

Pollen dispersal and genetic structure of the tropical tree *Dipteryx panamensis* in a fragmented Costa Rican landscape

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Abstract

In the face of widespread deforestation, the conservation of rainforest trees relies increasingly on their ability to maintain reproductive processes in fragmented landscapes. Here, we analysed nine microsatellite loci for 218 adults and 325 progeny of the tree *Dipteryx panamensis* in Costa Rica. Pollen dispersal distances, genetic diversity, genetic structure and spatial autocorrelation were determined for populations in four habitats: continuous forest, forest fragments, pastures adjacent to fragments and isolated pastures. We predicted longer but less frequent pollen movements among increasingly isolated trees. This pattern would lead to lower outcrossing rates for pasture trees, as well as lower genetic diversity and increased structure and spatial autocorrelation among their progeny. Results generally followed these expectations, with the shortest pollen dispersal among continuous forest trees (240 m), moderate distances for fragment (343 m) and adjacent pasture (317 m) populations, and distances of up to 2.3 km in isolated pastures (mean: 557 m). Variance around pollen dispersal estimates also increased with fragmentation, suggesting altered pollination conditions. Outcrossing rates were lower for pasture trees and we found greater spatial autocorrelation and genetic structure among their progeny, as well as a trend towards lower heterozygosity. Paternal reproductive dominance, the pollen contributions from individual fathers, did not vary among habitats, but we did document asymmetric pollen flow between pasture and adjacent fragment populations. We conclude that long-distance pollen dispersal helps maintain gene flow for *D. panamensis* in this fragmented landscape, but pasture and isolated pasture populations are still at risk of long-term genetic erosion.

Keywords: genetic diversity, genetic structure, paternal reproductive dominance, paternity analysis, pollen dispersal, spatial autocorrelation

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Introduction

Habitat loss is widely recognized as the greatest threat to global biodiversity (Myers *et al.* 2000; Novacek & Cleland 2001). In tropical forests, home to more than two-thirds of the world's species (Raven 1988), deforestation rates are estimated at 12 million hectares per year (Food and Agriculture Organization 2000). While large-scale agriculture and development projects can lead to complete habitat conversion, forest clearing often occurs more sporadically, leaving behind a mosaic of pastures, small farms and forest

patches (Myers 1984). Maintaining biodiversity within these fragmented landscapes presents a major challenge for policy makers, natural resource managers and landowners. Keystone species, those affecting community structure at multiple trophic levels, may be particularly important in this setting, since a suite of associated species would be impacted by their loss (Diamond *et al.* 1976; Wilcox & Murphy 1985).

Keystone plants in tropical forests are often large trees vulnerable to increased windthrow, disrupted pollen and seed dispersal, and environmental changes associated with habitat fragmentation (Nason *et al.* 1998; Laurance *et al.* 2000). Many also have commercial value, exposing them to logging pressure and other human disturbances. Keystone trees exhibit a strong influence on other groups of

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organisms at various trophic levels, particularly through copious fruit production (Nason *et al.* 1998). Their extirpation has the potential to cause changes in habitat structure and community composition, with the potential for a cascade of local extinctions (Nason *et al.* 1998; Lennartsson 2002). Preserving viable populations of keystone trees is a fundamental aspect of biodiversity conservation in fragmented tropical landscapes, and requires detailed knowledge of their responses to habitat fragmentation.

Innovations in molecular genetics have led to new insights in the study of pollen dispersal and the reproductive ecology of plant populations (Nason & Hamrick 1997; Ward *et al.* 2005). By applying these techniques to tropical trees, we can track specific patterns of pollen-mediated gene flow in fragmented landscapes (e.g. Aldrich & Hamrick 1998; White *et al.* 2002) and better understand issues of connectivity, population viability and the long-term genetic consequences of habitat fragmentation (reviewed in Lowe *et al.* 2005). Emerging trends highlight lower outcrossing rates (Dick *et al.* 2003), signs of inbreeding (Aldrich & Hamrick 1998; Cascante *et al.* 2002), reduced reproductive output (Hall *et al.* 1996) and decreased progeny vigor (Nason & Hamrick 1997; Cascante *et al.* 2002) in fragmented populations. On the other hand, studies have also shown increased pollen dispersal distances (White *et al.* 2002; Dick *et al.* 2003) and higher seed production (Dick 2001) in fragmented landscapes. Two studies have noted reproductive dominance by a few fecund individuals (Aldrich & Hamrick 1998; Sezen *et al.* 2005), which could lead to genetic bottlenecks in fragmented or regenerating habitats. Also, regeneration in forest fragments has in some cases been dominated by outside sources, either trees in adjacent pasture (Aldrich & Hamrick 1998) or trees in distant fragments and continuous forest (Nason & Hamrick 1997). In a recent review, Lowe *et al.* (2005) note that few studies of tropical trees have found signs of decreased genetic diversity in fragmented populations, but that such changes could take generations to appear. Also, long-distance pollen dispersal may bolster some species against the negative genetic consequences of fragmentation (Dick *et al.* 2003). The impact of altered pollen flow on the genetic diversity and structure of progeny populations, and the applicability of observed trends to other species and landscapes, remain largely unexplored.

In this study, we examine pollen dispersal, genetic diversity and genetic structure for the canopy emergent tree *Dipteryx panamensis* (Fabaceae), a keystone species in the Atlantic lowland forests of Central America. Although reduced by harvest and forest clearing over much of its range, *D. panamensis* persists in fragments and pastures and remains a conservation priority. It is listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) as a CITES Appendix III species (UNEP-WCMC 2006) and was recently designated

as Vulnerable to extinction in an evaluation of Costa Rican plant species using International Union for the Conservation of Nature and Natural Resources (IUCN) Red List criteria (Estrada-Chavarria *et al.* 2005). Additionally, the endangered great green macaw (*Ara ambiguus*) depends on *D. panamensis* as nesting habitat and a critical food source (Juniper & Parr 1998), a relationship that figures prominently in environmental education and conservation efforts throughout the region. Understanding the effects of forest fragmentation on this species has important implications for prioritizing the conservation of remnant habitats and for maintaining connectivity in a human-dominated landscape.

Dipteryx panamensis adults and progeny are studied in four habitat types: continuous forest, forest fragments, pastures adjacent to fragments and pastures isolated from forest by > 1.5 km. Because the reproductive adults (> 30 cm diameter) at all sites date from a continuous, prefragmentation forest, comparisons between adult and progeny generations allow us to estimate the genetic impacts of postfragmentation reproductive conditions. The continuous forest site is assumed to be relatively unaltered, providing a control where no difference is expected between adults and progeny. Six questions are addressed: (i) Do pollen dispersal distances vary among habitat types? (ii) Do outcrossing rates vary between forest and pasture trees? (iii) Do the progeny in increasingly fragmented habitats show a loss of genetic diversity? (iv) Do genetic structure and spatial autocorrelation increase for the progeny in fragmented habitats? (v) Is paternal reproductive dominance evident in *D. panamensis* populations, and does it vary among habitat types? (vi) Is there evidence of habitat-level reproductive dominance (i.e. asymmetric pollen flow) between fragments and adjacent pastures?

