

Genetic rescue of remnant tropical trees by an alien pollinator

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Habitat fragmentation is thought to lower the viability of tropical trees by disrupting their mutualisms with native pollinators. However, in this study, *Dinizia excelsa* (Fabaceae), a canopy-emergent tree, was found to thrive in Amazonian pastures and forest fragments even in the absence of native pollinators. Canopy observations indicated that African honeybees (*Apis mellifera scutellata*) were the predominant floral visitors in fragmented habitats and replaced native insects in isolated pasture trees. Trees in habitat fragments produced, on average, over three times as many seeds as trees in continuous forest, and microsatellite assays of seed arrays showed that genetic diversity was maintained across habitats. A paternity analysis further revealed gene flow over as much as 3.2 km of pasture, the most distant pollination precisely recorded for any plant species. Usually considered only as dangerous exotics, African honeybees have become important pollinators in degraded tropical forests, and may alter the genetic structure of remnant populations through frequent long-distance gene flow.

Keywords: *Apis mellifera*; Amazonia; habitat fragmentation; microsatellite; *Dinizia excelsa*; paternity inference

1. INTRODUCTION

Lowland tropical rainforests are renowned for their species richness and ecological complexity. Woody plants, the main structural elements of tropical forests, occur in assemblages of 280 species in a single hectare (Phillips *et al.* 1994; De Oliveira & Mori 1999) and upwards of 1000 species in 25 ha of pristine forest (Bermingham & Dick 2001). Concomitant with high alpha diversity, tropical trees occur in low population densities, typically less than one individual per hectare (Hubbell & Foster 1983). Despite low population densities, self-fertilization is rare in natural settings. In most tropical trees, outcrossing is enforced either through self-incompatibility (Bawa *et al.* 1985a), dioecy or high genetic loads (Alvarez-Buylla *et al.* 1996). Animals, rather than wind, are the presumed pollen vectors for over 99% of lowland rainforest species (Bawa *et al.* 1985b). Animals also disperse the fruits and seeds of most tropical plants.

Human encroachment of tropical forests through logging and agriculture threatens to fragment the world's remaining rainforests (Laurance 1998). Forest fragmentation has two immediate effects on the breeding structure of rainforest plants. First, it increases the distances between potential mates. Second, it can alter the species composition, relative abundance and foraging patterns of pollinators. The disruption of pollinators can result in reduced fecundity of the host plants due to pollen limitation (e.g. Levin 1995; Ghazoul *et al.* 1998) and genetic erosion through drift (Young *et al.* 1996) if plants in forest patches become reproductively isolated.

Large trees left standing in tropical pastures represent an extreme case of ecological deprivation. They may be isolated from forest-dwelling pollinators and seed dispersers, or surrounded by habitat that is unsuitable for seed-

ling establishment. Pasture trees have been described as the 'living dead' (Janzen 1986), persisting by virtue of their longevity but contributing little to forest regeneration and therefore meriting minimal conservation attention. Nevertheless, several genetic studies have recorded pollen flow to pasture trees separated by hundreds of metres (Chase *et al.* 1996; Aldrich & Hamrick 1998), indicating that spatially isolated trees may not necessarily be reproductively isolated.

I examined the viability of remnant populations of *Dinizia excelsa* (Fabaceae), a prominent Amazonian tree, in light of the 'living dead' hypothesis. *D. excelsa* is a model species for studies of pollinator disruption because its generalist bee-pollination system is shared by ca. 30% of lowland rainforest species (Bawa *et al.* 1985b). Data were obtained on the pollination and reproductive performance of trees in pasture, forest fragments and pristine habitats. Microsatellite markers were then used to quantify outcrossing and gene flow and to map precisely the breeding structure of trees in a fragmented population.

