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ORIGINAL ARTICLE

Remnant *Pachira quinata* pasture trees have greater opportunities to self and suffer reduced reproductive success due to inbreeding depression

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Habitat fragmentation is extensive throughout the world, converting natural ecosystems into fragments of varying size, density and connectivity. The potential value of remnant trees in agricultural landscapes as seed sources and in connecting fragments has formed a fertile area of debate. This study contrasted the mating patterns of bat-pollinated *Pachira quinata* trees in a continuous forest to those in pasture through microsatellite-based paternity analysis of progeny. The breeding system was determined by analysis of pollen tube growth and seed production from controlled pollinations. Fitness of selfed and outcrossed seed was compared by germination and seedling growth. There was more inbreeding within pasture trees (outcrossing = 0.828 ± 0.015) compared with forest trees (0.926 ± 0.005). Pasture trees had fewer sires contributing to mating events, but pollen dispersal distances were greater than those in the forest. Paternity analysis showed variation in outcrossing rates among pasture trees with high proportions of external and self pollen sources detected. A leaky self-incompatibility system was found, with self pollen having reduced germination on stigmas and slower growth rate through the style. Controlled pollinations also showed a varied ability to self among trees, which was reflected in the selfing rates among pasture trees shown by the paternity analysis (0–80% selfing). Self pollination resulted in lower seed set, germination and seedling growth compared with outcrossing. While remnant trees in agricultural landscapes are involved in broader mating patterns, they show increased but varied levels of inbreeding, which result in reduced fitness.
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INTRODUCTION

Habitat fragmentation resulting from anthropogenic activities is pervasive throughout the world. Habitat fragmentation results directly in reduced population sizes and local densities, along with increased geographic isolation among fragments. There is theoretical and empirical evidence that small isolated fragments have an increased probability of local extinction (Hanski and Ovaskainen, 2000; Frankham *et al.*, 2002), with habitat fragmentation consequently cited as a major threat to the persistence of species in agroecosystems (IUCN-CMP, 2006; Hayward, 2009; Wilson *et al.*, 2011).

Ecologically functional agroecosystems are essential for the maintenance of some remnant native species and communities. In heavily deforested and fragmented ecosystems the small size of protected areas mean that in many cases they are inadequate for the long-term conservation of species. Small fragmented patches and scattered trees in pastures were labelled as the 'living dead' under the assumption of poor reproductive success or failing to contribute to mating in surrounding populations (Janzen, 1986a,b; Lowe *et al.*, 2005; Dawson *et al.*, 2009). Despite more recent work, often showing extensive connectivity across agroecosystems (Dick, 2001; White *et al.*, 2002), small remnants have been given low value for conservation and largely ignored in preference to the management of larger remnants.

Fragmentation can result in immediate alterations in mating patterns as opposed to losses of genetic variation, which may take several generations to occur (Lowe *et al.*, 2005). Although mating patterns are context and species dependent, predictions of the degree and direction of their change in remnant fragments can be based on life histories. Species capable of utilising components of the matrix, either through persisting or recruiting into abandoned pastures are predicted to have greater connectivity in fragmented landscapes. Altered levels of competition and abiotic conditions in pastures may increase individual tree size and flowering, and potential pollinators, compared with trees in dense, continuous habitats where competition limits resource availability (Severns, 2003). Pollinators with increased foraging range and mobility (for example, bats, birds and some bees) are more likely to make use of scattered trees in pastures (Bernard and Fenton, 2003; Pejchar *et al.*, 2008; Hadley and Betts, 2009). How pollinators respond to the landscape connectivity will influence the quantity and composition of pollen a tree receives, which will ultimately be acted upon by the plant's breeding system to determine reproductive success.

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Plant breeding systems are a continuum from complete selfing to obligate outcrossing. Selfing can ensure reproductive success where mates are limited (by low densities or pollen movement) (Baker, 1955); however, it can lead to reduced genetic variation and offspring fitness. Outcrossing can be achieved by a complex array of mechanisms reducing self-pollination and self-fertilisation (Barrett, 1998; Lord and Russell, 2002; Hiscock and McInnis, 2003; Takayama and Isogai, 2005). Self-pollination can be limited by pre-pollination mechanisms, such as temporal and spatial separation of male and female structures, and pollinator behaviour (Barrett, 1998). Self-incompatibility may act at various stages: blocking germination on the stigma, growth through the style, and penetration and fertilisation of the ovary (Lord and Russell, 2002; Takayama and Isogai, 2005). Neotropical trees are predominantly outcrossing, despite earlier predictions that selfing would dominate due to low tree densities in wet forests (Baker, 1959; Bawa *et al.*, 1985; Ward *et al.*, 2005). Further, pollination is largely facilitated by animals (for example, >98%, Bawa 1990), which places trees at greater risk of negative impacts from fragmentation as anthropogenic disturbance expands and intensifies.

This study aims to understand the impacts of fragmentation on the viability and functioning of remnant *Pachira quinata* (Malvaceae) tree populations in pastures, through a novel combination of field and laboratory techniques. Specifically, we study the influence of spatial separation and breeding system on *P. quinata* mating patterns and subsequent impacts on reproductive success and progeny fitness. In particular, we elucidate the mechanisms that permit some level of self-fertilisation in a primarily outcrossed species.

MATERIALS AND METHODS

Study species

Pachira quinata (Jacq.) (Alverson, 1994) (syn: *Bombacopsis quinata*) is a medium-to-large-sized deciduous tree, highly valued for its timber within its native range from Honduras in Central America to Colombia and Venezuela in South America. It is hermaphroditic, largely self-incompatible and principally bat-pollinated (*Glossophaga soricina*, Sandiford, 1998). Flower buds initiate singly or in an inflorescence of between 2 and 40 buds, producing large white-pink-coloured flowers (8–14 cm length), which produce copious amounts of sweet smelling nectar (typically 60–90 ml). Each flower remains receptive for one evening, with a single, synchronous anthesis event shortly after sunset (~19:00). After opening, anthers dehisce almost immediately, but stigma receptivity maybe delayed until stigmatic lobes are fully reflexed, giving on occasions a degree of temporal as well as physical separation between male and female parts (Sandiford, 1998). Stigmatic receptivity declines through the night, with the corolla and staminal tube dropped by midday of the following day (Sandiford, 1998). The seed capsule contains up to 100 small, round, wind-dispersed seeds attached to kapok-like fibres and released after capsule dehiscence (Sandiford, 1998).

Sites and sampling

Pachira quinata trees were mapped at two sites (Lomas Barbudal (LB), Hacienda Ciruelas (HC)) separated by 5 km, in Guanacaste Province, Costa Rica. Both sites are in the dry tropical forest life zone, characterised by a long dry season (≥5 months with <75 mm rainfall per month). The mapped Lomas Barbudal site consisted of 62 *P. quinata* trees within 36 ha (Figure 1a) of the most intact area of dry forest in the 2279 ha Lomas Barbudal Biological Reserve (10°27′N, 85°21′W). The mapped Hacienda Ciruelas site (10°30′N, 85°21′W) borders the Reserve and consisted of 20 remnant *P. quinata* trees within a flat area of ~140 ha, deforested at least 50 years ago as part of an extensive cattle pasture (Figure 1b).

