

EFFECTS OF POLLINATION BY BATS ON THE MATING SYSTEM OF *CEIBA PENTANDRA* (BOMBACACEAE) POPULATIONS IN TWO TROPICAL LIFE ZONES IN COSTA RICA¹

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The identity and behavior of pollinators are among the main factors that determine the reproductive success and mating system of plants; however, few studies have directly evaluated the relationship between pollinators and the breeding system of the plants they pollinate. It is important to document this relationship because the global decline in pollinators may significantly affect the breeding systems of many animal-pollinated plants, particularly specialized systems. *Ceiba pentandra* is a tropical tree that has chiropterophilic flowers and a variable breeding system throughout its distribution, ranging from fully self-incompatible, to a mixed system with different degrees of selfing. To determine if regional differences in pollinators may result in regional differences in the outcrossing rate of this species, we used systematic observations of pollinator behavior in two tropical life zones and high-resolution genetic analysis of the breeding system of populations from these two regions using microsatellites. We found a predominantly self-compatible system in regions with high pollinator visitation, while in environments with low pollinator visitation rates, *C. pentandra* changed to a mixed mating system with high levels of self-pollination.

Key words: bats; *Ceiba pentandra*; Costa Rica; mating system; microsatellites; pollination.

The identity and behavior of pollinators are among the main factors that determine the reproductive success and mating system of plants (Baker, 1955; Barrett et al., 1996; Herrera et al., 2001). These aspects of the mutualistic relationship between plants and their pollinators may differ among habitats, with subsequent consequences for reproduction and gene flow in plant populations. Most studies of this phenomenon either evaluate the relationship between pollinators and the reproductive success of plants (Aizen and Feinsinger, 1994; Fleming et al., 1994; Sahley, 1996; Slauson, 2000) or examine the evidence of mating systems obtained almost exclusively from genetic markers (Hamrick and Murawski, 1990; Fleming et al., 1994; Chang and Rausher, 1998; Franceschinelli and Kesseli, 1999; Franceschinelli and Bawa, 2000; Campbell et al., 2002; Dutech et al., 2002). Therefore, it is important to conduct studies that directly integrate the relationship between pollinators and plant mating systems.

Evidence from the literature indicates that there is plasticity in the mating system and fitness of plants in response to changes in pollinators. In the absence or loss of pollinators, changes in plant mating systems favor self-pollination. For example, a review that compared island and mainland plant populations demonstrated a predominance of self-compatible plants and a reduction in quantity and diversity of pollinators on islands

(Barrett, 1996). Similarly, lower levels of outcrossing are found in young successional stages and this is attributed to the different foraging patterns of pollinators in young successional stages compared to the older successional stages (Cheptou et al., 2002).

Geographic variation in the presence and abundance of pollinators may also affect the breeding system throughout a species range. Several studies support a trend from specialization to generalization in pollination along the continuum of tropical regions to extratropical deserts (Slauson, 2000; Molina-Freaner et al., 2003; Silva-Montellano and Eguiarte, 2003a, b; but see Ollerton and Crammer, 2002, for opposing view). For example, Silva-Montellano and Eguiarte (2003a) found a significant decline in the number of potential pollinator visits with increasing latitude in *Agave lechugilla*. Similar results were found for the columnar cactus *Pachycercus pringlei* along its geographic range (Molina-Freaner et al., 2003).

Only a few studies have directly evaluated the relationship between pollinators and the breeding system of the plants they pollinate (Barrett, 2003). It is particularly important to document this relationship because the global decline in pollinators (Kearns and Inouye, 1997; Kearns et al., 1998) may significantly affect the breeding systems of many animal-pollinated plants.

