



Published online before print 29 May 2013, doi:

10.3732/ajb.1200542

Am. J. Bot. June 2013 vol. 100 no. 6 1095–1101



Published online before print 29 May 2013, doi:

10.3732/ajb.1200542

Am. J. Bot. June 2013 vol. 100 no. 6 1095–1101

Long-term effects of habitat fragmentation on mating patterns and gene flow of a tropical dry forest tree, *Ceiba aesculifolia* (Malvaceae: Bombacoideae)¹

Mauricio Quesada^{2,7}, Yvonne Herrerías-Diego³, Jorge A. Lobo⁴, Gumerindo Sánchez-Montoya², Fernando Rosas⁵ and Ramiro Aguilar⁶

+ Author Affiliations

+ Author Affiliations

Received for publication 13 October 2012.

Accepted for publication 26 March 2013.

ABSTRACT

• *Premise of the study:* Tropical forest loss and fragmentation isolate and reduce the size of remnant populations with negative consequences for mating patterns and genetic structure of plant species. In a 4-yr study, we determined the effect of fragmentation on mating patterns and pollen pool genetic structure of the tropical tree *Ceiba aesculifolia* in two habitat conditions: isolated trees in disturbed areas (≤ 3 trees/ha), and trees (≥ 6 trees/ha) in undisturbed mature forest.

• *Methods:* Using six allozyme loci, we estimated the outcrossing rate (t_m), the mean relatedness of progeny (r_p) within and between fruits, the degree of genetic structure of pollen pools (Φ_{ft}), and the effective number of pollen donors (N_{ep}).

• *Key results:* The outcrossing rates reflected a strict self-incompatible species. Relatedness of progeny within fruits was similar for all populations, revealing single sires within fruits. However, relatedness of progeny between fruits within trees was consistently greater for trees in fragmented conditions across 4 yr. We found high levels of genetic structure of pollen pools in all populations with more structure in isolated trees. The effective number of pollen donors was greater for trees in undisturbed forest than in disturbed conditions.

• *Conclusions:* Our study showed that the progeny produced by isolated trees in disturbed habitats are sired by a fraction of the diversity of pollen donors found in conserved forests. The foraging behavior of bats limits the exchange of pollen between trees, causing higher levels of progeny relatedness in isolated trees.

Key words:

Bombacaceae forest fragmentation pollen genetic structure
progeny relatedness plant reproduction plant mating systems
tropical dry forest

↵1 This paper constitutes partial fulfillment of the requirements for the PhD Graduate Program in Biological Sciences of the National Autonomous University of México (UNAM) for Y.H.-D., who received a scholarship from the National Council of Science and Technology (CONACyT). This work was supported by the Inter-American Institute for Global Change Research (IAI) CRN II # 021, the U.S. National Science Foundation (GEO-0452325), CONACyT

(U50863Q, 2009-C01-131008, 2010-155016), SEMARNAT-CONACyT (2002-C01-0597 and 2002-C01-0544), and PAPIIT, UNAM (IN201011) grants to M.Q. F.R. acknowledges support from CONACyT (Fellowship number 54313) as a Postdoctoral Fellow at UNAM, Centro de Investigaciones en Ecosistemas (CIEco) and support from UC MEXUS-CONACyT program (fellowship no. FE-09-110) as a Postdoctoral Fellow at University of California Los Angeles. The authors are especially grateful to Lorena Ashworth, Martha Lopezaraiza, Silvana Marten-Rodríguez, and two anonymous reviewers for very thoughtful discussions and for comments that significantly improved this manuscript and to the Chamela Biological Station (UNAM) for collecting facilities. The authors thank Heberto Ferreira and Alberto Valencia for valuable technical assistance.

↵7 Author for correspondence (e-mail: mquesada@cieco.unam.mx)

Habitat loss and fragmentation are widespread processes affecting tropical forests and represent the major threat for maintenance and viability of natural plant populations (Heywood et al., 1994; Quesada and Stoner, 2004). Habitat destruction and fragmentation directly reduce the size and increase the spatial isolation of populations affecting reproduction, gene flow, and genetic diversity of trees (McCauley, 1995; Young et al., 1996; Nason and Hamrick, 1997; Aguilar et al., 2008). It is anticipated that a decline in population size may reduce the density of reproductive individuals, thereby limiting pollen availability and increasing the probability of inbreeding in self compatible species or reducing the number of pollen sources involved in seed production, particularly in outcrossing species (Templeton et al., 1990; Ellstrand, 1992; Ellstrand and Ellam, 1993; Sork et al., 1999; Cascante et al., 2002; Fuchs et al., 2003; Lowe et al., 2005; Aguilar et al., 2008). Simultaneously, spatial isolation may restrict connectivity because of low levels of gene flow between patches and in the long term may cause a loss of genetic variability in the remnant tree populations due to genetic drift or high levels of inbreeding (Barrett and Kohn, 1991; Menges, 1991; Young et al., 1996; Sork and Smouse, 2006).

Several studies have shown that tropical trees are particularly vulnerable to the effects of habitat fragmentation because they naturally occur at low density, have complex self-incompatible mechanisms, high outcrossing rates (Murawski et al., 1994; Hall et al., 1996; Cascante et al., 2002; Fuchs et al., 2003; Lowe et al., 2005), and specialized interactions with pollinators and seed dispersers (Didham et al., 1996; Dick et al., 2003; Ward et al., 2005). It has been shown that fragmentation negatively affects plant reproduction by reducing pollinator activity, pollen deposition, fruit set (Aizen and Feinsinger, 1994a, b; Ghazoul et al., 1998; Cunningham, 2000; Aguilar et al., 2006), outcrossing rates, and the number of sires in fragmented landscapes (Murawski et al., 1994; Aldrich et al., 1998; Cascante et al., 2002; Aguilar et al., 2008). However, empirical evidence shows contrasting results, in some species, and under certain conditions, forest fragmentation can increase gene flow and connectivity between fragmented populations, reducing their genetic differentiation and reducing the loss of genetic diversity in the long-term (Foré et al., 1992; Hamrick et al., 1992; Dick, 2001; White et al., 2002; Dick et al., 2003; Lowe et al., 2005). Such contrasting evidence may be due to the quality of dispersal by different pollen vectors and by the degree of isolation and the size of the remnant patch (Nason and Hamrick, 1997). Although pollen flow can persist in fragmented habitats, remnant forest patches may be at risk for loss of genetic diversity because of a reduced number of local and immigrant pollen sources (Sork and Smouse, 2006). Therefore, integration of estimated pollen-dispersal distance with data on the genetic diversity of pollen pools is required to understand whether isolation and reduction of population size due to fragmentation have negative effects on plant reproduction and mating patterns of tree populations.

