

# REPRODUCTIVE BIOLOGY OF THE DRY FOREST TREE *ENTEROLOBIUM CYCLOCARPUM* (GUANACASTE) IN COSTA RICA: A COMPARISON BETWEEN TREES LEFT IN PASTURES AND TREES IN CONTINUOUS FOREST<sup>1</sup>

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We compared the rate of pollen deposition, the likelihood of fruit production, the number of seeds per fruit, the outcrossing rate, and the progeny vigor of the tropical dry forest tree *Enterolobium cyclocarpum* for individuals in pastures vs. individuals in continuous forest. We found that flowers from trees growing in continuous forests were more likely to have pollen deposited on their stigmas than flowers from trees in pastures (52.1 vs. 32.3%, respectively). We also found that trees from continuous forests were almost six times more likely to set fruits and produce more seeds per fruit than trees in pastures. Moreover, progeny from trees in continuous forests were, on average, more vigorous than the progeny from trees in pastures, as indicated by 12 of 16 indicators of plant vigor. However, there was no significant difference in the multilocus estimate of the outcrossing rate between the two groups of trees ( $t_m = 1.00$  and  $0.99$  for trees from continuous forest and trees from pastures, respectively). But there are differences in the correlation of paternity between the progeny of the two groups, where the progeny from trees in pastures showed a lower correlation of paternity than progeny of trees from continuous forests ( $r_p = 0.104$  and  $r_p = 0.189$ , respectively). We argue that the mechanisms that regulate progeny vigor are disrupted in trees from pastures. We discuss the implications of these findings for the conservation of *E. cyclocarpum*.

**Key words:** correlated mating; fruit abortion; mixed mating; outcrossing rate; pollen deposition; polyads; seed abortion.

The reduction of continuous habitat into smaller spatially isolated patches represents a significant threat to the long-term survival of many plant species (Saunders, Hobbs, and Margules, 1991; Young, Boyle, and Brown, 1996; Nason, Aldrich, and Hamrick, 1997). It has been proposed that reproductive isolation is one of the important consequences that landscape fragmentation may have on the biota that remains in the smaller patches (Saunders, Hobbs, and Margules, 1991). Moreover, reproductive isolation is often associated with reduction in the size of plant populations, which in turn may result in drastic loss of genetic variability due to drift, reduced gene flow, elevated inbreeding, and inbreeding depression (Templeton et al., 1990; Young, Boyle, and Brown, 1996).

Few studies have addressed the impacts of changes in the landscape on the reproductive biology and the population genetics of plants (Jennersten, 1988; Templeton et al., 1990; Foré et al., 1992; Aizen and Feinsinger, 1994a, b). For example, Foré et al. (1992) studied the genetic structure of *Acer saccharum* after forest fragmentation and found that genetic differentiation among woodlots was greater among adult trees than among juveniles. They concluded that postfragmentation gene flow rates were higher than prefragmentation rates and pointed out that the potential for long-distance gene flow may counter the negative impacts of forest fragmentation. In con-

trast, Young and Merriam (1994), working with 1-yr-old seedlings of fragmented and continuous forests, found that forest fragmentation adversely affected the genetic structure of *Acer saccharum*. They found differences in the patterns of gene flow within (and possibly among) forest patches for *Acer saccharum* as a consequence of fragmentation.

Other authors have examined the effects of forest fragmentation on the rates of pollination and seed set in plants. Jennersten (1988) showed that habitat fragmentation resulted in a lower flower visitation rate and seed set in *Dianthus deltooides* when compared to nonfragmented habitats. Similarly, Aizen and Feinsinger (1994a) showed that pollination level and seed output decreased nearly 20% from forest to fragments in the Chaco region of the Republic of Argentina. These findings indicate that the reduction of continuous habitat can have a negative effect on the reproductive biology of plants.

The effects of forest fragmentation on tropical plants have been addressed by other authors (Hall, Walker, and Bawa, 1996; Murcia, 1996; Nason and Hamrick, 1997; Aldrich and Hamrick, 1998; Cascante, 1999). It has been reported that pollination biology of tropical plants was not affected by habitat fragmentation, as pollination was not different among forest remnants of different size (Murcia, 1996). Cascante (1999) reported that forest fragmentation did not affect the rate of pollen deposition in the tropical dry forest tree *Samaea saman*. However, he found that isolated trees or trees in small forest remnants had fewer pollen tubes growing along their styles and fewer seeds per fruit than trees in continuous forest. Nason and Hamrick (1997) reported that trees of *Spondias mombin* found in small forest patches suffered significant reduction in fruit production and seed germination relative to large fragments or continuous forest. They also reported that most of the seeds produced in small patches were sired by trees located in forest stands located >80–1000 m away. Similarly, Aldrich and Hamrick (1998) studied the reproductive success of trees

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of *Symphonia globulifera* from forest remnants and pastures. They reported that most seedlings found in forest remnants were sired by trees in adjacent pastures. Because of the few trees typically found in pastures, this situation creates a genetic bottleneck. In addition, they also found that the rate of selfing was higher for trees in pastures than trees in remnant or continuous forest.