The size, age group and seed capsule production of all trees were estimated at the time of sampling. Tree size was measured as diameter at breast height (dbh, 1.3 m above the ground). Age was based on bark and crown characteristics and recorded in four ordinal categories: 1 (young), 2 (young mature), 3 (mature) and 4 (senescent). Seed capsule production was estimated as 1 (low <20), 2 (medium, 20–50), 3 (high, 50–100) and 4 (very high >100).

Mature, closed seed capsules were collected directly from different parts of the crown of all mapped trees by climbing and using a pruner, avoiding diseased or damaged fruit, with at least 10 fruit sampled per tree where present. Capsule size was measured as length and width using callipers. The seed was extracted from each capsule after dehiscence with the seed from each tree then bulked, dried and stored at 4 °C in hermetically sealed containers. For genetic analysis, progeny arrays of 24 seed were sampled from each of 20 trees from the two sites. All mapped trees sampled at Hacienda Ciruelas and at Lomas Barbudal were representative of the size and spatial distribution of mapped trees.

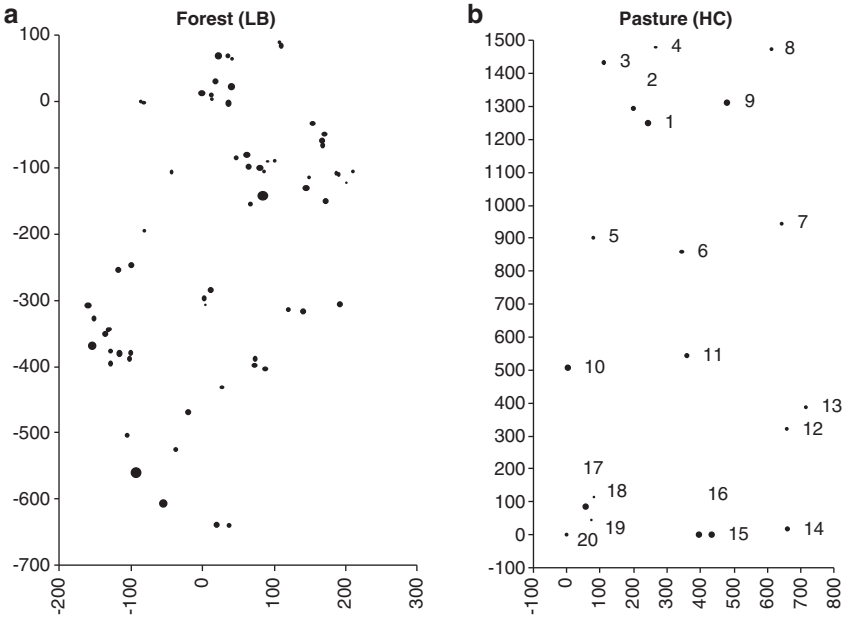

Figure 1 Maps of *Pachira quinata* trees in a continuous (**a**) forest at Lomas Barbudal (LB) and (**b**) pasture trees at Hacienda Ciruelas (HC), Costa Rica. The size of the point is proportional to the tree size (dbh). Units on the axis are metres. Number in panel **b** is the tree ID shown in Table 2.

Microsatellite analysis

DNA was extracted from seed using the QIAGEN DNeasy 96-well Plant Kit (Qiagen, Crawley, UK). Entire seed were ground in pre-warmed lysis buffer using the TissueLyser system (QIAGEN) for 3 × 1 min at 30 hz, with sterilized tungsten carbide beads. A 10-min incubation at 60 °C was included after lysis, with the rest of the extraction according to the supplier protocol for the dried plant tissue.

Seed were genotyped with eight nuclear microsatellite loci developed for *P. quinata* (Rymer *et al.* unpublished). Genotyping was conducted in multiplexed PCR reactions in two separate panels of four loci (eight loci in total) separated by fragment size and fluorescent primer label: 6-FAM from Eurofins MWG Operon (Ebersberg, Germany) and NED, VIC and PET from Applied Biosystems (Foster City, CA, USA). Each 10 µl reaction contained 1 × buffer and 0.5 units of YB-Taq polymerase (Yorkshire Biosciences, Ltd., York, UK), along with 2.5 µg BSA, 150 nM dNTPs, 20 nM each primer, 2 mM $MgCl_2$ and 1 µl of a 1:10 dilution of the DNA sample. PCR reactions were carried out on 96-well plates in a Bio-Rad DNA Engine (PTC-200) theromocycler at the following cycles: initial denaturation, 94 °C for 3 min; 30 cycles of 94 °C for 3 min, 60 °C for 30 s, 72 °C for 60 s; a final extension of 72 °C for 20 min and then held at 4 °C. Subsamples from the reactions were run on 1.5% agarose-TBE gels to confirm amplification and yield before analysis on an Applied Biosystems AB3700 sequence analyser (Department of Zoology, University of Oxford) with GeneScan 500 LIZ Size Standard (Applied Biosystems Inc.). Genotype spectral reads were scored and allele assignments were determined using the GeneMapper 4.0 software (Applied Biosystems Inc.). Five loci provided sufficient polymorphism, consistency of amplification, and no detectable null alleles to be used for later stages of analysis (Supplementary Table 1).

Genetic variation within each site was characterised by maternal genotypes, determined from the genotyped progeny arrays in MLTR (version 3.0). Standard estimates of genetic diversity (number of alleles, effective number of alleles, Shannon's information index, observed and expected heterozygosity, unbiased expected heterozygosity and fixation Index) were estimated. A 'global' spatial autocorrelation was performed using a multivariate approach to simultaneously assess the spatial signal generated by multiple loci (Smouse and Peakall, 1999). Pairwise, tree-by-tree genetic distance (for codominant data), and geographic distance (Euclidian distance) matrices were used to generate the autocorrelation coefficient (r) (Smouse and Peakall, 1999). Distance classes of 50 m and 100 m were examined for the forest and pasture separately and combined. Tests for statistical significance were conducted on the basis of 1000 random permutation and 1000 bootstrap estimates of r (performed in GenAlEx 6.0 package, Peakall and Smouse, 2006).

Mating system and pollen flow analysis

Mating patterns can be measured: (1) directly through paternity assignment/exclusion; and (2) indirectly through models that utilise allele and genotype frequencies across progeny arrays. As each method has its own biases because of differing approaches, assumptions and use of data (Burczyk and Chybicki, 2004; Sork and Smouse, 2006), it is worth using several methods to more clearly and accurately characterise mating patterns and the extent of pollen flow.

Using the genotyped progeny arrays, the mixed mating program MLTR (version 3.0) was used to estimate the multilocus outcrossing rate (tm), mean single locus outcrossing rate (ts), correlation of paternity (rp), correlation of outcrossing rate (rt) and effective number of pollen donors (ne, the inverse of the probability that an individual female is pollinated twice by the same male in a continuous plant population), with pollen and ovule allele frequencies not constrained to equal each other. The correlation of paternity (rp) is related to the number of outcross parents by $rp = 1/ne$, (Ritland, 1996). Biparental inbreeding is estimated as the difference between the multilocus and single-locus outcrossing rates ($tm - ts$). Standard deviations were calculated using 500 bootstraps, with families as the resampling unit.

