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Genetic Diversity in the Endangered Tropical Tree, *Guaiacum sanctum* (Zygophyllaceae)

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Abstract

Fragmentation of tropical forests has changed continuous tropical landscapes into a network of poorly connected fragments, reducing population sizes and potentially increasing genetic isolation. This study quantifies genetic diversity within and among the 7 extant populations of the endangered tropical tree *Guaiacum sanctum* in Costa Rica. We describe how genetic diversity differs between populations representing continuous and fragmented habitats. Allozyme analyses were conducted on adult samples from populations representing 2 geographic regions in northwestern Costa Rica. We found high levels of genetic diversity within the species ($H_{es} = 0.329$) and significant but relatively small differences in allele frequencies among populations ($G_{ST} = 0.101$) and between regions ($G_{ST} = 0.053$). We found no differences in genetic diversity between fragmented and continuous populations. Evidence for significant isolation-by-distance (IBD) was seen only when all populations were analyzed. If populations within the 2 regions were analyzed separately, IBD ceased to be significant. Our results suggest that the patterns of genetic diversity observed for *G. sanctum* may be caused by the ancient separation of populations into 2 disjunct geographic regions with extensive historical rates of gene flow among populations within each region. Recent forest fragmentation has not yet affected patterns of genetic diversity in this species.

Key words: *allozymes, forest fragmentation, genetic diversity, genetic structure, tropical dry forests,* Zygophyllaceae

Endangered species are often characterized by small population sizes, limited geographic distributions, and/or specific habitat requirements (Soulé 1986). Many endangered species have also suffered reductions in their population sizes due to exploitation and habitat reduction and fragmentation. Such changes can have both demographic and genetic consequences. Loss of genetic variation within populations of an endangered species may affect its ability to adapt to changing environmental conditions (Huenneke 1991). As a result, detailed analyses of the levels and spatial distribution of genetic diversity are important for the development of effective conservation strategies and management practices for endangered species (Hedrick and Miller 1992). Populations with higher levels of genetic diversity could serve as potential sources of propagules and reservoirs of genetic variation and, thus, are important conservation targets. Conversely, populations containing little genetic variability may be candidates for the introduction of novel genetic variation (Lande and Barrowclough 1987; Holsinger and Gottlieb 1991).

Fragmentation of tropical forests by deforestation and human expansion has changed once continuous tropical landscapes into a network of poorly connected fragments surrounded by agricultural fields, urban settlements, or abandoned sites. The immediate effects of fragmentation are a reduction of population sizes, local population extinction, and increased genetic isolation (Ellstrand and Elam 1993; Young et al. 1996). Reduction in population size may also lead to a loss of genetic diversity and the fixation of potentially deleterious alleles due to increased inbreeding (Alvarez-Buylla et al. 1996; Hedrick and Kalinowski 2000). In combination, these effects may increase the risk of extinction from stochastic and catastrophic events.

Tropical dry forests are threatened by deforestation and fragmentation and are considered among the most endangered tropical ecosystems (Janzen 1988). Tropical tree species are often more susceptible to forest fragmentation due to their long generation times, low densities, and commercial importance (Cascante et al. 2002). Despite their significance, only a few studies have quantified the effects of forest fragmentation on the genetic diversity of these species (Lowe et al. 2005, and references therein). Studies conducted on fragmented

populations of ecologically important tree species such as *Pachira quinata*, *Samanea saman*, and *Symphonia globulifera* (Aldrich and Hamrick 1998; Cascante et al. 2002; Fuchs et al. 2003) have shown that fragmentation alters pollinator visitation rates and the genetic composition of progeny.

Guaiacum sanctum L. (Zygophyllaceae) has a wide geographic distribution but is often locally scarce and is considered endangered due to overexploitation. Also known as Lignumvitae, *G. sanctum* has one of the densest woods known and was commonly harvested to manufacture propeller shafts for steam ships. Currently, even under the protection of the CITES convention (CITES 2000), *G. sanctum* is still used as an ornamental wood and for handicrafts. Additionally, *G. sanctum*'s bark produces a resin called *guaiacin*, which possesses antibiotic properties and has been used since the 1500s to cure sexually transmitted diseases, such as gonorrhea and syphilis (Voeks 2004). Its extensive use has led to a significant reduction in population sizes, and presently, large continuous populations are found almost exclusively within protected areas. Currently, there is little knowledge of the status of *G. sanctum* populations throughout its distribution. The continuous exploitation of this species requires an accurate description of genetic diversity to identify populations of special conservation concern.