In this study, we compared the reproductive biology of guanacaste trees (*Enterolobium cyclocarpum* Jacq.) growing in pastures with that of trees from continuous forest. In particular, we studied the rates of pollen deposition onto the stigma, the likelihood of fruit set, the number of seeds per fruit, the outcrossing rate, and progeny vigor between these two groups of trees.

## MATERIALS AND METHODS

**Study organism**—Guanacaste (*Enterolobium cyclocarpum* Jacq.) is a native leguminous tree, widely distributed throughout the Neotropics, ranging from Central Mexico to the northern part of South America (Pennington and Sarukhan, 1968; Little, Woodbury, and Wadworth, 1974; Janzen, 1983). It is most frequently found in lowland deciduous and semideciduous dry forests. In Costa Rica, it normally occurs at low densities in intact forests where they tend to be most abundant in areas of frequent local disturbance (Standley, 1937; Janzen, 1983; Zamora, 1991). This species is very important in the communities in which it is present because the few scattered trees are typically quite large (Holdridge and Poveda, 1975; Janzen, 1983; Francis, 1988).

Flowering occurs synchronously throughout the western lowlands of Costa Rica starting in early March, at a time when the tree is leafless or just expanding the first leaves. Each tree produces numerous inflorescences each year. The apetalous flowers are white and borne in loose heads. The florets open in the late afternoon and remain open all night. They do not wilt until the mid-morning heat of the following day, i.e., each flower <24 h. *Enterolobium cyclocarpum* disperses its pollen in polyads consisting of 32 pollen grains (Guinet, 1981; Rubick and Moreno, 1991). Pollen flow between guanacaste trees is believed to be mediated by moths, beetles, and other small nocturnal insects (Janzen, 1982), as well as diurnal insects such as bees (O. J. Rocha, personal observations).

Each inflorescence initiates between 0 and 3 fruits, but typically only one completes development. These minute fruits remain dormant throughout the upcoming rainy season (mid-May to mid-December) and then very rapidly expand to full size (4–15 cm in diameter) (Janzen, 1982; Zamora, 1991). The fruits ripen from March through May, about the time those flowers that will produce fruits the following year are opening. Mature fruits are smooth, shiny, indehiscent, relatively dry, and deep brown. Janzen (1982) reported that trees of this species produce as many as 18 seeds per fruit.

Mature seeds are hard, ovoid, brown, and weigh from 300 to 1000 mg (Janzen, 1983). At the beginning of the rainy season, seed germination is abundant as the hard seed coats have been weakened or broken by mechanical or thermal wear or by microorganism activity (Janzen, 1983). Often all of the seeds of a fruit will germinate before the fruit wall rots (O. J. Rocha, personal observation).

**Trees sampled**—A total of 47 trees (maternal families) were considered in this study. All of these families were used to estimate the outcrossing rate of *E. cyclocarpum*. Trees were grouped according to the two habitat where they were found: trees from continuous forest or trees from pastures. Pastures trees were only those trees that were at least 500 m away from other conspecific trees. Trees from continuous forest were trees that occurred in forest patches of <10 ha. However, special attention was given to 22 trees for which we conducted more detailed observations concerning the rates of pollen deposition, the likelihood of fruit set, and the number of seeds per fruit. Moreover, we only studied the rate of pollen deposition and likelihood of fruit set for 20 and 21 trees, respectively.

**Rates of pollen deposition**—To study the rate of pollen deposition on the

stigma, we collected inflorescences from 20 adult trees of *E. cyclocarpum* (ten from pastures and ten from continuous forest) the day after anthesis and fixed them in alcohol (70% v/v). Pistils of three flowers from each of ten inflorescences from each tree were later removed and softened and cleared in a 8.0 mol/L NaOH solution for 12 h at room temperature. The pistils were then rinsed with water, stained overnight in 1.0% aniline blue in a  $K_2PO_4$  solution, squashed under a cover slip, and observed with a fluorescence microscope. All pistils were scored for the presence of polyads, pollen germination, number of pollen tubes, and number of ovules in the ovary. In *E. cyclocarpum* pollen is shed in polyads that contain 32 pollen grains (Roubick and Moreno, 1991).

**Fruit set**—Likelihood of fruit set was estimated from three separate counts of 500 flower-bearing branches on each of the 21 trees. We were not able to include one tree in this portion of the study because the fruits initiated growth earlier than the other trees. Ten of the trees were from continuous forest, and the remaining 11 were trees left in pastures. In *E. cyclocarpum*, the inflorescences are borne on short branches. We scored as successful branches those 1-yr-old twigs bearing green fully matured fruits (i.e., before fruit maturation and shedding) and estimated fruit set as the proportion of successful branches in relation to the number of branches examined. In addition, we marked 50 reproductive branches from each of five trees (two in pastures and three in continuous forest) to determine the number of inflorescences per branch. We also collected ten inflorescences per tree to determine the number of flowers per inflorescence.

We conducted weighted least-square analyses to examine the effects of fragmentation on the likelihood of fruit set among flower-bearing branches. In these analyses, the proportion of successful branches in each replicate ( $p$ ) was weighted by its variance, where  $\text{Var}(p) = \text{Var}(y/n) = pq/n$  and weight =  $n/pq$ , where  $y$  = number of successful branches,  $n$  = total number of branches examined in each replicate, and  $q$  = proportion of failed branches ( $1 - p$ ). The analyses allow the calculation of least-square estimates of the mean proportion of successful branches among treatments. In addition, it also calculates the probability values for the pairwise comparison among the proportion of success (i.e.,  $H: p_i = p_j$ ) (GLM, option lsmean; SAS, 1985).