2. METHODS

(a) *Study species and site*

D. excelsa is one of the largest Amazonian trees, attaining 60 m in height and 2 m in girth (Ducke 1922); it is one of the region's most important timber species (Barbosa 1990). The species is endemic to Brazil, and occurs at natural densities of around one adult tree per 6 ha (Dick 2001). In the process of clearing forest to create pasture, *D. excelsa* are often left standing because of their value for timber and shade. The pale yellow-green flowers are small (calyx, 1–1.5 mm), hermaphroditic and borne on racemes. Small insects are attracted to their nectar and fragrance. Dormant seeds (ca. 1 cm) are borne in indehiscent pods and dispersed primarily by wind and gravity. The trees in this study were located in the reserve system of the Biological Dynamics of Forest Fragments Project (Lovejoy & Bierregaard 1990), located ca. 90 km north of Manaus, Brazil

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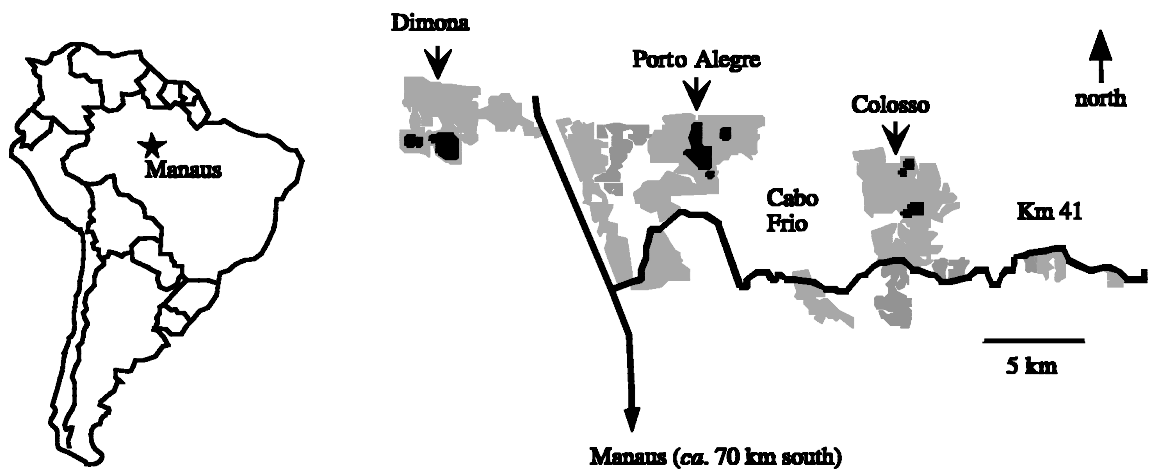


Figure 1. Reserves of the Biological Dynamics of Forest Fragments Project ($2^{\circ}30' S$, $60^{\circ} W$). *Dinizia excelsa* (≥ 40 cm diameter at breast height) were mapped in pasture (shaded areas) and forest fragments (black areas) in Dimona, Porto Alegre and Colosso ranches, and in undisturbed forest at Cabo Frio, Km 41 (unshaded) and Reserva Ducke (not shown, located ca. 70 km southeast of the Biological Dynamics of Forest Fragments Project reserves).



Figure 2. The author climbing pasture tree 'Col.06' for canopy observations (see § 2b and figure 4).

(figure 1). Trees were mapped in three habitat types: 15–20-year-old pasture of Colosso ranch, forest fragments in Colosso, and adjacent continuous forest west (Cabo Frio) and east (Km 41) of Colosso. Field studies were performed in 1995, 1996 and 1999.

(b) Canopy studies

Rope-climbing techniques (Laman 1994; Smith & Padgett 1996) were used to reach the canopy, at an average height of

Table 1. Microsatellite loci used in this study.

(The repeat motif is of the cloned sequence. The allele numbers shown are from 240 seed and tree genotypes from Colosso ranch.)

locus	repeat sequence	number of alleles
DE27	(AAG)8	4
DE37	(AC)20	12
DE44	(GT)13	7
DE48	(GA)27	34
DE54	(CT)39	23

25 m (figure 2). Flower-visiting insects were observed and collected on seven large densely flowering trees to assess changes in pollination. In addition, all mapped trees were observed from the ground with $\times 10$ binoculars during the course of phenological observations. The climbed trees were located in the following habitats: an isolated 10 ha reserve in Colosso ranch ($n = 2$), isolated 100 ha reserves in Porto Alegre and Dimona ($n = 2$), Colosso pasture ($n = 1$) and an undisturbed forest at site Km 41 ($n = 2$). The intensively studied tree 'Col.06' was separated from its nearest neighbour by over 600 m of open pasture (figure 4). Continuous-forest trees ('41.01' and '41.13') were situated 700 m apart.

