relatively short distances resulting in spatial genetic structure (SGS). Propagule dispersal is typically viewed as the key factor determining SGS, in the absence of variable selection pressures within populations. SGS has consequences for population demography and effective population size, and if strong enough, can impact local adaptation patterns (Fenster et al. 2003). In plants, it is important to distinguish between the relative roles of seed- and pollen-mediated gene dispersal in shaping SGS. The "spatial" distribution of related individuals depends primarily on seed dispersal patterns, maternal adult densities, and the distribution of suitable recruitment sites, whereas the "magnitude" of genetic relatedness within patches is a function of both seed and pollen movement, as well as maternal plant densities (i.e., seed shadow overlap; Dyer 2007). Although dispersal is a fundamental contributor to the evolutionary dynamics of natural populations, direct monitoring of seed and pollen movement in large

continuous populations and across multiple sites has proved difficult. Nevertheless, it is possible to infer historical dispersal rates and patterns from SGS analyses (Peakall and Beattie 1996; Smouse and Peakall 1999; Vekemans and Hardy 2004; Smouse et al. 2008).

In addition to restricted dispersal and natural selection, processes that contribute to the spatial location of genotypes within populations include demographic factors such as seedling establishment and mortality, the location of sites suitable for seedling establishment and growth, and whether related individuals are dispersed together (Levin 1981; Hamrick and Nason 1996). Plant species with limited and/or correlated seed dispersal should exhibit considerable genetic heterogeneity among patches of new seedlings, whereas overlapping seed shadows due to either long-distance seed dispersal and/or high adult densities should blur SGS. The density of maternal adults, human-generated habitat disruption, and foraging/deposition behavior of seed dispersal agents will all influence SGS (Hamrick et al. 1993; Hamrick and Nason 1996; Jordano et al. 2007). Additionally, local environmental heterogeneity and establishment history should also impact SGS (e.g., Knowles et al. 1992; Premoli and Kitzberger 2005). Undisturbed sites with low effective reproductive density often show significant SGS (Hamrick and Nason 1996; Hardesty et al. 2005), but the patterns expected after conversion of continuous forests into pastures or human settlements is unclear. Habitat disturbance could either enhance or blur natural spatial patterns of genetic relatedness by modifying the movement of dispersal vectors and significantly changing the number and distribution of recruitment sites. For example, conversion of forest into fenced pastures could curtail movement of potential seed dispersal vectors and restrict recruitment to fencerows, riparian areas or other topographic breaks within each pasture.

Specifically, we investigated SGS within Costa Rican populations of the Guanacaste tree, *Enterolobium cyclocarpum* (Jacq.) Griseb (Fabaceae, subfamily Mimosoideae). *Enterolobium cyclocarpum* is widely distributed across dry forest regions of Central America and northern South America (Janzen 1983), where it occurs from sea level to 900 m. Although *E. cyclocarpum* is widely distributed, it does not presently have any natural seed dispersal agents. Janzen and Martin (1982) proposed that its flat, indehiscent fruits were eaten by Pleistocene megafauna and subsequently dispersed in their dung. Some seeds are currently distributed by small mammals, but most contemporary seed movement in disturbed areas is by cattle and horses (Janzen 1982; Janzen and Martin 1982; Hamrick JL, personal observation).

Throughout the second half of the 20th century, tropical dry forests in Central America were extensively cleared for cattle grazing, but adult *E. cyclocarpum* were often left in pastures and adjacent to houses to provide shade (Rocha and Lobo 1996). In Costa Rica, *E. cyclocarpum* naturally occurs at relatively low densities in intact primary forest but can be more abundant in disturbed areas (Janzen 1983; Rocha and Lobo 1996). Thus, the modern distribution of *E. cyclocarpum* has been greatly impacted by anthropogenic disturbance at various spatial scales, and the question arises of how human activities have affected patterns of genetic variation within and among populations subjected to varying degrees of human disturbance.

Here, we explore whether different types and levels of human landscape disturbance have altered seed dispersal and recruitment patterns of *E. cyclocarpum* in ways that impact local SGS in Guanacaste Province, Costa Rica. We used multilocus, multiallele, spatial autocorrelation analyses originally developed by Smouse and Peakall (1999), a treatment that can be augmented to provide a formal test of the null hypothesis of homogeneous autocorrelograms for populations with different types and/or levels of habitat disruption (Smouse et al. 2008). Specifically, we address the following questions: 1) Is SGS within each population consistent with "isolation by distance" (IBD sensu Wright 1943)? 2) Are patterns of SGS similar across the 4 study sites? 3) Are differences in SGS associated with disturbance histories of the 4 locations?

