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LETTER

Genetic evidence of frequent long-distance recruitment in a vertebrate-dispersed tree

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Abstract

The importance of dispersal for the maintenance of biodiversity, while long-recognized, has remained unresolved. We used molecular markers to measure effective dispersal in a natural population of the vertebrate-dispersed Neotropical tree, *Simarouba amara* (Simaroubaceae) by comparing the distances between maternal parents and their offspring and comparing gene movement via seed and pollen in the 50 ha plot of the Barro Colorado Island forest, Central Panama. In all cases (parent-pair, mother–offspring, father–offspring, sib–sib) distances between related pairs were significantly greater than distances to nearest possible neighbours within each category. Long-distance seedling establishment was frequent: 74% of assigned seedlings established > 100 m from the maternal parent [mean = 392 ± 234.6 m (SD), range = 9.3–1000.5 m] and pollen-mediated gene flow was comparable to that of seed [mean = 345.0 ± 157.7 m (SD), range 57.6–739.7 m]. For *S. amara* we found approximately a 10-fold difference between distances estimated by inverse modelling and mean seedling recruitment distances (39 m vs. 392 m). Our findings have important implications for future studies in forest demography and regeneration, with most seedlings establishing at distances far exceeding those demonstrated by negative density-dependent effects.

Keywords

Barro Colorado Island, frugivory, microsatellites, Panama, parentage analysis, pollen movement, seed dispersal, seedling establishment, *Simarouba amara*, tropical forest.

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INTRODUCTION

The frequency and outcome of long-distance dispersal events is an important yet unsolved problem in dispersal biology and in biogeography (Anderson 1991; Eriksson & Jakobsson 1999; Ouborg *et al.* 1999), despite its fundamental role in forest community structure and dynamics (Clark *et al.* 1999; Hamilton 1999; Harms *et al.* 2000). Theoretical models show that even quite modest rates of long-distance dispersal can have large effects on rates of range expansion (Lewis 1997; Clark *et al.* 1999), and there are good phylogeographic data in several European and North American tree species supporting the importance of long-distance dispersal events during postglacial recolonization (e.g. Petit *et al.* 1997; McLachlan *et al.* 2005). Long-distance dispersal can also significantly influence community composition (Hubbell 2001; Chave & Leigh 2002) and β diversity (Condit *et al.* 2002).

Historically, evaluating the importance of dispersal has been hampered by the difficulty of measuring dispersal directly. Most attempts to measure seed dispersal in tropical forests have used indirect methods such as fruit removal rates by frugivore dispersers coupled with seed passage times (Holbrook & Smith 2000; Westcott & Graham 2000) or data on seed rain collected using traps (Muller-Landau 2001; Clark *et al.* 2001; Hardesty & Parker 2002). In the latter case, seed rain data have been fit with inverse modelling methods to estimate dispersal curves (Ribbens *et al.* 1994; Clark *et al.* 1999; Nathan & Muller-Landau 2000). Model fits have been applied with the greatest success to species whose seeds are dispersed by abiotic processes such as wind (Nathan & Muller-Landau 2000). With animal-dispersed tree species, these methods are fraught with difficulties because patterns of seed dispersal depend on the idiosyncratic, site-specific movement patterns of the dispersers and are often highly patchy. Moreover, for all dispersal syndromes, indirect methods tend to underestimate the frequency of long-distance dispersal events (Willson 1993).

Willson *et al.* (1989) estimated that 70–90% or more of all tropical tree species are animal-dispersed, mostly by vertebrates. Therefore, in order to understand the role played by dispersal agents in the population dynamics of tropical forests, better understanding of the outcome of dispersal in animal-dispersed tree species is required. Different animal dispersal agents may have different effects on the demography and population genetic structure of a given tree species, and may disperse seeds to particular sites (Fragoso 1997; Wenny & Levey 1998), spatially aggregate seeds in a non-random manner (Russo & Augspurger 2004; Grivet *et al.* 2005), drop seeds differentially underneath the canopy of the maternal trees, and have either a positive or negative effect on seed germination. Even when seed deposition is most dense under or near parent plants, seedlings may be more likely to recruit at arrival sites away from parent plants where natural enemies may occur in reduced numbers (Janzen 1970; Connell 1971). Whether recruitment is aggregated where seeds initially arrive depends not only on secondary dispersal and the strength of distance- and density-dependent survival in relation to the number of seeds that fall in a particular site, but also on predator satiation and the spatial scale over which density dependence occurs (Augspurger & Kitajima 1992; Russo & Augspurger 2004).