Using both maternal and progeny genotypes as input, the program TwoGener (Smouse *et al.*, 2001) conducts an analysis of molecular variance (AMOVA) of pollen pools partitioned among and within mothers. The variance among mothers is indicative of differences in the pollen pools sampled by mothers within the population (values of ΦFT statistically different from zero, $P < 0.05$). The effective pollination neighbourhood (Nep) can be determined from the relationship $\Phi FT \approx (2Nep) - 1$ (Smouse *et al.*, 2001). TwoGener analysis was run separately for the two sites using the GenAlEx 6.0 package (Peakall and Smouse, 2006). Estimates of dispersal distance, effective pollination neighbourhood and ΦFT, were compared with results from the other analytical methods mentioned above.

The program CERVUS (version 3.0; Kalinowski *et al.*, 2007) was used to assign paternity at the Hacienda Ciruelas site and thus directly identify discrete pollen flow events between trees. CERVUS assesses loci for the proportion of null alleles and calculates observed (Ho) and expected (He) heterozygosities from progeny data. CERVUS calculates 80% (relaxed) and 95% (stringent) confidence levels for paternity assignments based upon simulations. The allele frequencies present within the progeny arrays and the percentage of potential fathers within the mapped area for which genotypes are available are used in the simulations. Assignments with a delta criterion of zero were categorized as external pollen flow. Assignments below the 80% delta criterion but not mismatching at any loci were included as likely assignments, whereas any below the delta criterion and having one or more mismatches across loci were discarded as unassignable to either internal or external parents given the available data. Outcrossing rate was determined as the number of outcrossing events divided by the total number of assignments. Pollen dispersal distances were calculated as the Euclidean distance between the mother and most likely father assigned with stringent confidence. The number of pollen donors contributing to the progeny array of each mother tree was estimated as the number of different fathers assigned.

Multivariate analysis

Plant traits (plant size (dbh), age, seed capsule production (Seed) and capsule size (CapL)), density (distance to nearest tree (DistT), number of trees within 50 m, 250 m and 500 m (T50, T250, T500)) and genetic metric (heterozygosity (He), local relatedness (Smouse and Peakall, 1999) of the nearest trees (r) and five nearest trees (r5)) were modelled as explanatory variables for the mating parameter response variables (outcrossing rate (tm), biparental inbreeding (tm − ts), correlated paternity (rp)) in a multiple regression with stepwise simplification performed in R gui (R development team). Correlated variables identified by plotting pairs and estimating regression parameters (slope > 0.01 and $R^2 > 0.25$) were removed from the initial model. Variables likely to interact with the library tree and interaction terms were included in the initial model. The initial model was performed (ln('response variable' ~ Pop*Seed + Pop*CapL + Pop*r5 + Pop*log(DBH) + Pop*log(DistT))) followed by a model simplification function (step) and further manual stepwise removal of non-significant variables. Assumptions of normality and distributional patterns were tested and, where necessary, variables were transformed.

Controlled pollinations

Controlled pollinations were carried out at the La Soledad Field Station, Comayagua Valley, Honduras, to study (i) the presence and nature of any self-incompatibility mechanism and (ii) the relative ability of trees to self compared with outcross, and at Hacienda Ciruelas to study (iii) the influence of mating distance on the fitness of progeny, that is, whether biparental breeding related to spatial genetic structure reduces fitness.

Pollinations at both sites were conducted between 19:00 and 01:00 h each day during peak flowering. Flower buds expected to open on a given night were covered by pollination bags (PBS International, product PBSPF-3, transparent polyproplene with 1 mm holes) between 15:30 and 18:30 h to prevent pollen contamination. To obtain pollen, small branches with flowers likely to open that night were collected from the paternal tree between 15:30 and 18:30 h on the day of pollination, recut underwater and transferred to a glass bottle filled with water. After anthesis, the pistil was removed and the stamens gently tapped against a sheet of glass to dislodge the pollen. For each paternal parent, between one and five flowers were collected, the pollen was mixed and then placed in a 50 ml plastic tube (Sarstedt, No. 62.547.554) with a hole in the lid to hold a fine sable paint brush. Between pollen extractions, the glass sheet was cleaned with alcohol and dried. When pollinating, the pollination bag was removed, pollen was applied to the stigma via the paint brush, the flower was labelled with masking tape and the pollination bag was replaced.

Self-incompatibility mechanism. Controlled pollinations were carried out using floral buds removed from their parent trees and maintained in water, using the pollen collection and pollination techniques described above. Flowers were kept in a closed, draught-free area free of insects and potential pollinators. Twenty flowers were selfed and 20 flowers were crossed with a single unrelated pollen source on each of five trees over a period of 10 days (total of 200 flowers treated). The style and ovary of pollinated flowers were fixed in a 1:1:18 mixture of 40% formaldehyde, glacial acetic acid, 70% ethanol (FAA) at varying times. Five flowers of both the self- and outcross were then removed and fixed in FAA at between 4 and 120 h. The epidermis of the ovary was trimmed to assist infiltration of the fixative. Stylar material was removed from the FAA, washed in distilled water and softened in 10 M NaOH at 60 °C for 60 min. After softening, the styles were washed twice in deionised water and left to soak for 12 h before a final wash in deionised water. Softened stylar material was then placed in a leuco-aniline blue stain solution for an hour, then on a glass slide with one drop of a 1:1 mixture of aniline blue stain: 50% glycerol and firmly squashed and viewed under UV light (Zeiss Axiophlot epifluorescence microscope with incident lighting, UV filters, BG12, BG38). Pollen tubes and their callose plugs fluoresced brightly allowing estimation of pollen germination and growth. Ovaries were removed from the FAA, the epidermis further trimmed and then softened, washed and stained in the same manner as stylar material. The ovaries were then split, and the ovules were removed onto a glass slide by gentle scraping. A drop of 1:1 aniline blue and glycerol was placed onto the ovules that were then squashed and viewed under UV light as for stylar material. Remnants of fluorescing pollen tubes penetrating ovaries ('pollen tails') were clearly visible and allowed estimation of the timing of ovule penetration and number of ovules penetrated.

Selfs compared with outcrosses. The following pollination treatments were applied to 20 flowers per tree on the same eight trees. (1) Open (natural pollination): tagged, un-bagged and untreated. (2) Self (geitonogamy): no emasculation, pollen from different flower on same tree applied, flower then bagged. (3) Single (allogamy): no emasculation, pollen from single unrelated tree (source code = 11/88), flower bagged. (4) Bulk (mulitple allogamy): no emasculation, bulked pollen from five unrelated sources, flower bagged. Pollination bags were removed between 06:00 and 08:00 h the next morning as they can adversely affect seed set (Sandiford, 1998). When capsules approached maturity, they were protected by pollination bags to avoid predation by the insect *Dysdercus maculata* (Sandiford, 1998) and to facilitate collection.

Self, within and among site pollinations. Controlled pollinations were conducted to cover a range of distances from self, within (1 m–1 km) and among sites (1–5 km) at Hacienda Ciruelas. A total of 474 pollinations on nine maternal trees (5–85 pollinations per tree) were carried out over 10 nights (Billingham 1999). Capsules were similarly bagged before maturity, and, following collection, capsule width and length were measured to estimate volume. Seed were extracted, dried, the number of seed per capsule counted and then stored at 4 °C for subsequent use.