Ceiba pentandra is a pan tropical tree that has chiropterophilic flowers; however, the breeding system varies throughout its range. In southeast Asia and Africa it has been described as completely self-compatible (Toxopeus, 1948; Baker, 1955, 1965), whereas in Samoa (Gribel et al., 1999) and in Brazil (Gribel et al., 1999) it has been described as self-incompatible. In Panama, it has been classified as having a mixed breeding system (Murawski and Hamrick, 1992). The variability in the breeding system in *C. pentandra* provides an excellent opportunity to study breeding system plasticity as a function of environmental variables such as pollinator diversity and abundance.

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While studying the effect of forest fragmentation on the reproductive success of several species of Bombacaceae in Costa Rica and Mexico (Stoner et al., 2002; Munguía-Rosas, 2003; Quesada et al., 2003, 2004), we observed marked differences in the number of bat pollinators that arrived at flowers of *C. pentandra* in different life zones. This observation led to the hypothesis that regional differences in bat visitation may result in regional differences in the outcrossing rate of this species. The objective of our study is to test this hypothesis using systematic observations of pollinator behavior in the two regions and high-resolution genetic analysis of the breeding system using microsatellites previously described for this species (Brondani et al., 2003).

MATERIALS AND METHODS

Study sites—This study was conducted in two seasonal tropical forests on the Pacific coast of Costa Rica; they differ in precipitation regime, average temperature, and dry season duration (Lobo et al., 2003). The first site was located on the northern Pacific coast of Costa Rica in the province of Guanacaste (10°45' N, 85°30' W). This protected area is considered tropical dry forest (Holdridge et al., 1971). Average annual rainfall is 1440 mm, and the dry season extends from December through April (Maldonado et al., 1995). Most of the tree species are deciduous, and peak flowering occurs during the dry season (Frankie et al., 1974). This site will be referred to hereafter as Guanacaste.

The second site was located on the Osa Peninsula in the southern Pacific coast of Costa Rica (8°26' N, 83°30' W) within the Osa Conservation Area (ACOSA). This area is considered tropical moist forest (Holdridge, 1971), and average annual rainfall is 3000 mm in the lowlands and 4000 mm in the uplands, with a marked dry season from December through April (Hartshorn, 1983). Most tree species are evergreen, and only a few drop their leaves during the dry season (December–March; Allen, 1956). This site will be referred to hereafter as Osa.

The trees sampled in this study were distributed along roads in Guanacaste and Osa and are remnants of the original populations of *C. pentandra* in these disturbed areas. The characteristics used to select trees were (1) Reproductive adults with massive flowering the year of study; (2) minimum distance 20–30 km between sampled trees; and (3) at least one branch low enough to allow accessibility for filming and fruit collection. Trees studied in Guanacaste were distributed along 100 km of the Panamerican Highway between the towns of Esparza and Cañas and were separated by at least 30 km. These trees were found in small fragments of forest along the highway or as isolated individuals in pastures, and generally they were immersed in a disturbed matrix of scattered human populations and agriculture development (rice and pastures). Trees in Osa were distributed along 70 km of rural highway between La Palma and Puerto Jiménez and were separated by at least 20 km. These trees were principally found in forest patches along the road but were immersed within a matrix of agricultural areas (mainly forest plantations, cattle, and African palms). Two trees in Osa were located inside the mature forest of Corcovado National Park (a 40000-ha forest reserve) and were separated by 50 km from trees along the highway. Density of *C. pentandra* in the two regions was estimated by systematically searching for trees along the highway stretch that had the two furthest trees, respectively for Osa y Guanacaste. Five hundred meters were searched on each side of the highway with binoculars and the total number of individuals found was divided by the area covered. The estimated density of *C. pentandra* in Guanacaste was 0.01 individuals/ha and 0.03 individuals/ha in Osa.