In companion studies of pollen-mediated gene flow (Hamrick JL, in preparation; Smouse PE, in preparation), we document widespread pollen movement, suggesting that pollen flow should contribute little to genetic substructuring within populations. Therefore, genetic relatedness at relatively short distances should primarily result from seed dispersal and recruitment patterns, barring natural selection. The morphology, size, and weight of *E. cyclocarpum* fruits and seeds leads to an expectation of short-distance dispersal and potentially strong SGS in undisturbed sites because seeds lacking natural dispersal vectors would not move very far from maternal trees; thus, we should expect an IBD pattern within populations. However, conversion of original forests to pastures has introduced cattle and horses as seed dispersers and has limited seedling recruitment to areas protected from grazing. The SGS observed should then be a direct function of the number and proportion of maternal individuals contributing offspring to these recruitment sites. Finally, in landscapes with a long history of human settlement, we should see the impact of multiple effects (i.e., both animal and human mediated seed movement, more even spacing of individuals, limited access of livestock to suitable recruitment sites, tree removal, etc.), resulting in reduced SGS in urban–rural mixed habitat. Thus, SGS should decline with increasing history of disturbance and the presence of introduced cattle and human settlement.

Materials and Methods

Study Sites

Study sites are located in Guanacaste Province in northwestern Costa Rica (Figure 1, Table 1). This area is characterized by a 6-month dry season, during which very little of the 1500 mm of annual precipitation occurs. Natural habitats containing *E. cyclocarpum* range from thorn-shrub forest occupying heavy clay soils to mesic riparian gallery forest distributed along permanently flowing streams (Janzen 1983). We sampled 4 locations, selected for their divergent

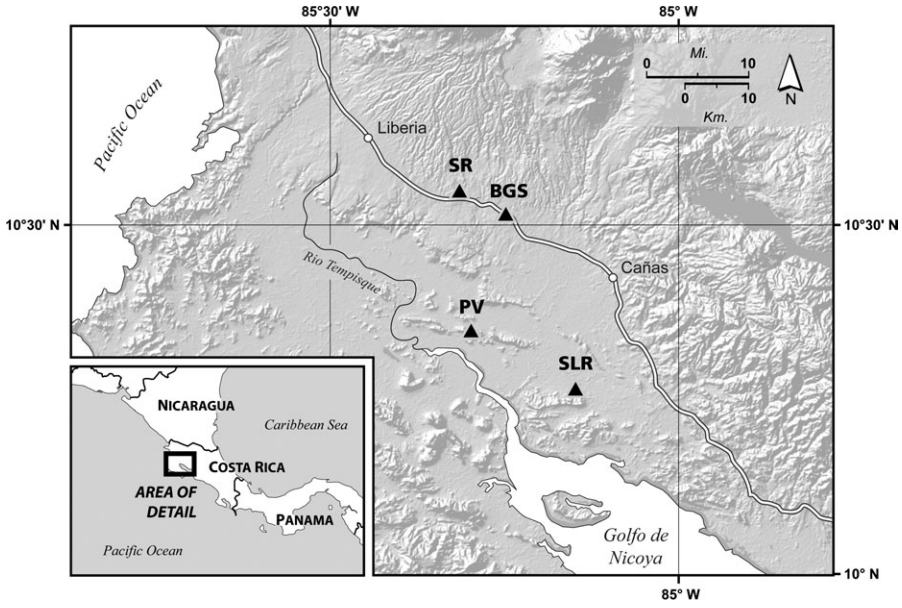

Figure 1. Geographic locations of the 4 study sites in Guanacaste Province, Costa Rica; locations are indicated by triangles: PV National Park, SR, SLM, and BGS.

histories of anthropogenic disturbance: Palo Verde National Park (PV), Stewart Ranch (SR), Hacienda Solimar (SLM), and Bagaces (BGS).

Palo Verde National Park

Prior to the late 1970s, when the area was designated a national park, flatter thorn-shrubs areas were heavily grazed. Steep rocky slopes retain their original dry forest vegetation, although some of the more valuable tree species (e.g., *Swietenia macrophylla*, *Pachira quinata*, and *Guaiacum sanctum*) have been harvested. Contemporary vegetation is a mosaic of primary forest on the steeper slopes and secondary forests on the flatter, previously grazed areas. *Enterolobium cyclocarpum* primarily occupy flatter sites and lower hillsides. Large adults are generally widely dispersed (>500 m), and there are a few clusters with 2–6 small to moderate sized (presumably younger) trees separated by about 20–100 m. These clusters of smaller adults are typically found in former pastures that are undergoing secondary succession.

Stewart Ranch

Stewart Ranch represents one of the oldest and largest cattle ranches in Guanacaste Province (established in the 1920s). Most of the area was cleared in the 1920s and 1930s and is now heavily grazed pasture with well-developed forest vegetation along permanent spring-fed streams and secondary forest on the steeper, rocky slopes. A few (1–3) large *E. cyclocarpum* trees are found in open pastures, but most reproductive individuals are clustered in gallery forests or on small rocky outcrops. Clusters of trees have from 3 to 6 individuals within 100 m of each other.

Hacienda Solimar

Until the mid-1950s, this area supported well-developed upland tropical dry forest. The area was cleared for pastures during the mid-1950s, but several large *E. cyclocarpum* were left for shade. Over the last half century, hillsides as well as areas immediately adjacent to seasonally dry streams have undergone secondary succession (Pacheco O, personal communication). Pastures on lower, flatter areas are today separated by strips of second-growth canopy trees along stream courses with little or no understory vegetation. Currently, individual pastures have several large *E. cyclocarpum* (3–6), but the highest densities of *E. cyclocarpum* are found on steep stream banks, where small to medium sized adults occur in strings of 5–25 individuals extending over several hundred meters.