Fragmentation impacts on pollinator communities and the spacing and density of adult trees are expected to influence pollen dispersal distances and the genetic diversity of progeny. We predict that pollen dispersal distances will increase in fragmented areas, but that pollen will move less often and among fewer individuals, leading to lower outcrossing rates for pasture trees and lower genetic diversity for their progeny. We expect that reduced outcrossing and smaller breeding populations in pastures will cause increased genetic structure and spatial autocorrelation in pasture progeny. Finally, we expect more reproductively dominant fathers in pastures, where reduced competition and abundant light should allow greater allocation of resources to reproductive output.

Methods and materials

Study area

Study sites for this project lie in Cantón Sarapiquí, Heredia Province, Costa Rica, at elevations of 30–70 m. The natural

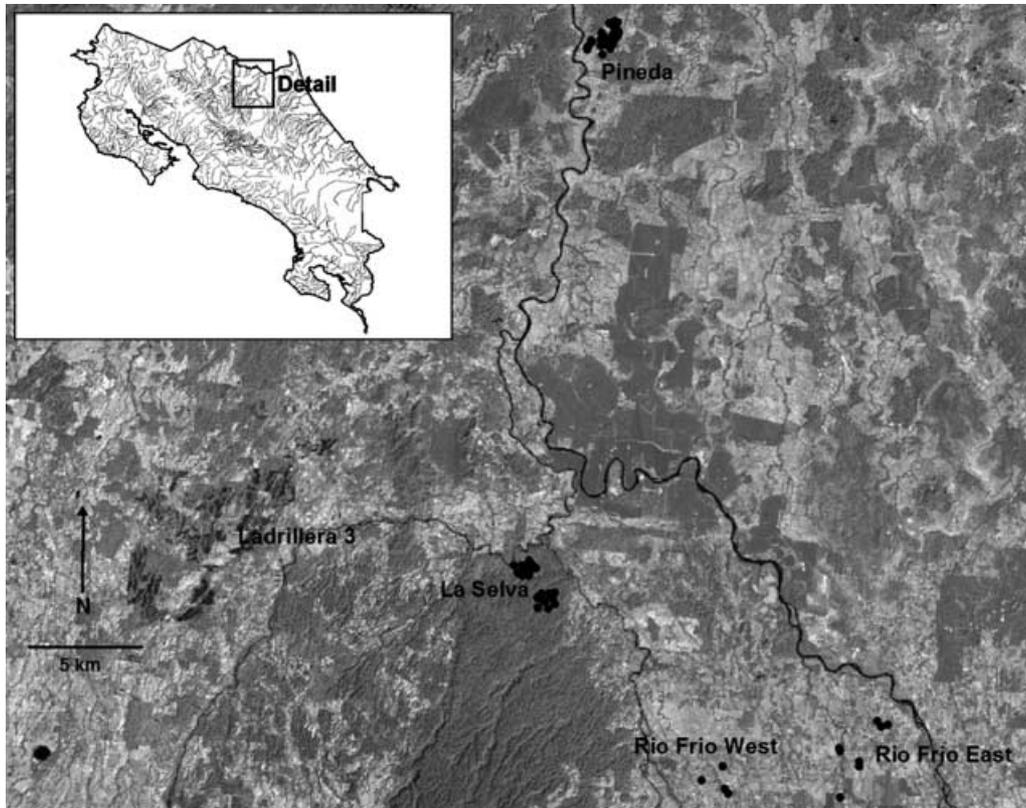


Fig. 1 Landsat (2001) image showing study sites, adult *Dipteryx panamensis* (•) and major features in the fragmented landscape of Cantón Sarapiquí, Heredia Province, Costa Rica.

vegetation community consists of lowland wet tropical forest with a mean annual rainfall of ~4000 mm (measured at La Selva Biological Station) (McDade *et al.* 1994). Since roads first penetrated the area in the early 1950s, rapid settlement has replaced much of the original forest cover with a network of fragments and wooded riparian strips, set in an agriculturally dominated matrix of pastures, banana, pineapple and palm plantations, small towns and family farms (Butterfield 1994).

This study was carried out in a private forest reserve (La Selva), two forest fragments exposed to hunting and selective logging (Ladrillera 3; Pineda), two actively grazed pastures adjacent to the forest fragments, and two actively grazed pasture areas isolated from forests by > 1.5 km (Rio Frio) (Fig. 1). Study sites were separated by a mean distance of 18.4 km (range; 0.5–32 km) and were grouped for analysis into four habitat types: continuous forest, forest fragments, pastures and isolated pastures.

La Selva Biological Station is a 2500-ha private reserve that includes 700 ha of lowland rainforest protected from logging and hunting for at least the last 20–30 years. It adjoins Braulio Carrillo National Park, forming the largest tract of intact habitat in the region (McDade *et al.* 1994). We established two 65-ha focal study areas in the unlogged interior portion of this forest, separated from one another

by 500 m and containing populations of mature *Dipteryx panamensis* at 0.97/ha and 0.68/ha.

Pineda (40 ha) and Ladrillera 3 (52 ha) are privately held remnants surrounded by pastures and managed under sustainable forestry principles in partnership with the Fundación para el Desarrollo y Conservación de la Cordillera Volcánica Central (FUNDECOR), a local nongovernmental organization. Ladrillera 3 consists of primary forest that was selectively logged in 2002 at an intensity of 1.5 trees/ha for a total volume of 351.2 m³ (FUNDECOR, unpublished data). In the Pineda fragment, 15.5 ha were selectively logged in 2001 at an intensity of 4.32 trees/ha for a total volume of 291.93 m³ (FUNDECOR, unpublished data). Additional area in Pineda was selectively logged during the 1990s, but no specific harvest data are available. Both fragment sites maintain a diverse forest canopy with populations of adult *D. panamensis* at 0.58/ha in Pineda and 0.21/ha in Ladrillera 3. Pastures surrounding both fragments are actively grazed and contain remnant *D. panamensis* as shade trees at densities of 0.19/ha for Pineda and 0.25/ha for Ladrillera 3.

The Rio Frio sites (east and west) include small populations of isolated remnant trees in pastures located > 1.5 km from continuous forest or forest fragments, as determined by extensive ground surveys, interviews with landowners,

and examination of 2001 Landsat satellite imagery (1000 m²/pixel). Density of adult *D. panamensis* in the Rio Frio landscape is < 0.04/ha.

Study species

Dipteryx panamensis (Pittier) Record & Mell (Fabaceae; Papilionoideae) is a large canopy emergent tree occurring in lowland wet tropical forests of the Caribbean slope, from Colombia to Nicaragua (Flores 1992). Considered a keystone species for its copious fruit production, *D. panamensis* bears single-seeded drupes for an extended period during the low-rainfall season, outside the peak fruiting period for this forest type (Frankie *et al.* 1974). Sixteen species of mammals (Bonaccorso *et al.* 1980) and approximately 100 species of birds (Flores 1992) have been observed in and around fruiting trees. Suspected pollinators include 19 different medium to large native bees (Thiele 2002), while seed dispersal is carried out by frugivorous bats, and through the scatterhoarding habits of several small mammals (Bonaccorso *et al.* 1980). Evidence from the same sites used in this study suggests that seed dispersal remains relatively robust in fragmented areas (Hanson *et al.* 2007). The tree is monoecious and has been suggested as an obligate outcrosser (Perry & Starrett 1980), although our results suggest otherwise. Trees flower asynchronously for 6-week periods from May through August (Frankie *et al.* 1974; Perry & Starrett 1980), and individuals mature and begin bearing fruit at approximately 30 cm diameter (T. Hanson, personal observation). The ploidy level of *D. panamensis* has not been previously described and its chromosome number remains unknown. Over the course of this study, eight of nine microsatellites behaved like tetraploid loci, while one showed additional signs of gene doubling. The Amazonian congener *Dipteryx odorata* also exhibits signs of tetraploidy with multisomic inheritance (C. Vinson, personal communication).