Due to the increased destruction of tropical forests in recent years, it is imperative to study the consequences of forest fragmentation and habitat disturbance for the preservation of tropical tree species. Several studies have evaluated the effects of spatial and temporal isolation due to fragmentation on gene flow and mating patterns (Aldrich et al., 1998; Cascante et al., 2002; White et al., 2002; Dick et al., 2003; Fuchs et al., 2003; Quesada et al., 2003, 2004), but most of these studies have only analyzed a single reproductive event in long-lived plants. Few studies have assessed year to year variation on reproductive output and pollination behavior under fragmented conditions (Herrerías-Diego et al., 2006), indicating that this variation can have an effect

on mating patterns, number of pollen donors (Irwin et al., 2003), and progeny performance. In contrast to 1-yr evaluations, studies of gene flow on long-lived plants that include several reproductive events across multiple years will provide more accurate descriptions of patterns of pollen movement, genetic diversity, and structure of populations.

In this study, we determined the effects of spatial isolation and the reduction of tree density due to habitat fragmentation on pollen movement, pollen pool genetic structure, and on degree of progeny relatedness of the tropical tree *Ceiba aesculifolia* over a 4-yr period. To accomplish our general objective, we compared the following variables between undisturbed and disturbed populations: (1) the frequency of outcross matings (t_m), (2) mean relatedness (r_p) of seeds within and between fruits, and (3) TwoGener estimates of pollen distance movement (δ) and structure of pollen pool (Φ_{ft}) sampled by seed parents.

MATERIALS AND METHODS

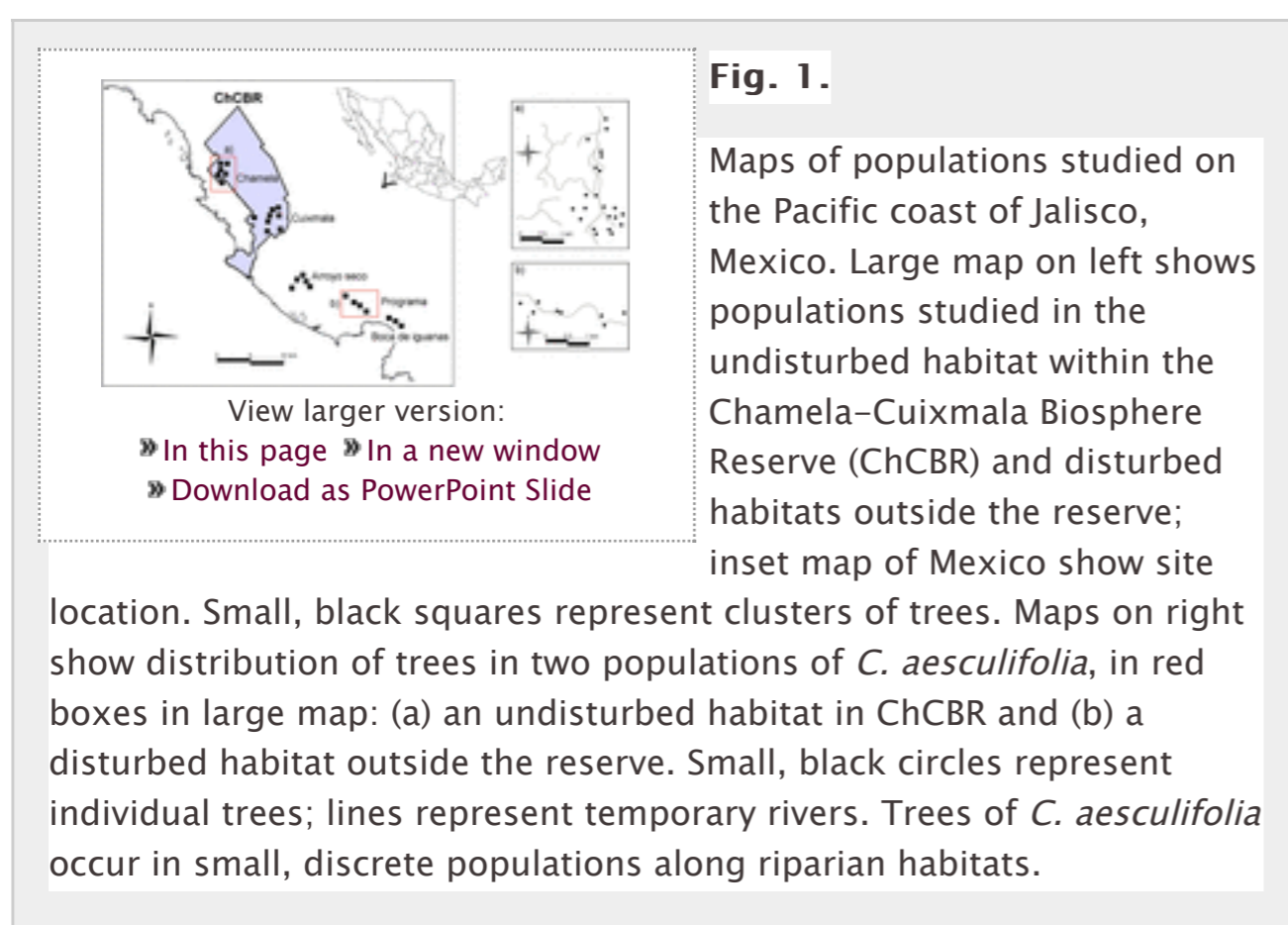
Study species

Ceiba aesculifolia (Malvaceae: Bombacoideae) is a neotropical tree species distributed from Mexico to northern Costa Rica (Cascante-Marin, 1997). Adult trees grow up to 20 m tall and have a diameter at breast height (dbh) of 20 to 50 cm. *Ceiba aesculifolia* has large (10–16 cm) hermaphroditic flowers with five brown, pubescent petals. Styles are on average 15 cm long and surpass the stamens by 2 cm. In the tropical dry forest of Mexico and Costa Rica, *C. aesculifolia* flowers from June to July, and all the fruits mature during the following dry season (Lobo et al., 2003; Quesada et al., 2004). *Ceiba aesculifolia* has a predominantly outcrossing mating system and is effectively pollinated by two bat species, *Glossophaga soricina* and *Leptonycteris curasoae* (Quesada et al., 2004).