Genotyping seedlings and matching them to their maternal source provides a means to resolve long-standing questions in dispersal ecology about the spatial relationships between parent trees and their established offspring. For this study, we used microsatellite genetic markers to study effective dispersal (i.e. seedling recruitment distances from parents) in a naturally occurring population of an animal-dispersed tropical tree, *Simarouba amara* Aubl. (Simaroubaceae). It should be noted that genotyping seedlings and assigning them to their maternal parent characterizes the recruitment kernel rather than solely the dispersal kernel. It combines the net effects of dispersal, post-dispersal survival and germination of seeds, with establishment of seedlings. We address the following facets of recruitment in *S. amara*: (1) the distances seeds disperse and successfully recruit as seedlings; (2) the frequency of long-distance (> 100 m) recruitment, which necessarily involves vertebrate dispersers; (3) the relative contribution of seeds and pollen to gene movement; and (4) the relative fit of the recruitment kernel determined by genetic markers to dispersal kernels obtained from inverse modelling methods.

MATERIALS AND METHODS

Study site and focal species

The study was conducted in the 50 ha Forest Dynamics Project (FDP) plot on Barro Colorado Island (BCI), Panama (9°10′ N, 79°51′ W) (Hubbell & Foster 1983). Within the FDP, all *c.* 240 000 free-standing woody plants ≥ 1 cm diameter at breast height (dbh) have been tagged, mapped and identified to species, including our focal species, *S. amara*. The first census was completed in 1982 and the FDP has been re-censused every 5 years beginning in 1985. *Simarouba amara* is a dioecious, vertebrate-dispersed tree that grows to 35 m and has a maximum reported dbh of 70 cm on BCI (Croat 1978). The floral syndrome is consistent with pollination by small generalist insects such as bees and moths (Bawa 1990), which have been observed visiting *S. amara* flowers (BDH, pers. obs.). This moderate-to-high light requiring, out-crossing tree typically flowers from the end of January to April. The pale, yellowish, unisexual flowers are presented in terminal panicles. *Simarouba amara* produces clusters of large-seeded fleshy drupes up to 17 mm long, with a seed size of 10–14 mm (Croat 1978). Observed dispersers include chachalacas, flycatchers, motmots and thrushes (Croat 1978), tamarins (BDH, pers. obs.), and howler and spider monkeys (Hladik & Hladik 1969). The 2000 BCI FDP census contained 1230 individuals ≥ 1 cm dbh.

Sampling

From 2001 to 2003 we collected leaf tissue from the canopy layer of all reproductive individuals and from any individual ≥ 20 cm dbh within the BCI 50 ha FDP ($n = 134$; comprising 50 females, 38 males, and 46 non-reproductive or of unknown reproductive status trees). Although most *S. amara* individuals do not reproduce below 30 cm dbh, to ensure we did not miss any reproductive trees and because *S. amara* can grow rapidly, we assessed any individual of ≥ 20 cm dbh or more for reproductive output (dbh based upon the 2000 census). In addition, we mapped and collected tissue from all ≥ 20 cm dbh *S. amara* within a 100 m 'buffer zone' around the FDP. The buffer increased the total area sampled for adults by 34 ha and added 84 reproductive sized individuals: 24 females, 23 males and 37 unknown gender/non-reproductive trees to the study. We added the buffer to increase the likelihood of determining the parentage of seedlings on the plot that may have derived from parents located outside the FDP. We sexed trees based upon floral morphology and presence of fruit. If trees did not fruit in any a particular year, they were not considered for maternal assignment to seedlings germinated in that year. Hence, we used 49, 56 and 70 female trees for maternal matching in 2001, 2002 and 2003, respectively, including all 'buffer zone' trees ($n = 74$ females collectively). Trees identified as male were used for testing paternity, and individuals with > 20 cm dbh whose reproductive status was 'unknown' but which could have been reproductive were also considered as potential pollen parents.

From 2001 to 2003, *c.* 40 ha were exhaustively searched for *S. amara* seedlings, generally avoiding the plot edge

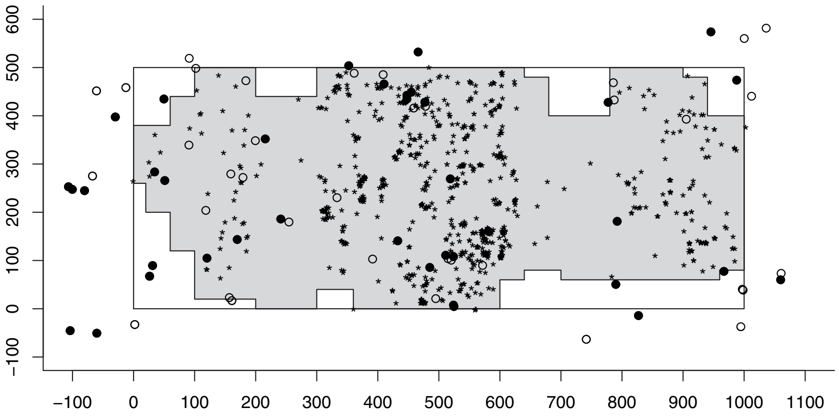

Figure 1 Spatial locations of genotyped seedlings (stars), female reproductive trees (open circles) and genetic mothers (closed circles). Grey indicates areas searched for seedlings.