Over 52 h were spent (on 17 visit days) in canopy observation during the mornings (06.00–12.00), when insect visitation was at its peak. During the afternoon and evening periods, 10 h were spent in observation (on 4 days). Observations were divided between trees in pristine forest (27 h) and trees in disturbed habitats (26.5 h). The putative pollinators were captured with nets, mounted and identified by specialists (Dick 2001).

(c) Reproductive performance

Fecundity and regeneration were used as indicators of reproductive performance. Fecundity was calculated as the total number of pods and seeds produced per tree. The pods were counted from the ground with binoculars in Cabo Frio and Km 41 and in the pastures, gallery forests and 10 ha reserve at Colosso. Juvenile trees were excluded from the analyses. Colosso pasture and fragment populations were analysed separately, and

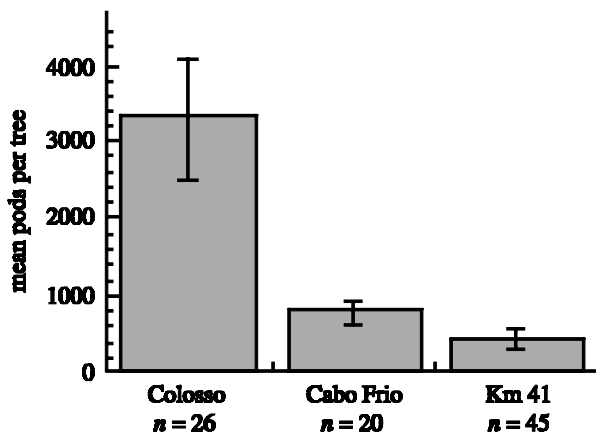


Figure 3. Mean pod set of remnant trees in Colosso ranch and adjacent forest (Cabo Frio and Km 41).

then combined for comparison with undisturbed forest. Seeds were counted from 2177 fallen pods from trees in Colosso ($n = 25$ trees), Km 41 ($n = 45$) and Cabo Frio ($n = 23$) to obtain estimates of mean seed set per pod. Seedling regeneration was surveyed and mapped in secondary vegetation throughout Colosso ranch.

(d) Genetic analyses

Leaf tissue was collected from all of the mapped adult trees in Colosso ($n = 36$). DNA was extracted from adult leaves and from 11 seed families (25–55 seeds per family) collected below maternal trees located in the different habitat types. Genotypes were obtained for five microsatellite loci using primers developed for *D. excelsa* (Dick & Hamilton 1999). A total of 80 alleles in the seed and adult populations provided an average exclusion probability of greater than 99% (table 1). The outcrossing rate (t) for each maternal tree was calculated as the proportion of its seed array that contained non-maternal alleles. The minimum number of sires for each seed family was calculated by dividing the number of paternal alleles for the most variable locus by two, since the species is diploid. Seed paternity was assigned on the basis of multilocus segregation probabilities using the program CERVUS (Marshall *et al.* 1998).

3. RESULTS

(a) Pollinator shifts

Only native insects were observed on the densely flowering trees in continuous forest. These included stingless bees (tribe Meliponini), predatory wasps and small beetles (2–5 mm in length) (Dick 2001). Stingless bees (14 species, 12 genera) were judged to be the primary outcross pollinators of these trees, as they were the only insects observed foraging among branches and flying away from the trees. More than 10 species of small beetles (from eight families) were observed in the floral cups of *D. excelsa*. These beetles were not observed moving beyond individual racemes, but may be a vector for self-pollination.