Trees

Selection of trees

To examine the effects of forest fragmentation on the genetic structure of the progeny of *C. aesculifolia*, we compared reproductive adult trees in disturbed and undisturbed habitat conditions. A tree was considered to be in disturbed habitat when ≤ 3 trees per ha were surrounded by agricultural fields or pastures. Trees in disturbed habitats were systematically selected in sites along Federal Road no. 200 (Barra de Navidad to Puerto Vallarta) near the Chamela–Cuixmala Biosphere Reserve and were grouped in three populations (Arroyo Seco, Programa, and Boca de Iguanas) to control for the variation between sites within condition. Populations in undisturbed habitats consisted of groups individuals of ≥ 6 trees per ha surrounded by undisturbed mature forest and were located within the Chamela–Cuixmala Biosphere Reserve. To include the largest possible area within the reserve, we selected two populations from two different watersheds within the reserve that were separated from each other by more than 8 km (Chamela and Cuixmala). The number of individuals of *C. aesculifolia* sampled varied among years due to variation in the number of reproductive trees (see Herrerías-Diego et al., 2006). Figure 1 shows the location of tree populations.



Genetic data

To determine the effects of forest fragmentation on the genetic structure of the progeny, we conducted an allozyme analysis in six polymorphic loci using

starch gel electrophoresis. We collected 5–10 fruits from each tree in undisturbed and disturbed populations (Table 1) and randomly selected five seeds from each fruit. Enzyme extraction, gel buffers, and staining protocols followed [Alfnas et al. \(1991\)](#) and [Soltis and Soltis \(1989\)](#). We analyzed five enzyme systems: shikimate dehydrogenase (SKDH, 1.1.1.25), phosphoglucosomerase (PGI, 5.3.1.9), aspartate aminotransferase (AAT, 2.6.1.1.), esterase (EST, 3.1.1.1.), and isocitrate dehydrogenase (IDH: 1.1.1.41). The AAT system showed two polymorphic loci; therefore, we used six polymorphic loci for genetic analysis. The mean number of seeds sampled for each tree was 25, which allows the identification of the maternal genotype with high confidence. Maternal genotypes were estimated from progeny genotypes using the method of [Brown and Allard \(1970\)](#).

View this table: » In this window » In a new window	Table 1. Number of <i>Ceiba aesculifolia</i> trees and offspring sampled and analyzed for each population in disturbed and undisturbed habitats in each of 4 yr.
--	--

Spatial genetic structure of adults was analyzed with the method of [Smouse and Peakall \(1999\)](#), using the software GenAlEx 6 ([Peakall and Smouse, 2006](#)). This method was used because in cases of high genetic structure of adult tree populations estimation of pollen flow through F_{st} statistics can be misleading ([Austerlitz and Smouse, 2001](#)). Spatial genetic structure occurs when there is correlation between spatial distance and genetic relatedness of individuals in a population. Such correlation may occur as a result of limited seed dispersal, low pollen flow, and selection. To study the spatial genetic structure of adult trees, we estimated spatial and genetic pairwise distances between individuals. The software GenAlEx conducts a random shuffling of all individuals among the geographic locations to estimate a 95% confidence interval of the distance between individuals under the assumption of a lack of spatial structure. We used the multiloci distance (Codom–genotypic genetic distance) proposed by [Smouse and Peakall \(1999\)](#) to estimate the genetic distance between individuals. We estimated mean genetic distances between pairs of trees located at 5 km intervals (i.e., 0–5 km, >5–10 km, >10–15 km, etc.).

We estimated multilocus outcrossing rate (t_m) of progeny in undisturbed and disturbed populations using the program MLTR ([Ritland, 2002](#)). The standard error of the estimates was calculated by bootstrapping with 1000 repetitions.

We calculated two coefficients of mean relatedness for seeds of each tree: the mean relatedness of seed pairs (1) within and (2) between fruits. Estimates of mean relatedness for trees within population and year were calculated with the computer program RELATEDNESS 5.0 ([Queller and Goodnight, 1989](#); [Goodnight and Queller, 1990](#)). Standard errors of these estimates were calculated using jackknife statistical procedure with 1000 repetitions.

To obtain estimates of the genetic differentiation between paternal alleles of seeds from different progenies, we conducted a TwoGener analysis ([Smouse et al., 2001](#)) using the software GenAlex ([Peakall and Smouse, 2006](#)). This analysis estimates the statistic Φ_{ft} , a variation of the Φ_{st} statistic in AMOVA ([Excoffier et al., 1992](#)) except that the focus of the analysis is on populations of male gametes of different progenies instead of adults. The parameter Φ_{ft} can have values between 0 and 1. High values of Φ_{ft} indicate the population is highly selfing, contains few pollen donors, and/or little pollen movement. Low values of Φ_{ft} indicate the population is highly outcrossing, contains many pollen donors, and/or great pollen movement. To estimate the most robust Φ_{ft} with the lowest variance, we grouped trees within sampling year under disturbed and undisturbed conditions as described already. We also estimated the effective number of pollen donors contributing to the progeny of the average mother tree (N_{ep}), which was obtained directly from the estimates of Φ_{ft} for each population and year as $N_{ep} \approx 1/2 \Phi_{ft}$, as well as the parameter δ (i.e., the average distance of realized pollen dispersal), assuming a normal distribution of pollen dispersal around trees ([Austerlitz and Smouse, 2002](#); [Austerlitz et al., 2004](#)). We estimated δ from Φ_{FT} assuming that the effective density of reproductive trees was similar to the observed density of adult trees in the field.

RESULTS

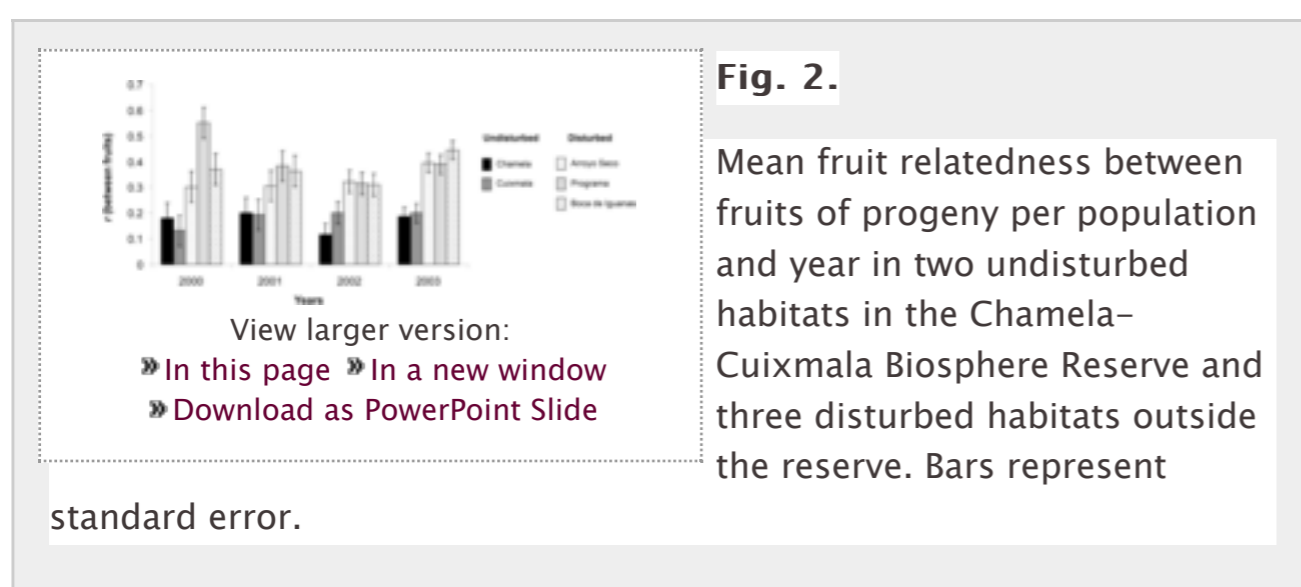
Allelic frequencies of progenies from undisturbed and disturbed habitats were