Study species—*Ceiba pentandra* is distributed in the Neotropics from Mexico to the Amazonian basin and in the paleotropics throughout western Africa (Hartshorn, 1983). In Costa Rica and Mexico this species is found in many different habitats in the tropical life zone within 200 m above sea level (a.s.l.). It is a tall (40 m) emergent deciduous tree that may reach diameters (dbh) of more than 200 cm. Flowers are small (3–3.5 cm length of corolla) with five

stamens around a protruding style with white to pink petals (Cascante-Marín, 1997). Anthesis is nocturnal and occurs at approximately sunset (19:00–19:30 h). Nectar is available at the time of anthesis, and pollen dehiscence occurs approximately one hour later. *Ceiba pentandra* frequently experiences patterns of supra-annual flowering with different individuals flowering during different years; however, within any one year, the individuals that flower are highly synchronized and do so within a relatively short period of six weeks (Frankie et al., 1974; Gribel et al., 1999; Lobo et al., 2003). Along the Pacific coast of Mexico and Central America flowering occurs at the beginning of the dry season in January and February (Lobo et al., 2003). Dehiscent wind-dispersed fruits are produced approximately one month later during February and March. The range of fruit production per individual varies from 500 to 4000 (Baker, 1983), and fruit set is relatively low (<5%; Munguía-Rosas, 2003). The mating system of *C. pentandra* has been described as mixed, based on genetic studies (Murawski and Hamrick, 1992) and manual crosses (Baker, 1955, 1965); however, Gribel et al. (1999), using manual crosses, concluded that this species is self-incompatible.

Abundance and activity of pollinator visits—The abundance and activity of nocturnal visitors were recorded during the dry season, January–February 2002, with a Sony Digital camcorder (Handycam DCR-TRV18) using a technique similar to Quesada et al. (2003). Because many of the inflorescences were found higher than 15 m, we used an infrared flash (Sony HVL-IRH2) and a 2× teleconverter (Sony VCL-HG2030X) to improve visibility. Videotaping began at sunset immediately before anthesis and continued for 4 h, which corresponds to peak foraging times of most phyllostomid bats (Fenton and Kunz, 1977). The camera was placed to observe 5–10 inflorescences with 3–10 flowers each. When height permitted, two cameras were used each night to film two groups of inflorescences. Two hundred inflorescences were filmed for 4 h a night over a period of 32 h (eight nights) in seven trees in Osa, and 193 inflorescences were filmed for 4 h a night in five trees over a period of 28 h (seven nights) in Guanacaste. Filming was conducted one or two nights a week during the flowering period, avoiding 3 nights before and after full moon due to the potential negative effect of moonlight on bat foraging activity (Morrison, 1978a, b). The average number of bat visits per hour was calculated for each inflorescence filmed.

Each night of filming, we also placed two 12-m mist nets in a natural corridor near the flowering tree to document which species were visiting *C. pentandra*. Each bat was identified to species using the key of Timm and LaVal (1998). Pollen samples were collected from the bodies and faces of all frugivorous and nectarivorous species captured using the glycerine-fucsin gelatin technique of Beattie (1971).

Hand pollinations—To determine the incompatibility system of *Ceiba pentandra*, 1140 hand pollinations were performed on four trees (280 pollinations per tree) in Guanacaste. Outcrossed flowers were obtained by artificially pollinating 140 pristine flowers on each tree using pollen from at least three different donors. Similarly, 140 pristine flowers were self-pollinated on each tree using pollen from the same tree. Flowers were marked and covered before anthesis with mosquito net bags to prevent pollinator visitation. Both self- and outcross pollen were collected in plastic cups one hour after anthesis. Hand pollinations were performed by gently saturating the stigma with pollen (500 pollen grains per stigma) using a soft paintbrush. Each marked flower was followed until fruit maturation.

Fruit collection—Mature and immature fruits were collected in February–March 2002. Fruits were collected from seven trees in Osa and eight trees in Guanacaste; whenever possible, fruits were collected from trees that were filmed. Approximately five to ten fruits were collected per tree depending on accessibility. Due to the height of most mature fruits (45 m in Osa and 30 m in Guanacaste), most of the fruits were collected using a giant sling-shot (BigShot, Sherrill Arborist Supplies, 200 East Seneca Road, Greensboro, NC). In a few cases fruits on lower branches were harvested with a tree pruner. In the laboratory seeds were extracted from the fruits; immature seeds were frozen, and mature seeds were stored in individual bags.