Sampling

All adult (reproductively mature) trees (> 30 cm diameter) at Pineda (forest *n* = 26; pasture *n* = 26), Ladrillera 3 (forest *n* = 14; pasture *n* = 36) and Rio Frio (west *n* = 5; east *n* = 7) were located and global positioning system (GPS) mapped through exhaustive ground surveys and interviews with landowners during site visits in August 2004 and January–April, 2005 (Table 1). All adult trees at La Selva (west *n* = 63; east *n* = 41) had been previously mapped as part of an ongoing demographic study (e.g. Clark & Clark 1987, 1999) and were revisited for this project in 2005 (Table 1). Samples of leaf or cambium tissue were collected from every adult at each site and stored immediately in silica gel for later extraction and analysis. Progeny arrays (*n* = 65) were gathered concurrently and during additional visits in 2006

Table 1 Maximum likelihood paternity analysis for 325 *Dipteryx panamensis* progeny from continuous forest, forest fragments, pastures adjacent to fragments and pastures isolated from forest by > 1.5 km in Cantón Sarapiquí, Costa Rica

Site	Habitat*	Adults N	Progeny N (arrays)	Locit	Missing data	Exclusion probabilities (paternity/percentage)	Delta criterion (95% confidence threshold from 10 000 simulations)	Paternity assignments			
								1 father	2 potential fathers	Father outside population	
La Selva West	CF	63	21 (5)	51	1.89%	(84.5%/96.3%)	2.08	6	8	0	7
La Selva East	CF	41	29 (6)	47	1.16%	(85.1%/96.8%)	2.29	11	5	0	13
Pineda Forest	FF	26	74 (15)	49	0.34%	(86.7%/97.4%)	2.02	17	25	1	31
Ladrillera 3 Forest	FF	14	49 (10)	47	1.37%	(86.1%/97.1%)	2.34	26	7	0	16
Pineda Pasture	P	26	50 (10)	49	0.56%	(86.3%/97.4%)	2.26	23	11	1	15
Ladrillera 3 Pasture	P	36	58 (12)	47	1.47%	(86.1%/97.1%)	1.92	21	13	11	13
Rio Frio West	IP	5	15 (3)	38	1.75%	(83.5%/96.2%)	1.52	4	0	11	0
Rio Frio East	IP	7	29 (6)	40	1.42%	(74.7%/90.1%)	1.21	13	2	14	0
totals:								121 (37.2%)	71 (21.8%)	38 (11.7%)	95 (29.2%)

*CF (continuous forest), FF (forest fragment), P (pasture), IP (isolated pasture); †dominant loci from transformed tetraploid microsatellite data (Rodzen *et al.* 2004).

and consisted of five seeds or the leaves of seedlings gathered from directly beneath focal maternal trees (total progeny $n = 325$) (Table 1). Focal maternal trees (3–15 per site) were chosen randomly from the reproductive adults for which usable seeds or seedlings were available. The assumption of maternity (i.e. that the progeny had not been dispersed to beneath that tree from another adult) was supported by genetic analysis: alleles present in the maternal tree were also present in the progeny at all loci. The maternity assumption also corresponds to feeding habits of the bat *Artibeus lituratus*, the primary *D. panamensis* seed disperser, which transports fruit to remote feeding roosts rather than moving them among adult trees (Bonaccorso *et al.* 1980; Morrison 1980).

Genetic markers

We used nine microsatellite primer pairs designed from loci developed for the Amazonian species *D. odorata* (Vinson 2004). Extraction protocols, sequencing methods and polymerase chain reaction (PCR) characteristics are fully described elsewhere (Hanson *et al.* in press). For this study, genotypes were determined for 218 adults and 325 progeny. Error rates were calculated by re-analysing 90 samples (16% of all samples) for all nine loci.

The application of microsatellite markers in a polyploid species is limited by the challenge of identifying true genotypes for partial heterozygotes. In a tetraploid like *D. panamensis*, for example, the genotype ABBC produces the microsatellite result ABC, and cannot be reliably differentiated from AABC or ABCC. The analysis software TETRASAT computes all possible allele combinations for partial heterozygotes and reports mean values for metrics including expected Hardy–Weinberg heterozygosity (H_E) and Nei's measure of population differentiation (G_{ST}) (Markwith *et al.* 2006). Similar techniques do not exist, however, for paternity analysis or spatial autocorrelation. For these analyses, we transformed our codominant microsatellite data into a dominant data set following the methods of Rodzen *et al.* (2004). This technique treats microsatellite alleles as loci, creating binary genotypes that can be used with a variety of programs designed for dominant markers. This approach involves an inherent loss of information (heterozygosity), but has been used successfully for parentage analysis (e.g. Rodzen *et al.* 2004) as well as studies of genetic diversity and structure (Mengoni *et al.* 2000).

Pollen dispersal distances

Paternity analysis and estimates of pollen pool structure (e.g. TWOGENER) have been used to determine pollen dispersal distances for tree species (Smouse & Sork 2004). Analytic tools often rely, however, on codominant allelic frequency data unavailable for polyploids (see discussion

of partial heterozygotes above). Given the uncertainties inherent in polyploid microsatellite data, we chose to perform maximum likelihood paternity analyses for the progeny at each site, using our dominant-transformed data set with the dominant algorithms and default settings of the software FAMOZ (Gerber *et al.* 2003). For each population, we used the delta criterion (Marshall *et al.* 1998) from 10 000 simulations to assign paternity among multiple likely fathers with strict 95% confidence. All potential fathers were sampled in each study area. Where only one father was identified in the population, we determined an exact pollen dispersal distance between the maternal and paternal trees using Euclidian distance matrices calculated from their GPS locations. When no likely father was present (e.g. the progeny contained alleles not found in any potential father within the population), we assumed that the pollen came from outside the population and measured a minimum pollen dispersal distance to the nearest edge of the sampled area. In many cases ($n = 71$), the analysis determined that one of two trees was the father (both had delta scores above the 95% confidence threshold). We called these instances 'two-father assignments', and used the closest tree as a minimum dispersal distance and the farthest tree as a maximum dispersal distance. Progeny with ambiguous paternity (i.e. likely father present, but without a delta score above the 95% confidence threshold) were left unassigned.