(Fig. 1). We collected leaf tissue from each seedling, except in 5 × 5 m quadrats where ≥ 5 seedlings were encountered. Here, we sampled tissue from every third seedling in that quadrat. We could determine if seedlings were produced in the current year because seedlings retain their cotyledons (up to 6 months post-germination), and the stem base is distinctly purplish in colour (BDH, pers. obs). We completely genotyped 782 seedlings, 540 of these were 'cohort of a particular year'.

In the field we kept leaf tissue in a cooler, and upon return to the laboratory, the tissue was either flash frozen in liquid nitrogen and stored in a −50 °C freezer prior to DNA extraction, or stored in a refrigerator for no more than 2 days prior to DNA extraction. We extracted DNA using the Qiagen DNEasy Plant kit (Qiagen Sciences, Inc., Germantown, MD, USA) and PCR protocols followed those described in Hardesty *et al.* (2005), using nuclear microsatellite markers developed by Rodriguez von Platen *et al.* (2000). We genotyped samples on an MJ Basestation automated DNA analyzer (MJ Research Inc., Waltham, MA, USA). By multiple loading of differentially labelled primers, we were able to genotype large numbers of individuals at multiple loci efficiently and cost-effectively. To ensure reliability in scoring gels and avoid genotyping errors (see review in Bonin *et al.* 2004), we extracted DNA, performed PCR, and genotyped *c.* 20% of all potential parents and 10% of seedlings at least twice.

Spatial analysis

We used the Ripley's K function minus πr^2 to test for spatial aggregation of (1) all FDP individuals, (2) seedlings (using an edge correction to account for the irregular shape of the area sampled for seedlings) (Ripley 1988; Baddeley & Turner 2005), (3) reproductive male, and (4) reproductive female trees across the FDP (Ripley 1988). Because πr^2 is the expected value of Ripley's function for a Poisson process (complete spatial randomness), $K - \pi r^2 > 0$ indicates aggregation (more points than expected compared with complete spatial randomness), and $K - \pi r^2 < 0$ indicates a regular distribution (fewer points than expected from a Poisson process). With a finite number of points (the number of individuals we are testing), even for a Poisson process, values may not be equal to 0. Hence, we used 500 random simulations to test if the observed distributions fell within or outside envelopes drawn from the random simulations. For seedlings, the observed distribution was compared with the theoretical expectation of the Poisson process, based upon 500 random simulations, accounting only for areas searched (Fig. 1).

Genetic analyses

Because *S. amara* seeds do not experience dormancy (Camargo *et al.* 2002), seedlings germinate in their dispersal year. Parentage analyses were conducted against the pool of reproductive adults in each year for seedlings of that year. For older seedlings, parentage analyses were conducted against the entire pool of potential parents and included the localities of dead adults. If a dead adult was the nearest possible parent, we assumed parentage by that individual to make a conservative estimate of effective dispersal distance. We inferred seed immigration from outside the extended FDP (84 ha) if a maternal parent for a seedling could not be identified, and gene flow via pollen from outside the FDP if a male parent could not be identified.

Maternity assignment was conducted by simple exclusion comparing multilocus genotypes of seedlings to those of candidate females. Potential mothers were rejected if alleles at any of the five loci were incompatible to those of the seedling in question, and hence, they were not an 'exact multilocus match'. All reproductive female trees within the population had unique genotypes, with the exception of three trees (separated by > 100 m) that shared an exact genotype. For cases in which multilocus matching resulted in 2+ suitable candidate mothers for a seedling, we assigned the nearest female to be the mother, thereby conservatively estimating the maternal–offspring recruitment distance. As

Table 1 Number of alleles at each locus (*k*), and exclusion probabilities for single parent analyses, and second parent analysis when one parent is known (paternity analysis) for *S. amara*. Total values represent pooled values for first and second parent exclusion probability

Locus	*k*	First parent exclusion	Second parent exclusion
SA02	15	0.167	0.327
SA05	10	0.327	0.502
SA06	14	0.034	0.136
SA27	16	0.509	0.678
SA29	10	0.145	0.317
Mean	13.0	–	–
Total	65	0.772	0.936

the female parent was not definitive for seedlings with > 1 potential mothers, we did not perform parent-pair analyses for these seedlings, although we were able to test separately for paternity (as first parent, Table 1). We also calculated a conservative dispersal estimate for seedlings whose parents were not located within the 84 ha study area: the minimum distance from the seedling to the nearest edge of the expanded plot.