Genetic analysis—Genotypes of the seeds were determined using micro-satellite markers developed by Brondani et al. (2003) for *C. pentandra*. DNA was extracted using the cetyltrimethylammonium bromide (CTAB) procedure (Doyle and Doyle, 1987). Five fruits for each tree and four seeds for each fruit were analyzed. The methods of polymerase chain reaction (PCR) were the same used by Brondani et al. (2003), except that we used 25 μ L PCR reaction volumes instead of 12.5 μ L. The sequences of the primers for the loci *CP1*, *CP12*, *CP13*, *CP15*, *CP19*, and *CP22* were based on those reported by Brondani et al. (2003) with forward primers labeled with one of three fluorescent dyes (HEX, NED, and 6FAM). The amplified products were separated using capillary electrophoresis on an automated DNA sequencer (ABI 310), and alleles were sized by comparison with Gene Scan Rox 500 DNA standard.

Gene frequencies and expected heterozygosity by locus for Osa and Guanacaste were calculated with the MLTR computer program (Ritland, 2002). This program estimates population gene frequencies with progeny data, using bootstrapping to calculate gene frequency standard errors.

We estimated multilocus outcrossing rate (t_m), paternity correlation (r_p), and correlation of outcrossing (r) within trees for the population of trees in Guanacaste and Osa. The paternity correlation is also equivalent to the probability that any two randomly chosen outcrossed seeds were sired by the same father. The paternity correlation is inversely related to the number of outcross parents (n) by $r_p = 1/n$, where n is the effective number of pollen donors (i.e., unrelated sires; Ritland, 1989). The correlation of outcrossing indicates the level of correlation between the probability of selfing within progenies. High levels of this parameter are obtained when the probability of selfing is not randomly distributed among trees, but is concentrated in certain individuals of the population (Ritland, 1989, 2000).

We calculated genetic parameters using the models proposed by Ritland (1989) and the MLTR computer program (Ritland, 2002). The standard error was calculated by bootstrapping with 1000 repetitions by resampling individual seeds within trees (Ritland, 2002). To compare the genetic parameters for the populations of trees in the two different regions, we determined the difference in outcrossing rates and paternity correlation for each of the 1000 bootstraps for trees. The normality of these differences was verified, and the probability of obtaining a different value statistically different from zero was tested with a Student's t test. In order to study the inter-tree variation in mating system within each population, we also obtained individual estimates of t_m and r_p for each tree.

We determined the levels of mean relatedness for the progenies of trees in Guanacaste and Osa using seed pairs within individual trees, both within fruits and between fruits. We used a regression measure of relatedness (Queller and Goodnight, 1989; Lynch and Ritland, 1999) as an estimate of the standard coefficient of relationship, in which an estimate of 0.25 indicates a half-sibling relationship and 0.5 indicates a full-sibling relationship. Regression estimates of relatedness were calculated with the computer program Relatedness 5.0 (Goodnight and Queller, 1990). Standard errors and 95% confidence intervals for relatedness estimates were calculated by jackknifing over loci (Goodnight and Queller, 1990). The same procedure was used to calculate standard errors and confidence intervals for differences between estimates of relatedness for Osa and Guanacaste.