In Costa Rica, *G. sanctum* is locally rare and is only found in the tropical dry forests of Guanacaste province in northwestern Costa Rica. To our knowledge, there are only a few remnant populations, some are protected within national parks; others are located within fragmented or disturbed habitats under private ownership. These populations are of special interest because they comprise the southernmost distribution of the species (Jiménez 1993). It is generally accepted that these peripheral populations are at greater risk due to smaller population sizes and reduced potential for gene flow, making them prone to extinction through stochastic processes (Lesica and Allendorf 1995).

This study quantifies genetic diversity within the 7 extant *G. sanctum* populations in Costa Rica and describes how this diversity is structured among populations. We also describe how genetic diversity differs between populations located within relatively continuous national park habitats and populations located in fragmented sites within agricultural/urban settings. Because populations of *G. sanctum* in Costa Rica are at the southern extreme of the species' range and deforestation has fragmented its populations, we expect to observe low levels of genetic diversity within its remaining populations. We also hypothesized that due to restricted gene flow, genetic differentiation among populations should be correlated with geographic distance as suggested by the isolation-by-distance (IBD) model (Rousset 1997). Finally, trees within disturbed habitats should have less genetic diversity and more evidence of inbreeding than populations in more protected habitats, as a direct consequence of smaller population sizes and reduced gene flow.

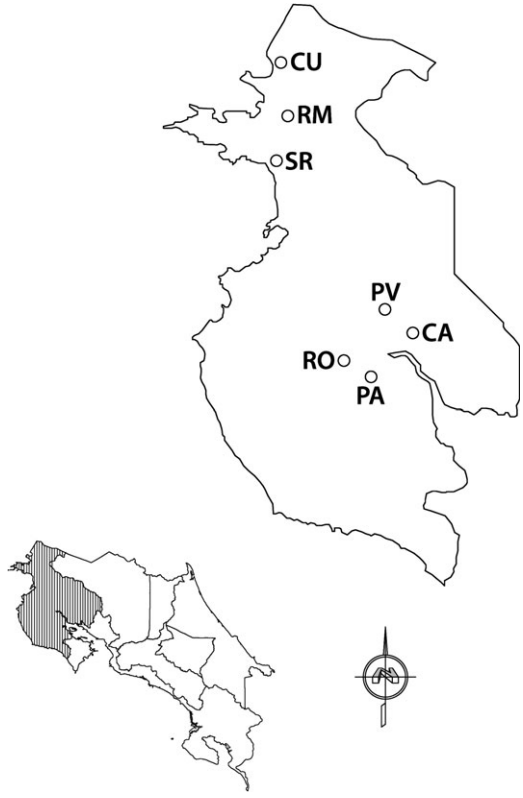

Figure 1. Location of sampled *Guaiacum sanctum* populations in Costa Rica. Population codes are shown in Table 1.

Materials and Methods

Study Species

Guaiacum sanctum L. (Sapindales: Zygophyllaceae) is a slow growing tropical and subtropical dry-forest tree, distributed from southern Central America to northern Mexico and Florida and throughout the Greater Antilles (Holdridge and Poveda 1975). Trees grow to 10–15 meters and are usually found in the subcanopy of tropical dry forests (Gonzalez-Rivas et al. 2006). Trees are evergreen with opposite compound leaves about 7–12 cm long, with oblong to lanceolate leaflets. *Guaiacum sanctum* has conspicuous perfect blue–purple flowers that open between March and July. In Costa Rica, the most important visitors are introduced Africanized bees, but native wasps and solitary bees have also been observed visiting flowers (Jiménez 1993). Fruiting occurs from May to November; fruits are 5-lobed ovoid capsules, with one arilloid seed per locule. Seeds are dark brown to black, oblong and are 1–2 cm long. Mature seeds are covered by a bright red aril that attracts birds, the main dispersers (Wendelken and Martin 1987).