Although the species composition and the abundance of native insects were similar in continuous forest, 10 ha and 100 ha forest fragments, African honeybees occurred only in disturbed habitats, where they greatly outnumbered all native insects (Dick 2001). Honeybees were virtually the only pollinating insects on the pasture tree Col.06, which was devoid of beetles and visited rarely by

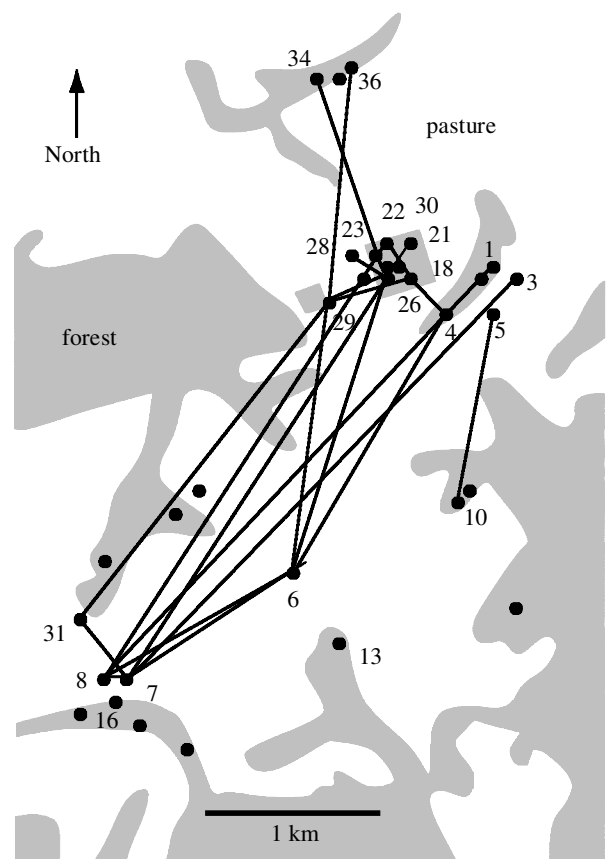


Figure 4. Map of Colosso ranch showing the mating patterns quantified in table 3. Pasture and secondary vegetation is shown in white; the shaded areas represent primary forest. The 10 ha fragment harbours trees 18–26 and 30. *Dinizia excelsa* seedlings were mapped near trees 03, 06, 07, 08, 11, 13, 27 and 29.

native bees. African honeybees outnumbered the native bees on the pasture tree by thousands. No behavioural interactions were observed between African honeybees and native insects on any of the trees. The co-occurrence of native and exotic bees on flowers in the forest isolates suggests that African bees did not displace native insects from pasture trees. Rather, the long distance of open pasture probably inhibits foraging by native bees, which tend to nest in standing forest (Roubik 1989; Aizen & Feinsinger 1994).

(b) Reproductive performance

The trees in pasture and forest fragments ($n = 26$) produced more than three times as many pods per tree as did trees from the adjacent continuous-forest populations ($n = 20$ and $n = 45$; Welsch test, $p < 0.01$; figure 3). No significant difference in fecundity was observed between trees in pasture ($n = 10$) and trees in forest fragments ($n = 16$) ($p > 0.05$). Senescent pasture trees increased the variance of the Colosso population. The mean seed set of *ca.* three seeds per pod across habitats indicates that pods are an accurate proxy for seed set. The mean seed set at Colosso was *ca.* 10 000 seeds per tree, with the most isolated pasture tree, Col.06, at the mean.

Although *D. excelsa* regeneration was rarely observed in undisturbed forest, seedlings were abundant in abandoned pastures near remnant trees, with stem diameters

Table 2. Genetic diversity of seed arrays.

(t , multilocus outcrossing rate (see §2d). The numbers below each locus indicate the number of unique paternal alleles in the seed array, which are summed across loci under 'total alleles'. The bottom row describes the allelic diversity of Colosso adult genotypes. Asterisks indicate samples of fewer than 10 seeds. Seed arrays are from the 1995 flowering, except for Col.06'93' and Col.07'93' from 1993. Canopy observations were made on the trees indicated in *italic*.)