RESULTS

We observed a total of 137 visits by bats to the inflorescences of trees in Guanacaste and zero visits in Osa. Each inflorescence in Guanacaste received between one and 25 visits, and all trees in this region were visited. Two bat species, *Glossophaga soricina* and *Phyllostomus discolor*, were captured with *C. pentandra* pollen on them and thus appear to be the main pollinators of this species in Guanacaste. In contrast, the flowers of trees from Osa were never visited by bats; however, we did observe on two occasions a kinkajou (*Potus flavus*) drinking nectar from the flowers of a tree in Corcovado National Park. In spite of its relatively large size (4 kg), the kinkajou appeared not to damage flowers and may be a pol-

linator, but because it was observed only twice, it is unlikely to be an effective pollinator. Some of the trees in Osa and Guanacaste also received a few visits by small unidentified moths, but they did not come in contact with the reproductive parts and are not considered to be potential pollinators. The results of hand pollinations indicate that, of the 560 outcross pollinations performed, 40% developed into mature fruits. However, none of the self-pollinations developed into fruit.

Allele frequencies from which to estimate genetic parameters were calculated from six loci with seven to 15 alleles each (Table 1). Overall mean expected heterozygosity of *C. pentandra* in the Costa Rican populations was 0.705 (Table 1). Similar high levels of expected heterozygosity were estimated for progenies from both the Guanacaste ($H_e = 0.636 \pm 0.173$) and Osa populations ($H_e = 0.652 \pm 0.144$). *Ceiba pentandra* has a mixed mating system with a mean multilocus outcrossing rate of 0.623. The proportion of outcrossed seeds was significantly greater for trees in Guanacaste than for trees in Osa (Fig. 1, $t = 67.65$, $df = 200$, $P < 0.001$). The paternity correlation was significantly lower for trees in Guanacaste than Osa ($t = 72.08$, $df = 200$, $P = 0.001$; Fig. 1) suggesting that the individuals from Osa were more likely to produce full-sibling progeny within trees than were individuals from Guanacaste. The paternity correlation estimated that a mean of 2.82 outcross pollen donors sired the progeny of trees from Guanacaste populations, whereas usually only one outcross pollen donor sired the progeny of trees from Osa. These results are confirmed by the analysis of mean relatedness within fruits and between fruits within trees in Osa and Guanacaste (Fig. 2). Mean relatedness within fruits was significantly greater for progeny of trees in Osa than for progeny of trees in Guanacaste. The mean difference between regions was 0.171 (95% confidence interval ± 0.165). Mean relatedness between fruits within trees was also significantly greater for progenies of trees in Osa (0.507) than for progenies of trees in Guanacaste (0.254). In this case, the mean difference between relatedness coefficients was 0.252 (95% confidence interval of the difference ± 0.209). Therefore, most of the fruits from Guanacaste were sired by multiple pollen donors; however, this phenomenon was rare in Osa where a single pollen donor per fruit, or completely selfed fruits, were more frequent.

DISCUSSION

Direct observations indicate that there is a significant geographic difference in pollinator visits. In the tropical dry forest of Guanacaste, *Glossophaga soricina* and *Phyllostomus discolor* were the principal visitors observed, whereas in Osa, no bats were observed at all. Furthermore, our observations indicate that each inflorescence in Guanacaste was visited at least once during each night. This marked difference in foraging behavior of bats in the two regions may be a consequence of the intense dry season that is characteristic of Guanacaste. Previous studies have documented that nectar, in particular the nectar of *C. pentandra*, is a very important resource for nectar-feeding bats in Guanacaste during January and February when both food resources and water are scarce (Heithaus et al., 1975; Lobo et al., 2003).

Our study documents that geographic variation exists in both the quantity and diversity of pollinators in populations of tropical trees of the same species found within a relatively close distance (<600 km). Although Osa and Guanacaste are near each other, their floristic composition, and intensity and

TABLE 1. Allele frequencies, standard errors, and expected heterozygosity for each microsatellite locus in *Ceiba pentandra* populations from Costa Rica.