Study Sites

We located all extant populations of *G. sanctum* in northwestern Costa Rica (Figure 1 and Table 1). Populations were separated into 2 regions: Northern populations: Santa

Table 1 Geographic location, sample size, and Habitat type for 7 populations of *Guaiacum sanctum* in Costa Rica

Site	Code	*N*	Habitat type	Region	Latitude (N)	Longitude (W)
Cuajiniquil	CU	32	Fragmented	North	11°00′	85°41′
RaboMico	RM	30	Fragmented	North	10°55′	85°38′
Santa Rosa	SR	40	Continuous	North	10°18′	85°39′
Rosario	RO	35	Continuous	South	10°16′	85°23′
PozoAgua	PA	27	Fragmented	South	10°17′	85°20′
Palo Verde	PV	30	Continuous	South	10°21′	85°20′
Catalina	CA	47	Continuous	South	10°20′	85°15′

N, number of individuals sampled.

Rosa (SR), Cuajiniquil (CU), and RaboMico (RM) and are separated by at least 70 km from the 4 southern populations; Rosario (RO), Pozo Agua (PA), Palo Verde (PV), and Catalina (CA) (Figure 1). Populations were also grouped into continuous and disturbed (fragmented) habitats. Populations PV, CA, SR, and RO are located within national parks or forest reserves and have been protected since the late 1960s or 1970s. Remaining populations are located within pastures, fields, or are part of living fences or gardens. Within each population, we located, marked, and mapped all adults with a Global Positioning System. During Spring, 2006, 2 mature leaves were collected from each adult, placed in liquid nitrogen, and transported to the University of Georgia for allozyme analysis. Between 27 and 47 individuals were sampled from each population (Table 1).

Allozyme Electrophoresis

Leaves were crushed in chilled mortars and pestles with liquid nitrogen in a phosphate buffer according to Wendel and Weeden (1989). Protein extracts were absorbed onto 4 × 6–mm paper wicks (Whatmann 3M chromatography paper) and stored at −70 °C until needed for electrophoretic analyses. We performed starch-gel electrophoresis (10%) on 5 buffer systems. We scored 15 polymorphic loci from 11 enzyme systems. Buffer and stain recipes followed Soltis et al. (1983) except uridine triphosphate (UTP)-glucose-1-phosphate (UGPP) and diaphorase, which were adapted from Manchenko (1994). Buffer system numbers are also derived from Soltis et al. (1983). We resolved triosephosphate isomerase (TPI1, TPI2, EC 5.3.1.1) in system 8; malate dehydrogenase (MDH1, MDH2, EC 1.1.1.37), phosphoglucomutase (PGM, EC 5.4.2.2) and shikimic acid dehydrogenase (SKDH, EC 1.1.1.25) on system 4; aspartate aminotransferase (AAT1, AAT2, EC 2.6.1.1), diaphorase (DIA1, DIA2, EC 1.6.99), and superoxide dismutase (SOD, EC 1.15.1.1) on system 7; and UTP-glucose-1-phosphate (UGPP, EC 2.7.7.9) and isocitric acid dehydrogenase (IDH, EC 1.1.1.42) on a morpholine-citrate buffer. System 6 was used to score phosphoglucoisomerase (PGI, EC 5.3.1.9) and colorimetric esterase (CE, EC 3.1.1.-). Banding patterns were consistent with expectations based on Wendel and Weeden (1989), and we considered a locus polymorphic if at least 2 alleles were observed.

Genetic Analysis

Genetic diversity parameters and allele frequencies were calculated within populations (subscript "p"), at the regional level (subscript "r"), and pooled over all populations (subscript "s"). Genetic diversity was also analyzed by averaging across populations belonging to "continuous" or "fragmented" habitats (subscript "h"). Standard measures of genetic diversity were calculated: proportion of polymorphic loci (%*P*), mean number of alleles per locus (*A*), number of alleles per polymorphic locus (AP), effective number of alleles (A_e), mean observed heterozygosity (H_o) and expected heterozygosity (H_e). These statistics were calculated separately for each locus and averaged over loci. Pooled estimates and means across populations were used to estimate diversity parameters for the different regions (i.e., North and South) and for different habitat types (i.e., Continuous and Fragmented). All calculations were performed using the Lynsprog program created by Loveless MD and Schnabel A (University of Georgia, unpublished program) and GenAlEx 6.1 (Peakall and Smouse 2006).