**Number of seeds per fruit**—Fruits from 21 trees (10 from continuous forest and 11 from pastures) were collected in 1997 and 1998. The number of seeds per fruit was determined for at least 20 open-pollinated fruits from each tree. All seeds from each fruit were kept in separate paper envelopes and stored in the dark at room temperature until used for genetic analyses. Variation in the number of seeds per fruit was analyzed using analysis of variance with habitat where the trees were found (pasture or continuous forest) considered as the main effect (GLM; SAS, 1985).

**Mating systems**—*Isozyme electrophoresis procedure*—To study the outcrossing rate of *E. cyclocarpum*, we determined the genotype of at least 25 seeds from each tree. Only one seed was taken from each fruit. All seeds were germinated before enzymes were extracted. To facilitate seed germination, the seedcoat of each seed was weakened with a file, and the seed was soaked in water for 24 h, placed in a glass petri dish, and incubated at 25°C. Seeds were examined twice a day until radicle emergence, which typically occurred between 48 and 72 h. Enzymes were extracted when the germinating seedlings were at least 2 cm long, i.e., between 72 and 96 h after the emergence of the radicle, according to the methods described by Rocha and Lobo (1996). We determined the genotype for five polymorphic enzyme systems markers: EST, PGI, PGM, IDH, and DDH (DIA). Electrophoresis was performed using a continuous histidine-citrate buffer system, with an electrode buffer of 0.065 mol/L L-histidine, 0.019 mol/L citric acid, pH 6.7. The gel buffer was a 1 : 7 dilution of the electrode buffer (Wendel and Weeden, 1989). For multiple isozymes, the most anodal isozyme was arbitrarily numbered "1," with the remaining isozymes numbered sequentially. The different electromorphs (alleles) for each isozyme (locus) were identified in a similar fashion; the most anodal allele was arbitrarily identified as "A," with the remaining alleles identified sequentially. Seeds from 47 trees (maternal families)

TABLE 1. Number of flowers of *Enterolobium cyclocarpum* with and without polyads deposited onto their stigma for trees from pastures and trees from continuous forest.

| Treatment                    | Flowers     |                |                |
|------------------------------|-------------|----------------|----------------|
|                              | With polyad | Without polyad | Total examined |
| Trees from continuous forest | 156         | 144            | 300            |
| Trees from pastures          | 98          | 205            | 303            |
| Overall                      | 254         | 349            | 603            |

Note: chi-square test = 23.892, df = 1,  $P < 0.0001$ .

were included in this study; however, we paid special attention to those 21 families from which we had collected data on the reproductive biology.

**Data analyses**—The outcrossing rate was conducted using the method of Ritland and Jain (1981) and the MLT program provided by Ritland (1990). This method is based on the mixed-mating model proposed by Ritland and Jain (1981), where the mating parameters are estimated based on the assumption that the seed crop of a tree is made up of a proportion,  $t$ , of outcrossed seeds, and a proportion,  $1 - t = s$ , of self-fertilized seeds. It also assumes that each tree receives a sample of pollen with the allele frequencies representative of the population. Moreover, it also assumes that gene frequencies in the pollen pool, as well as the outcrossing rates, are constant from one tree to another in the same population. According to this model, any increase in the number of homozygotes in the progeny, in relation to a totally outcrossed progeny, is due to selfing. For this reason,  $s$  is an estimate of the effective selfing rate, which includes true selfing, as well as biparental inbreeding (Ritland, 1989). The analysis uses the electrophoretic data from each family to calculate multilocus estimates of outcrossing,  $t_m$ , as well as the coefficient of inbreeding,  $F$ , for the population, and the gene frequencies for all alleles of each of the loci examined. Moreover, it can also provide separate estimates of the gene frequencies in the pollen pool and the ovules.

**Correlated mating**—Data were also analyzed using the correlated mating model proposed by Ritland (1989). This model introduces a modification to the mixed mating model, where the outcrossing rate is not expected to be the same for each tree and the gene frequencies in the pollen that each tree receives are allowed to differ. This modification allows one to investigate the proportion of matings occurring with the same sire, as well as the correlation of mating within progenies. Under this model, the observations are taken from the genotypes of pairs of progenies drawn from a random sample of all genotypes in the progeny of each maternal tree. With this new arrangement of our data, we consider the probability of obtaining pairs of genotypes in a model where they are distributed according to the type of mating that produced each pair of progeny, i.e., self-self, self-outcrossed, and both outcrossed. The last group could be further subdivided according to the identity of the sire in fullsibs and halfsibs. The proportion of fullsibs among the outcrossed progeny is measured by the correlation of paternity,  $r_p$ , and it serves as an indicator of the variation in the gene frequencies of the pollen received by each plant. This is expected in natural populations where trees differ in their flowering time, their degree of isolation, and the species composition and behavior of the pollinators that visit them (see Ritland, 1989).