Table 1. Locations and sample sizes for the 4 *Enterolobium cyclocarpum* Study sites in Guanacaste Province, Costa Rica

Study site	Current habitat type	Latitude	Longitude	Number of trees sampled	Proportion of population sampled (%)
PV	Forest	10°21′N	85°20′W	67	90
SR	Pasture	10°33′N	85°19′W	45	100
SLM	Pasture	10°16′N	85°09′W	251	90
BGS	Pasture and urban	10°31′N	85°15′W	47	85

Bagaces

Bagaces is a small town of ~5000 inhabitants that was established at least 100 years ago. The area immediately around Bagaces consists of relatively small, heavily grazed pastures, many of which are undergoing early secondary succession. Reproductive *E. cyclocarpum* occur along the Rio Bagaces, in vacant lots, along roadsides within the town itself, and along the margins of abandoned pastures. In many areas, houses are being built in abandoned pastures, often in the shade of established *E. cyclocarpum*. In Bagaces, there are no clusters of adult trees and only a few pairs of younger adult trees, perhaps because sites suitable for recruitment were generally small and evenly spaced (i.e., backyards and vacant lots). Generally, most of the neighboring pairs of young adult trees are in different pastures and are separated from one another by one or more fences and a road.

Although sampling was not exhaustive at each site, we sampled most (85% BGS–100% SR, Table 1) of the adult trees within each site, permanently tagged them, and recorded their geographic location with GPS. Within clusters, trees were mapped relative to each other with a compass and range finder. We constructed a matrix of pairwise geographic distances among trees within each site and used it to characterize their spatial distribution by determining distance to the nearest neighbor and average distances among the 5 and 10 nearest trees. We used these measurements as a proxy for tree density because of the strongly patchy distribution of trees within PV, SR, and SLM.

Lab Analyses

We used starch-gel electrophoresis to determine allozyme diversity and the multilocus genotype of each tree within the 4 study sites. We extracted enzymes by grinding fresh seedling leaf tissue with a mortar and pestle and adding the extraction buffer of Wendel and Parks (1982) to solubilize and stabilize enzymes. The extract was absorbed onto chromatography paper wicks and stored at −70 °C until electrophoretic analyses were conducted. We employed 4 gel-electrode buffer combinations to resolve 14 putative polymorphic loci on 11% starch gels: Buffer 4: IDH, PGM-1, PGM-2; Buffer 6: DIA, FE-2, PGI; Buffer 8: AAT-1, CE-1; Buffer 9: MDH-1, MDH-2, ACO-1, ACO-2, UGPP-1, UGPP-2. Stain recipes for AAT and DIA follow Cheliak and Pitel (1984), UGPP from Manchenko (1994); all other stains follow Soltis et al. (1983). Polymorphic loci possessed 2–5 alleles.

Data Analyses

Genetic Characterization

We inferred the multilocus genotype of each adult by analyzing genotypes of a minimum of one seed from each of 16 fruits from each maternal tree. Because we utilize the 4 study sites for a long-term study of mating patterns (Hamrick JL, in preparation), genotypes of most adults are based on much larger progeny arrays. *Enterolobium cyclocarpum* disperses its pollen in polyads of 32 pollen grains, resulting in correlated paternity among all seeds within each fruit. Only approximately 0.2% of the fruits have more than one pollen donor (Hamrick JL, personal observation). We used 200 full-sib progeny arrays (i.e., seeds within a fruit) to calculate correlation coefficients (described below). This value, obtained from known full-sib progeny, allows us to translate correlation coefficients obtained from spatial autocorrelation analyses (ranging from −1 to +1) into biological interpretations of average "relationship coefficients." The correlation coefficient for these full-sib sets ($r = 0.473$) was very close to expectation ($r = 0.500$), so to a first approximation, autocorrelations can be viewed as relationship coefficients.

Genetic Structure Analyses

We assessed genetic diversity within each population and the divergence among populations with several standard measures; the proportion of polymorphic loci (P), the number of alleles per polymorphic locus (AP), expected heterozygosity (H_e), inbreeding coefficients (F_{IS}) for each locus, and the average multilocus inbreeding coefficient for each population. To compute F_{ST}, we used an analysis of molecular variance (Excoffier et al. 1992) to partition genetic variation among populations, among individuals within populations, and alleles within individuals, an approach analogous to that of Weir and Cockerham (1984), but using significance tests computed via random permutation ($n = 1000$), rather than bootstrapping across loci. We estimated these standard population genetic measures to evaluate overall levels of genetic variation and its distribution within and among study sites.

Spatial Autocorrelation

To assess SGS within populations, we performed an autocorrelation analysis with GENALEX version 6.1 (Peakall and Smouse 2006). Analyses were based on pairwise genetic distance measures of genetic similarity for diploid individuals and codominant markers (Peakall et al. 1995; Smouse and Peakall 1999). We used multilocus assessments to improve replication for a pair of individuals and provide a more precise analysis of genetic pattern (Excoffier et al. 1992; Peakall et al. 1995; Smouse and Peakall 1999; Epperson 2004). Smouse and Peakall (1999) showed that one could begin with an $N \times N$ pairwise interindividual distance matrix $\mathrm{D} = \{d^2_{gh}\}$ and could construct a hyperdimensional (multilocus) covariance matrix (Gower 1966), from which it was possible to derive a spatial genetic autocorrelation analysis for individuals. We updated that earlier approach to a multiple-population analysis that allows us to gauge the degree of homogeneity/heterogeneity of SGS within separate populations (Smouse et al. 2008).