Germination and growth of seed produced from controlled pollinations were studied in both common garden field and greenhouse trials. In all, 915 seed were sown in soil from Hacienda Ciruelas, at a nursery located in Bagaces, Guanacaste, Costa Rica. Plastic root trainers ($3 \times 3.3 \times 10\,cm^3$; Rannoch 'B' Rootrainers, Ronaash Ltd., Kelso, TD5 8HH, Scotland) were used to germinate the seed, with a single seed in each cavity. Germination was recorded 24 days after sowing. In all, 1262 seed from pollinations were sown in a compost/sand 2:1 mix in plastic trays with cavities ($4 \times 4 \times 5.3\,cm^3$) in a greenhouse at the Department of Plant Sciences, University of Oxford, UK. Seed of the same pollination were planted in a tray row (*ie* five cavities, with five seed in total), although seed from different pollinations were planted in rows within trays to reduce the effect of tray position on germination. Percentage emergence was determined 1 month after sowing.

Plant growth was measured under glasshouse and common garden conditions. A random subset of 292 seedlings from all available pollinations established in the glasshouse were transplanted into 10-cm diameter pots of 10 cm depth and maintained in the greenhouse. Plants were randomised on a monthly basis to reduce the effects of location within the greenhouse. Diameter, 5 cm above the soil, and height to the terminal bud were measured 4 months after sowing. All plants were then re-potted into 18-cm diameter pots of 18 cm depth 5 months after sowing and re-measured 8 months after sowing for diameter (5 cm above the soil) and height to the terminal bud. Seedling volume was estimated by multiplying the cross sectional area with height. Seedlings established in the randomised block common garden design at La Soledad Field Station, Comayagua, Honduras were measured for height and diameter at 9 and 21 months.

Data analysis

Results were blocked into distance categories to examine differences indicative of pollination events in forest and pasture systems. Self-pollination (0 m), nearest neighbours (pollinations from 1–100 m, 50 m category), forest tree density (101–400 m, 250 m category), pasture tree density (401 m–1 km, 700 m category) and adjacent sites (1.01–5 km, 3 km category). These blocking distances maximised sample sizes; however, similar results were obtained from testing a range of categories.

The number of capsules formed, in the different distance categories, together with germination (ie both binomial data sets) were analysed using the χ^2-tests. When the analysis indicated differences between distance categories, the pair-wise *post hoc* χ^2-tests were performed. The discrete variables (number of seed per capsule) and continuous variables (capsule volume, seedling height, diameter and volume) were analysed using the non-parametric Kruskal–Wallis tests, as untransformed, square root and log transformed data did not consistently produce normal distributions nor homogeneous variances. When significant differences were identified using the Kruskal–Wallis test, pair-wise *post hoc* Mann–Whitney U tests were conducted to identify which distance categories were different.

RESULTS

Microsatellite analysis

Multilocus genotypes were generated for 1013 seed based on five variable microsatellite loci. Twenty progeny arrays from known mothers with 17 to 47 seed (mean ± s.e. 25.3 ± 1.1) were genotyped from both the forest (LB 475 seed) and pasture (HC 538 seed). Maternal genotypes were reconstructed based on site allele frequencies and progeny array genotypes using a maximum likelihood approach in MLTR. The number of alleles detected per locus ranged from 5 in Bq19 to 23 in Bq31 in the maternal trees (mean 10.8 ± 3.2) (Supplementary Table 1) and increased in the progeny arrays, ranging from 6 to 30 alleles/locus (15.0 ± 4.0; data not shown). Genetic diversity was similar in pasture (HC) and forest (LB) parental trees: effective number of alleles (Ne = HC 4.764 ± 1.090, LB 4.781 ± 1.325), Shannon's index (I = HC 1.663 ± 0.245, LB 1.634 ± 0.246) and unbiased expected heterozygosity (UHe = HC 0.770 ± 0.047, LB 0.758 ± 0.051) (Supplementary Table 1). The fixation index indicated complete outcrossing in trees from both sites (F = HC −0.013 ± 0.084, LB −0.150 ± 0.026). There were no significant deviations from Hardy–Weinberg equilibrium for any locus or site ($P > 0.389$).

Inbreeding estimates

Estimated levels of outbreeding based on MLTR analysis of the progeny arrays were significantly lower in the pasture (HC, mean ± 1s.d., 0.828 ± 0.015) than the forest (LB 0.926 ± 0.005) (*t*-test df = 38, $P = 0.036$). The correlation of multilocus outcrossing estimates was moderate for both pasture and forest (HC 0.636 ± 0.147, LB 0.429 ± 0.166). Bi-parental inbreeding was negligible for both sites (Table 1).

The paternity analysis of progeny arrays from pasture trees provided a detailed understanding of mating patterns (Table 2). The five SSR markers had a combined exclusion probability of >0.99. More than half of the progeny could be assigned as self- or internal-

mapped pasture tree pollination events (Table 2). Paternity analysis (CERVUS) estimated outcrossing in the pasture (HC) to be 0.830 ± 0.054 (similar to the MLTR analysis). There was variation in the mating patterns among pasture trees in which the level of selfing ranged from 0 to 81.8%, with six trees showing no selfing (0%), three trees with low levels of selfing (0.1–4.9%), five trees with low-to-moderate selfing (5–19.9%), three trees with moderate selfing (20–49.9%) and three tress with high selfing (>50%) (Table 2).

Inbreeding depression can be predicted by the difference between inbreeding levels in parental trees and progeny arrays (Ritland 1990). The estimates of inbreeding were similar for the pasture and forest trees (F_{IS} = 0.000 and −0.003, respectively), indicating complete outcrossing for adult trees. Inbreeding estimates from the progeny arrays were significantly greater for the pasture (HC 0.172 ± 0.015) than the forest (LB 0.074 ± 0.005) (Inbreeding = 1 − outcrossing; Table 1). On the basis of Ritland's estimate for inbreeding depression $w = 2\left[\frac{(1-s)F}{s(1-F)}\right]$ (where, s is the inbreeding (HC 0.172, LB 0.074), F is the inbreeding coefficient of parents (LB and HC 0.001)) the relative fitness (w) of seed produced by forest trees (w = 0.025) is estimated to be almost four times that of the pasture trees (w = 0.096) (Ritland 1990).

Table 1 Mating parameters estimated for trees in pasture and continuous forest contexts using direct and indirect methods of analysis

Mating parameter	*Analysis*	*Pasture (HC)*		*Forest (LB)*	
Outcrossing	MLTR	0.828	± 0.015	0.926	± 0.005
	CERVUS	0.830	± 0.054	NA	
Bi-parental inbreeding	MLTR	0.001	± 0.020	0.027	± 0.019
Pollen donors	MLTR	3.448	± 0.065	3.676	± 0.030
	TwoGener	3.13		4.55	
	CERVUS	4.05	± 0.08	NA	
Dispersal distance	TwoGener	158.2 m		47.9 m	
	CERVUS	438.0 m	± 51.7	NA	

Abbreviation: NA, not applicable.
Values are mean ± s.e.

Pollen dispersal

The number of pollen donors contributing to pollination of trees was marginally fewer in pasture (HC 3.45 ± 0.07) than the forest (LB 3.68 ± 0.03) (t-test df = 38, P = 0.035). A greater difference was found by TwoGener analyses (Table 1), while CERVUS estimated a larger number of pollen donors for the pasture than the other methods (Table 1). The pollen dispersal distance was estimated to be greater in the pasture based on a TwoGener analysis (HC 158.2 m LB 47.9 m).