**Progeny vigor**—To compare the vigor of progeny from trees in pastures and trees from continuous forests, we conducted a greenhouse experiment. We took five seeds from each of six fruits from each tree. The seeds from the two groups of trees were sown in 3-L plastic bags containing organic soil, randomly assigned to a greenhouse bench, and watered as necessary. For each seed, we recorded the number of days to emergence, the number of days from emergence to the production of the first true leaf, and stem diameter, plant height, and number of leaves per plant at 21 d after emergence. Finally, at 60 d after emergence, we recorded stem diameter, plant height, and number of leaves for each plant. In addition, we measured leaf area using a leaf area meter (model 3000; LI-COR, Lincoln, Nebraska, USA) and harvested the plants to measure the dry mass of roots, stems, and leaves.

TABLE 2. Number of inflorescences of *Enterolobium cyclocarpum* examined and number of inflorescences with pollen in at least one of the three flowers examined for trees from pastures and trees from continuous forest.

| Treatment                    | Inflorescences |                |                |
|------------------------------|----------------|----------------|----------------|
|                              | Pollinated     | Not pollinated | Total examined |
| Trees from continuous forest | 104            | 16             | 120            |
| Trees from pastures          | 72             | 50             | 122            |
| Overall                      | 176            | 66             | 242            |

Note: chi-square test = 23.318, df = 1,  $P < 0.0001$ .

## RESULTS

**Rates of pollen deposition**—We examined a total of 603 flowers from 21 trees of *Enterolobium cyclocarpum*. We found that 48.12% of the flowers examined had at least one polyad deposited onto their stigmas (Table 1). However, we found significant differences in the proportion of pollinated flowers among trees from the pastures and trees from continuous forest (chi-square test = 23.892, df = 1,  $P < 0.0001$ ). In general, more flowers from trees growing in continuous forest had polyads onto their stigmas (52.1%) than pastures trees (32.3%).

We also studied the likelihood of pollination among inflorescences (Table 2). Our data revealed that inflorescences from trees located in continuous forest were more likely to have at least one flower with pollen than those of pasture trees (chi-square test = 23.318, df = 1,  $P < 0.0001$ ). Overall, 86.7% of the inflorescences from continuous forest received pollen while only 59.0% of the inflorescences from trees in pastures did so. These findings indicate that inflorescences on trees in continuous forest are more likely to be pollinated than inflorescences on trees from pastures, but when pollinated, the number of flowers with pollen on their stigma is similar.

We also examined variation in the number of polyads deposited on the stigma among pollinated flowers (Table 3). Most of the stigmas had only one polyad and the proportion of flowers with one or more polyads was not different between trees from different habitats (chi-square test = 3.638, df = 2,  $P < 0.162$ ). This finding demonstrates that, in spite of the difference in the likelihood of pollination, the amount of pollen deposited on pollinated flowers is the same for the two groups of trees studied (pastures vs. continuous forest).

**Fruit set**—We studied the probability of fruit set in *E. cyclocarpum* in ten trees from continuous forest and ten trees from pastures. Weighted analysis of variance showed that the proportion of inflorescences bearing branches that set fruits was higher for trees in continuous forest than for trees in pastures (Table 4). Overall, inflorescences on trees from continuous forest were almost seven times more likely to set fruits

TABLE 3. Frequency of the number of flowers of *Enterolobium cyclocarpum* with pollen per inflorescence in the sample for trees from pastures and trees from continuous forest.

| Treatment                    | Number of flower with pollen per inflorescence |     |       | Total no. flower |
|------------------------------|--|-----|-------|------------------|
|                              | One  | Two | Three |                  |
| Trees from continuous forest | 50   | 14  | 8     | 72               |
| Trees from pastures          | 58   | 26  | 20    | 104              |
| Overall                      | 108  | 40  | 28    | 176              |

Note: chi-square test = 3.638, df = 1,  $P < 0.162$ .

TABLE 4. (A) Results of the weighted analysis of variance for the likelihood of fruit set among flower bearing branches in *Enterolobium cyclocarpum*. (B) Least square means estimate of proportion of fruit bearing branches per 500 reproductive branches examined.

| A) Source of variance        | df | F                                     | P      |
|------------------------------|----|---------------------------------------|--------|
| Habitat                      | 1  | 357.99                                | 0.0001 |
| Trees within habitat         | 18 | 5.05                                  | 0.0001 |
| B) Treatment                 |    | Proportion of<br>branches with fruits | SE     |
| Trees from continuous forest |    | 0.093                                 | 0.004  |
| Trees from pastures          |    | 0.014                                 | 0.002  |

than trees from pastures (likelihood of fruit set, 0.093 vs. 0.014, respectively). In addition, we found that fruit set varied significantly among trees within each population.

**Number of seeds per fruit**—For the analysis of variation in the number of seeds per fruit between trees in continuous forest and trees in the pasture, we used 868 fruits from all 22 trees of *E. cyclocarpum* described above. An analysis of variance revealed that the fruits from trees in continuous forests bore, on average, significantly more seeds than fruits from trees in pastures (12.43 vs. 10.96, respectively; see Table 5). In addition, we also found significant variation in the number of seeds per fruit among trees. These findings indicate that, on average, trees in pastures produce fewer seeds per fruits than trees in continuous forest.