We used Wright's fixation index (*f*) to measure the deviation of observed heterozygosity from Hardy–Weinberg expectations (Wright 1969). Significance of the *f* values was assessed by 10 000 permutations among loci, using *F*-stat (http://www2.unil.ch/popgen/softwares/fstat.htm). A mean fixation index for the entire species (f_s) was calculated by averaging across loci and populations. We estimated Nei's G_{st} statistic to determine genetic structure among populations.

To assess differences in pooled estimates of genetic diversity (H_e) between regions and between habitat types, we performed a permutation test conducted in R (Ihaka and Gentleman 1996). Differences in genetic diversity between regions or habitats were calculated from 10 000 permutations of individuals among categories. The proportion of values smaller than the calculated difference was used to estimate α (i.e., critical value). Average estimates were compared with a Mann–Whitney test due to small sample sizes.

Genetic structure was also analyzed by means of hierarchical *F*-statistics (Weir and Cockerham 1984) using an analysis of molecular variance (AMOVA) to test differences in allele frequencies among regions, among populations within regions, and within populations. These analyses were performed using Arlequin 3.1 (Excoffier et al. 2005). Differences in allele frequencies were also assessed by AMOVA between fragmented and continuous populations. Statistical significance of *F*-statistics was attained by 30 000 permutations of individuals or populations depending on the level of the analysis.

Spatial Analysis of Genetic Diversity

To test whether population differentiation is caused by dispersal limitation (i.e., IBD), we estimated the correlation between log geographic distance and genetic differentiation $F_{st}/(1 - F_{st})$ between populations as suggested by Rousset (1997). Pairwise values were calculated for all population

Table 2 Genetic diversity estimates for *Guaiacum sanctum* at the species, region, and population levels

	%*P*	*A*	AP	A_e	H_o[a]	H_e	*f*[a]
Overall	100	3.33	3.33	1.63	—	0.329 (0.057)	—
Regions							
North	100	2.93	2.93	1.58	—	0.296 (0.053)	—
Mean	95.6	2.70	2.62	1.55	0.253	0.287 (0.024)	0.094 (0.028)
South	100	3.00	3.00	1.61	—	0.327 (0.049)	—
Mean	93.3	2.64	2.74	1.59	0.254	0.311 (0.020)	0.174 (0.051)
Populations							
CU	93.3	2.67	2.85	1.69	0.284	0.332 (0.056)	0.137 (0.147)
RM	100	2.73	2.73	1.49	0.247	0.280 (0.048)	0.084 (0.107)
SR	93.3	2.47	2.57	1.47	0.227	0.249 (0.054)	0.060 (0.185)
RO	100	2.67	2.67	1.65	0.264	0.335 (0.053)	0.180 (0.382)
PA	100	2.80	2.80	1.69	0.299	0.356 (0.047)	0.230 (0.386)
PV	86.7	2.47	2.69	1.49	0.250	0.286 (0.045)	0.085 (0.162)
CA	86.7	2.60	2.85	1.52	0.204	0.268 (0.060)	0.201 (0.242)
Mean	94.3	2.63	2.74	1.57	0.254 (0.012)	0.301 (0.015)	0.139 (0.025)
Fragmentation							
Continuous	100	2.87	2.87	1.57	—	0.302 (0.051)	—
Mean	91.7	2.55	2.70	1.53	0.236 (0.013)	0.285 (0.018)	0.132 (0.084)
Fragmented	100	3.27	3.27	1.68	—	0.348 (0.051)	—
Mean	97.8	2.73	2.77	1.62	0.277 (0.015)	0.322 (0.022)	0.156 (0.132)

Diversity statistics were also computed for populations in fragmented and continuous habitats. %*P*, percent polymorphic loci; *A*, average number of alleles; AP, alleles per polymorphic locus; A_e, effective number of alleles; H_o, observed heterozygosity; H_e, Hardy–Weinberg equilibrium expected heterozygosity; *f*, inbreeding coefficient. (Standard errors are shown in parenthesis).