We coupled the exact multilocus matching for maternity with likelihood estimates of parentage for paternity assignment. We used CERVUS (Marshall *et al.* 1998) to perform paternity analyses so we could compare gene movement via both pollen and seed. Exclusion probabilities differ depending on whether the analysis seeks to identify a single parent (when neither parent is known) or whether the user has previously assigned a maternal genotype and paternal exclusion is the goal (Table 1). Probability scores are estimated for the set of parents and offspring selected. We limited our analyses and discussion to seedlings for which we have high confidence (80% or higher, per CERVUS) in our ability to assign the paternal parent. Among candidate fathers, one pair of multilocus genotype matches occurred for two trees separated by > 100 m. For seedlings for which we could separate gene movement from pollen vs. seed, we determined the relative contribution of each to the population of seedling recruits within the BCI 50 ha FDP.

RESULTS

Spatial analyses

The spatial distribution of *S. amara* on the FDP ($n = 1230$) differs from complete spatial randomness (Fig. 2a). The mean distance between nearest females was 36.5 m, whereas nearest males were separated by 42.0 m on average; both demonstrated a spatial distribution different from random (Fig. 2b,c, respectively). The spatial distribution of seedlings also suggests a non-random pattern (Fig. 2d). The average distance was 11.3 m between nearest seedling pairs. It appears that as numbers of individuals increase in our sampling (whether all FDP individuals or seedlings), *S. amara* diverges more strongly from spatial randomness.

Maternity analyses (seedling recruitment)

In total, we detected 65 alleles among the five microsatellite loci sampled across all individuals (range 10–16 alleles per locus, Table 1). Of 782 seedlings completely genotyped, 328 seedlings could be matched to zero, one, two or three potential mothers using multilocus matching. For 132 seedlings, there was no genetic maternal match within the 84 ha study area. For these seedlings, we estimated an average minimum recruitment distance (i.e. distance from seedling to the extended plot edge) of 210.3 ± 65.6 m (SD) (range 93.1–343.5 m). The mean effective dispersal distance for the 94 seedlings with a single candidate mother was 391.6 ± 234.6 m (SD) (range 9.3–1000.5 m) (Fig. 3, solid bars). For 98 seedlings we found two to three maternal matches. We conservatively assigned the nearest of these as the 'actual' maternal parent. For these seedlings, the mean recruitment distance was 235.4 ± 162.3 m (SD) (range 11.4–803.6 m) (Fig. 3, hashed bars).

Collectively, the 196 seedlings for which we were able to assign one to three candidate mothers were progeny from 40 of 74 possible mothers. For these seedlings, only 8.2% ($n = 16$) were produced by the nearest reproductive female. Overall, distances from seedlings to the nearest reproductive female tree averaged 50.5 ± 35.5 m (SD) (range 0.76–153.1 m), contrasting sharply with the distances to genetic mothers (see Appendix S1 for 10th, 25th, 50th, 75th and 90th percentile recruitment distances).

For the 454 seedlings with 4+ exact multilocus match candidate mothers, we also assigned the nearest of all genetically possible mothers as the 'actual' mother. With this minimum-distance assignment, the mean recruitment distance dropped to 129.8 ± 101.3 m (range 3.6–576.0 m). However, we still find that for the majority (73.7%) of these seedlings, the nearest reproductive female was still not the assigned maternal parent.

Paternity analyses (pollen dispersal)

For first parent paternity analyses, the overall exclusion probability was 77%. For seedlings with a known mother, the probability of assignment increased to 94% (Table 1). We were able to make high confidence pollen parent assignments for 100 seedlings. The mean distance between the paternal parent and offspring was 373.2 ± 243.1 m (SD) (range 1.4–1005.8 m) (Fig. 4, hashed bars). Twenty-five (of 75 potential) fathers sired one to 18 seedlings each.