Pollen dispersal distances from one-father, two-father or no-father (outside population) assignments were compared among habitat types in analyses of covariance (ANCOVA), using the Tukey–Kramer procedure (JW Tukey, Princeton University, Princeton, NJ, unpublished manuscript; Kramer 1956) for multiple comparisons and the least-square means method (Ott & Longnecker 2001) to compare individual treatments. To account for the proximity of potential fathers around maternal trees, we included the median distance to the five nearest reproductive adults as a covariate for each pollen dispersal assignment. This covariate helped control for variation associated with near fathers (e.g. even in low-density populations, a maternal tree may happen to be located within a tight group of reproductive adults) rather than the habitat-level variation of interest. To test and calibrate the covariate, we repeated all analyses using the median distance to the 10 nearest reproductive adults as a covariate.

Outcrossing rate

Two analyses were performed to determine if outcrossing rates varied for trees in different habitats. The multilocus outcrossing rate (t_m) was measured as the proportion of progeny in each array that contained nonmaternal alleles. The assignment outcrossing rate (t_a) was measured as the proportion of paternity assignments attributed to non-maternal trees. (Both of these calculations should be viewed as minimum outcrossing rates, as matings between closely

related individuals could produce genotypes without nonmaternal alleles.) Assignments outside the population (no father present) were considered outcrossing events for the determination of t_a . Calculations for both t_m and t_a accounted for genotyping error rates and were compared between habitat types using the Kruskal–Wallis test (Kruskall & Wallis 1952) for t_m and a chi-squared test for t_a . Because of the low incidence of self-pollination events in continuous forest and forest fragments, habitat types were combined for a comparison of all forest and forest fragment trees vs. trees in pastures and isolated pastures.

Genetic diversity

We compared mean Hardy–Weinberg H_E and allelic richness (A) between adult (prefragmentation) and progeny (postfragmentation) generations in all habitat types. H_E values were generated using the program TETRASAT (Markwith *et al.* 2006). Intense computational demands, however, limited the number of partial heterozygotes that can be processed at one time (Markwith *et al.* 2006). We therefore performed 10 iterations of each analysis on random subsamples (10 individuals, with replacement) from each population, using the program's default subsampling routines and settings. This subsampling protocol also accounted for unequal sample sizes among habitats. We calculated A as the mean number of alleles per microsatellite locus.

H_E and A values were blocked by locus and compared between adult and progeny generations using paired t -tests for normal data and Wilcoxon signed-rank sum tests if the data failed a Shapiro–Wilk test for normality (Shapiro & Wilk 1965). The t -tests were two-tailed for continuous forest populations, but one-tailed for other habitat types where we expected lower diversity and higher structure for the progeny generation.

Genetic structure

Nei's population differentiation (G_{ST}) was calculated between the adult populations and between the progeny populations within each habitat type using the program TETRASAT and the subsampling protocol outlined above. To determine if genetic structure increased among progeny populations, mean G_{ST} values were compared between generations in each habitat using paired t -tests, blocked by locus.

Spatial autocorrelation

Spatial autocorrelation explicitly combines spatial and genetic data, allowing the examination of genetic structure at finer scales than G_{ST} . We performed spatial autocorrelation analysis on the parental and progeny generations in each

habitat type using our dominant data set in the program GENALEX (Peakall & Smouse 2006). GENALEX follows the multivariate approach described by Smouse & Peakall (1999), and tests for significance by establishing upper and lower 95% confidence boundaries for the autocorrelation coefficient (r) based on 999 random simulations of the null (no autocorrelation) model. Similar confidence intervals are established around the estimate of r using 999 bootstraps, and structure is inferred for nonoverlapping positive ranges. We chose the multiple distance class option in GENALEX to output graphs of the combined mean autocorrelation coefficient (r_c) for increasing distance size classes (100–2500 m). The spatial limit of genetic structure is indicated by the size class at which r_c is no longer significant. Rather than a traditional correlogram, which can be strongly affected by the interplay between true genetic structure, the chosen distance classes, and unequal samples within those classes, the cumulative model is less prone to parameter errors and may provide a more reliable estimate of the spatial extent of genetic structure (Double *et al.* 2005).

Paternal reproductive dominance

Reproductive dominance can be paternal, through overrepresentation of individual pollen donors in progeny cohorts, or maternal, through the overrepresentation of seeds. Here, we identified the reproductively dominant pollen donors at all sites and compared the prevalence of dominance in progeny arrays among habitat types. To identify dominant individuals, a reproductive index (R) was calculated for each adult tree, based on the number of outcrossing events assigned to them during paternity analysis ($R = \text{single-father assignments} + \frac{1}{2} \text{two-father assignments}$). Individuals whose reproductive index was higher than 95% of their peers (> 2 SD above mean) were considered dominant. To compare the prevalence of reproductive dominance among habitats, the ratio of observed fathers to the number of fathers expected under a random, equal-contribution mating model was calculated for each progeny array and compared among habitats using a Kruskal–Wallis test (Kruskall & Wallis 1952).

Habitat dominance

Habitat-level reproductive dominance occurs when individuals in one habitat type contribute disproportionately (via pollen or seeds) to progeny cohorts in another habitat. We examined relative pollen flow between adjacent habitats at the two sites where pastures and forest fragments occurred side-by-side (Ladrillera 3 and Pineda). From the results of our paternity analysis, we used chi-squared tests to compare the proportion of pollination events that originated from within the fragment or adjacent pasture, and vice versa.