[a] Pooled estimates include Wahlund effect and are thus omitted.

pairs, using Arlequin 3.1. A Mantel correlation was performed between the geographic and genetic distance matrices, and its significance was assessed by 10 000 permutations in R. Given the geographic distances between northern and southern populations, we analyzed the effect of region on IBD by means of a partial Mantel test, controlling for populations belonging to the same region. Significance was attained by 10 000 permutations using the Vegan package in the R statistical language.

To determine if genetic differences between populations correspond to geographic distribution patterns, genetic distance (*D*) of Nei (1972) was used to construct an unrooted phenogram with the neighbor joining (NJ) algorithm. Calculations and graphics were produced using the APE (Analysis in Phylogenetics and Evolution) library in R.

Results

We resolved 15 allozyme loci with an average of 3.4 alleles (Table 2). Three loci (TPI2, CE, and IDH) were monomorphic in at least one population. Two loci were highly polymorphic (CE and UGPP) with 5 alleles each, and AAT1 and PGI each had 4 alleles. On average, most loci in the sample had at least 3 alleles ($A = 3.33$; standard deviation = 0.211).

Pooling across populations resulted in all loci being polymorphic (%$P = 100$). Across populations the percentage of polymorphic loci ranged between 87% and 100%, with a mean of 94.3% (Table 2). Two southern populations (RO and PA) had 100% polymorphism, whereas the other 2 southern populations (CA and PV) had the lowest %*P* values (87%). At the regional level, the mean percentage of polymorphic loci was slightly higher in the northern populations than in southern populations. Pooled estimates show no difference among regions, with both having 100% polymorphism. On average, individuals located in fragmented populations had a slightly higher proportion of polymorphic loci compared with populations in relatively undisturbed sites (Table 2).

Across populations, allele number per locus ranged between 2.47 and 2.85. On average, the number of alleles did not differ significantly (Mann–Whitney's $U = 7$, $P = 0.685$) between regions nor did pooled estimates. A similar pattern was observed for number of alleles at polymorphic loci and effective number of alleles. All pooled estimates of allelic diversity were slightly higher in fragmented than in continuous populations (permutation test, $P = 0.057$). Trees in fragmented habitats have on average 2 private alleles, contrasted to 3 in continuous habitats. Averages across populations in fragmented and continuous habitats echo the results for pooled samples.

Observed heterozygosity (H_{op}) across loci ranged between 0.033 and 0.760. The highest average for a population was found in PA ($H_{op} = 0.299$) and the lowest in CA ($H_{op} = 0.204$), both southern populations. Average observed heterozygosity values for populations within regions were not significantly different ($U = 5$; $P = 0.85$). Although populations in fragmented habitats had higher observed values, no statistical differences were found for mean observed heterozygosity across habitat types ($U = 2$; $P = 0.23$).

Genetic diversity (H_e) estimates for each locus ranged between 0.033 and 0.716 (CEF: give loci), the pooled value was $H_{es} = 0.329$, and mean genetic diversity across populations was $H_{ep} = 0.301$. The population with the highest average was PA, whereas SR had the lowest estimate of genetic diversity (Table 2). Across regions, southern populations exhibited higher expected heterozygosity values than northern populations ($U = 1$; $P = 0.042$). No differences were found in H_e values between fragmented and continuous populations for pooled estimates (permutation test; $P = 0.42$) or averages across populations ($U = 3$; $P = 0.4$).

The mean inbreeding coefficient (0.139 ± 0.025) estimated as an average across loci and populations, indicated a significant deficit of heterozygotes (f_s; $P < 0.05$). F_{IS} values ranged between -0.095 and 0.465, with most loci displaying an excess of homozygotes. Unusually, high F_{IS} values (CE: $F_{IS} = 0.465$) were observed in loci with low genetic diversity (i.e., CE = 0.05), if these loci were removed from the analysis, an average heterzygote deficiency was still observed. Fragmented and continuous habitats showed no significant heterozygote deficiency.