One can plot genetic affinity against the logarithm of spatial separation, and the decay at evolutionary equilibrium is expected to be linear, beyond some minimum "proximal" distance (Rousset 2000). The equilibrium assumption is almost surely not credible in heavily disturbed habitats, and the most proximal distance class is where we would expect to see the highest relatedness, but we nevertheless found it useful to define linear distance classes on a logarithmic scale. An important consideration for spatial autocorrelation analysis is the choice of distance classes because this choice can influence the outcome and interpretation (Peakall et al. 2003; Vekemans and Hardy 2004). In our analyses, we

adopted distance classes that allowed relatively even sample size per distance class and comparability across localities, as outlined below. We also tested other distance class options and found they revealed similar patterns. We used the same distance classes for all 4 locations: Class-1 (1–64 m), Class-2 (65–128 m), Class-3 (129–256 m), Class-4 (257–512 m), Class-5 (513–1024 m), Class-6 (1025–2048 m), and Class-7 (2049–4096 m). The larger (PV and SLM) sites have additional distance classes, but our analyses indicated that the pattern had "played itself out" within 4 km. Thus, we present the results up to 4 km at all 4 sites. The choice of these distance classes resulted in low numbers of pairs at the 2 shortest distance classes (0–64 m and 65–128 m) for the 3 low-density sites (PV, SR, and BGS), reducing our ability to demonstrate significant differences between correlograms from those sites. Two steps could help rectify this situation and increase the statistical robustness of our analyses. First, we could increase the proportion of trees sampled at PV and BGS (Table 1). However, this would have had little impact on the shortest distance classes because the mean distance between trees within these 2 sites is much larger than 128 m. Also, the low number of pairs within these distance classes is due to the low number (PV and SR) or lack of (BGS) clusters in these 3 sites. Second, we could have pooled the pairs into a single 0–128 m distance class. We chose not to use this approach as it would mask relatedness of near neighbors and would compromise our ability to compare these 3 sites with SLM.

As described in more detail in Smouse et al. (2008), we conducted a separate autocorrelation analysis for each of the 4 locations and tested correlation values with permutational procedures (Smouse and Peakall 1999). Using the tail probabilities (P values) for each of 7 distance classes ($h = 1, \ldots, 7$), we computed Fisher's combined probability criterion Ω as a gauge of the departure of the entire correlogram from the null hypothesis of no spatial structure at any distance,

$$\Omega = -2 \bullet \sum_{h=1}^{7} \log_e(p_h), \qquad (1)$$

and evaluated Ω with bootstrapping (Equation 1). Finally, we explored the question of whether the 4 sites exhibited similar or divergent SGS patterns, using the methods in Smouse et al. (2008) and GENALEX 6.1. To address the issue of unequal sample sizes among the 4 sites, we subdivided SLM into 4 subplots, each containing numbers of trees similar to the other 3 sites but found that this subdivision did not affect the outcome of the analyses (results not shown). These new procedures are freely available in GENALEX 6.2 (Available from the Australian National University, Canberra, Australia. http://www.anu.edu.au/BoZo/GenAlEx/).

Results

Genetic Variation within and Population Divergence among Populations

The average proportion of polymorphic loci within populations (P) was 85.7% (range: 78.6–100%; Table 2).

Table 2. Genetic diversity within 4 *Enterolobium cyclocarpum* study sites in Guanacaste Province, Costa Rica

Location	P (%)	AP	H_e	F_{IS}
PV	100	2.43	0.218	−0.041[a]
SR	78.6	2.14	0.212	−0.039
SLM	85.7	2.29	0.217	−0.094
BGS	78.6	2.29	0.247	−0.107
Mean	85.7	2.29	0.220	−0.070
Total	100	2.79	0.222	—

P, % polymorphic loci; AP, number of alleles per polymorphic locus; H_e, expected heterozygosity; F_{IS}, departure from panmixia within populations.
[a] All F_{IS} values were not significantly different from random expectations.

For the 14 polymorphic loci, we observed 39 alleles, of which 17 were rare in at least one population (frequency <0.05); 7 alleles were restricted to one population. Allele frequencies are available from J.L.H. on request. Expected heterozygosity (H_e) within populations ranged from 0.212 to 0.247 (Table 2). Mean F_{ST} is very small (0.009), though significant ($P < 0.01$), due to large sample sizes. Fixation indices (F_{IS}) were predominantly negative for individual loci, ranging from −0.341 to 0.262, with overall multilocus values ranging from −0.039 to −0.107, indicative of a nonsignificant excess of heterozygous adults in all 4 sites (Table 2).