**Outcrossing rate**—The analyses of the breeding system of the 40 maternal families of *E. cyclocarpum* using the mixed-mating model are shown in Table 6. These findings indicate that this species is predominantly outcrossed, as the multilocus estimators of the outcrossing rates were all high for both groups of trees, i.e., trees from pastures and continuous forest. Our results also showed that the biparental inbreeding, as shown by the difference between the multilocus estimate and the mean single locus estimate, was very low (Ritland, 1989). Overall, there was no significant difference in the outcrossing rates ( $t_m$ ) between trees from pastures and trees from continuous forest.

We tested for heterogeneity between the gene frequencies in the pollen and ovules as proposed by Weir (1996) and James et al. (1998). We found heterogeneity in the allele frequencies between pollen and ovules for only one locus, namely EST (chi-square test,  $P < 0.01$ ). In addition, we found significant heterogeneity in the pollen frequencies estimated from the successful pollination (pollinations that resulted in a mature seed) of each maternal family (Table 6).

Estimates of the correlation of paternal parentage,  $r_p$ , showed important differences among the trees from the two habitats. The correlation of paternal parentage describes the mean probability of finding seeds sired by the same tree in a given seed crop. In general, progeny from trees in pastures showed lower correlation of paternity than progeny from trees in a continuous forest, which appears to indicate that continuous forest trees have more of their fruits pollinated by one or few pollen donors, perhaps a near neighbor. This finding is also supported by the variation in the estimates of pollen frequencies estimated for each tree.

**Progeny vigor**—Results from the greenhouse experiment showed that, on average, progeny from continuous forests

TABLE 5. (A) Results of the analysis of variance for the number of seeds of *Enterolobium cyclocarpum* produced per fruit for trees from continuous forest and trees from pastures. (B) Least-square means and standard error for the number of seeds per fruit.

| A) Source of variance        | df | F               | P      |
|------------------------------|----|-----------------|--------|
| Habitat                      | 1  | 13.75           | 0.0002 |
| Trees within habitat         | 21 | 12.09           | 0.0001 |
| B) Treatment                 |    | Number of seeds | SE     |
| Trees from continuous forest |    | 12.43           | 0.35   |
| Trees from pastures          |    | 10.96           | 0.18   |

were more vigorous than progeny from pasture trees (Table 7). Multivariate analyses of variance test revealed that there was a significant habitat effect for 12 of the 15 indicators of vigor considered in this study. In general, progeny from trees in continuous forest took less time to germinate and to produce the first leaves than progeny from trees in pastures. Moreover, progeny from forests were taller at 15 and 60 d and had more leaves, larger stem diameter, and more leaf area at 60 d after emergence than progeny from pastures. The multivariate analyses of variances for the indicators of vigor also showed that seed mass had a significant effect on most of the measures of vigor. Only stem diameter was not affected by seed mass. In addition, the analyses of variance also showed that there was significant variation among maternal families for most indicators of progeny vigor.

## DISCUSSION

The goal of this study is to compare the reproductive biology of trees of *Enterolobium cyclocarpum* from pastures with that of trees in continuous forests. We found that flowers on trees from pastures are less likely to receive pollen than flowers on trees from continuous forest. We also found that inflorescence bearing branches on trees from pastures are less likely to set fruits than those on trees from continuous forest. Moreover, fruits produced on trees from pastures contain fewer seeds than fruits produced on trees from continuous forests. However, we also found that the outcrossing rate among the two groups of trees was similar, but the correlation of paternity among progeny was higher for trees from continuous forest than for trees from pastures. Finally, we found that the vigor of seedlings from trees in continuous forests was higher than that of seedlings from trees in pastures.

Only a few studies have addressed the impact of the size of the patch on the rate of pollination of tropical flowering plants (Murcia, 1996; Cascante, 1999). Cascante (1999) proposed that his findings with *Samanea saman* support the notion proposed by Frankie (1976), that geitonogamy could explain the similarity in the level of pollination between the trees in pastures and continuous forests. But, Janzen (1971) proposed that insects may serve as long-distance pollinators in tropical areas. This long-distance pollen dispersal mechanism might explain the arrival of foreign pollen observed in *Samanea saman* and *Enterolobium cyclocarpum* trees in pastures.

Others have also determined whether fruit production is limited by the availability of pollen (Bradbury, 1929; McDade and Davidar, 1984; Calvo, 1990; Niesenbaum, 1992; Spira et al., 1992; also see Stephenson, 1981, 1992). Lee and Bazzaz (1986), working with *Cassia fasciculata*, found that hand-pollination of flowers in the field did not increase the number of mature fruits, suggesting that fruit production is not pollen

TABLE 6. Breeding estimates for the 47 maternal families of *Enterolobium cyclocarpum* considered in this study. (A) Outcrossing and correlated mating estimates for trees from pastures and continuous forest. (B) Gene frequencies estimates for pollen and ovules of the five polymorphic loci. Numbers in parentheses indicate standard errors. (C) Heterogeneity tests for the five polymorphic loci.