Locus	Frequency	H_e
<i>CPI</i>		0.818
168	0.014 ± 0.014	
169	0.266 ± 0.053	
171	0.040 ± 0.027	
173	0.063 ± 0.023	
175	0.019 ± 0.014	
177	0.251 ± 0.047	
183	0.001 ± 0.001	
184	0.014 ± 0.015	
187	0.189 ± 0.045	
191	0.062 ± 0.021	
193	0.020 ± 0.015	
199	0.014 ± 0.014	
203	0.043 ± 0.021	
205	0.001 ± 0.001	
209	0.005 ± 0.001	
<i>CPI2</i>		0.664
65	0.009 ± 0.009	
80	0.366 ± 0.062	
82	0.173 ± 0.056	
84	0.414 ± 0.059	
86	0.005 ± 0.001	
88	0.023 ± 0.017	
92	0.006 ± 0.001	
94	0.005 ± 0.001	
<i>CPI3</i>		0.692
104	0.010 ± 0.002	
106	0.471 ± 0.061	
108	0.052 ± 0.022	
112	0.048 ± 0.020	
114	0.001 ± 0.001	
116	0.216 ± 0.052	
118	0.186 ± 0.046	
120	0.013 ± 0.013	
124	0.004 ± 0.001	
<i>CPI5</i>		0.728
103	0.043 ± 0.025	
105	0.048 ± 0.023	
107	0.038 ± 0.023	
109	0.005 ± 0.001	
111	0.019 ± 0.019	
121	0.001 ± 0.001	
74	0.019 ± 0.018	
86	0.019 ± 0.019	
88	0.463 ± 0.074	
90	0.085 ± 0.039	
94	0.200 ± 0.064	
96	0.062 ± 0.026	
98	0.001 ± 0.001	
<i>CPI9</i>		0.584
245	0.043 ± 0.022	
247	0.625 ± 0.052	
249	0.091 ± 0.036	
251	0.089 ± 0.028	
255	0.059 ± 0.024	
259	0.060 ± 0.024	
265	0.005 ± 0.001	
<i>CP22</i>		0.744
239	0.018 ± 0.013	
241	0.165 ± 0.046	
243	0.442 ± 0.056	
247	0.040 ± 0.024	
251	0.149 ± 0.043	
253	0.068 ± 0.033	
257	0.046 ± 0.024	
259	0.031 ± 0.018	
262	0.041 ± 0.020	
Mean Heterozygosity		0.705

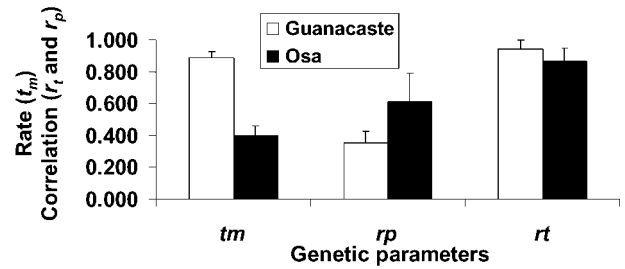


Fig. 1. Mean outcrossing rate (t_m), paternity correlation (r_p), and correlation of outcrossing (r_t), with their respective standard errors of *Ceiba pentandra* populations from Osa and Guanacaste, Costa Rica.