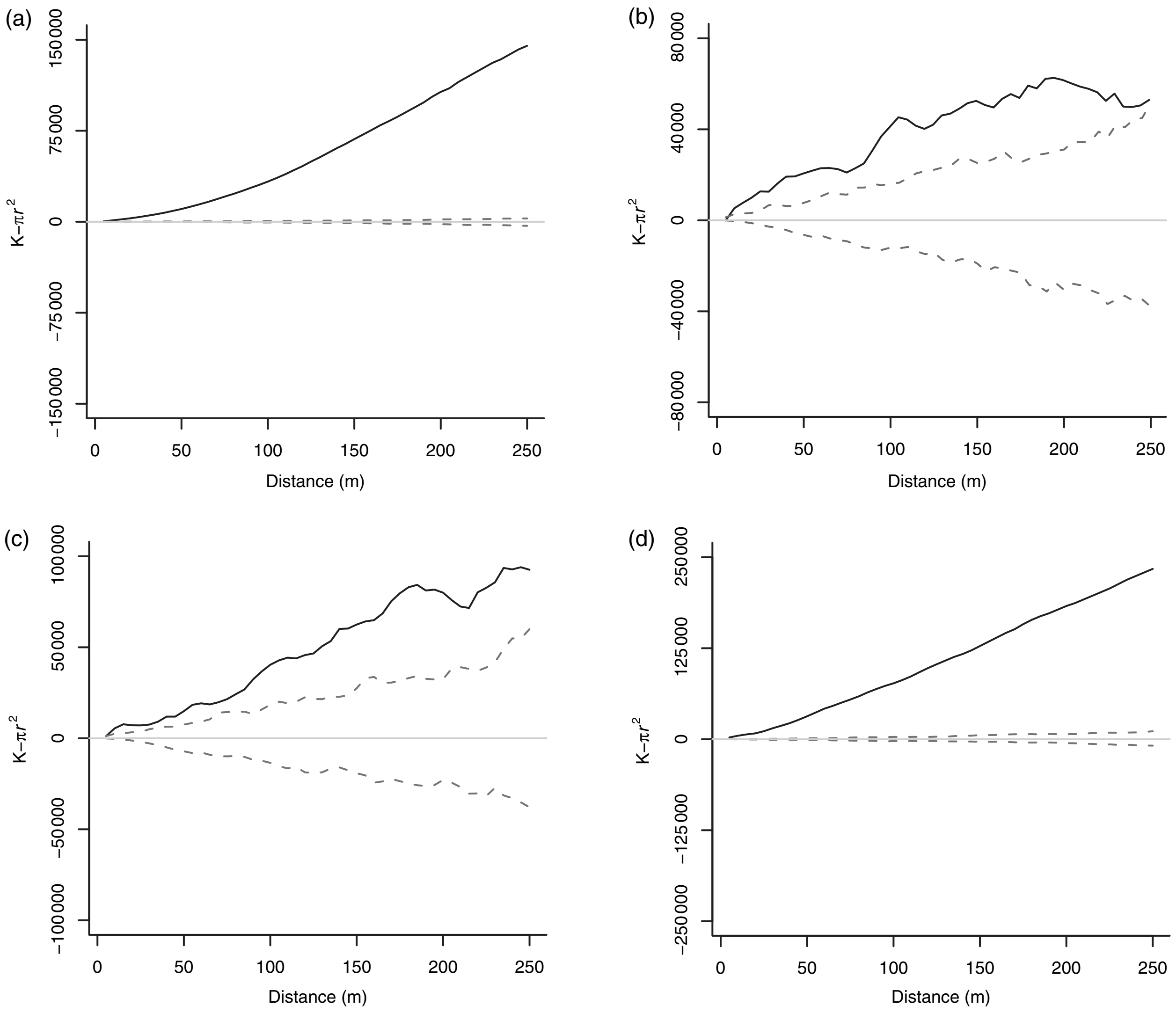

Figure 2 Ripley's $K - \pi r^2$ used to test for spatial aggregation of *S. amara*. Observed values (solid line) outside the envelope (500 simulations, dashed line) > 0 indicate aggregation, whereas values < 0 demonstrate a regular distribution for (a) the 1230 *S. amara* within the FDP; (b) reproductive females ($n = 74$); (c) reproductive males ($n = 61$) and (d) seedlings ($n = 782$) (dashed line represents the Poisson distribution).

We assigned the pollen parent unambiguously (only one likely father) for 33 of the 94 seedlings with a single mother. For these full parentage seedlings, the mean distance between parent pairs, and thus, the average distance for genetically measured direct pollen movement, was 334.4 ± 231.0 m (SD) (range 8.0–1063.2 m, Fig. 4, solid bars). In contrast, the mean distance between nearest male–female pairs was 54.1 ± 35.6 m (SD) (range 2.3–145.6 m). Thus, seedlings were seldom the result of pollination between nearby reproductive trees of the opposite sex. The average distance between fathers and seedlings for parent-pairs was 326.3 m, slightly less than the 349.7 m distance between mothers and seedlings for the two-parent seedlings.