| A) Population                     | $t_m$            | $t_s$            | $t_m - t_s$      | $r_p$            |                  |
|-----------------------------------|------------------|------------------|------------------|------------------|------------------|
| Trees from pastures               | 0.999<br>(0.055) | 0.980<br>(0.067) | 0.019<br>(0.026) | 0.104<br>(0.036) |                  |
| Trees from forest                 | 1.000<br>(0.035) | 0.981<br>(0.036) | 0.019<br>(0.026) | 0.189<br>(0.068) |                  |
| Alleles                           |                  |                  |                  |                  |                  |
| B) All trees                      | <i>Est-1</i>     | <i>Gpi-1</i>     | <i>Pgm-1</i>     | <i>Idh-1</i>     | <i>Dia-1</i>     |
| Pollen                            | 0.478<br>(0.031) | 0.821<br>(0.020) | 0.583<br>(0.031) | 0.772<br>(0.025) | 0.075<br>(0.013) |
| Ovule                             | 0.543<br>(0.054) | 0.809<br>(0.034) | 0.596<br>(0.053) | 0.734<br>(0.041) | 0.084<br>(0.029) |
| C) Test for heterogeneity         |                  |                  |                  |                  |                  |
| Pollen and ovule frequencies      | ***              | NS               | NS               | *                | NS               |
| Pollen frequencies among families | ***              | ***              | ***              | ***              | ***              |

Note: NS =  $P > 0.05$ ; \*  $P < 0.05$ ; \*\*\*  $P < 0.001$ .

limited. Similar results have been reported for other species (Stephenson, 1980; Guth and Weller, 1986). However, others have found that fruit production is pollen-limited (Bierzychudek, 1981; Calvo, 1990; Robertson and Wyatt, 1990; Bertness and Shumway, 1992; Karoly, 1992; Schmidt and Antlfinger, 1992). Our results showed that in *E. cyclocarpum*, more flowers are pollinated than fruits mature. In addition, a single polyad should provide enough pollen grains to fertilize all ovules in the ovary; therefore, factors other than pollination should determine the likelihood of fruit maturation.

Others have suggested that the source of pollen could influence the probability of fruit maturation (Janzen, 1977; Charnov, 1979; Stephenson and Bertin, 1983; Bookman, 1984; Lee, 1988). The effect of pollen source on fruit maturation has been well documented for self-incompatible species, where fertile hermaphroditic plants fail to produce fruits upon self-pollination (Stephenson, 1981; Barrett, 1988; Lee, 1988). The mechanisms of self-incompatibility may operate before fertilization (prezygotic incompatibility) or after fertilization (postzygotic incompatibility) (Lee, 1988). But, self-incompatibility is sometimes only partial, with at least a small amount of fruit production occurring after selfing. However, fruits from self-pollinated flowers tend to have fewer seeds and are more likely to abort than fruits from cross pollinated flowers (Stephenson, 1981; Stephenson and Bertin, 1983; Bookman, 1984; Lee, 1988; Vander Kloet, 1991; Becerra and Lloyd, 1992). Frankie (1976) and Cascante (1999) suggested that geitonogamy may be important in tropical trees after habitat fragmentation and this might explain the lower likelihood of fruit maturation observed among trees of *E. cyclocarpum* from pastures. Two trees from pastures, which were not included in this study, flowered earlier than most of the *E. cyclocarpum* trees in three consecutive years. Under these circumstances of temporal and spatial isolation, fruit production in these trees was limited to only a few pods, indicating that self-incompatibility is very strong.

In this work, we found significant differences in the likelihood of pollination between trees of *Enterolobium cyclocarpum* from pastures and continuous forests. The flowers and inflorescences from trees in continuous forests were more likely to be pollinated than those from trees in pastures. These results support the findings and conclusions of other authors that the reduction of continuous habitat reduces the rate of

visitation and likelihood of pollen deposition (Aizen and Feinsinger, 1994a, b; Murcia, 1996). However, we did not find differences in the number of polyads deposited on the stigma of flowers of *E. cyclocarpum*. Our finding suggests that factors such as competition among developing fruits, within inflorescences and between inflorescences, and/or geitonogamy, might play an important role in determining the likelihood of fruit maturation.

Differential maturation may also occur among fruits initiated with pollen from different donors (Bertin, 1982, 1988; Bookman, 1984; Marshall and Ellstrand, 1985, 1988; Lee, 1988). For example, Bookman (1984) showed that in *Asclepias speciosa*, certain pollen donors always produced more fruits than others across a number of maternal recipients. This indicates that a genetic factor (paternal effect) can affect the number of fruits sired by donors. Bertin (1982) showed that in *Campsis radicans*, pollen recipients differentially matured fruits sired by certain donors and that donors that are more successful in siring fruits on one recipient plant may not be as successful on others. Thus, fruit production may also be a consequence of genotype interactions between paternal and maternal parents. Similar findings have been reported for *Raphanus sativus* (see Marshall and Ellstrand, 1985, 1988). In our study, we found higher values for the correlation of paternity among the seed crop of trees of *E. cyclocarpum* from continuous forests than that of trees from pasture. This finding suggests that selective abortion of fruits might also be related to differences in the likelihood of fruit maturation among fruits sired by different pollen donors.