calculated from three alleles for each of three loci *Aat1*, *Icd*, *Fe*; two alleles from locus *Aat2*; and six alleles for locus *Pgi*. The total mean expected heterozygosity for all populations was $H_e = 0.403$, $SD = 0.084$. Levels of expected heterozygosity for progeny were $H_e = 0.414$, $SD = 0.084$, and $H_e = 0.388$, $SD = 0.085$ for undisturbed and disturbed populations respectively, throughout the 4 yr of the study.

There was no significant correlation between genetic and spatial distance between individuals, indicating a lack of spatial genetic structure. Values of r (Codom-genotypic genetic distance) ranged between upper and lower 95% confidence intervals values expected under the assumption of a lack of genetic structure.

The multilocus outcrossing rate calculated on tree populations from both habitat conditions over the 4 yr ($t_m \approx 1$) indicates that *Ceiba aesculifolia* presents a predominantly outcrossing breeding system.

Estimates of relatedness of progeny within fruits from trees in both habitat conditions were approximately 0.5 (range 0.42–0.58) over four consecutive years, indicating a full-sib relationship and one single donor per fruit. In contrast, progeny of trees from undisturbed populations showed consistently lower levels of relatedness between fruits (≈ 0.25) than progeny from disturbed populations (≈ 0.5) across the 4 yr (Fig. 2). This result indicates that number of pollen donors siring seeds is higher on trees of undisturbed populations.



The Φ_{ft} values were high (0.12–0.35) throughout the 4 yr in both habitat conditions (Table 2), indicating high genetic structure of pollen pools. Nevertheless, populations in disturbed habitats presented greater Φ_{ft} values than undisturbed populations during all years except year 2000 (Table 2). Mean pollen dispersal distances (δ) were consistently greater in disturbed populations than in undisturbed populations throughout the 4 yr. Dispersal distances ranged from 271 to 316 m in disturbed populations and from 166 to 217 m in undisturbed populations. The effective number of pollen donors (N_{ep}), derived from Φ_{FT} values was greater in undisturbed populations, with the exception of year 2000. The number of effective pollen donors ranged from 2 to 4 in undisturbed populations and from 1 to 2 in disturbed populations.

View this table:
[» In this window](#) [» In a new window](#)

Table 2. Estimated values of genetic structure (Φ_{FT}), average distance of pollen flow (δ), and the effective number of pollen donors per maternal parent (N_{ep}) for each population by habitat condition and year.

DISCUSSION

Our unique multiyear study shows that this bat-pollinated tree species frequently experienced reduced gene flow through pollen in remnant trees compared to trees surrounded by continuous forest. Overall, the genetic structure of the pollen pool was high for all studied populations and varied across the 4 yr of the study. The spatial isolation and decline of tree density due to fragmentation clearly reduced pollen sources contributing to siring seeds and increased the degree of relatedness of progeny in disturbed conditions. Such decline of pollen donors are observed even though distance of pollen movement is increased in disturbed habitat conditions. Our results clearly showed that fragmentation does impact pollen flow not by imposing a barrier for pollen movement between remnant patches, but by reducing the diversity of pollen sources siring seeds. Thereby, a loss of genetic diversity of progeny may negatively affect the long-term viability of tree populations

remaining in fragmented tropical forests.

The present study shows that outcrossing rates in *C. aesculifolia* were similar in disturbed and undisturbed habitat conditions across the 4 yr, indicating a sustained strict self-incompatible mechanism of the species across the landscape. Studies of mating systems of other species of the family Bombacaceae suggest that levels of outcrossing can be influenced by pollinators, tree density, or levels of forest fragmentation (Murawski and Hamrick, 1992a, b; Gribel et al., 1999; Fuchs et al., 2003; Lobo et al., 2005). For example, *Pachira quinata* was completely outcrossing in undisturbed forest to partially outcrossing in disturbed populations (Quesada et al., 2001; Fuchs et al., 2003). *Ceiba pentandra* shows varying levels of outcrossing depending on the year and habitat (Murawski and Hamrick, 1992a; Lobo et al., 2005), and *Cavanillesia platanifolia* responds to changes in population density with changes in outcrossing rates (Murawski and Hamrick, 1992b). However, such flexibility does not seem to be present in the self-incompatibility system of *C. aesculifolia*.

This study shows that mean relatedness within fruits in all populations studied was not statistically different from 0.5, indicating that all the seeds of a fruit are full sibs independent of habitat condition, population, or year. One explanation to this pattern is that enough pollen is transferred from a given donor by pollinators to saturate the stigma surface, preventing multiple paternity of seeds within fruits. Another explanation is that pollinators deposit pollen from multiple donors, but only one successfully sires the seeds due to pollen competition (Quesada et al., 1991, 1993, 2001; Mazer et al., 2010). Conversely, mean relatedness of progeny among fruits was greater for seeds of trees from disturbed populations across four consecutive years. This variation in levels of relatedness between habitat condition may be attributed to changes in patterns of foraging behavior of pollinators due to differences in the distribution of floral resources: reproductive trees in disturbed populations have lower conspecific density but higher number of flowers per tree compared to trees in undisturbed populations (Herrerías-Diego et al., 2006). Thus, in disturbed habitats, pollinators might use a concentrated resource found on individuals with large numbers of flowers, thereby obtaining large quantities of nectar and pollen of a single donor, possibly optimizing the use of energy. The landscape of undisturbed populations is more complex and has greater density of trees in bloom but with smaller number of flowers per tree, forcing bat pollinators to visit more pollen and nectar sources in a given night which may explain more pollen donors siring progeny in these trees (Herrerías-Diego et al., 2006; Quesada et al., 2004).