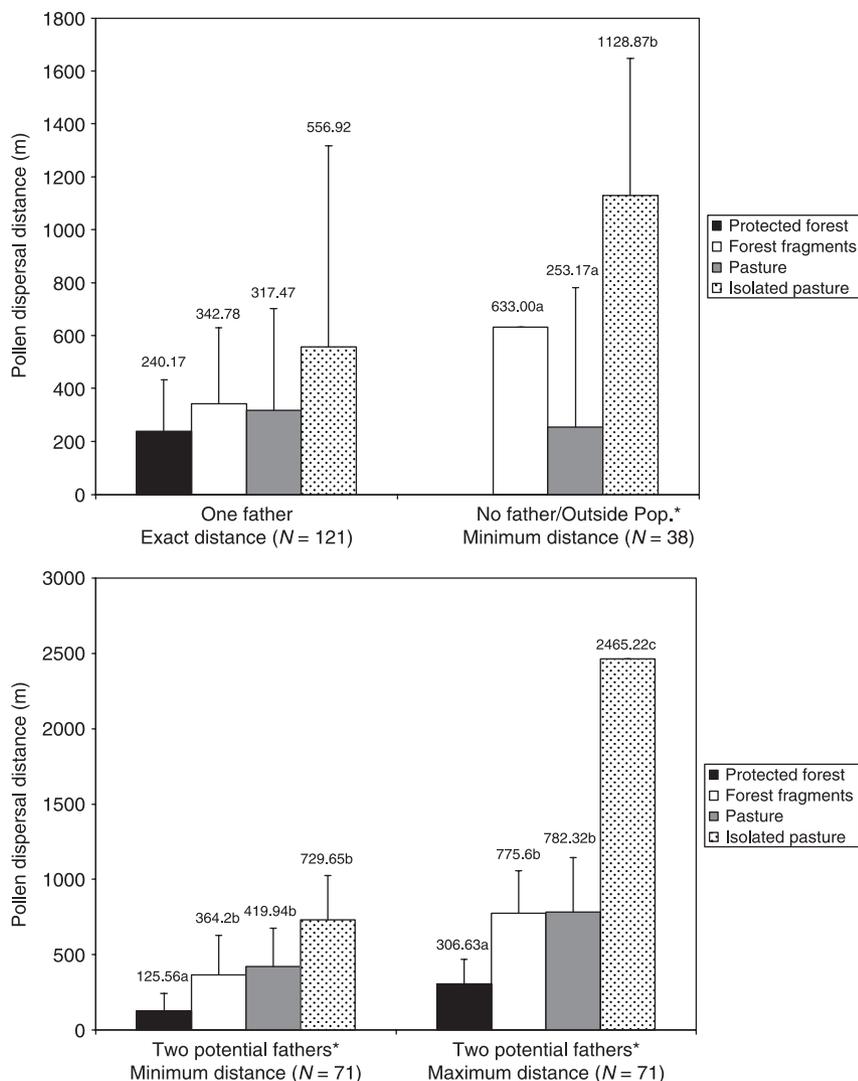


Fig. 2 Mean pollen dispersal distances (+SD) for *Dipteryx panamensis* progeny assigned by paternity analysis to one father (exact distance) or two potential fathers (minimum and maximum distances), or with no likely fathers in the population (minimum distance to nearest edge of site). All assignments made with 95% confidence; *ANCOVA results indicate a significant effect of habitat type ($P < 0.01$), means with different letters differ significantly from each other (LSM comparisons, $P < 0.01$).

Statistical analyses

We used SAS version 9.1 (© 2002–2003, SAS Institute Inc.) for all standard statistical tests (e.g. ANCOVA, *t*-tests). Nonparametric tests (e.g. Kruskal–Wallis) were used only when data or log-transformed data failed tests of normality.

Results

Sampling and genetic markers

A total of 218 adults were located, mapped and sampled from the four habitat types, as well as 325 progeny representing 65 maternal trees. All tissue samples were analysed at nine microsatellite loci, with a mean genotyping error rate of 1.47%. Details of microsatellite loci performance are fully described elsewhere (Hanson *et al.* 2008).

Pollen dispersal distance

Maximum likelihood paternity analysis assigned a single father or two likely fathers for more than half of all progeny tested (Table 1). An additional 38 progeny had no potential fathers within the population and were assigned minimum dispersal distances to the edge of the sampling area, leaving less than 30% of the progeny without pollen dispersal estimates (Table 1). We found the shortest pollen dispersal distances in continuous forest, moderate distances in forest fragments and adjacent pastures, and distances of up to 2.3 km among isolated pasture trees. This pattern was consistent for exact distances determined from one-father assignments as well as the minimum (nearest tree) and maximum (farthest tree) distances from two-father assignments (Fig. 2). Mean pollen dispersal distances from one-father assignments were 240.1 ± 192.6 m for continuous forest, 342.8 ± 286.9 m for forest fragments, 317.5 ± 385.7 m

for pastures adjacent to fragments, and 556.9 ± 761.1 m for isolated pastures (Fig. 2). The variance (SD) for these estimates followed the same pattern, increasing with increased fragmentation (Fig. 2). Distances from no father/ outside population assignments were less clear because of a lack of data from continuous forest, where all assignments were made within the population (Fig. 2). We documented pollen moving among trees within habitats and also found pollen moving from forest fragments to pastures (up to 901.3 m), from pastures to fragments (up to 1000.3 m) and between fragments across an intervening pasture (427.8 m).

The difference in dispersal distances among habitats was significant in ANCOVA tests of no father/ outside assignments ($F = 14.9$, $P < 0.001$) and two-father minimum ($F = 4.6$, $P = 0.005$) and maximum ($F = 11.1$, $P < 0.001$) assignments, but not for the single father assignments ($F = 1.86$, $P = 0.14$) (Fig. 2). Where tests were significant, least square means comparisons found that pollen travelled shorter distances in continuous forests than in all other habitats ($P < 0.01$ in all comparisons), and that there was no difference between dispersal distance in forest fragments and adjacent pastures ($P > 0.70$ in all comparisons). Pollen generally travelled farther in isolated pastures than other habitats, but this trend was not significant in all comparisons. The covariate accounting for the proximity of potential fathers was significant only for distances from no father/ outside population assignments ($F = 5.9$, $P = 0.005$), which may reflect the large contribution of widely spaced (< 0.04 adults/ha) isolated pasture trees to this model (25 of 38 assignments). Otherwise, pollen dispersal distances appeared largely unaffected by the proximity of potential fathers near focal maternal trees, confirming habitat-level characteristics as significant sources of variability. Interaction effects between the covariate and habitat were not significant in any test ($P > 0.46$ for all models), upholding the ANCOVA assumption of equality of slopes for treatment and covariate effects. The use of the median distance to the nearest 10 potential fathers, instead of the nearest five, produced similar ANCOVA results in all tests, with the exception of the no father/ outside population analysis, where the covariate was no longer significant ($F = 2.80$; $P = 0.1032$).

Outcrossing rate

Although outcrossing was prevalent, we found evidence of self-pollination in all habitats, refuting earlier suggestions of *Dipteryx panamensis* as an obligate outcrosser. Outcrossing rates were lower for pasture and isolated pasture trees than for trees located in continuous forest and forest fragments (Fig. 3). The statistical differences were marginal ($P < 0.10$) for both the multilocus outcrossing rate (t_m) ($\chi^2 = 2.7418$, d.f. = 1, $P = 0.0978$), and the assignment outcrossing rate (t_a) ($\chi^2 = 3.4583$, d.f. = 1, $P = 0.0629$).

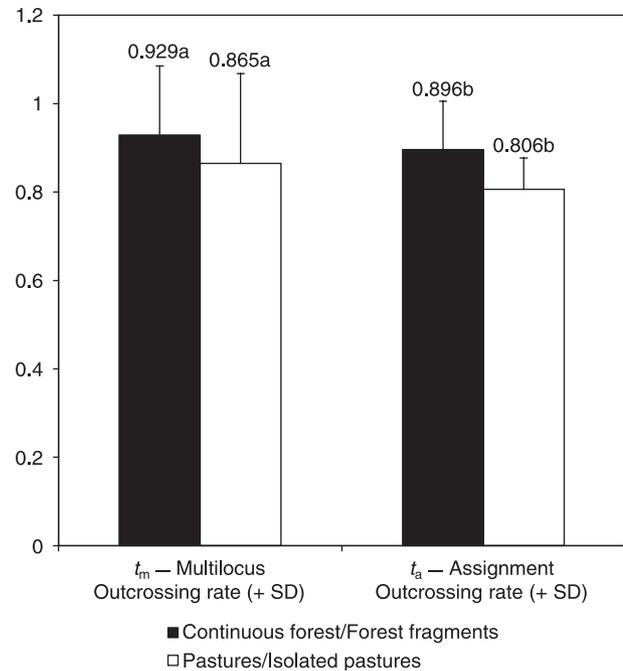


Fig. 3 Two measures of outcrossing rate for *Dipteryx panamensis* in continuous forest and forest fragments vs. pastures and isolated pastures. The multilocus outcrossing rate (t_m) is derived from the presence of nonmaternal alleles in progeny arrays. The assignment outcrossing rate (t_a) is the proportion of paternity assignments ascribed to nonmaternal trees. (a: $P = 0.0978$; b: $P = 0.0629$).