The paternity analysis indicated a mean of 44.6% of progeny likely to be the result of external pollination (father not present within the sampled pasture area, Table 2). The progeny assigned to fathers within the pasture was used to calculate the pollen dispersal distance to the known mother. Mothers had between one and ten fathers from within the site contributing to mating events. The mean pollen dispersal distance was 438.0 m (± 1s.e. 51.7 m), which ranged from 80.8 m to 969.3 m (Table 2). The mean distance among trees within the pasture was 748.6 m (minimum 38.1 m, maximum 1516.9 m).

Influence of tree and site parameters

The forest and pasture are significantly different in tree size and density. Trees are significantly larger in the pasture (dbh = HC 97.25 ± 9.03 cm, LB 70.90 ± 6.76 cm; t-test, df = 38, P = 0.012) with increased distances among trees (distance to the nearest neighbour =

Table 2 Characterisation of the size and density of *Pachira quinata* trees in the pasture, and their corresponding mating parameters estimated from the CERVUS paternity analysis

Tree ID	*Size dbh(cm)*	*Capsule production*[a]	*Distance to nearest tree*	*Number of trees*		*Array analysed*	*% external*	*% self*	*% internal cross*	*% siring success*	*Dispersal distance*[b]
				<250 m	*250–500 m*						
1	159	3	62.5	4	5	22	9.1	81.8	9.1	11.1	631.8 ± 113.5
2	115	2	62.5	3	6	24	0.0	70.8	29.2	2.9	126.8 ± 53.4
3	110	1	162.8	5	3	23	69.6	0.0	30.4	3.4	280.7 ± 57.9
4	56	1	162.8	5	4	24	25.0	29.2	45.8	1.4	223.5 ± 29.9
5	87	1	270.0	0	7	24	37.5	8.3	54.2	10.6	513.2 ± 29.3
6	87	3	270.0	0	7	51	37.3	51.0	11.8	2.4	675.4 ± 67.2
7	61	1	312.5	0	7	28	60.7	0.0	39.3	1.9	514.9 ± 47.4
8	65	2	209.0	1	7	24	41.7	0.0	58.3	4.3	230.1 ± 26.4
9	157	2	209.0	2	9	22	54.5	13.6	31.8	8.7	333.7 ± 70.0
10	143	1	269.6	0	7	48	58.3	2.1	39.6	4.8	612.6 ± 76.9
11	96	1	316.3	0	9	24	87.5	0.0	12.5	3.8	389.7 ± 0.0
12	78	1	89.0	3	6	25	32.0	0.0	68.0	3.4	158.5 ± 62.9
13	62	2	89.0	3	6	48	60.4	2.1	37.5	14.9	611.3 ± 57.2
14	94	3	228.2	1	6	26	69.2	7.7	23.1	0.5	751.0 ± 365.3
15	131	2	42.7	2	6	23	56.5	4.3	39.1	0.5	969.3 ± 0.0
16	147	1	42.7	1	7	25	60.0	0.0	40.0	2.4	482.9 ± 28.1
17	152	4	38.1	3	4	24	50.0	29.2	20.8	17.8	521.1 ± 89.0
18	35	1	38.1	3	4	24	20.8	12.5	66.7	1.4	385.7 ± 279.2
19	32	2	43.6	3	4	24	25.0	8.3	66.7	1.9	80.8 ± 72.1
20	78	2	88.2	3	3	25	36.0	20.0	44.0	1.9	267.8 ± 190.5
mean	97.3	1.8	150.3	2.1	5.9	27.9	44.6	17.0	38.4	5.0	438.0
s.e.	9.0	0.2	22.7	0.4	0.4	2.1	4.9	5.4	4.0	1.1	51.7

[a]1 (low <20), 2 (medium, 20–50), 3 (high, 50–100), 4 (very high >100).
[b]distance (m) from maternal to sire tree ± s.e.

HC 150.3 m ± 22.7, LB 26.9 m ± 5.4; *t*-test, df = 38, $P < 0.0001$). Seed capsule production was greater in the forest (2.35 ± 0.25) than the pasture (1.80 ± 0.20); however, this was not significant (*t*-test, df = 38, $P = 0.097$). Genetic relatedness among neighbouring trees (r5) and the level of heterozygosity (He) did not differ between pasture and forest trees (r5 = HC 0.61 ± 0.01 LB 0.61 ± 0.01; He = HC 0.77 ± 0.04 LB 0.74 ± 0.05). There was, however, significant spatial autocorrelation detected in the pasture with the nearest neighbours (0–50 m and 0–100 m distance classes) more closely related than expected by chance (Supplementary Figure 1 presents the 50 m distance class, which was similar to the 100 m class results not presented here).

Association of mating parameters with plant and site characteristics

Multiple regression analysis revealed significant associations of MLTR mating parameters with plant and site characteristics. Outcrossing rates differed significantly between sites even when plant size, reproductive output and plant densities were included in the model. Site ($P = 0.0068$) and seed capsule production ($P = 0.0231$) remained significant following a stepwise reduction in the multivariate model (R^2 adj = 0.29, df = 4 and 35, $P = 0.0022$) with a significant interaction of genetic relatedness (of the five closest trees (r5)) with site ($P = 0.0113$). Outcrossing rate in forest and pasture trees was associated with different factors: genetic relatedness explained 32% of the variance in the pasture (R^2 adj = 0.32, df = 1 and 18, $P = 0.0058$), and seed capsule production explained 16% of the variance in the forest (R^2 adj = 0.16, df = 1 and 18, $P = 0.0463$) (Supplementary Figures 2a and b).

Biparental inbreeding (tm −ts) was lightly associated with seed capsule production ($P = 0.0740$), but strongly with genetic relatedness ($P = 0.0063$), and distance to the nearest neighbour ($P = 0.0248$) in the multivariate analysis (R^2 adj = 0.26, df = 3 and 36, $P = 0.0029$). There were no simple associations with biparental inbreeding for forest trees; however, genetic relatedness explained over half the variance in biparental inbreeding for pasture trees (R^2 adj = 0.50, df = 1 and 18, $P = 0.0003$) (Supplementary Figures 2c and d).

The number of sires (1/rp) was associated with site ($P = 0.0317$) and tree size ($P = 0.0098$) explaining 16% of the variance in the model (R^2 adj = 0.16, df = 2 and 36, $P = 0.0150$). A complex interaction with seed capsule production, genetic relatedness and distance to the nearest neighbour was only found in pasture trees, with a simple association of siring with tree size in the forest (R^2 adj = 0.47, df = 1 and 17, $P = 0.0006$) (Supplementary Figures 2e and f).

The mating patterns derived from paternity analysis in the pasture site largely support the multivariate analysis based on indirect estimates from MLTR (presented above). The direct estimates of selfing showed a significant positive correlation with seed capsule production (R^2 adj = 0.232, $P = 0.0183$), while correlated paternity had a weak relationship with tree size (R^2 adj = 0.083, $P = 0.1158$). Mean pollen dispersal distance was negatively correlated with the percentage of internal cross pollinations (R^2 adj = 0.173, $P = 0.0382$) and number of trees within 250 m (R^2 adj = 0.104, $P = 0.0899$), whereas it was positively correlated with tree size (R^2 adj = 0.102, $P = 0.0920$).