Significant heterogeneity in allele frequencies was found across populations ($G_{st} = 0.101$). G_{st} values across loci ranged between 0.016 (CE) and 0.252 (AAT1); loci with low values were characterized by very skewed allele frequencies (frequency of most common allele >0.90). A hierarchical AMOVA revealed significant structure among regions ($\theta_r = 0.070$; $P = 0.022$) and among populations within regions ($\theta_p = 0.064$; $P < 0.001$). Our results showed no significant effect of habitat type on allele frequencies ($\theta_h = -0.014$; $P = 0.7725$).

Populations within regions were separated by an average distance of 11.5 km, but separation in northern populations (16.7 km) was significantly greater than interpopulation distances among southern populations (7.2 km, $U = 2$; $P = 0.033$). A significant IBD pattern was observed when genetic differentiation (i.e., $F_{st}/[1 - F_{st}]$) was correlated with the natural logarithm of pairwise spatial distances among populations ($r = 0.653$; $P = 0.0061$). This correlation was significant only when all populations were analyzed regardless of region. The correlation ceased to be significant, however, when region was taken into account (Mantel–Partial correlation: $r = 0.035$; $P = 0.437$); within regions there is no correlation between genetic differentiation and geographic separation (i.e., no IBD).

The unrooted NJ phenogram created with Nei's distances clearly depicts 2 clades separated by a large distance, corresponding to the northern and southern regions (Figure 2). Within the southern region, 2 smaller clusters correspond to the PV–CA populations, located within 10 km of each other in Palo Verde National Park. The second group consists of RO and PA, located on the Nicoya Peninsula west of the Tempisque River (Figure 1). A comparison of the phenogram (Figure 2) and the geographic position of populations (Figure 1) show a marked correspondence (with the exception of RM) between geographic location and genetic differences. Average genetic identity for populations within a region ($I = 0.963$) was significantly higher than the average identity among populations in different regions ($I = 0.929$; $t = 5.94$, $P < 0.001$).

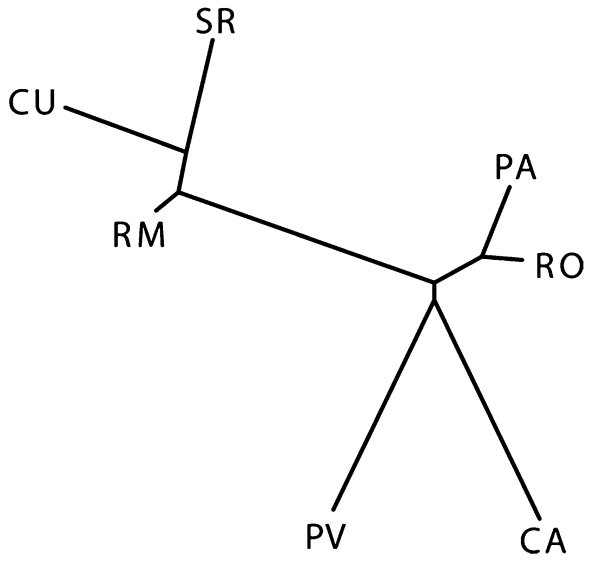

Figure 2. NJ phenogram based on Nei's genetic distance, depicting populations of *Guaiacum sanctum* in Costa Rica. Population codes are shown in Table 1.