Genetic diversity

We found moderately high levels of H_E and slight variation among populations, ranging from 0.604 in the adults of the Rio Frio isolated pasture to 0.520 in the progeny of Rio Frio East (Table 2). No significant differences were found in H_E between parental and progeny generations in any habitat, although the trend was lower for progeny in pastures and isolated pastures. A ranged from 4.44 for isolated pasture progeny to 5.56 for continuous forest adults (Table 2), but did not vary significantly between generations in any comparison.

Genetic structure

Mean pairwise Nei's differentiation index (G_{ST}) indicated moderate structure among all groups in the study area ($G_{ST} = 0.0540$) (Table 3). Comparisons of G_{ST} between adult and progeny cohorts did not vary in continuous forest ($t = -0.4548$, $P = 0.6630$), forest fragments ($t = 0.5609$, $P = 0.5923$) or pastures ($t = -0.6507$, $P = 0.5360$), but there was a significant (one-tailed) increase in differentiation among progeny in isolated pastures ($t = -2.5495$, $P = 0.0381$).

	<i>N</i>	$H_E \pm SD^\dagger$	$A \pm SD$
Populations by habitat			
Continuous forest adults	104	0.5893 ± 0.0152	5.56 ± 2.35
Continuous forest progeny	50	0.5775 ± 0.0164	5.44 ± 2.07
Forest fragment adults	40	0.5671 ± 0.0335	5.44 ± 2.01
Forest fragment progeny	129	0.5678 ± 0.0193	5.33 ± 2.23
Pasture adults	62	0.5608 ± 0.0442	5.22 ± 2.28
Pasture progeny	108	0.5358 ± 0.0522	5.11 ± 2.37
Isolated pasture adults	12	0.5848 ± 0.0205	4.56 ± 2.40
Isolated pasture progeny	44	0.5496 ± 0.0351	4.44 ± 2.19
Populations by site (habitat*)			
	<i>N</i>	$H_E \pm SD^\dagger$	$A \pm SD$
La Selva West adults (CF)	63	0.5984 ± 0.0080	5.33 ± 2.74
La Selva East adults (CF)	41	0.5802 ± 0.0157	5.11 ± 2.03
La Selva West progeny (CF)	21	0.5754 ± 0.0150	4.56 ± 2.19
La Selva East progeny (CF)	29	0.5797 ± 0.0189	5.00 ± 1.73
Pineda adults (FF)	26	0.5916 ± 0.0281	5.22 ± 1.64
Ladrillera 3 adults (FF)	14	0.5426 ± 0.0153	5.00 ± 2.73
Pineda progeny (FF)	75	0.5707 ± 0.0281	5.11 ± 2.37
Ladrillera 3 progeny (FF)	54	0.5648 ± 0.0116	4.67 ± 2.06
Pineda pasture adults (P)	26	0.5252 ± 0.0310	5.22 ± 2.28
Ladrillera 3 pasture adults (P)	36	0.5490 ± 0.0574	4.44 ± 2.51
Pineda pasture progeny (P)	50	0.5226 ± 0.0478	5.00 ± 2.40
Ladrillera 3 pasture progeny (P)	58	0.5916 ± 0.0281	4.78 ± 2.33
Rio Frio West adults (IP)	5	0.6040 ± 0.0029	4.44 ± 2.01
Rio Frio East adults (IP)	7	0.5657 ± 0.0045	3.78 ± 2.05
Rio Frio West progeny (IP)	15	0.5791 ± 0.0173	4.75 ± 2.12
Rio Frio East progeny (IP)	29	0.5200 ± 0.0171	4.22 ± 2.05

*CF (continuous forest), FF (forest fragment), P (pasture), IP (isolated pasture); † expected Hardy-Weinberg heterozygosity from 10 iterations of TETRASAT analysis on equal subsamples from each population.

Table 3 Nei's population differentiation (G_{ST}) for adult and progeny populations of *Dipteryx panamensis* in continuous forest, forest fragments, pastures and isolated pastures in Cantón Sarapiquí, Costa Rica. (**significant difference ($P < 0.05$) between generations)

Populations	$G_{ST} \pm SD§$
All samples	0.0558 ± 0.0023
Continuous forest adults	0.0499 ± 0.0139
Continuous forest progeny	0.0495 ± 0.0084
Forest fragment adults	0.0657 ± 0.0090
Forest fragment progeny	0.0700 ± 0.0221
Pasture adults	0.0676 ± 0.0165
Pasture progeny	0.0842 ± 0.0180
Isolated pasture adults	0.0460 ± 0.0035**
Isolated pasture progeny	0.0649 ± 0.0069**

Spatial autocorrelation

Significant spatial autocorrelation existed in adult populations in continuous forest, forest fragments and adjacent pastures, but no pattern was detected in isolated pastures (Fig. 4). In continuous forest, the combined autocorrelation coefficient (r_c) measured 0.034 in the first 100 m and remained

Table 2 Hardy-Weinberg expected heterozygosity (H_E) and allelic richness (A) from microsatellite data for adult and progeny populations of *Dipteryx panamensis* in continuous forest, forest fragments, pastures and isolated pastures in Cantón Sarapiquí, Costa Rica

significant to 300 m. In fragments, r_c reached 0.077 at 100 m and maintained significance to 400 m. Pasture trees showed no autocorrelation at 100 m, perhaps due to small sample size in that distance class. Their r_c values resembled forest fragment trees at 400 m (0.031), but remained significant at very low magnitude (< 0.01) up to 1000 m. The lack of signal in isolated pastures probably stemmed from small sample size and spatial distributions beyond the scale of the measurable genetic structure.

Progeny populations in all habitats displayed significant spatial autocorrelation (Fig. 5). The peak magnitude and scale of the autocorrelation rose in increasingly fragmented habitats, from a peak r_c value of 0.184 (100 m) and significance up to 500 m in continuous forest to magnitude 0.274 at 100 m and significance to 2000 m in isolated pastures.

Paternal reproductive dominance

Dominant pollen donors were present in continuous forest ($n = 4$), forest fragments ($n = 2$) and pastures adjacent to fragments ($n = 3$), but none were detected in isolated pastures. At the Ladrillera 3 forest fragment, one tree contributed pollen to 23.1% of all assigned outcrossing events ($n = 26$). Collectively, these nine dominant individuals accounted