Male and female reproductive success (measured by % siring success and seed capsule production, respectively) only trended towards significance (R^2 adj = 0.143, $P = 0.0562$). The relationship, however, was non-linear, and a positive quadratic function explained 37.6% of the variance. Removal of three mother trees with high (>50%) selfing further improved the fit ($y = 0.023x^2 - 0.308x + 2.062$, $R^2 = 0.566$).

Self-incompatibility mechanism

Significant differences in seed capsule production between the selfed, open pollinated and control pollinated treatments were detected (Kruskall–Wallis. H = 8.61, d.f. = 3, $P = 0.035$). Using the Mann–Whitney test, the proportion of capsules set from open, bulk and single cross pollinations were similar (W = 76.5, $P = 0.397$) but all were significantly greater than capsule set from self-pollinations ($P = 0.05$, Figure 2). Controlled pollinations also revealed a varied ability to self among trees, with 50% of trees failing to self, whereas 12.5% showed high capsule set (>25%) from self-pollination.

From 200 crosses, only 23% of the selfed crosses had germinated pollen 20 h after pollination compared with 79% of unrelated crosses ($\chi^2 = 125.49$, $P < 0.001$). When selfed pollen germinated, the pollen tubes grew significantly more slowly down the style than those from unrelated crosses (6.8 vs 14 mm in 12 h, $P < 0.001$, $n = 23$–26 styles). Pollen tubes from selfed crosses were normal (that is, no swollen, ruptured, reversed or spiralling ends) and did reach the ovary, although significantly more slowly than pollen tubes from unrelated crosses (Table 3). The number of pollen tubes that had reached the ovary 120 h after pollination in selfed and unrelated crosses was, however, not significantly different (Table 3). Pollen tubes from both selfed and unrelated crosses were seen to penetrate the micropyles of ovules, although selfed pollen tubes penetrated ovules in significantly lower numbers compared with pollen tubes from unrelated crosses (37% compared with 63%).

This variability in self-incompatibility among trees influences the extent to which low tree densities result in inbreeding. Low tree density is no guarantee of selfing, but does allow its expression in

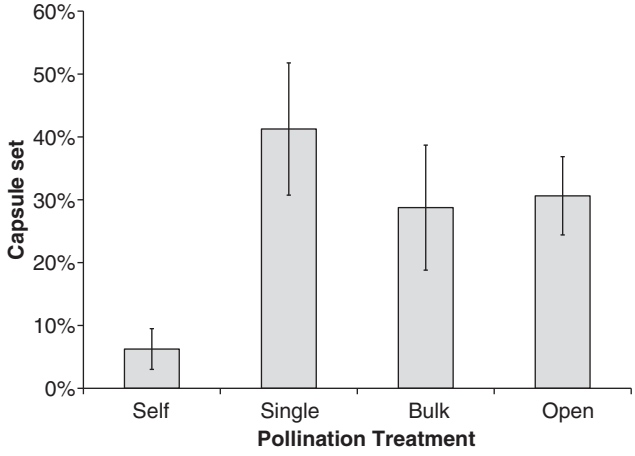

Figure 2 Percentage of capsules set from controlled pollination treatments of *Pachira quinata* clones in trial E02/88, Comayagua Valley, Honduras. Self = pollen from up to five different flowers of the same tree. Single = pollen sourced from a single tree (ID 11/88). Bulk = pollen from five different trees. Open = natural pollination. Twenty flowers were pollinated on eight trees for each treatment. Values are mean ± s.e.

Table 3 Percentage of samples in which pollen tubes in self- and unrelated crosses of *Pachira quinata* had reached the ovary

Hours after pollination	*Self cross*	*Unrelated cross*	χ^2	P-*value*
48	15%	56%	8.699	0.003
72	64%	90%	5.404	0.020
120	89%	90%	0.009	0.923

All pollinations made at 20:00 hours on eight clones. Number of samples (*n*) varies from 19–32.

those trees with the capacity to self. Outcrossing rates for self-compatible pasture trees showed a strong negative relationship with tree density, whereas the higher tree density in continuous forest meant there was no such relationship (Figure 3).

Pollination distance: reproductive success, germination and growth

A total of 112 capsules were formed from 474 pollinations (23.6% capsule formation) at Hacienda Ciruelas. Significantly more capsules were set with pollen from 700 m than all other distance categories, all of which were not significantly different from each other (Table 4). Selfed pollinations formed the smallest capsules and pollen from 250 m the largest capsules, although there was no trend of increasing or decreasing capsule volume with increasing pollination distance (Table 4). Similarly, the number of seed produced per capsule was the lowest for selfed pollinations and greatest for pollinations from 250 m away, with no apparent distance trend (Table 4).

Seeds from selfed pollinations had significantly lower germination than those from all other pollination distances ($P<0.001$), regardless of the germination conditions (Table 4). Pollination distances between 50 m and 3 km had over 65% germination under the range of conditions, with the highest levels at 250 m and 700 m (Table 4).

Seedling growth was the lowest for selfed seed under both glasshouse and common garden conditions from 4 to 21 months (Table 5). In the glasshouse, seedling volume after 4 months growth

Table 4 Reproductive success and germination response from self (0 m), nearest neighbour (50 m), within forest (250 m), within pasture (700 m), and surrounding sites (3 km) pollination treatments

Fitness response	*0 m*	*50 m*	*250 m*	*700 m*	*3 km*
Reproductive success					
Seed per capsule***	$n=4$	$n=3$	$n=7$	$n=4$	$n=19$
	11.2 (4.9)	64.0 (21.6)	111.2 (10.7)	71.4 (15.6)	77.1 (8.1)
Capsule volume**	$n=7$	$n=4$	$n=7$	$n=5$	$n=20$
	13.4 (4.4)	39.6 (9.9)	57.5 (5.8)	26.5 (5.3)	41.7 (2.6)
Proportion of capsules set*	$n=59$	$n=31$	$n=29$	$n=26$	$n=55$
	0.118 (0.040)	0.126 (0.057)	0.239 (0.079)	0.594 (0.090)	0.228 (0.054)
Germination					
Hacienda Ciruelas soil***	$n=14$	$n=30$	$n=80$	$n=21$	$n=85$
	0.219 (0.109)	0.806 (0.067)	0.898 (0.039)	0.673 (0.105)	0.780 (0.041)
Common garden***	$n=14$	$n=35$	$n=95$	$n=25$	$n=100$
	0.138 (0.081)	0.655 (0.083)	0.744 (0.045)	0.875 (0.063)	0.770 (0.042)
Glasshouse***	$n=18$	$n=40$	$n=105$	$n=30$	$n=114$
	0.225 (0.104)	0.846 (0.065)	0.906 (0.026)	0.898 (0.063)	0.859 (0.031)

Values are mean (s.e.). n=sample size. Level of significance *$P<0.05$, **$P<0.01$, ***$P<0.001$ by χ^2 analysis.