Discussion

Genetic Diversity

We observed high levels of genetic diversity for *G. sanctum*, even though these populations are located at the southern margin of its Central American range. All loci were polymorphic in the pooled sample, with only 2 loci monomorphic in some populations. Heterozygosity for the pooled populations ($H_{es} = 0.329$) was higher than the mean for species with similar life-history characteristics. Even though our populations represent a restricted geographic area, *G. sanctum* exceeds the mean values for long-lived tropical woody species ($H_{es} = 0.191$; Hamrick et al. 1992) and for long-lived species with animal-dispersed seeds ($H_{es} = 0.225$; Hamrick and Godt 1996). Although no genetic studies have been conducted on other *Guaiacum* species, genetic diversity estimates for *G. sanctum* are consistent with values for 6 other Zygophyllaceae (species mean $H_{es} = 0.234$) (Cortes and Hunziker 1997; Lia et al. 1999; Ge et al. 2003; Duran et al. 2005; Cuevas et al. 2006). These results contrast markedly with our initial expectation that this endangered, uncommon tree would have low genetic diversity. However, tree species with wide geographic distributions, predominantly outcrossing mating systems and long-distance seed and pollen dispersal generally have high levels of genetic diversity (Hamrick and Murawski 1990; Hamrick et al. 1993). Although *G. sanctum* is locally scarce, it is widely distributed (Holdridge and Poveda 1975) and has the potential for long-distance pollen and seed dispersal (Fuchs EJ, Hamrick JL, unpublished data).

There is a consistent deficiency of heterozygotes, which is usually indicative of some form of inbreeding. Analyses of the mating system in 2 *G. sanctum* populations (PV and CU) show that outcrossing rates were $t_m = 0.72$ and 0.94, respectively, suggesting that *G. sanctum* is a mixed-mating species (Fuchs EJ, Hamrick JL, unpublished data). Additionally, a temporal Wahlund effect may contribute to the observed excess of homozygotes. By using all adults within a population, different reproductive cohorts may be grouped together. Ignoring this temporal subdivision may result in a slight increase in homozygosity.

Genetic Structure

The G_{st} values suggest a low but significant structuring of genetic diversity among populations ($G_{st} = 0.101$; $P < 0.001$). This value is slightly lower than that observed for 26 long-lived tropical woody species ($G_{st} = 0.119$; Hamrick et al. 1992). Because statistics such as G_{st} and AMOVA's θ measure the results of historical levels of gene flow, these results suggest that prior to fragmentation *G. sanctum* experienced high to moderate levels of gene flow.

However, it is likely that *G. sanctum* was never continuously distributed throughout its range in Costa Rica. Currently, its populations occur on thin, xeric soils located on limestone hills, which arose during the early Pleistocene (Burnham and Graham 1999). Based on data from Costa Rica's digital Atlas (Provided by Costa Rica's National Geographic Institute www.mopt.go.cr/ign) only 2 areas have these conditions: the Tempisque River basin, where Palo Verde National Park is located and northwestern Guanacaste province (populations in the northern region). Furthermore, herbarium collections suggest that these 7 populations are the only known sites for *G. sanctum* in Costa Rica.

High levels of historical gene flow between populations within regions seems to be a better explanation for the current patterns of genetic structure in *G. sanctum*. Pollination of *G. sanctum* is performed mostly by bees and wasps, which are capable of transporting pollen over large distances (Janzen 1971; Frankie et al. 1976). We have data from CU that indicates that pollen routinely dispersed more than 4 km (Fuchs EJ, Hamrick JL, unpublished data). Seed dispersal is mostly performed by birds belonging to the Momotidae, Trogonidae, and Tyranidae (Fuchs EJ, personal observation). Populations within regions are separated by less than 20 km, well within the flying capabilities of pollen and seed dispersers (Fleming 1992). Previous work conducted on a population of *G. sanctum* within Palo Verde National Park has shown that seed dispersal over distances of 150 m is commonplace (Fuchs EJ, Hamrick JL, unpublished data). Pairwise F_{st} values among populations within a region (mean = 0.04) suggest that interpopulation migrations may frequently occur. Other studies conducted in tropical dry-forest tree species have shown similar results, with high rates of pollen movement between populations located in fragmented habitats (Apsit et al. 2001; White et al. 2002; Hamrick and Apsit 2004).

More than 50% of the variation among populations is due to regional differences in allele frequencies, indicating that gene flow between regions is more limited than gene flow within regions. The 2 regions are separated by 70 kilometers, probably beyond the normal range of pollinators and seed dispersers. Alternatively, differences between regions may be explained by historical vicariance events. Populations in the northern and southern regions may have been separated since the late Pliocene (Burnham and Graham 1999). This separation, coupled with limited contemporary gene dispersal among regions, may have produced the genetic differences observed. The NJ phenogram (Figure 2) corroborates these conclusions by clustering populations in almost perfect association with their geographic locations. The most evident separation is between northern and southern clades, indicating that although gene flow occurs frequently enough to minimize genetic drift, gene exchange occurs predominantly among populations located within a few kilometers (~10 km) of each other.