The degree of genetic relatedness among fruits was higher for the progeny of isolated trees than that of trees in continuous forest; however, relatedness within fruits was similar between habitat conditions. This indicates that spatial isolation and density decline of reproductive trees affect the pattern of pollen movement among flowers within individual trees, but not the likelihood of uniparental paternity within individual flowers. The two main pollinators of *C. aesculifolia* at our study sites are the nectarivorous bats *Glossophaga soricina* and *Leptonycteris curasoae* (Quesada et al., 2004); *C. aesculifolia* is their principal source of food from April to June when trees are blooming (Lobo et al., 2003; Quesada et al., 2004). These bat pollinators essentially depend on *C. aesculifolia* during this time of the year, and their foraging behavior is determined by the blooming pattern of this species. In particular, low density of pollen sources and higher flower production due to fragmentation may hinder the capability of pollinators to transfer pollen between trees, likely modifying the dynamics of pollen deposition and the paternity of progeny. Some studies showed a limited transfer of compatible pollen between reproductive individuals in fragmented habitats (Ghazoul et al., 1998; Cunningham, 2000). Similarly, Fuchs et al. (2003) found that in contrast to continuous forest, trees of *Pachira quinata* in forest fragments have greater levels of relatedness between fruits.

Our study showed that populations of *C. aesculifolia* presented high genetic structure of pollen pools (Φ_{ft}) in both habitat conditions, but it is consistently higher in disturbed conditions across all years except 2000. The observed values are high and similar to the maximum estimated Φ_{ft} values reported in the literature for tropical plants where maximum values range from 0.173–0.205 (e.g., Dyer and Sork, 2001; Sork et al., 2002). This reveals that forest fragmentation modifies the pollen pool structure of the bat-pollinated tree *C. aesculifolia*, particularly indicating that pollen flow of trees in fragmented patches is limited within populations and most of the seeds are sired by a few pollen donors. Similarly, in the tree *Symphonia globulifera*, the number of pollen donors mating with seed trees in fragmented landscapes (average N_{ep}

= 3.27) is considerably lower than in continuous forest (average $N_{ep} = 9.55$) (Carneiro et al., 2007).

Based on estimates of theoretical distributions of pollen flow (Austerlitz et al., 2004), high differentiation between pollen pools indicates that pollen dispersal occurs within flowering trees located within discrete neighborhoods. While one has to be careful in estimates of pollen distances using TwoGener because pollen dispersal may not fit a binormal distribution, it is interesting to note that, an average estimate of the mean pollen dispersal distance of 300 m and 198 m was found for trees in disturbed and undisturbed habitats, respectively. Such distances correspond to the nearest neighbor mean distance between trees. Similar effects of spatial structure of reproductive trees on patterns of pollen dispersal have been reported. For example, Stacy et al. (1996) found a predominance of local mating in populations of clumped reproductive trees, whereas a reduction of the density of reproductive trees is associated with an increase of pollination distance on three species of tropical trees. On the basis of bat behavior, we originally predicted that these pollinators of *C. aesculifolia* would fly long distances, dispersing and carrying pollen over long distances from many pollen donors (Horner et al., 1998; Law and Lean, 1999). However, the observed high differentiation between pollen pools indicates that gene flow patterns may explain paternity better than just the maximum range of bat movement reported in the literature. Our results together with previous findings (Herrerías-Diego et al., 2006) indicate that the spatial distribution of reproductive trees, the number of flowers per tree and the presence of food sources during a given time of the year are as important in determining the movement and foraging patterns of bats as their large home ranges and maximum travel distances (Law and Lean, 1999). *Leptonycteris curasoae* can cross open disturbed areas, has large home ranges, and may travel up to 50 km to arrive at a foraging area (Horner et al., 1998; Quesada et al., 2004). In contrast, the other pollinator of *C. aesculifolia*, *Glossophaga soricina*, is a much smaller bat, with a relatively small foraging range and is territorial (Heithaus et al., 1975; Lemke, 1984, 1985). Both pollinators had the same rate of visitation to flowers in undisturbed forest, but *L. curasoae* visited significantly more flowers of disturbed tree populations (Quesada et al., 2004). Therefore, we could expect that the large pollinator, *L. curasoae*, would have moved pollen from many donors and over large distances, reducing differentiation of the genetic structure of populations, particularly in disturbed areas, and the opposite for *G. soricina*. However, both species of bats tended to limit pollen flow to a restricted number of pollen donors within specific neighbors maintaining discrete gamete structures.

Our results indicate that seeds produced by trees in conserved forest were less related to each other than were seeds from trees in fragmented areas, likely because two times more pollen sources contributed to sire progeny in undisturbed habitats. This negative alteration of mating patterns due to fragmentation was observed during 3 of 4 yr of study, even though the average distance of pollen movement was 1.5 times higher in disturbed conditions. Therefore, pollen movement across greater distances does not necessarily imply greater number of sires; we found that while isolated trees experienced pollination at higher distances, they received fewer pollen donors. However, there is controversy in the literature concerning the interpretation of pollen flow, pollen movement distance, and the genetic diversity and number of pollen donors found in the pollen pool in fragmented landscapes. Hamrick (2004) proposed that trees may be resilient to fragmentation impacts because of their longevity, high standing genetic diversity, and extensive pollen movement. Some empirical studies indicate that a high level of pollen flow may counteract the negative impacts related to fragmentation (Aldrich et al., 1998; Nason et al., 1998; White et al., 2002; Dick et al., 2003). On the contrary, other studies have shown negative effects of habitat fragmentation by a reduction in pollen flow among pasture trees (Lowe et al., 2005), reduction in the number of pollen donors, and/or genetic diversity found in the progeny of isolated trees or fragmented patches (Cascante et al., 2002; Fuchs et al., 2003; Fernández-M and Sork, 2007; Rosas et al., 2011). A meta-analysis review found that in most plants studied, forest fragmentation decreased genetic diversity patterns, increased inbreeding, and altered mating patterns in most plant species (Aguilar et al., 2008).

In conclusion, our 4-yr study showed that the progeny produced by isolated trees located in disturbed habitats are consistently sired only by a fraction of the diversity of pollen sources represented in trees in conserved forests across years. The degree of relatedness of the progeny within trees was consistently greater for trees in fragmented conditions across 4 yr. Similarly, the effective number of pollen donors was greater for trees from undisturbed forest (2-4) in contrast to that on disturbed conditions (1-2). We propose that the foraging

behavior of bats limits the exchange of pollen between trees, causing higher levels of progeny relatedness in isolated trees. Future studies need to evaluate the fitness consequences on the progeny relatedness and their ability to respond to environmental changes related to habitat deterioration.