Sib distances

Of the 94 seedlings matching a unique mother, we found that pairwise seedling distances for half and full sibs generally exceeded 100 m [median = 165.5 m, mean = 197.7 ± 165.5 m (SD), range = 0.81–801.6 m] and sib distances were significantly different ($P < 0.0001$, *t*-test) from nearest seedling distances across all seedlings [median nearest seedling distance = 6.2 m, mean = 11.3 ± 15.8 (SD), range = 0.34–132.8 m]. These sibs were the collective progeny of 30 female trees whose numbers of genotyped offspring ranged from 1 to 10 seedlings. Overall, the variance in the number of genotyped offspring produced by

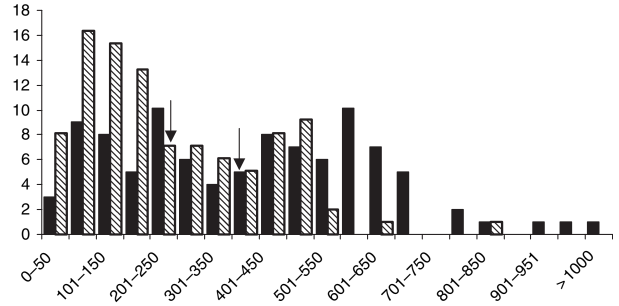

Figure 3 Frequency distribution (per cent total) of *Simarouba amara* seedlings at each distance class (m) to maternal parent for seedlings with unique maternal assignment (solid bars) and seedlings with two to three potential mothers; seedling assigned to nearest of possible mothers (hashed bars). Arrows indicate mean distances (m).

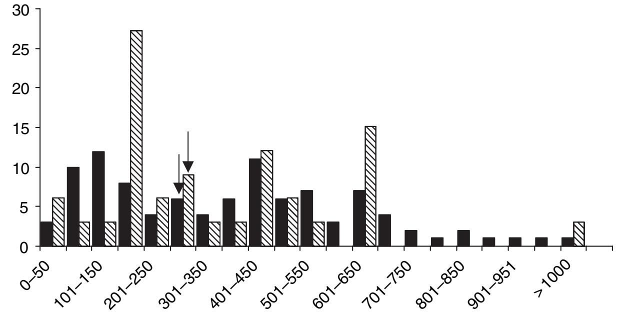

Figure 4 Frequency distribution (per cent total) of *Simarouba amara* pollen movement events at each distance class (m) from paternal parent to seedlings (hashed bars) and direct pollen movement (paternal to maternal parent) (solid bars). Arrows indicate mean distances (m).

females was 8.0, while the variance for male reproductive success was nearly threefold larger, 23.9.

DISCUSSION

Seed dispersal impacts both the local genetic structure and the maintenance of genetic diversity within and among plant populations. Animal-generated seed shadows can affect local population genetic structure (Hamrick *et al.* 1993), which will subsequently influence gene movement and recruitment patterns (Jordano & Godoy 2002). As pollen is haploid and seeds are diploid, if all else is equal, seeds contribute to two-thirds of the genetic neighbourhood size, and variance in seed movement contributes twice as much as the variance in pollen movement (Hamilton 1999). Molecular techniques provide an opportunity to measure the elusive 'tail' of the dispersal curve (Clark *et al.* 1999, Ouborg *et al.* 1999) and to improve our estimates of dispersal and recruitment distances.

Our findings suggest that Janzen–Connell effects influence the distribution of *S. amara* seedlings in the BCI population. Seeds and seedlings may be less likely to survive under parent trees because of high predation or pathogens; in spite of an abundance of immature and ripe fruits being deposited beneath reproductive females (BDH, unpubl. data). Thus, even though it may appear that the majority of seeds are dropped beneath a reproductive tree, one should be cautious about assigning those seeds or seedlings to nearby adults. Our genetic data clearly demonstrate that seldom are germinated seedlings produced by the nearest or even nearby reproductive adults.

Where seeds are deposited is an important determinant in seedling establishment and survival. Dispersal away from both adult and seedling conspecifics may increase post-dispersal survivorship, particularly if strong density- and distance-dependent mortality is present (Harms *et al.* 2000). It is worth noting that in *S. amara*, the average distance of seedlings to the nearest conspecific adult in the FDP was only 39.0 m. Given the long distances at which seeds regularly established away from parent plants, it seems that seeds arriving some distance away from parents may be more likely to recruit, regardless of the proximity of nearby non-parent adults. Similar results for seed arrival were found by Jordano & Godoy (2002) in the Mediterranean tree, *Prunus mahaleb*. Microsatellite markers revealed that many seeds are deposited beneath the canopy of conspecific adults other than the maternal parent as dispersers move from tree to tree. These results and ours suggest that the causal mechanisms behind the observed seed deposition and seedling recruitment patterns are more complex than simple non-genetic distance- and density-related Janzen–Connell effects would predict.

The spatial aggregation of seedlings and long-distance dispersal away from parent plants demonstrated by this study also may reflect disperser movement patterns. Primate dispersers of *S. amara* are known to deposit seeds in clumps and to maintain large home ranges which may exceed the total sample area of this study (Milton 1980; Russo & Augspurger 2004). Perhaps offspring experience higher survivorship away from their parents as Augspurger (1983) showed with *Platypodium elegans*, which may help promote or maintain genetic diversity (Schmitt & Antonovics 1986). Our results from Ripley's *K* analysis also provide an independent estimate of dispersal. The non-random spatial pattern of *S. amara* is undoubtedly the result of the combined effects of both dispersal and mortality.