Spatial Distribution of Trees

The 4 sites varied in the spatial distribution of *E. cyclocarpum*. The continuous PV forest had the lowest density of adult trees, indicated by the greatest average distances to the nearest neighbor (247 m) and to the 5 and 10 nearest neighbors (703 m and 1329 m, respectively; Table 3). The urban site (BGS) had the next lowest density (193 m, 412 m, and 589 m, respectively). The 2 pasture sites (SR and SLM) had higher densities and closer spacings than PV or BGS. Distance to the nearest neighbor at SR was twice that at SLM (105 m and 52 m, respectively). The pasture sites also had more and denser clusters of smaller adult trees, as measured by average distance between the 5 and 10 nearest neighbors (Table 3).

Spatial Genetic Structure

Patterns of SGS within populations vary among the 4 sites for the shortest distance class. We found significant SGS in 3 populations (PV, SR, and SLM), with a general trend of declining relatedness among near neighboring trees in the order PV > SR > SLM > BGS (Table 4, Figure 2). Sites also differed in the spatial scale over which autocorrelation was significantly greater than zero. In PV, relatedness was highest ($r = 0.321$) in the first distance class and remained positive (although not statistically different from zero) at all distances up to 1 Km (Figure 2a). Relative to PV, we detected lower autocorrelation in both pasture populations (SR and SLM, Figure 2b,c) and no spatial autocorrelation in the urban population (BGS, Figure 2d). Pasture populations generally displayed declining SGS to 500 m; in SR,

Table 3. Spatial distribution of *Enterolobium cyclocarpum* trees within the 4 study sites

	Distance to the nearest tree (m)				Distance to the nearest 5 trees (m)				Distance to the nearest 10 trees (m)			
Site	PV	SR	SLM	BGS	PV	SR	SLM	BGS	PV	SR	SLM	BGS
Mean	247	105	52	193	703	330	108	412	1329	490	162	589
Median	82	70	31	111	412	191	88	308	1005	268	148	480
Range	18–2297	11–586	2–635	26–1220	154–3424	48–794	11–843	136–1377	494–5544	122–1354	24–985	253–1648

significant autocorrelation was observed in the first distance class as well as around 250 m, whereas in SLM, autocorrelation declined gradually and remained significant to 500 m (Figure 2b). Although the mean correlation coefficient in the first distance class of SR (0.165) was higher than that of SLM (0.064), due to the small number of pairs in SR, this difference was not significant ($P = 0.294$). In BGS, the correlation among neighboring trees was negative, though nonsignificant, and oscillated around zero throughout the sampled area, again a consequence of the small number of pairwise comparisons in the first distance class. The small sample size is also reflected in stochastic variation that is quite evident in BGS and SR.

Test of Heterogeneous SGS among Populations

Overall, correlograms for the 4 sites were not statistically heterogeneous, but the heterogeneity test did result in statistically significant differences for 2 pairwise comparisons (Table 5) for the first distance class [1–64 m]: PV vs. BGS [$P < 0.008$], and PV vs. SLM ($P < 0.007$). A significant difference also occurred between SLM and SR ($P < 0.034$) for the third distance class (129–256 m). With 6 pairwise contrasts for each of 7 distance classes, one might expect 2 significant tests at the $\alpha = 0.05$ level, but at $\alpha = 0.01$, we would expect less than one. Even if we attribute the third distance class result for SLM and SR to the vagaries of sampling, the significant first distance class results are compelling. Departures from a homogeneous spatial pattern are most likely to be seen in the shortest distance class, in terms of the small-scale impact of historically recent changes in seed dispersal by livestock and humans, compounded with restrictions imposed by permissive "safe site" survival conditions for new recruits. Over a longer time period, the altered isolation by distance pattern might be expected to "ripple outward," probably accentuated by pollen flow in future generations, but there has not yet been time for that process to play out, except at Bagaces, where human-mediated disturbance has a longer history, and where there is no SGS.

Table 4. Single distance-class evaluation of the null hypothesis of no autocorrelation, with number of pairs (n) single class autocorrelation r-estimates, P values, as well as multiclass test criteria (Ω), sample sizes (N) and P values for: 1) PV National Park; 2) SR; 3) SLM; 4) BGS

		Location of study			
Distance class interval (m)	Construct values	PV	SR	SLM	BGS
0–64	Number of pairs (n)	17	14	232	8
	r-estimate	+0.321	+0.165	+0.064	−0.078
	Tail probability	0.001*	0.023*	0.003*	0.749
65–128	Number of pairs (n)	17	17	313	7
	r-estimate	+0.087	+0.051	+0.053	−0.032
	Tail probability	0.147	0.256	0.003*	0.607
129–256	Number of pairs (n)	30	56	830	33
	r-estimate	+0.039	+0.143	+0.031	+0.073
	Tail probability	0.280	0.001*	0.005*	0.078
257–512	Number of pairs (n)	45	88	2475	88
	r-estimate	+0.043	+0.007	+0.005	−0.031
	Tail probability	0.188	0.421	0.213	0.761
513–1024	Number of pairs (n)	89	101	7275	222
	r-estimate	+0.039	−0.017	+0.001	−0.008
	Tail probability	0.124	0.755	0.372	0.702
1025–2048	Number of pairs (n)	254	160	12774	460
	r-estimate	−0.013	+0.001	−0.002	+0.009
	Tail probability	0.754	0.466	0.831	0.174
2049–4096	Number of pairs (n)	280	369	6510	259
	r-estimate	+0.010	−0.029	−0.011	−0.002
	Tail probability	0.272	0.985	1.000	0.592
Multiclass comparison	Sample size (N)	67	45	251	47
	Fisher's (Ω)	33.11	38.39	56.26	20.28
	Overall P value	0.009*	0.004*	0.001*	0.139