Our findings indicate that the breeding patterns of trees in continuous forest and pasture trees differ significantly. The higher correlation of paternity in the forest trees could be due to the presence of nearby trees that contribute abundant pollen to fertilize several pods. Alternatively, selective abortion of fruits could also result in an overrepresentation of one or few donors in the seed crop of a tree. Because of limited arrival of foreign pollen, trees in pastures mature may lack the conditions for selective abortion among different pollen donors, thus maturing many normally abortive fruits. This lack of selective abortion on the basis of pollen source could contribute to a lower correlation of paternity and might also explain the lower number of seeds per fruit found in pasture trees.

Many plant species produce more ovules than mature seeds

TABLE 7. (A) Least square means and standard errors for all measures of vigor for progeny from pastures and progeny from continuous forest. (B) Results of multivariate analyses of variance (Proc. GLM, SAS Institute, 1985) for the greenhouse performance of a sample of progeny produced from trees in pastures and trees in continuous forest. Differences in germination were tested using contingency table analysis (Proc Catmod, SAS Institute, 1985).

|   | Seed mass (g)      | % germination   | Time to emergence (d) | Time to first leaf (d) | Number of leaves (15 d) | Plant height (cm) (15 d) | Height epicotyl (cm) (15 d) | Stem diameter (mm) (15 d) | Number of leaves (60 d) | Plant height (cm) (60 d) | Stem diameter (mm) (60 d) | Leaf area (cm <sup>2</sup> ) (60 d) | Root biomass (g) (60 d) | Stem biomass (g) (60 d) | Leaf biomass (g) (60 d) |
|---|--------------------|-----------------|-----------------------|------------------------|-------------------------|--------------------------|-----------------------------|---------------------------|-------------------------|--------------------------|---------------------------|-------------------------------------|-------------------------|-------------------------|-------------------------|
| <b>A. Indicators of plant vigor</b>                           |                    |                 |                       |                        |                         |                          |                             |                           |                         |                          |                           |                                     |                         |                         |                         |
| Pastures  | 0.5483<br>(0.0055) | 47.7<br>(1.2)   | 9.99<br>(0.09)        | 6.01<br>(0.10)         | 2.50<br>(0.10)          | 14.06<br>(0.15)          | 7.41<br>(0.09)              | 2.04<br>(0.06)            | 6.67<br>(0.06)          | 24.75<br>(0.34)          | 3.67<br>(0.08)            | 141.01<br>(4.09)                    | 0.61<br>(0.24)          | 0.48<br>(0.19)          | 0.83<br>(0.29)          |
| Forest  | 0.6423<br>(0.0059) | 60.2<br>(1.8)   | 9.65<br>(0.08)        | 5.97<br>(0.09)         | 2.35<br>(0.09)          | 15.10<br>(0.14)          | 7.97<br>(0.08)              | 2.12<br>(0.06)            | 7.26<br>(0.05)          | 27.41<br>(0.32)          | 4.06<br>(0.07)            | 165.92<br>(3.74)                    | 0.70<br>(0.22)          | 0.68<br>(0.13)          | 1.04<br>(0.23)          |
| <b>B. Significance test for each indicator of plant vigor</b> |                    |                 |                       |                        |                         |                          |                             |                           |                         |                          |                           |                                     |                         |                         |                         |
| <b>Source of Variation</b>                                    |                    |                 |                       |                        |                         |                          |                             |                           |                         |                          |                           |                                     |                         |                         |                         |
| Seed mass   | —                  | —               | 3.29<br>*             | 6.96<br>***            | 5.67<br>**              | 130.89<br>*****          | 29.73<br>*****              | 3.78<br>*                 | 36.99<br>*****          | 118.33<br>*****          | 0.02<br>NS                | 50.86<br>*****                      | 5.01<br>**              | 6.77<br>***             | 14.57<br>*****          |
| Treatment   | 134.07<br>*****    | 134.56<br>***** | 7.24<br>***           | 0.08<br>NS             | 1.02<br>NS              | 25.02<br>*****           | 19.46<br>*****              | 0.74<br>NS                | 48.69<br>*****          | 29.84<br>*****           | 11.35<br>*****            | 18.82<br>*****                      | 10.36<br>***            | 18.80<br>*****          | 47.97<br>*****          |
| Tree (treatment)  | 15.82<br>*****     | —               | 5.56<br>*****         | 5.42<br>*****          | 2.03<br>***             | 7.19<br>*****            | 6.64<br>*****               | 0.79<br>NS                | 8.75<br>*****           | 11.03<br>*****           | 8.75<br>*****             | 6.65<br>*****                       | 2.13<br>***             | 5.39<br>*****           | 8.43<br>*****           |

Note: NS =  $P > 0.05$ ; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ ; \*\*\*\*  $P < 0.0001$ .