LITERATURE CITED

Aguilar B., J. Achwarth, J. Galotto, and M. A. Aizen. 2006. Plant reproductive susceptibility to habitat fragmentation: Review and synthesis through a meta-analysis. *Ecology Letters* 9: 968–980. » [CrossRef](#) » [Medline](#)

» [Web of Science](#) » [Google Scholar](#)

Aguilar B., M. Quacado, J. Achwarth, V. Herrerías, Diego, and J. Lebo. 2008. Genetic consequences of habitat fragmentation in plant populations: Susceptible signals in plant traits and methodological approaches. *Molecular Ecology* 17: 5177–5188. » [CrossRef](#) » [Medline](#) » [Web of Science](#)

» [Google Scholar](#)

Aizen M. A. and B. Feinsinger. 1994a. Forest fragmentation, pollination, and plant reproduction in a Chaco dry forest, Argentina. *Ecology* 75: 330–351.

» [CrossRef](#) » [Web of Science](#) » [Google Scholar](#)

Aizen M. A. and B. Feinsinger. 1994b. Habitat fragmentation, native insect pollinators, and feral honey bees in Argentine Chaco Serrano. *Ecological Applications* 4: 378–392. » [CrossRef](#) » [Web of Science](#) » [Google Scholar](#)

Alfonso A. C., J. Peters, W. Bruce, and C. C. Baccador. 1991. Electroforesis de proteínas e isoenzimas de fungos e orquídeas forestais. Universidad Federal de Viçosa, Viçosa, Brazil. » [Google Scholar](#)

Aldrich B., J. L. Hamrick, B. Chavarriaga, and C. Kochert. 1998. Microsatellite analysis of demographic genetic structure in fragmented populations of the tropical tree *Symphonia globulifera*. *Molecular Ecology* 7: 933–944.

» [CrossRef](#) » [Medline](#) » [Web of Science](#) » [Google Scholar](#)

Austerlitz E., C. W. Dick, C. Dutoch, E. K. Klein, S. Oddou-Muratorio, B. E. Smouse, and V. L. Sork. 2004. Using genetic markers to estimate the pollen dispersal curve. *Molecular Ecology* 13: 937–954. » [CrossRef](#) » [Medline](#)

» [Web of Science](#) » [Google Scholar](#)

Austerlitz E. and B. E. Smouse. 2001. Two-generation analysis of pollen flow across a landscape. III. Impact of adult population structure. *Genetical Research* 79: 271–280. » [CrossRef](#) » [Medline](#) » [Web of Science](#)

» [Google Scholar](#)

Austerlitz E. and B. E. Smouse. 2002. Two-generation analysis of pollen flow across a landscape. IV. Estimating the dispersal parameter. *Genetics* 161: 355–363. » [Abstract/FREE Full Text](#)

Barrett S. C. H. and J. B. Kohn. 1991. Genetic and evolutionary consequences of small population size in plants: Implications for conservation. In D. A. Falk and K. E. Holsinger [eds.] *Genetic and conservation of rare plants*. 2–30. Oxford University Press, New York, New York, USA. » [Google Scholar](#)

Brown A. H. D. and B. W. Allard. 1970. Estimation of mating system in open-pollinated maize populations using isozyme polymorphisms. *Genetics* 66: 133–140. » [FREE Full Text](#)

Carneiro E. da Silva, A. Sebbenn, M. Kanashiro, and B. Degen. 2007. Low inter-annual variation of mating system and gene flow of *Symphonia globulifera* in the Brazilian Amazon. *Biotropica* 39: 628–636.

» [Google Scholar](#)

Casante A., M. Quacado, J. A. Lebo, and E. J. Eucher. 2002. Effects of dry tropical forest fragmentation on the reproductive success and genetic structure of the tree, *Samanea saman*. *Conservation Biology* 16: 137–147.

» [Google Scholar](#)

Casante, María A. 1997. La familia Bombacaceae (Malvales) en Costa Rica. *Brenesia* 47–48: 17–36. » [Google Scholar](#)

Cunningham S. A. 2000. Depressed pollination in habitat fragments causes low fruit set. *Proceedings of the Royal Society of London, B, Biological Sciences* 267: 1149–1152. » [Abstract/FREE Full Text](#)

Dick C. W. 2001. Genetic rescue of remnant tropical trees by an alien pollinator. *Proceedings Biological Sciences* 268: 2391–2396.

» [Abstract/FREE Full Text](#)

Dick C. W., C. Etcheberry, and E. Austerlitz. 2002. Pollen dispersal of tropical trees (*Dinizia excelsa*, Fabaceae) by native insects and African honeybees in pristine and fragmented Amazonian rainforest. *Molecular Ecology* 12: 753–764. » [CrossRef](#) » [Medline](#) » [Web of Science](#) » [Google Scholar](#)

Didham R. K., J. Chazoual, N. E. Stork, and A. J. Davis. 1996. Insects in fragmented forests: A functional approach. *Trends in Ecology & Evolution* 11: 255–261. » [Medline](#) » [Google Scholar](#)

Dyer B. J. and V. L. Sork. 2001. Pollen pool heterogeneity in chestleaf pine, *Pinus echinata* Mill. *Molecular Ecology* 10: 859–866. » [CrossRef](#) » [Medline](#)

» [Google Scholar](#)

Ellstrand N. C. 1992. Gene flow of pollen: Implications for plant conservation genetics. *Oikos* 63: 77–86. » [CrossRef](#) » [Web of Science](#) » [Google Scholar](#)

Ellstrand N. C. and D. R. Ellam. 1993. Population genetic consequences of

small population size: Implications for plant conservation. *Annual Review of Ecology and Systematics* 24: 217-242. » [CrossRef](#) » [Web of Science](#)

» [Google Scholar](#)

Excoffier L., D. E. Smouse, and J. M. Quattro. 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131: 479-491.

» [Abstract/FREE Full Text](#)

Fernández M. I. L. and V. L. Sork. 2007. Genetic variation in fragmented forest stands of the Andean oak *Quercus humboldtii* Bonpl. (Fagaceae). *Biotropica* 39: 72-78. » [CrossRef](#) » [Web of Science](#) » [Google Scholar](#)

Forsé S. A., J. Hickey, J. L. Markat, S. L. Cuttman, and B. L. Schaefer. 1992. Genetic structure of a forest fragmentation: A landscape ecology perspective on *Acer saccharum*. *Canadian Journal of Botany* 70: 1659-1668.

» [Google Scholar](#)

Fuchs E., J. A. Lobo, and M. Quesada. 2002. Effects of forest fragmentation and flowering phenology on the reproductive success and mating patterns on the tropical dry forest tree *Bachira quinata* (Bombacaceae). *Conservation Biology* 17: 149-157. » [CrossRef](#) » [Web of Science](#) » [Google Scholar](#)

Chazoul L., K. A. Lister, and T. J. P. Boyle. 1998. Disturbance induced density dependent seed set in *Shorea siamensis* (Dipterocarpaceae), a tropical forest tree. *Journal of Ecology* 86: 462-473. » [CrossRef](#)

» [Web of Science](#) » [Google Scholar](#)

Goodnight K. E. and D. C. Queller. 1999. Relatedness. E. O. Kirk Center for Computational Biology, Rice University, Houston, Texas. Available from <http://www.gsoftnet.us/GSoft.html> [accessed March 2010].