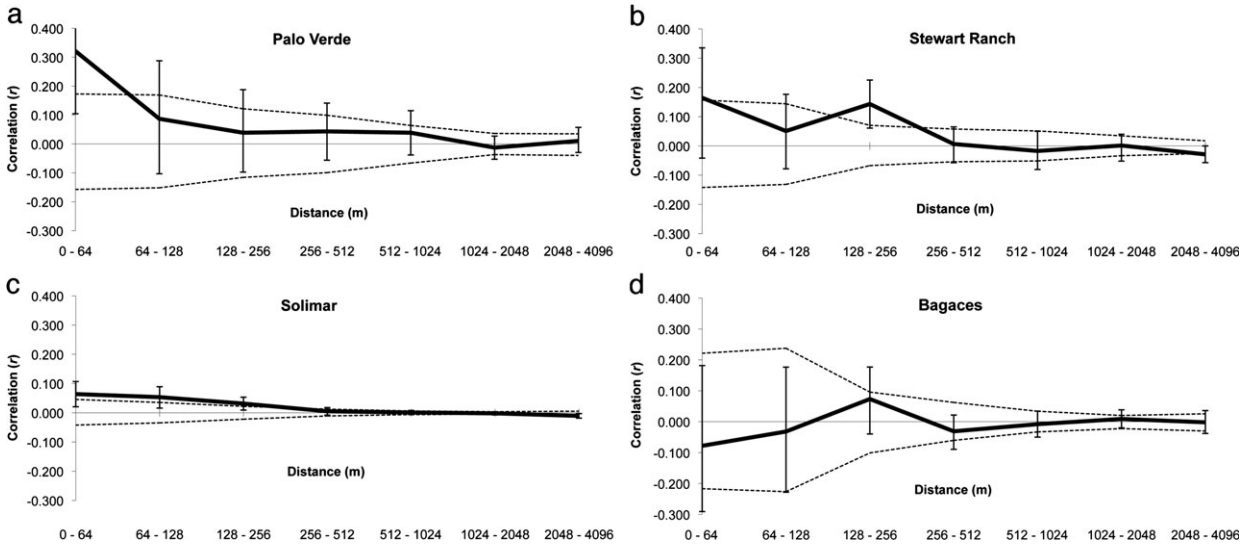

Figure 2. Spatial genetic autocorrelograms showing average correlation coefficients between pairs of individuals, plotted against geographic distances, subdivided into 7 distance classes. Dashed lines represent 95% confidence bands under the null hypotheses that genotypes are randomly distributed across the landscape. Error bars represent 95% confidence intervals around each mean correlation coefficient. (**a**) PV National Park; (**b**) SR; (**c**) SLM; (**d**) BGS.

Discussion

Divergence among and Variation within Populations

Consistent with comparable surveys of animal-pollinated tropical trees (Hamrick and Godt 1989; Hamrick 1994) and with a study of *E. cyclocarpum* in Costa Rica (Rocha and Lobo 1996), most of the genetic diversity resides within populations, rather than among geographically distant sites. Low genetic divergence among these 4 populations is consistent with *E. cyclocarpum*'s predominantly outcrossing mating system (Rocha and Aguilar 2001) and its potential for long-distance pollen movement (~3 km, Hamrick JL, unpublished data) via nocturnal moth pollinators. In many areas within Guanacaste Province, individuals are rarely separated by more than a kilometer, a distance that is within the documented range of pollen movement (Apsit et al. 2001). Furthermore, in highly disturbed landscapes, semi-isolated trees may serve as "stepping stones" or "bridges" that facilitate connectivity by pollen-mediated gene flow between otherwise fragmented populations (Hamrick 1994; White et al. 2002; Fuchs E and Hamrick JL in preparation). Negative F_{IS} values indicate that adult *E. cyclocarpum* are slightly more heterozygous than Hardy–Weinberg expectations. This trend has been observed in other tree species (e.g., Strauss 1986; Alvarez-Buylla and Garay 1994; Hufford and Hamrick 2003).

Table 5. Probability of heterogeneity of r values for the first (1–64 m) and third (129–256 m) distance classes for all pairs of the 4 study sites; *P* values for the first distance are presented above the diagonal; *P* values for the third distance class are presented below the diagonal

First	PV	SR	SLM	BGS
PV	—	0.204	**0.007**	**0.008**
SR	0.157	—	0.294	0.124
SLM	0.901	**0.034**	—	0.255
BGS	0.676	0.345	0.472	—

Significant comparisons are underlined and in boldface.