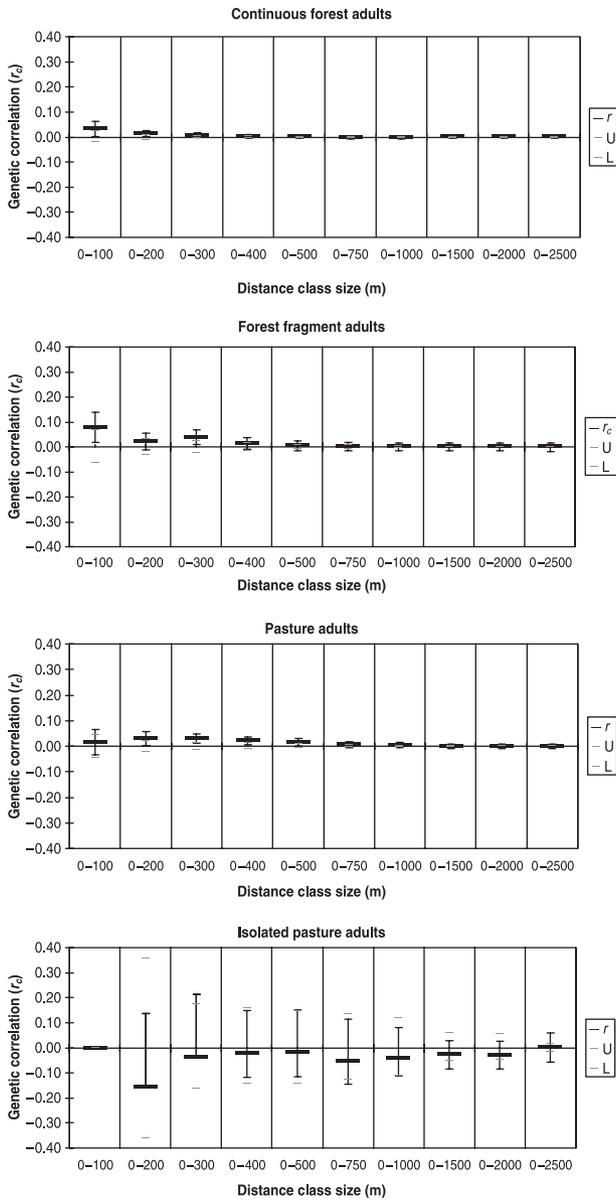


Fig. 4 Combined spatial autocorrelation coefficient values (r_c) for adult populations of *Dipteryx panamensis* across increasing size classes in continuous forest, forest fragments, pastures and isolated pastures. Upper and lower 95% confidence boundaries from 999 simulations; 95% confidence bars around r_c from 999 bootstraps.

for 24.0% of all outcrossing events recorded at their sites ($n = 148$). We found no difference among habitats in the ratios of observed fathers to fathers expected under random equal mating ($\chi^2 = 0.9954$, d.f. = 3, $P = 0.8024$), suggesting similar rates of reproductive dominance across habitats.

Habitat dominance

Where pastures and forest fragments were adjacent, there was asymmetric pollen flow between habitats ($\chi^2 = 8.6722$,

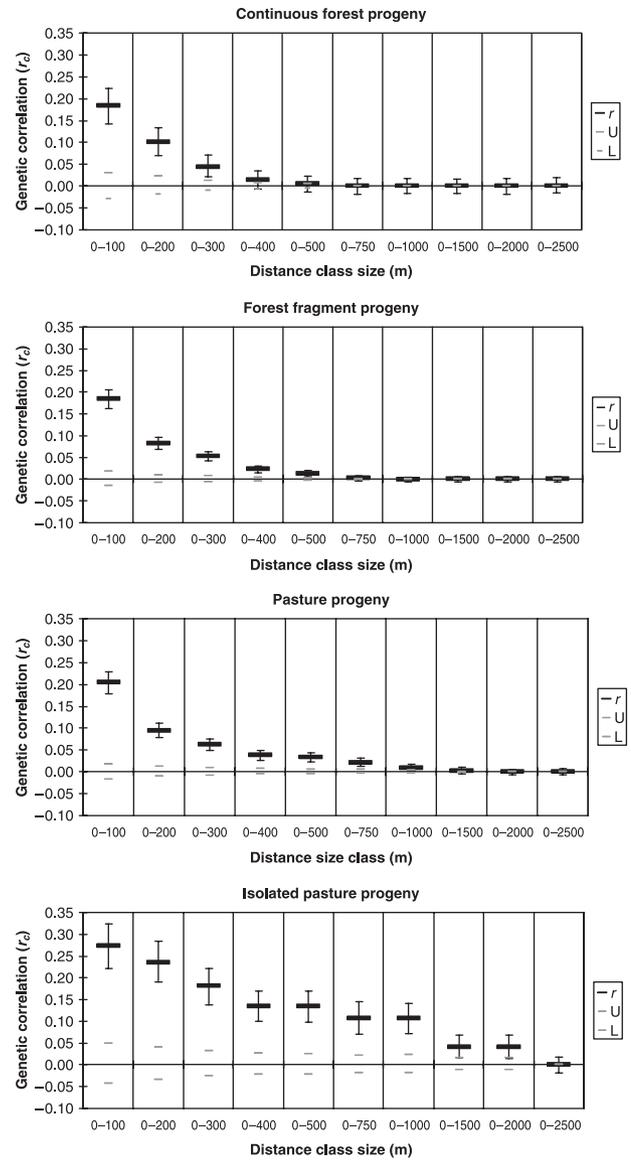


Fig. 5 Combined spatial autocorrelation (r_c) for progeny populations of *Dipteryx panamensis* across increasing distance class sizes in continuous forest, forest fragments, pastures and isolated pastures. Upper and lower 95% confidence boundaries from 999 simulations; 95% confidence bars around r_c from 999 bootstraps.

d.f. = 1, $P = 0.0032$). In fragments, 41.9% of outcrossed progeny ($n = 43$) were pollinated by trees in neighbouring pastures, while in pastures only 13.6% of outcrossed progeny ($n = 44$) received pollen from fragment trees.

Discussion

Pollen dispersal and outcrossing rate

Pollen dispersal patterns for *Dipteryx panamensis* generally matched our predictions, with increased dispersal distances

in fragments and pastures compared to continuous forest, and the longest distances in isolated pastures. Several factors suggest caution in interpreting these results, however. Limited sample size of maternal trees and progeny makes our estimates vulnerable to anomalous pollination events or conditions, while the presence of nonsampled adults nearby our study areas could have led to inaccuracy in paternity assignments. These limitations, or the inherent loss of resolution in transformed polyploid data, may explain why dispersal trends and habitat differences were not significant in all analyses. Still, our results resemble findings from other fragmented populations of bee-pollinated tropical trees (Dick 2001; White *et al.* 2002; Dick *et al.* 2003). The lower density and wider spacing of reproductive adults in disturbed habitats, particularly pastures, must inherently lead to longer pollination distances if outcrossing is to take place. In a study of two Amazonian tree species, however, Cloutier *et al.* (2007) found that adult density alone does not account for all the variability in pollen dispersal distances. Density varied by habitat in our analysis, but our ANCOVA accounted for the proximity of the nearest fathers, suggesting that other factors also contributed to habitat-level differences in dispersal distance. A major shift in pollinator behaviour or the community of pollinators in fragmented areas would help explain this trend. In Brazil, Dick (2001) observed non-native Africanized honeybees (*Apis mellifera*) dominating the pollinator community of *Dinizia excelsa* in fragmented landscapes. He and colleagues (2003) also documented longer pollen dispersal distances among isolated trees than in continuous forest where honeybees were largely absent. Similar processes may be at work in our study area, where *A. mellifera* is widespread in fragmented areas (Rincon *et al.* 2000), but has not been observed visiting *D. panamensis* flowers in our continuous forest site (Thiele 2002). The increased variance we found in fragments and pastures is also suggestive of altered, less regular pollination conditions.