Grihal B., P. E. Gibbs, and A. J. Queiroz. 1999. Flowering phenology and pollination biology of *Caiba pentandra* (Bombacaceae) in Central Amazonia. *Journal of Tropical Ecology* 15: 247-263. » [CrossRef](#) » [Web of Science](#)

» [Google Scholar](#)

Hall B., S. Walker, and K. S. Bawa. 1996. Effect of forest fragmentation on genetic diversity and mating system in a tropical tree *Dioscoreophyllum* *elegans*. *Conservation Biology* 10: 757-768. » [CrossRef](#) » [Web of Science](#)

» [Google Scholar](#)

Hamrick J. L. 2004. Response of forest trees to global environmental changes. *Forest Ecology and Management* 197: 323-335. » [CrossRef](#)

» [Web of Science](#) » [Google Scholar](#)

Hamrick J. L., M. J. O. Godt, and S. J. Sherman. 1992. Factors influencing levels of genetic diversity in woody plants species. *New Forests* 6: 95-124.

» [CrossRef](#) » [Google Scholar](#)

Haithaus E. B., T. H. Fleming, and B. A. Opler. 1975. Foraging patterns and resource utilization in seven species of bats in a seasonal tropical forest. *Ecology* 56: 841-854. » [CrossRef](#) » [Web of Science](#) » [Google Scholar](#)

Herreras-Diego V., M. Quesada, J. A. Lobo, and K. E. Stoner. 2006. Effect of forest fragmentation on phenological patterns and reproductive success of the tropical dry forest tree *Caiba aciculifolia*. *Conservation Biology* 20: 1111-1120. » [CrossRef](#) » [Medline](#) » [Web of Science](#) » [Google Scholar](#)

Hewwood V. H., C. M. Mace, D. M. May, and S. M. Stuart. 1994. Uncertainties in extinction rates. *Nature* 368: 105. » [Google Scholar](#)

Horner M. A., T. H. Fleming, and C. T. Sabely. 1999. Foraging behaviour and energetics of a nectar-feeding bat *Lantonycteris curacaoe* (Chiroptera: Phyllostomidae). *Journal of Zoology* 244: 575-586. » [CrossRef](#)

» [Web of Science](#) » [Google Scholar](#)

Invin A. J., J. L. Hamrick, M. J. W. Godt, and D. E. Smouse. 2002. A multiview estimate of the effective pollen donor pool for *Albizia julibrissin*. *Heredity* 90: 187-194. » [CrossRef](#) » [Medline](#) » [Web of Science](#) » [Google Scholar](#)

Law R. S. and M. Lean. 1999. Common blossom bats (*Syconycteris australis*) as pollinators in fragmented Australian tropical rainforest. *Biological Conservation* 91: 201-212. » [CrossRef](#) » [Web of Science](#) » [Google Scholar](#)

Lemke T. O. 1994. Foraging ecology of the long-eared bat *Glossophaga soricina* with respect to resource availability. *Ecology* 65: 538-548.

» [CrossRef](#) » [Web of Science](#) » [Google Scholar](#)

Lemke T. O. 1995. Pollen carrying by the nectar-feeding bat *Glossophaga soricina* in a suburban environment. *Biotropica* 17: 107-111. » [CrossRef](#)

» [Web of Science](#) » [Google Scholar](#)

Lobo J. A., M. Quesada, and K. E. Stoner. 2005. Effects of pollination by bats on the mating system of *Caiba pentandra* (Bombacaceae) populations in two tropical life zones in Costa Rica. *American Journal of Botany* 92: 370-376.

» [Abstract/FREE Full Text](#)

Lobo J. A., M. Quesada, K. E. Stoner, E. J. Fuchs, V. Herreras-Diego, J. Rojas, and C. Sabaris. 2002. Factors affecting phenological patterns of bombacaceous trees in seasonal forest in Costa Rica and Mexico. *American Journal of Botany* 90: 1054-1063. » [Abstract/FREE Full Text](#)

Lowe A. J., D. Boshier, M. Ward, C. E. E. Bagles, and C. Navarro. 2005. Genetic resource impacts of habitat loss and degradation: reconciling empirical evidence and predicted theory for neotropical trees. *Heredity* 95: 255-273.

» [CrossRef](#) » [Medline](#) » [Web of Science](#) » [Google Scholar](#)

Meyer S. L., A. A. Howe, B. S. Miller, and M. Barbet. 2010. The joint evolution of mating system and pollen performance: Predictions regarding male gametophytic evolution in selfers vs. outcrossers. *Perspectives in Plant Ecology, Evolution and Systematics* 12: 31–41. »[CrossRef](#) »[Web of Science](#) »[Google Scholar](#)

McCaulley D. E. 1995. Effects of population dynamics on genetics in mosaic landscapes. In: L. Hansson, I. Fahrig, and C. Merriam [eds.] *Mosaic landscape and ecological processes*, 178–198. Chapman and Hall, London, UK. »[Google Scholar](#)

Menges E. S. 1991. Seed germination percentage increases with population size in a fragmented prairie species. *Conservation Biology* 5: 158–164. »[CrossRef](#) »[Web of Science](#) »[Google Scholar](#)

Murawski D. A., I. A. H. M. Gunatilleke, and K. S. Pawa. 1994. The effects of selective logging on inbreeding in *Shorea rostrata* (Dipterocarpaceae) from Sri Lanka. *Conservation Biology* 8: 997–1002. »[CrossRef](#) »[Web of Science](#) »[Google Scholar](#)

Murawski D. A. and I. L. Hamrick. 1992a. Mating system and phenology of *Coiba pentandra* (Bombacaceae) in Central Panama. *Journal of Heredity* 83: 401–404. »[Abstract/FREE Full Text](#)

Murawski D. A. and I. L. Hamrick. 1992b. The mating system of *Cavanillesia platanifolia* under extremes of flowering tree density: A test of predictions. *Biotropica* 24: 99–101. »[CrossRef](#) »[Web of Science](#) »[Google Scholar](#)

Nason J. D. and I. L. Hamrick. 1997. Reproductive and genetic consequences of forest fragmentation: Two case studies of neotropical canopy trees. *Journal of Heredity* 88: 264–276. »[Abstract/FREE Full Text](#)