Because we were interested in the role of dispersers in successful recruitment events at long distances from the parents, we ensured sampling for seedlings in all areas ≥ 100 m from any potential parent tree. Although we were initially concerned this could skew our results, we found only 44 seedlings with a > 100 m minimum distance to the nearest possible mother for which we ascertained maternal matches; this accounts for only 14% of the total seedlings sampled and 1 ha of sample area. Removing these 44 seedlings from analysis did not significantly alter our findings; the mean distance between nearest seedling pairs is nominally reduced (to 11.1 m) and seedlings remain aggregated.

One might expect that reproductive adults located near high-density seedling areas would be overrepresented as parents because of their proximity to nearby seedlings. The frequency of long-distance dispersal and the relative infrequency of assigning as parents the reproductive adults closest to matched seedlings suggested this was quantitatively unimportant. Also, *a priori* one might expect that seedlings near the edge of the plot would be more likely to have maternal parents predominately located among adults outside of the 100 m buffer zone that were not genotyped. However, we found no evidence to indicate that seedlings in any particular area of the FDP were more or less likely to have assigned parents.

We chose a conservative approach for maternal assignment in cases of seedlings with multiple potential mothers, by assigning seedlings to the nearest of the genetically possible mothers. In doing so, we almost certainly underestimate recruitment distances, an assertion consistent with our finding that dispersal distances for seedlings with single mothers were notably longer compared with distances of seedlings with ≥ 1 possible mother. Notwithstanding our conservative assignment of seedlings to adult females, few seedlings belonged to the nearest reproductive female tree – 10% for unambiguously assigned maternal parents; 16% across all seedlings. Despite this, our average seedling establishment distance exceeded 390 m, more than 10-fold greater than the 39 m mean seed dispersal distance estimated for *S. amara* using inverse modelling (Muller-Landau 2001).

Inverse models that focus on seed arrival rather than successful seedling establishment (*sensu* Clark *et al.* 1999, Muller-Landau 2001) did not account well for our findings of seedling establishment distances for a vertebrate-dispersed tree. However, inverse models fit well to genetic data of seed arrival for the wind-dispersed tree *Jacaranda copaia* on BCI (Jones *et al.* 2005), although the best-fitting model differed among years and the authors state there was 'considerable uncertainty in the tail' of their dispersal kernels. Even maximum likelihood recruitment models (LePage *et al.* 2000; Uriarte *et al.* 2005) likely underestimate true seedling recruitment distances, and may ignore long-distance dispersal altogether (Ribbens *et al.* 1994). Although seed dispersal models may not be a good proxy for seedling recruitment distances, it is possible to reconcile the contrasting findings if distance- and density-dependent mortality of seeds is quite strong near parent plants.

Our results also indicate that insect pollinators are moving hundreds of metres and perhaps several kilometres. While it is certainly possible that nearby parent-pairs produced seeds and resulted in germinated seedlings, in our analyses we were unable to discern any such pairings, perhaps because we did not exhaustively map, genotype, and match every seedling in the FDP. We found only three instances of parent-pair distances < 100 m, although the average distance between nearest possible parent pairs was 54 m. The significant spatial autocorrelation observed for *S. amara* adults at distances to 40 m (Hardesty *et al.* 2005) may help to explain this pattern if pollination between neighbouring related adults results in aborted (inbred) embryos or leads to rapid demographic thinning. Alternately, it is possible that there was no phenological overlap among neighbouring adults. Data from 16 years of seed traps suggests this is unlikely as *S. amara* experiences a long flowering period and neighbouring trees are likely to have phenological overlap for at least a portion of the flowering duration (S. J. Wright and O. Calderon, unpubl. data).

Few studies have estimated both pollen and seed dispersal directly for vertebrate-dispersed tropical trees (Table 2). Although Aldrich & Hamrick (1998) reported plant recruitment distances and Sezen *et al.* (2005) reported pollen movement and recruitment distances, each focused on a species within a disturbed landscape, either fragmented or second-growth forest. In addition, these two studies took place at spatial scales of 30 and 38.5 ha, less than half of the area we sampled for adults. Furthermore, the authors were unable to distinguish between male and female parent trees. Whereas Aldrich & Hamrick (1998) and Sezen *et al.* (2005) reported reproductive dominance by few individuals in their study sites, we found that 54% of the reproductive females and 34% of the known males contributed progeny in this study. Using microsatellite markers, Hufford (2000) found that > 75% of possible fathers sired progeny for *Platypodium elegans* on the BCI FDP. The difference in genetic dominance in cases of altered landscape compared with intact forest may have a large long-term effect on genetic diversity and species persistence through time. Although where forests are fragmented genetic variability may be maintained by long-distance pollen flow in tropical trees such as *Dinizia excelsa* (e.g. Dick 2001), gene movement via seed appears equally important in *S. amara*, at least in the intact forest where this study took place.