Table 5 Growth (height, diameter and volume) response in glasshouse and common garden trials from self (0 m), nearest neighbour (50 m), within forest (250 m), within pasture (700 m), and surrounding sites (3 km) pollination treatments

Fitness response	*0 m*	*50 m*	*250 m*	*700 m*	*3 km*
Growth in glasshouse					
Seedling size at 4 months	$n=4$	$n=15$	$n=35$	$n=15$	$n=56$
Height (cm)	19.8 (5.4)	27.1 (2.0)	26.4 (1.0)	24.5 (2.1)	25.1 (1.0)
Diameter (mm)	4.8 (0.6)	5.7 (0.4)	5.8 (0.2)	5.4 (0.4)	5.5 (0.2)
Volume (cc)	3.16 (1.28)	6.66 (1.16)	6.18 (0.54)	5.28 (0.76)	5.53 (0.41)
Seedling size at 8 months	$n=2$	$n=7$	$n=23$	$n=7$	$n=26$
Height (cm)	40.0 (9.9)	56.2 (8.6)	49.6 (4.0)	61.7 (7.8)	51.7 (3.8)
Diameter (mm)	7.7 (0.01)	9.7 (0.9)	8.8 (0.5)	9.1 (1.0)	8.8 (0.4)
Volume (cc)	18.5 (2.6)	47.9 (13.5)	35.9 (5.0)	48.6 (12.4)	37.4 (4.8)
Growth in common garden					
Seedling size at 9 months	$n=2$	$n=20$	$n=51$	$n=25$	$n=90$
Height (m)**	0.66 (0.12)	0.82 (0.05)	0.82 (0.02)	0.68 (0.03)	0.77 (0.01)
Diameter (mm)	29.2 (3.9)	30.8 (1.4)	30.5 (0.1)	29.4 (1.1)	29.6 (0.1)
Volume (cc/100)	4.8 (0.3)	7.4 (0.7)	6.7 (0.4)	6.3 (0.7)	7.3 (0.4)
Sapling size at 21 months	$n=2$	$n=20$	$n=51$	$n=25$	$n=90$
Height (m)	2.33 (0.01)	2.85 (0.01)	2.91 (0.01)	2.76 (0.09)	2.8 (0.01)
Diameter (mm) **	43.0 (4.5)	54.5 (1.7)	56.8 (0.2)	49.7 (2.5)	52.9 (0.1)
Volume (cc/100)	35.3 (7.7)	78.7 (5.3)	81.4 (4.5)	71.8 (5.6)	91.7 (7.5)

Values are mean (s.e.). n=sample size. Level of significance **$P<0.01$ by χ^2 analysis.

from selfed seed was <50% that of the longer pollination distances, decreasing to ~40% volume by 8 months (volume growth 4–8 m = volume selfed/average volume (50 m to 3 km) = 0.42). Seedling height and diameter were also lower in selfed plants (73–85% other pollinations), with a marginal reduction in growth from 4 to 8 months (growth 0.70–0.84). A similar pattern was observed in the common garden trial with smaller plants and lower growth rate for selfed plants (Table 5). This relationship was, however, non-significant because of the low survival of selfed plants resulting in a small sample size.

DISCUSSION

This study shows that although remnant trees in pastures can contribute to broader mating patterns in agroecosystems, this ability is strongly influenced by the nature, strength and variability of any self-incompatibility system. Reproductive success in *P. quinata* is maintained in low tree density pastures, partly by pollinator movement leading to increased pollen dispersal distances. Selfing also provides reproductive assurance in the pasture trees facilitated through a 'leaky' self-incompatibility mechanism, although the fitness of selfed seed was reduced compared with outcrossed seed.

Variable selfing

Plant breeding systems are highly variable from complete selfing to outcrossing controlled by floral structure and self-incompatibility (SI) mechanisms (Lande and Schemske, 1985; Barrett, 1998; Takayama and Isogai, 2005). Variation among species and populations has been ascribed evolutionary and ecological significance (Rymer *et al.*, 2005; Ward *et al.*, 2005). Although the majority of neotropical tree species are self-incompatible (>80% of species studied in Bawa *et al.*, (1985)), approximately a third of species produce some seed from experimental self-pollinations (Bawa *et al.*, 1985). *Pachira quinata* was found to be predominantly self-incompatible (Sandiford, 1998; Quesada *et al.*, 2001), fitting with some other bat-pollinated tree species in the Malvaceae (Murawski *et al.*, 1990; Murawski and Hamrick, 1992; Gibbs *et al.*, 1999; Gribel and Gibbs, 2002). There is, however, significant variation in the Malvaceae, with a number of species exhibiting mixed mating systems (Ward *et al.*, 2005). Notably the genus *Ceiba* has shown outcrossing and mixed mating varying both within and among species (Quesada *et al.*, 2004). Controlled pollinations in this study showed a varied ability to self among *P. quinata* trees, with 50% of trees failing to self, whereas 12.5% showed high seed set under selfing. This concurs with the paternity analysis from the pasture site where 30% of the trees failed to self and 15% showed high levels of selfing. Interestingly, the 'leaky' SI mechanism described here for *P. quinata* differs from other studied Malvaceae species, where no stigma or style SI mechanism has been detected. *Ceiba* and *Pseudobombax* produce no viable seed from self pollination due to late-acting, postzygotic, SI and/or inbreeding depression. Thus, the varied capacity between trees, populations and species to self appears to be controlled by different SI mechanisms or distributions of deleterious alleles (Barrett, 1998; Hiscock and McInnis, 2003; Charlesworth *et al.*, 2005; Takayama and Isogai, 2005).

Plant mating patterns are largely dictated by the interaction of their breeding system with the quantity and quality of pollen received. Mate availability and pollinator behaviour have been shown to dramatically alter pollination and mating patterns (Grant, 1994), which has led to predictions that the immediate effect of habitat fragmentation will be reduction in reproductive success, outcrossing rates and plant fitness (Lowe *et al.*, 2005). The majority of studies are in line with this prediction (7/10 reproduction, 6/8 inbreeding and 6/6 fitness in Lowe *et al.*, (2005)), with variation in effect explained largely by breeding system, rarity status and time since disturbance (Aguilar *et al.*, 2008). Our study confirms the findings of Fuchs *et al.* (2003), with reproductive success and outcrossing rates for *P. quinata* significantly greater in continuous forest than isolated trees. In that study, trees were isolated by >500 m and the outcrossing rate was even lower (0.777), although the number of sires was larger in our study at both forest and pasture sites (3.7 LB, 3.4 HC compared with 2.2 in forest and 1.4 in isolated trees; Fuchs *et al.*, 2003). We have also found a similar pattern in a fragmented pasture landscape, where high levels of outcrossing are maintained at high tree densities (Honeycutt, 2007). Therefore, the patterns of reduced reproductive success and outcrossing in isolated trees seem to be consistent among sites and studies and are likely to hold for the species as a whole. It remains to be seen whether the findings can be extrapolated across similar life histories and in particular in species with similar self-incompatibility systems.

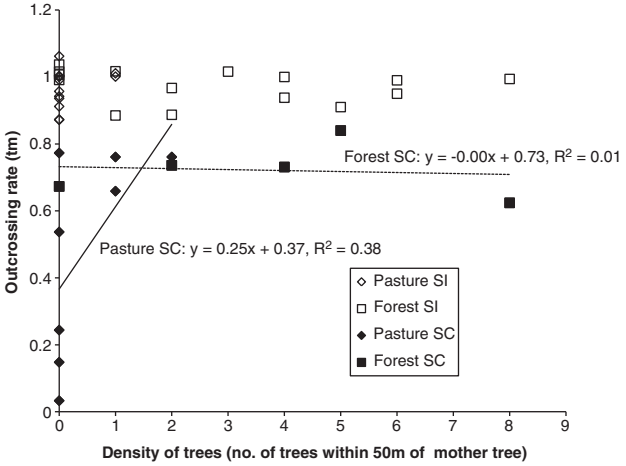

Figure 3 The expression of among tree variability in self-incompatibility at low tree densities in agroecosystems. *Pachira quinata* trees vary in their breeding system, a proportion being obligatory outcrossing (SI) and others capable of producing seed from self-pollinations (SC). The relationship between outcrossing rates from natural pollinations and tree density for pasture and continuous forest are explored for SC trees.