duration of their dry seasons are quite distinct (Lobo et al., 2003). Nevertheless, no evidence exists to suggest that the density of bat pollinators differs in these two regions. *Phyllostomus discolor* and *G. soricina* are both frequently captured in mist nets in Guanacaste (Heithaus et al., 1974, 1975; Stoner, 2001) and Osa (K. E. Stoner, Universidad Nacional Autónoma de México, unpublished data). In a comparative study of the phenology of several species of Bombacaceae, Lobo et al. (2003) showed that *C. pentandra* is one of the first species of Bombacaceae to flower in the dry season in both Guanacaste and Osa, contributing to its importance as a resource for nectar-feeding bats. Nevertheless, the attractiveness of nectar as a resource may be quite different in the two regions because water does not appear to be a limiting resource in Osa at any time. Average monthly rainfall in Guanacaste in January is 0 mm, whereas it is 85 mm in Osa. Furthermore, at the end of the rainy season in December, average rainfall in Guanacaste is only 75 mm, whereas it is 600 mm in Osa. Another factor that may influence the attractiveness of *C. pentandra* nectar as a resource in each region is the availability of other potential resources for *G. soricina* and *P. discolor* in January. Both of these species are considered generalists that consume fruit, nectar, and insects (Nowak, 1994). In January in Guanacaste, *Pseudobombax septenatum* and *Ochroma lagopus* are the only other chiropterophilic species flowering, whereas in Osa, approximately nine species are available as potential nectar resources for bats, including *Caryocar costaricense*, *Ochroma pyramidale*, *Parkia pendula*, and several species of *Inga* (Fleming, 1988; Quesada et al., 1997). In addition, at this time, fewer fruit species that are consumed by bats are available in Guanacaste than in Osa, three vs. 17, respectively (Fleming,

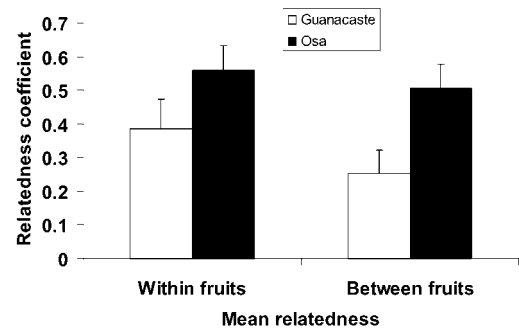


Fig. 2. Mean relatedness (Queller and Goodnight, 1989) within progenies of *Ceiba pentandra* trees in Osa and Guanacaste, Costa Rica. Relatedness is calculated both between pairs of seeds from one fruit and between pairs of seeds from different fruits for individual trees.

1988; Quesada et al., 1997). In sum, the importance of nectar as a water resource as well as a food resource in Guanacaste in January likely results in *C. pentandra* flowers being a particularly attractive resource for generalist bats such as *G. soricina* and *P. discolor*. This is the most plausible explanation for the high visitation rate observed in the tropical dry forest of Guanacaste compared to the tropical wet forest of Osa.

The difference in bat visitation rate in the two regions appears to be correlated with the breeding system and the levels of relatedness of the progeny produced in *C. pentandra* in these areas. In Guanacaste the progeny of all trees tend to be predominately outcrossed, whereas the progeny of the trees in Osa do not show this pattern. The number of sires from outcrossed progenies was significantly greater in seeds from Guanacaste that received bat visits than in seeds from Osa that did not receive bat visits. As a result, relatedness of seeds both within fruits and between fruits was greater in progenies from Osa. In spite of the difference in average outcrossing between the two regions, high outcrossing correlation rates (r_i) indicate that the breeding system is not homogeneous across individuals. A few individuals in Osa are completely outcrossing and some individuals in Guanacaste have high levels of selfing; however, the frequency of these extreme values is quite different in the two regions.

Higher levels of outcrossing in the Guanacaste trees may be related to the movement of pollen by nectarivorous bats in this region. One study in the tropical dry forest of northern Colombia indicated that the average feeding rate of *G. soricina* at flowers of *Agave* spp. was 189 visits/h and was as high as 1000 visits/h for some plants (Lemke, 1984). Furthermore, *G. soricina* may fly up to 2 km between foraging sties (Fleming et al., 1972) and thus have the capacity to move pollen relatively long distances and promote outcrossing (Heithaus et al., 1975). The movement of pollinators can also be affected by changes in the habitat, thereby affecting the mating system of *C. pentandra*. In a continuous population of *Pachira quinata* located in forest, Quesada et al. (2001) showed, using hand pollination experiments and genetic analysis of seeds produced under natural pollination, that this population was predominantly outcrossing. However, in another study of the same species, the progeny of trees from continuous populations experienced higher levels of outcrossing and more sires than trees in isolation, suggesting that a change in pollinators due to forest fragmentation can have an effect on the mating patterns of this tropical dry forest tree (Fuchs et al., 2003). Similarly, in *Asclepias incarnata*, swamp populations are capable of self-fertilizing, whereas natural populations found in other environments are mainly outcrossing (Ivey et al., 1999). In addition, work on other populations of *C. pentandra* have reported that these species have low outcrossing rates under conditions of isolation due to geitonogamy (Murawski and Hamrick, 1992). Although we did not observe any potential pollinators in the trees in Osa except for two rare visits of kinkajous, other potential pollinators such as noctuid moths, nocturnal wasps and wind would principally result in inter-crown pollinations and thus contribute to a high rate of geitonogamy, as observed in this population.