Godoy & Jordano (2001) suggest that 'distance limited spatial aggregation of seed shadow' is typical of fleshy-fruited animal-dispersed plant species. However, we found successful long-distance recruitment to be common, at least at the scale of several hundreds of metres. Although they report on seed arrival distances and we evaluated seedling

Table 2 Some published seed dispersal and recruitment studies of tropical trees and palms employing molecular tools. Reference, mode of seed dispersal, growth form, habitat, life stage, molecular marker employed, dispersal/recruitment distances, and pollen movement distances provided

Study	Dispersal mode	Growth form	Habitat type	Life stage studied	Marker used	Dispersal/recruitment distances (m)	Pollen distances
Aldrich & Hamrick (1998)	Vertebrate	Tree	Fragmented forest	Seedlings/saplings	Microsatellites	'Hundreds of metres' actual distances not reported	Not reported
Jones *et al.* (2005)	Wind	Tree	Intact forest	Seeds	Microsatellites	Range 2.7–421.8 m, mean 40.1 ± 65.9 (2000); range 2.9–710.5 m, mean 58.8 ± 101.3 (2002)	Not reported
Sezen *et al.* (2005)	Vertebrate	Palm	Second-growth forest	'Founder generation' (breeding adults)	AFLP	Median 270 m, max 875 m	Median 100 m, max < 220 m
This study	Vertebrate	Tree	Intact forest	Seedlings	Microsatellites	Range 3.6–1000.5 m, mean 391.6 m ± 234.6 m (see Appendix S1)	Range 8.0–1063.2 m, mean 334.4 m ± 231.0 m

recruitment from maternal parents, we find a remarkably similar proportion of immigrant seed rain, 18% and 17%, respectively. We detected dispersal distances comparable to, although longer than, recruitment distances reported by Sezen *et al.* (2005) with the tropical palm *Iriartea deltoidea*. In contrast to their findings that pollen moved considerably shorter distances between maternal trees and established founders, pollen and seed moved similar distances for *S. amara* in Panama.

There is no reason to expect that the pattern observed in *S. amara* is unique to this species. *Simarouba amara* is pollinated by numerous generalist insect species, and several species of frugivores are known to disperse its seeds. It seems likely that as genetic studies on dispersal of other frugivore-dispersed tropical trees are conducted, similar patterns of frequent long-distance seed arrival and seedling survival will emerge. The seedling recruitment distribution we observed is more similar to patterns generated by the seed dispersal models of Holbrook & Smith (2000) – which incorporate animal movement rates and gut passage times of seeds for large frugivorous birds – than it is to the patterns generated by inverse models using *S. amara* adults and seed trap data within the FDP. We urge researchers to use caution in presuming nearby reproductive adults are the parent plants, especially in the absence of genetic confirmation. If our results depict a general pattern, such an assumption will lead to a considerable underestimate of both seed dispersal and seedling recruitment distances.

In summary, we found that gene flow via seed and pollen was of a similar magnitude, in contrast with other studies of tropical trees, which generally attribute gene movement to long-distance pollen flow (Aldrich & Hamrick 1998; Nason *et al.* 1996; Hamrick & Loveless 1989; Konuma *et al.* 2000; Latouche-Hallé *et al.* 2003). Furthermore, our estimates of seed and pollen movement are conservative owing to the possible false assignment of an offspring to a parent whose true parent may reside at some distance outside the study site (Devlin & Ellstrand 1990). Seedling establishment in the FDP was frequent near non-parent trees rather than adjacent to maternal parents, and seedling establishment 500 m or more from the maternal parent regularly occurred, providing compelling evidence that long-distance dispersal and subsequent seedling establishment is not a rare event. Whereas previous studies have focused on seed arrival in traps or have coupled radio-tracking of frugivore dispersers with seed passage trials to estimate dispersal distances, we can now be more precise and garner exact data on seed arrival (*sensu* Jordano & Godoy 2002; Jones *et al.* 2005) and seedling establishment distances from parent trees using molecular techniques. Our findings of frequent long-distance dispersal and subsequent recruitment provide strong evidence that vertebrate dispersers are indeed playing a critical role by actively dispersing seeds of *S. amara*.

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SUPPLEMENTARY MATERIAL

The following supplementary material is available online for this article from http://www.Blackwell-Synergy.com:

Appendix S1 Minimum, 10th, 25th, 50th, 75th, 90th percentile, and maximum dispersal distances for seedlings with 1–4+ potential maternal parents, and results from all genotyped seedlings (pooled).

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