Mulitvariate analyses, in this study, revealed that genetic relatedness among neighbouring trees was a major explanatory variable for the level of outcrossing and biparental inbreeding in pasture trees. This pattern is supported by controlled crosses (Table 4) and the finding that pollinations between half-sibs formed fewer capsules than unrelated crosses (Sandiford, 1998). Reproductive success was negatively correlated with outcrossing rate (Supplementary Figure 2), indicating that selfing provides some reproductive assurance. In fact, as the availability of unrelated pollen diminishes with the intensification of land use in agroecosystems, mating patterns are likely to shift towards increased selfing (Aguilar *et al.*, 2008), although this will depend on the ability to self within the species concerned (Figure 3).

Inbreeding depression

Altered mating patterns in fragmented landscapes have important consequences for plant fitness. Theoretical models and empirical studies have shown selfing in otherwise outcrossing species reduces plant fitness through inbreeding depression resulting from the expression of recessive deleterious alleles (Lande and Schemske, 1985; Schemske and Lande, 1985). Given neotropical trees are

predominantly outcrossing (Ward *et al.*, 2005), and extensive fragmentation in the region has in many cases increased selfing (Lowe *et al.*, 2005), the predicted fitness consequences of inbreeding depression are high. Indirect methods have commonly been used, where inbreeding coefficients estimated by co-dominant genetic markers are compared for multiple stages of development (ovary, seed, seedling to adult). The findings, however, have been mixed with comparisons between seedling and adult trees having similar F_{IS} values in some cases (Dayanandan *et al.*, 1999), while others have elevated coefficients of inbreeding in seedlings (Hall *et al.*, 1994; Aldrich *et al.*, 1998), indicative of inbred seedlings being purged from the population before developing into adults. Estimating outcrossing rates for progeny arrays is a more accurate approach than inbreeding coefficients, with self-pollinated ovules purged following fruit abortion, maturation and seedling germination (tm = 0.79, 0.82 and 0.91, respectively Hufford and Hamrick, 2003). Direct estimates of fitness have been investigated for open pollinated seed from different landscape contexts. Fitness estimates are often limited to seed production (Boshier *et al.*, 2004), germination (Nason and Hamrick, 1997) or seedlings (Cascante *et al.*, 2002), but a few studies have followed through to the sapling stage (Stacy, 2001; Navarro and Hernández, 2004). Interestingly, the review by Lowe *et al.*, (2005) found all studies showed a significant impact of fragmentation on fitness, with reduction in outcrossing rates. Although fitness losses may be driven by mate limitation (Naito *et al.*, 2005), the exact cause is unclear and may vary by species and landscape context. The implications and significance of such fitness losses will depend on the extent to which inbred material survives regeneration. This necessitates culling of inferior stock in the nursery to avoid high mortality or reduced growth in restoration plantings.

The fitness costs of altered mating patterns, through changes in selfing and biparental inbreeding rates from alterations in tree density and spatial genetic structure, can perhaps best be unravelled through experimental pollinations over a range of distances (Fischer and Matthies, 1997). Few studies of tropical trees have explored the relationship between pollination distance and progeny fitness (Billingham, 1999; Stacy, 2001). This study is one of the first to accurately determine mating patterns in continuous forest and isolated trees in pasture, while characterising seed production, germination, seedling and sapling growth for seed resulting from selfing, within and among population crosses. The most striking finding is that self-pollinations produce fewer seed, with lower germination, survivorship and growth, indicative of inbreeding depression. Experimental crosses at the pollen dispersal distances estimated within forests (c. 50 m) showed marginally lower reproductive success, germination and growth than that in greater pollination distances. Lower reproductive success may be due to biparental inbreeding, as genetic relatedness was a strong explanatory variable even though the relationship with distance was weak (Supplementary Figures 2c and d). Significant genetic spatial autocorrelation was detected in the pasture trees at 0–50 m distance class but not in the forest trees (Supplementary Figure 1). The larger distances between trees (150 m) and increased pollen dispersal (150–450 m), estimated directly and indirectly through microsatellite genotyping, in pasture trees may avoid much of the potential for biparental inbreeding. Further, almost half of the pollination events in pasture trees came from outside the known potential sires, potentially from isolated conspecific trees 500 m from the pasture or the nearest population almost 3 km away. The experimental crosses had similar relative fitness from 250 m to 3 km pollination distances, indicating that the mating events detected from outside the pasture would result in high quality seed. Despite this increase in pollination distances for outcrossing events in pasture trees compared with forests, the general increase in selfing in pasture trees is expected to result in reduced seedling establishment due to inbreeding depression.

Functional agroecosystems

Predictions of the effects of habitat fragmentation have largely been based on the island biogeography theory, which predicts species extinction probabilities based on land area size and isolation from large island or mainland source populations (MacArthur and Wilson, 1967). Islands are surrounded by a matrix of water that prevents terrestrial organisms from establishing and reproducing. Habitats in a fragmented landscape, on the other hand, are surrounded by a complex mosaic of different land-use types, which vary in the level of hostility. While some land-uses prevent pollen flow, reproduction or establishment (for example, industrial land), others may be more benign and functional (for example, some agroecosystems; Lander *et al.*, 2011). Remnant pasture trees are isolated and immersed in a matrix modified by the removal of the surrounding shrub and tree layer. Long-term persistence depends largely on their ability to survive in environments with altered light and water availability, along with changes in biotic interactions, such as pollinators and herbivores.

Scattered trees in agroecosystems may be of disproportionate value in highly fragmented or deforested ecosystems (Fischer *et al.*, 2010). Pasture trees offer habitat for organisms across trophic levels, thereby harbouring biodiversity. They may be also critical in maintaining connectivity across anthropogenically modified landscapes (White *et al.*, 2002; Lander *et al.*, 2010). Remnant *Pachira quinata* trees in pastures were found to significantly contribute to pollen movement across the landscape (mean pollen dispersal distance 438.0 ± 51.7 m within the pasture and up to 50% external pollinations). Despite increased levels of inbreeding, pasture trees had moderate levels of reproductive success. Natural regeneration is largely absent in pastures due to grazing and weed competition; however, silviculture techniques are well established in *P. quinata,* creating the potential for assisted regeneration. Appropriate seed sourcing practices will be critical, such as avoiding isolated trees where inbred seed are more likely and focusing on large populations. Alternatively, rigorous selection of seedlings in the nursery can ensure that inbred material is eliminated, as is likely under natural regeneration conditions. With the global decline in remnant trees in farmlands, changes in management strategies are urgently required to value and conserve scattered trees in modified landscapes.

DATA ARCHIVING

Data deposited in the Dryad repository: doi:10.5061/dryad.6f3q5.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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