SGS within Populations

Tropical tree species tend to have spatially clumped distributions, in part, due to limited seed dispersal (Jones et al. 2005). Theoretical models (e.g., IBD) predict high genetic relatedness among trees in close proximity under locally restricted seed dispersal, particularly in situations with low adult densities. The same models predict an absence of SGS when seed-mediated gene flow is extensive, relative to distances separating reproductive trees, because overlapping seed shadows lower SGS. These IBD models usually assume that seed dispersal occurs concentrically around maternal individuals, with fewer seeds deposited at increasing distances. In reality, however, progeny recruitment may only be possible in patchily distributed safe sites, located at some distance from maternal individuals (e.g., treefall gaps in continuous forest). Such recruitment sites may receive progeny from several maternal individuals, depending on each individual's fecundity and dispersal kernel. As a result, the genetic composition of established progeny may be quite variable among sites, depending on the number and proximity of contributing maternal trees (e.g., Jones et al. 2006; Jordano et al. 2007). Disturbed landscapes, such as managed ranchland, may provide an extreme example of patchily distributed recruitment sites because germinating seedlings are protected from grazing (i.e., inaccessible stream banks, rocky outcrops, and fenced roadsides, etc.).

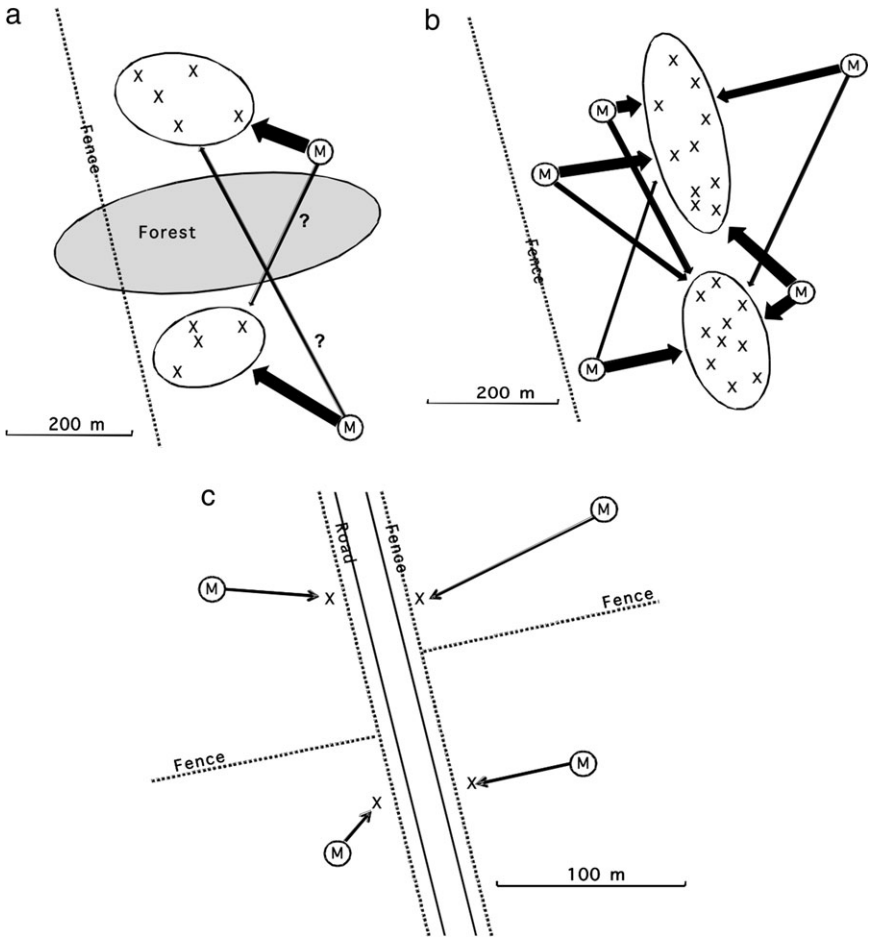

Figure 3. Schematic illustrations of seed dispersal and recruitment patterns for the 4 study sites. (**a**) Recruitment patterns in abandoned pastures (PV); (**b**) Recruitment patterns in contemporary pastures, (SR and SLM); (**c**) Patterns of recruitment at BGS. The widths of the arrows indicate the relative contribution of each maternal tree (M). An "x" indicates a more recent recruitment.

The relative proportion of surviving progeny contributed by each maternal individual should therefore be a function of its distance to the safe site(s) and its fecundity, relative to the distance and fecundity of other maternal trees.

The strength of SGS at PV, SR, and SLM is consistent with the density of older trees at each site and with the number and distribution of clusters of smaller (probably younger) trees (Figure 3a,b). The small clusters (2–6 individuals) at PV are located in previous pastures, contain relatively younger trees, and are separated from other clusters by several hundred meters of primary forest or more advanced second-growth forests (Figure 3a). *Enterolobium cyclocarpum* in these clusters probably established after PV became a national park in the late 1970s. The low densities and scattered distribution of older *E. cyclocarpum* at PV, coupled with older forests serving as partial barriers to cattle dispersal, would limit the number of maternal individuals contributing progeny to each recruitment site. On average, observed correlations for the first distance class ($r = 0.321$) are consistent with a single maternal individual contributing progeny to each cluster. At SLM (Figure 3b) there are older, larger trees in the pastures that almost certainly predate forest clearing (1955), with the more numerous smaller trees found primarily along seasonally dry ravines. With higher densities of older trees at SLM (3–8 per pasture), seeds from several adults could have been dispersed by cattle along the linear ravines. Water movement within the ravines during periods of heavy rainfall could have further mixed successful recruits from different maternal plants. At SR, on the other hand, age structure is not as clear (Figure 3b). Streamside trees are at least 30 years older than those at SLM, due to this site being cleared for pastures in the 1920s and 1930s. There are also fewer (1–3) adults in open pastures at SR, which is consistent with higher first distance class correlations than at SLM, though the difference is not significant. Significant r values at SR for the 1–64 m and 128–256 m distance classes are probably due to separate clusters of trees within a pasture that share the same maternal parents (Figure 3b). Levels of relatedness at PV, SR, and SLM depend primarily on the number of older trees that disperse seeds into each location's recruitment sites. Dispersal distance per se is probably not an issue at SLM and SR because pastures (<100 hectares) are