Nason J. D., E. A. Herre, and I. L. Hamrick. 1998. The breeding structure of a tropical keystone plant resource. *Nature* 391: 685–687. »[CrossRef](#) »[Web of Science](#) »[Google Scholar](#)

Peakall B. and P. E. Smouse. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6: 288–295. »[CrossRef](#) »[Web of Science](#) »[Google Scholar](#)

Queller D. C. and K. E. Goodnight. 1989. Estimating relatedness using genetic markers. *Evolution* 43: 258–275. »[CrossRef](#) »[Web of Science](#) »[Google Scholar](#)

Quesada M., E. I. Fuchs, and I. A. Lobo. 2001. Pollen load size, reproductive success and progeny kinship of naturally pollinated flowers of the tropical dry forest tree *Bachira quinata* (Bombacaceae). *American Journal of Botany* 88: 2113–2118. »[Abstract/FREE Full Text](#)

Quesada M., C. D. Schlichting, J. Winsor, and A. C. Stephenson. 1991. Effects of genotype on pollen performance in *Cucurbita pepo*. *Sexual Plant Reproduction* 4: 208–214. »[Web of Science](#) »[Google Scholar](#)

Quesada M. and K. E. Stoner. 2004. Threats to the conservation of the tropical dry forest in Costa Rica. In: C. H. Frankie, A. Mata, and S. B. Vinson [eds.] *Biodiversity conservation in Costa Rica: Learning lessons in a seasonal dry forest*, 266–280. University of California Press, Berkeley, California, USA. »[Google Scholar](#)

Quesada M., K. E. Stoner, I. A. Lobo, V. Herrero-Diego, C. Palacios-Cuevara, M. A. Murcuía-Rosas, and K. A. Ocaquera-Salazar. 2004. Effects of forest fragmentation on pollinator activity and consequences for plant reproductive success and mating patterns in bat pollinated bombacaceous trees. *Biotropica* 36: 131–138. »[CrossRef](#) »[Web of Science](#) »[Google Scholar](#)

Quesada M., K. E. Stoner, V. Rosas, Cuarrero, C. Palacios-Cuevara, and I. A. Lobo. 2002. Effects of habitat disruption on the activity of nectarivorous bats (Chiroptera: Phyllostomidae) in a dry tropical forest: Implications for the reproductive success of the neotropical tree *Coiba grandiflora*. *Oecologia* 135: 400–406. »[Medline](#) »[Web of Science](#) »[Google Scholar](#)

Quesada M., I. A. Winsor, and A. C. Stephenson. 1993. Effects of pollen competition on progeny performance in a heterozygous cucurbit. *American Naturalist* 142: 684–706. »[Google Scholar](#)

Ditland K. 2002. Extensions of models for the estimation of mating systems using *n* independent loci. *Heredity* 88: 221–228. »[CrossRef](#) »[Medline](#) »[Web of Science](#) »[Google Scholar](#)

Rosas E. M., Quesada, I. A. Lobo, and V. Sork. 2011. Effects of habitat fragmentation on pollen flow and genetic diversity of the endangered tropical tree *Suicidia humilis* (Meliaceae). *Biological Conservation* 144: 3082–3088. »[CrossRef](#) »[Web of Science](#) »[Google Scholar](#)

Smouse P. E., E. J. Dyer, P. D. Westfall, and V. J. Sork. 2001. Two generation analysis of pollen flow across a landscape I. Male gamete heterogeneity among females. *Evolution: International Journal of Organic Evolution* 55: 260–271. »[CrossRef](#) »[Medline](#) »[Web of Science](#) »[Google Scholar](#)

Smouse P. E. and B. Peakall. 1999. Spatial autocorrelation analysis of multi-allele and multi-locus genetic micro-structure. *Heredity* 82: 561–573. »[CrossRef](#) »[Medline](#) »[Web of Science](#) »[Google Scholar](#)

Soltis D. E. and P. S. Soltis. 1989. *Isoczymes in plant biology*. Dioscorides Press, Portland Oregon, USA. »[Google Scholar](#)

Sork V. L., F. W. Davis, P. E. Smouse, V. J. Apsit, J. Dyer, J. Fernández, and

- B. Kuhn. 2002. Pollen movement in declining populations of California Valley oak, *Quercus lobata*: Where have all the fathers gone? *Molecular Ecology* 11: 1657–1668. » [CrossRef](#) » [Medline](#) » [Web of Science](#) » [Google Scholar](#)
- Sork V. L., J. Nason, D. P. Campbell, and J. Fernández. 1999. Landscape approaches to historical and contemporary gene flow in plants. *Trends in Ecology & Evolution* 14: 219–224. » [CrossRef](#) » [Medline](#) » [Web of Science](#) » [Google Scholar](#)
- Sork V. L. and B. E. Smouse. 2006. Genetic analysis of landscape connectivity in tree populations. *Landscape Ecology* 21: 821–836. » [CrossRef](#) » [Web of Science](#) » [Google Scholar](#)
- Stacy F. A., J. L. Hamrick, J. D. Nason, S. B. Hubbell, B. B. Foster, and B. Condit. 1996. Pollen dispersal in low density populations of three neotropical tree species. *American Naturalist* 148: 275–298. » [CrossRef](#) » [Web of Science](#) » [Google Scholar](#)
- Templeton A. R., K. Shaw, E. Bouman, and S. K. Davis. 1990. The genetic consequences of habitat fragmentation. *Annals of the Missouri Botanical Garden* 77: 13–27. » [CrossRef](#) » [Web of Science](#) » [Google Scholar](#)
- Ward M., C. W. Dick, B. Cribel, and A. J. Lowe. 2005. To self or not to self: A review of outcrossing and pollen-mediated gene flow in neotropical trees. *Heredity* 95: 246–254. » [CrossRef](#) » [Medline](#) » [Web of Science](#) » [Google Scholar](#)
- White C. M., D. H. Baskin, and W. Bayell. 2002. Increased pollen flow counteracts fragmentation in a tropical dry forest: An example from *Suaeda humilis* Zuccarini. *Proceedings of the National Academy of Sciences, USA* 99: 2038–2042. » [Abstract / FREE Full Text](#)
- Young A., T. Boyle, and T. Brown. 1996. The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology & Evolution* 11: 413–417. » [CrossRef](#) » [Medline](#) » [Web of Science](#) » [Google Scholar](#)

Articles citing this article

Following Darwin's trail: Interactions affecting the evolution of plant mating systems

Am. J. Bot. June 1, 2013 100:999–1001

» [Abstract](#) » [Full Text](#) » [Full Text \(PDF\)](#)

A multidimensional approach to understanding floral function and form I

Am. J. Bot. June 1, 2013 100:1102–1104

» [Abstract](#) » [Full Text](#) » [Full Text \(PDF\)](#)