(Stephenson, 1981; Wiens, 1984; Lee, 1988; Rocha and Stephenson, 1990, 1991a). Sometimes, ovules fail to produce seeds because pollination/fertilization has not occurred or as a result of external factors such as seed predation (Stephenson, 1981; Lee and Bazzaz, 1986; Nakamura, 1986; Lee, 1988). But a few recent studies have shown that a large fraction of the fertilized ovules often fail to mature seeds in many species (Nakamura, 1983; Casper, 1984; Guth and Weller, 1986; Rocha and Stephenson, 1991b). For example, all four ovules of *Crypthanta flava* are typically fertilized; however, most fruits contain only one seed (Casper, 1984). Pechan and Webster (1986) reported that all ovules examined in *Phaseolus vulgaris* were fertilized, but they found a discrepancy between the number of ovules fertilized and the final seed number per pod. In addition, Nakamura (1983, 1986) reported that the basal ovules within the ovary of *P. vulgaris* are less likely to mature seeds. However, these normally aborted ovules develop viable embryos when cultured in vitro. Lee and Bazzaz (1986) reported that 95% of the ovules of *Cassia fasciculata* were fertilized, but only 53% of the ovules matured seeds. They also pointed out that the ovules near the base of the fruit were less likely to mature seeds than ovules at the stylar end. These studies concluded that such difference between fertilized ovules and seed matured are primarily due to the abortion of fertilized ovules and seeds (also see Bawa and Webb, 1984). In addition, Nakamura (1983, 1986) and Lee and Bazzaz (1986) suggested that resources limit seed maturation per fruit and promote seed abortion (also see Stephenson, 1981, 1992; Nakamura and Stanton, 1987; Lee, 1988; Stephenson, Devlin, and Horton, 1988).

We found that fruits from trees in pastures produce, on average, fewer seeds than fruits from trees in continuous forest. This finding is hard to explain because pollen is dispersed in polyads of 32 pollen grains. However, differences in pollen viability, age of the polyad, age of the flower, and geitonogamy could result in insufficient fertilization that might be invoked to explain such differences. But, if the number of seeds per fruit determines the likelihood of fruit abortion in *E. cyclocarpum*, this finding suggests that otherwise abortive fruits are being developed on trees from pastures. Moreover, this also means that there are more resources for seed production among the developing fruits. However, the trees from pastures produce fewer seeds than trees from continuous forests. These findings suggest that there is selective abortion of seeds in *E. cyclocarpum*. But these findings also suggest that seed abortion must be coupled to fruit abortion in order to have a significant impact on progeny vigor, as the progeny from pastures are less vigorous than progeny from continuous forest.

In this study, we found that reproductive branches from trees in continuous forests are more likely to set fruits than reproductive branches from trees in pastures. Moreover, we also found that, on average, fruits produced on trees from continuous forests bear more seeds than fruits produced on trees from pastures. It might be argued that these findings might be explained on the basis of the high level of geitonogamy that trees from pastures might experience.

There is ample evidence to suggest that in plants, in which reproduction is resource limited, the patterns of flower production and fruit and seed abortion are not random. Even though plants have little or no control over the number or the genotype of the pollen grains deposited onto their stigmas, it is becoming increasingly clear that plants can regulate, at least to some degree, the quality of their progeny (Stephenson,

1981; Marshall and Ellstrand, 1985, 1986, 1988; Stephenson and Winsor, 1986; Winsor, Davis, and Stephenson, 1987; Bertin, 1988; Lee, 1988; Rocha and Stephenson, 1991a, b). Other authors have reviewed the possible role of the overproduction of flowers, fruits, and seeds in plants (Stephenson, 1981; Lee, 1988). Some have argued that the overproduction of fruits and ovules followed by nonrandom abortion of fruits/seeds is a mechanism that increases the average quality of the seed crop (Janzen, 1976; Charnov, 1979; Westoby and Rice, 1982; Bawa and Webb, 1984; Bookman, 1984; Stephenson and Winsor, 1986; Winsor, Davis, and Stephenson, 1987). This hypothesis does not, however, assume that plants actually choose which fruits and seeds to mature and which to abort. The fruits and seeds are resource "sinks" competing with one another as well as with vegetative "sinks." The success of each fruit and each seed within a fruit is a function of their ability to compete for maternal resources (see Lee, 1988; Stephenson, 1992). The results presented here strongly suggest that habitat fragmentation may disrupt the mechanisms through which plants regulate the quality of their progeny by increasing the rates of self-pollination in isolated trees and thus causing the pasture trees to produce fewer viable fruits. Because only a few fruits are being produced, competition among developing fruits is low, and normally abortive fruits develop. For that reason, trees in pastures produce progeny that are less vigorous than progeny from continuous forest. This should be considered as another adverse effect of forest fragmentation.

**Implications for conservation**—The results presented here may have important implications for the adequate conservation of genetic resources of tropical trees (Ledig, 1988; Barrett and Kohn, 1991; Ellstrand and Elam, 1993). First, they show that trees in pasture play an important role in the movement of pollinators in fragmented habitats. Similar findings were already reported by Aldrich and Hamrick (1998), who reported that trees of *Symphonia globulifera* from pastures are overrepresented in the seed crop of trees in adjacent forests. They proposed that their findings result in a severe bottleneck for these populations and, hence, should be anticipated for the development of conservation strategies.

Second, it shows that seed from pastures are a poor source of seeds for the establishment of commercial plantations, as the resulting progeny are likely to be less vigorous than that from trees in continuous forests. This reduction in the vigor of the progeny results from a disruption of the mechanisms that plant use to control the quality of their offspring (Rocha and Stephenson, 1991; Stephenson, 1992). This finding is by no means unique to *E. cyclocarpum*, as Cascante (1999) reported similar results for *Samaea saman*.

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