Long-distance pollen flow may increase the genetic neighbourhood of trees in fragmented areas and counteract some negative effects of habitat degradation (Lowe *et al.* 2005). In *D. panamensis*, however, we also observed lower outcrossing rates for pasture trees (0.806–0.865) than forest trees (0.896–0.929). So while pollen is moving farther, it appears to be moving less often. This could reflect lower incidence of pollination in general, or different pollinator behaviour (e.g. spending complete foraging bouts in one tree). Our results contradict earlier suggestions of obligate outcrossing in *D. panamensis* (Perry & Starrett 1980), but reflect similar changes in outcrossing rates observed in other pasture trees (Cascante *et al.* 2002; Dick *et al.* 2003). Increases in pollen-mediated gene flow for fragmented *D. panamensis* populations must therefore be interpreted in the context of increased inbreeding, which can have an

immediate impact on heterozygosity and fitness (Nason & Hamrick 1997; Lowe *et al.* 2005).

Genetic diversity

The moderately high levels of H_E in adult populations (0.53–0.60) indicate high rates of historic (prefragmentation) gene flow throughout the study area. There was no difference in H_E or A between adult and progeny generations in any habitat, although a nonsignificant trend towards lower H_E was evident for pasture and isolated pasture progeny. The long-term genetic consequences for these populations is unclear, and depends in part on whether long-distance pollen flow can offset the effects of increased selfing. Because the adults in our study date from the prefragmentation period, their progeny represent the first generation to reflect postfragmentation reproductive conditions. Only a severe bottleneck would be expected to produce large declines in diversity in one generation (Frankham *et al.* 2002), but microsatellite data can often detect even moderate bottleneck events (Spencer *et al.* 2000). Simulations indicate that a breeding population of 20 trees with an outcrossing rate of 0.80, similar to our isolated pasture sites, would produce declines of < 10% in allelic richness and expected heterozygosity in one generation, but would lead to more than 50% loss of alleles and a 25% drop in heterozygosity after 10 generations (Lowe *et al.* 2005). As a tetraploid, *D. panamensis* should fare better in preserving allelic diversity by carrying more copies per individual – this may account for the lack of significant differences in H_E and A between adult and progeny generations. Tetraploids are still vulnerable to genetic erosion in fragmented settings, however (e.g. Buza *et al.* 2000), and our data do show a trend towards lower H_E and A in the small, isolated pasture populations (Table 2). While the individual adults in this study maintain diversity levels of a prefragmentation forest, their effective breeding population may be reduced in pasture settings despite long pollen dispersal distances. Studies have documented the loss of rare alleles in fragmented tree populations (White *et al.* 1999), but most analyses of diversity have been inconclusive (Lowe *et al.* 2005). Research spanning multiple generations, a challenge for long-lived tree species, may be necessary before the true genetic impacts of fragmentation can be known.

Genetic structure and spatial autocorrelation

Limited gene flow in isolated pastures appears to cause greater differentiation (G_{ST}) among postfragmentation populations. Spatial autocorrelation analysis produced a similar but more revealing trend, as the magnitude of the correlation coefficient for progeny increased steadily with increasing fragmentation, from continuous forest to fragments, pastures and isolated pastures. The scale of the

correlation increased as well, a reflection of the longer pollen dispersal distances in fragmented habitats. These patterns follow theoretical predictions for the genetic effects of fragmentation: an increase in differentiation among populations over successive generations (Nason & Hamrick 1997). We are unaware of other studies examining spatial autocorrelation for tropical trees in forests and pastures, but Pither *et al.* (2003) did document higher differentiation among naturally fragmented *Terminalia amazonia* populations in Belize, while Cascante *et al.* (2002) found higher levels of relatedness within seed arrays of isolated *Samanea saman* individuals in a fragmented Costa Rican dry forest. The dispersal of seeds also impacts spatial genetic structure, but recent evidence from the same landscape found no fragmentation effects on seed dispersal (Hanson *et al.* 2007), pointing to a stronger role of pollen dispersal in the patterns observed here.

Paternal reproductive dominance

We expected paternal dominance to be more prevalent in fragmented areas but in fact found no difference among habitats. Collectively, the dominant pollen donors in continuous forest, forest fragments and adjacent pastures comprised only 4.3% of the adults at those sites but accounted for 24.0% of the outcrossing events. In any habitat, this trend reduces the effective population size for *D. panamensis*, but it may be particularly important in pasture settings where lower outcrossing rates and smaller breeding populations may already be affecting genetic diversity. Regenerating forest (e.g. abandoned pastures) could also be strongly affected if conditions allowed heavy recruitment from a single seedling cohort. Individual productivity, pollinator activity and other reproductive factors can vary widely from year to year (e.g. Kenta *et al.* 2004), however, which may ameliorate the effects of paternal reproductive dominance in a single season. Our data represent only a snapshot, but complement earlier observations and add to the evidence that the effects fecund individuals merit further study in tree populations.

Habitat dominance

The high rates of pollen flow we observed from pastures to adjacent fragments echo the findings of Aldrich & Hamrick (1998), where pasture trees were responsible for the majority of regeneration in fragments. High fecundity in pasture trees may be a response to reduced competition and high light conditions, allowing a greater allocation of resources to flowering and fruit production. Such dominance can effectively cause a bottleneck, sharply reducing the effective number of breeding adults in the population (Aldrich & Hamrick 1998). For *D. panamensis*, however, we observed pollen arriving not from a dominant few but from many

pasture individuals (15 at Pineda and 10 at Ladrillera 3) which may in fact enhance the genetic diversity of fragment populations, underscoring the importance of pasture trees to landscape-level gene flow and diversity.

Limitations

Although many of our expectations were met, we view the results with some caution due to the sample size limitations and the inherent loss of resolution involved in dominant transformation of polyploid data (see Hanson 2006). Also, additional seasons would be required to capture long-term variability in reproductive output, pollinator activity, and associated genetic trends. Finally, although eight loci for in this study behaved in a tetraploid fashion, the ploidy history and inheritance patterns for *D. panamensis* remain unconfirmed. In reality, most polyploid species fall somewhere on a continuum between true allopolyploids and autopolyploids, since hybridization events are typically between closely related species with similar genomes. We therefore present these results with the caution that in addition to assumptions of Hardy–Weinberg equilibrium, we have assumed random chromosomal segregation at all loci.

Conclusions

Low population densities and animal-mediated pollen and seed dispersal make many tropical trees vulnerable to habitat disturbance and fragmentation (Nason *et al.* 1997). On the other hand, their natural ability to maintain gene flow over wide areas may help them persist in fragmented settings (Hamrick 2004; Sork & Smouse 2006), provided that systems for pollen and seed dispersal can adapt to the new conditions. Pollen dispersal distances for *Dipteryx panamensis* appear to increase dramatically in fragmented areas and measures of genetic diversity in progeny remain generally high. Long-distance pollination among isolated pasture trees and between pastures and forest fragments emphasize the importance of remnant trees in maintaining landscape-level gene flow, but this trend will be ephemeral unless pasture populations are replanted or allowed to regenerate. Despite heightened pollen flow, however, we observed lower outcrossing rates for pasture trees as well as slightly lower diversity and increased genetic structure among their progeny, suggesting that fragmented populations are still at risk of long-term genetic erosion.

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