easily crossed by cattle within few minutes. At PV, with areas of primary forest constraining cattle movements, dispersal limitations may have played a more prominent role.

BGS is unique in several ways. First, it is the most highly disturbed landscape, consisting of a mosaic of established urban development, newly developed urban sites, roadsides, and heavily grazed pastures. It also has a relatively low density of *E. cyclocarpum*, and there are no clusters of smaller individuals in the shorter distance classes (mean and median distance between nearest neighbors of 193 m and 111 m; Table 2). This scattered distribution of individuals is probably due to the limited number and small size of recruitment sites available in older urban settings. Also, volunteer seedlings and saplings are sometimes cut by homeowners (Hamrick JL, personal observation) or are intentionally planted for shade. Such human activities may have prevented the clusters of related individuals that produce the higher levels of genetic relatedness observed in the other 3 sites. Within BGS, 5 of 8 pairs within the 64 m distance class consist of individuals growing on opposite sides of roads, separated by the roadway and 2 fences (Figure 3c). As a result, cattle movement is greatly restricted, relative to SR and SLM, and it is unlikely that neighboring pairs would be siblings. Furthermore, we (Hamrick JL, personal observation) have never observed pods from trees close to the roadsides being passively dispersed across roadways into nearby pastures (Figure 3c).

Our finding of SGS that declines with increasing distance is consistent with other studies of tree species (e.g., reviewed in Hardy et al. 2006). Most published studies of tropical trees report minimal SGS within continuous, closed canopy forests. Lowe et al. (2003) reported uniform patterns of SGS up to 100 m for multiple plots in Costa Rican populations of *Swietenia macrophylla*. Dutech et al. (2002) found that kinship coefficients in a *Vouacapoua americana* population were significantly higher than expected over distances less than 100 m but that low kinship coefficients indicated that progeny of several adults were represented within any one area. Similarly, Hardesty et al. (2005) found weak SGS for a *Simarouba amara* population in Panama. Other studies have indicated strong genetic structure for saplings of 2 wind-dispersed canopy trees (*Platypodium elegans* and *Alseis blackiana*) but weaker SGS for saplings of the much denser bird-dispersed treelet, *Swartzia simplex* (Hamrick et al. 1993; Hamrick and Nason 1996). Weak or nonexistent SGS is typically explained by long-distance seed dispersal, seed-shadow overlap, and/or extensive pollen flow.

Previous studies have emphasized the interaction of seed dispersal distances, adult tree densities, and mating patterns in interpreting the levels and distribution of observed SGS (Jones et al. 2006). They generally assume that established progeny are centered around the adult, but there are many situations in both natural and disturbed landscapes where this is not a realistic assumption (Dyer 2007; Holderegger and Wagner 2008). Our results indicate that an additional factor must be considered in the interpretation of SGS patterns; the distribution of sites where progeny can successfully establish. If these sites are rare and/or patchily distributed, the spatial distribution of adults, relative to the recruitment sites, must also be considered. If there are several trees whose dispersal kernels allow their progeny to reach these sites, levels of relatedness among individuals within them would be low (i.e., SLM). If, on the other hand, only one or a few adults are available, relatedness within recruitment sites would be higher (i.e., PV). The former scenario is analogous to the migrant pool model developed for landscapes with extinction–recolonization dynamics (Slatkin 1977, Hamrick and Nason 1996), whereas the latter situation is more analogous to a propagule pool model.

Our results indicate that genetic structure within populations of *E. cyclocarpum* is significantly influenced by both past and current human activities. Clearing continuous forests for pastureland and urban development produced recruitment sites for *E. cyclocarpum*, although such sites may be few and patchily distributed. The number of these sites, their spatial relationship to maternal trees, and the number of available reproductive adults influence levels of genetic relatedness among trees within recruitment sites. Seed dispersal distances and the availability of recruitment sites are currently impacted by the location of man-made barriers (fences, roads, ownership patterns, etc.) that limit movement of dispersal vectors (i.e., cattle and horses). In our study areas, such barriers limit the number of adults that contribute seed to an individual recruitment site, consequently increasing genetic relatedness over relatively short distances. These results clearly illustrate that interpretations of SGS should take into account the disturbance history of the site as well as the reproductive biology of the species in question (Double et al. 2005; Dyer 2007) and should not be overly dependent on continuous mathematical models of seed dispersal.

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