Effects of Fragmentation

Guaiacum sanctum located in disturbed or fragmented habitats had very similar levels of genetic diversity to populations in national parks or forest reserves (Table 2). Although this is in contrast to population genetics theory (e.g., Young et al. 1996; Hedrick and Kalinowski 2000), this result should not be surprising because most dry-forest fragmentation has occurred within the lifetime of adult trees (Lowe et al. 2005, and references therein). Although *G. sanctum* has been actively used and extracted due to its valuable wood and medicinal uses since the 1500s (Voeks 2004), fragmentation of tropical dry forests in Costa Rica has primarily occurred in the last 100 years with a drastic increase in the second half of the 20th century (Quesada and Stoner 2003). Because *G. sanctum* is a long-lived species with individuals surviving up to a 1000 years (Wilson and Eisner 1968), it is likely that the adult trees analyzed in this study were alive before most of the habitat fragmentation occurred. Therefore, factors such as genetic drift and inbreeding would not have had enough time since European intervention to have changed genetic diversity within these 7 populations. Habitat fragmentation influences genetic diversity by reducing effective population sizes within populations and reducing gene flow into populations (Frankham 1996). Our results indicate that historically gene flow was probably not limited between populations separated by less than 20 km. The elimination of continuous habitat between populations may alter the behavior of pollen or seed vectors and, thus, modify gene movement between fragments (Hall et al. 1996). Thus, the impacts of forest discontinuity may become more obvious in progeny produced by trees found in low densities in disturbed habitats (Fuchs et al. 2003). Finally, many national parks and forest reserves in the dry forest region of Costa Rica were previously ranches or were logged prior to protection. Populations PV and CA, for example, were subject to selective logging and periodic fires until the 1970s when PV National Park was created. Although harvesting

may have occurred, forest cover was not eliminated. Nevertheless, selective logging in these sites may have reduced allelic diversity somewhat, perhaps explaining the lack of observed differences between continuous and fragmented populations.

Implications for Conservation

Results of the present study can be used to directly infer conservation strategies for *G. sanctum*. Populations RO and PA display somewhat higher levels of genetic diversity, making them appropriate candidates for in situ conservation. Additionally, trees in fragmented habitats within this region are commonly found next to gravel roads, in gardens, agricultural fields, or as part of living fences (Fuchs EJ, personal observation); areas susceptible to further perturbation. Guanacaste province has the only remaining tropical dry forest in Costa Rica, currently estimated to represent less than 0.01% of its original cover (Janzen 1988). This area continues to be threatened due to tourism related developments and agriculture. Guanacaste Province has the second largest rural population growth rate (Estado de la Nación 2005) and one of the highest urbanization rates relative to the rest of Costa Rica. Therefore, extant populations of *G. sanctum* in Guanacaste Province are threatened and should receive high priority for conservation.

Low levels of genetic differentiation among populations have direct implications for ex situ conservation strategies. A large number of individuals may be collected from fewer populations, properly representing the species' genetic diversity. Because populations such as CU and PA have high levels of genetic diversity and are threatened by human activities, seedlings and vegetative clones should be collected from these sites and grown in nurseries or plantations. This may ensure the preservation of important allelic diversity found in these populations.

Finally, because we have documented substantial levels of gene flow over at least 4 km (Fuchs EJ, Hamrick JL, unpublished data), it is likely that pollen immigration can occur into fragmented populations located within 10 km of continuous habitats (Dick 2001; Dick et al. 2003). Trees in fragments and isolated trees may also function as stepping-stones between larger populations and, thus, are important components of the population dynamics of endangered tropical trees (Fuchs EJ, Hamrick JL, unpublished data). Conservation strategies should provide sufficient protection for these trees to ensure their continued presence in disturbed landscapes.

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