Similarly, a geographic study on *Agave lechuguilla* along a latitudinal gradient showed that flowers are less frequently visited in northern than in southern populations and that there is lower genetic differentiation in the southern populations, suggesting high levels of gene flow between populations (Silva-Montellano and Eguiarte, 2003a, b). Apparently, the northern

populations are more isolated, and geitonogamous self-pollination among flowers within the same individual and transfer of pollen within genets is responsible for higher inbreeding in these populations (Silva-Montellano and Eguiarte, 2003b).

Even though Gribel et al. (1999) and our hand pollination experiments indicate that *C. pentandra* is apparently self-incompatible, our genetic analysis using high-resolution molecular markers and a larger sample size shows that in environments with low pollinator visitation rates, this species changes to a mixed mating system with high levels of selfing. A similar facultative breeding system has been reported for other bombacaceous species (Baum, 1995; Quesada et al., 2001; Fuchs et al., 2003). Because *C. pentandra* is capable of selfing facultatively, the low frequency of selfing reported in some populations is not likely due to high embryo mortality caused by genetic load (Gribel et al., 1999; Gribel and Gibbs, 2002). In fact, Lobo et al. (2003) showed that overall fewer individuals of *C. pentandra* set fruit in Guanacaste than in Osa during the same year these observations were made. This indicates that higher levels of selfing do not necessarily reduce fruit production, although it is unknown whether progeny vigor is affected.

Our results agree with other studies that have indicated that self-incompatibility systems can be flexible. Genetic factors associated with pleiotropic effects of modifying genes (Levin, 1996; Ivey et al., 1999; Good-Avila and Stephenson, 2002, 2003), polyploidy or gene duplication (de Nettancourt, 2001), or temporal plasticity in the expression of self-incompatibility (Richardson et al., 1990; Vogler et al., 1998) can maintain selfing in natural populations. Good-Avila and Stephenson (2002) found that the genes responsible for increasing self-fertility in *Campanula rapunculoides* have both additive and mildly recessive effects, but that populations of *C. rapunculoides* are not likely to become completely self-fertile because of the high levels of inbreeding depression in this species.

Even though high levels of fruit are produced in *C. pentandra* in Osa and Guanacaste (Lobo et al., 2003), inbreeding depression may possibly purge self-seeds and prevent this species from becoming fully self-fertile, thereby maintaining a mixed mating system. Nevertheless, in the absence of pollinators, self-pollination assures reproduction and maintains a minimum threshold on female reproductive success in *C. pentandra*. Future studies should evaluate and compare progeny vigor among populations with different levels of outcrossing to determine if populations with higher inbreeding show lower progeny vigor. Furthermore, our results suggest that the global decline in pollinators (Kearns and Inouye, 1997; Kearns et al., 1998) may differentially affect the breeding systems of many specialized animal-pollinated plants depending upon the life zone they occupy. The long-term consequences of this phenomenon are unknown. Finally, future studies with other species of tropical trees should further evaluate the plasticity of mating systems using high resolution genetic markers and quantifying pollinator activity to determine the frequency of this phenomenon in other species.

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