maternal tree	habitat	number of seeds	t	locus					total alleles	minimum number of sires
				DE27	DE37	DE44	DE48	DE54		
<i>Col.06'95'</i>	pasture	35	0.84	1	5	3	7	11	27	6
<i>Col.06'93'</i>	pasture	20	0.80	1	3	4	3	5	16	3
<i>Col.07'95'</i>	pasture	25	0.80	1	4	1	5	5	16	3
<i>Col.07'93'</i>	pasture	25	0.87	1	4	3	9	8	25	5
Col.08	pasture	25	0.96	1	2	3	6	4*	16	3
Col.29	pasture	25	0.80	0	4	3	7	1*	15	4
Col.13	gallery	25	0.92	3	4	3	12	11	33	6
<i>Col.18</i>	10 ha	25	1.00	2	4	1	9	8	24	5
<i>Col.26</i>	10 ha	25	0.96	2	5	4	14	12	37	7
Duk.10	forest	28	0.91	0	3	3	8	11	25	6
Duk.32	forest	25	0.96	1	4	3	12	8	28	6
<i>Km 41.13</i>	forest	25	0.90	1	4	3	8	9	25	5
CF.26	forest	25	1.00	1	4	0	7	5	17	4
Colosso adults ($n = 36$)				3	6	6	21	18	54	n/a

Table 3. Paternity assignments (see also figure 4).

(Maternal tree, single non-excluded male ('sire'), number of identical parentage events ('seeds'), distance between parents and the exclusion probability given the maternal and seed genotypes. In the case of more than one event, the range of exclusion probabilities is shown. Exclusion probabilities greater than 0.995 are rounded to 1.0. Canopy observations were performed on the mother trees indicated in *italic*.)

maternal tree	sire	seeds	distance between parents (m)	exclusion probability
<i>Col.06</i>	Col.07	1	1053	0.98
<i>Col.06</i>	Col.08	1	1181	1.0
<i>Col.06</i>	Col.04	1	1600	0.99
<i>Col.06</i>	Col.26	2	1500	0.98–1.0
<i>Col.06</i>	Col.29	1	1600	0.98
Col.07	Col.08	7	128	0.99–1.0
Col.07	Col.31	6	400	0.97–0.99
Col.07	Col.06	1	1053	0.99
Col.07	Col.21	1	2800	1.0
Col.07	Col.03	5	3200	0.92–1.0
Col.08	Col.07	4	128	0.98–0.99
Col.08	Col.22	1	2900	1.0
Col.08	Col.01	3	3000	0.98–0.99
Col.10	Col.03	2	400	1.0
<i>Col.18</i>	Col.29	2	500	1.0
<i>Col.18</i>	Col.22	2	250	1.0
<i>Col.18</i>	Col.04	1	300	1.0
<i>Col.18</i>	Col.30	1	270	1.0
<i>Col.18</i>	Col.01	1	400	0.99
<i>Col.26</i>	Col.34	1	1100	0.95
<i>Col.26</i>	Col.28	1	300	1.0
<i>Col.26</i>	Col.36	1	1100	1.0
Col.29	Col.03	1	1000	1.0
Col.29	Col.21	1	500	0.88
Col.29	Col.31	4	2200	0.97–1.0
Col.29	Col.36	2	1200	0.92–0.97
Col.29	Col.23	1	450	0.97

ranging from 1 mm to over 6.5 cm and with heights of up to 8 m. Over 30 seedlings were mapped in 2 year old secondary vegetation between the 1 ha and 10 ha fragments in Colosso. Over 40 saplings were mapped on the forest edges near Col.13, Col.18 and Col.16. Dozens of seedlings were found near Col.03, Col.06, Col.07 and Col.08. These observations indicate that demographic growth is possible in the disturbed habitats.

(c) Genetic results

Outcrossing rates were high (*ca.* 95%) and did not differ between trees in forest fragments and trees in continuous forest ($p > 0.05$, $n = 7$). However, pasture trees experienced 10% higher rates of self-fertilization (t -test, $p < 0.01$, $n = 6$; table 2). Increased selfing has been observed for other canopy species in pasture (Aldrich & Hamrick 1998) and selectively logged forest (Murawski *et al.* 1994).

Gene flow within the fragmented population was extensive. Paternity inference of seed genotypes from seven maternal trees in Colosso yielded paternity assignments with high exclusion probabilities for 77 out of 240 seeds (table 3), indicating that about two-thirds of the seeds were sired by unmapped trees in the surrounding forest. Out of the 77 inferred pollinations, 22 resulted from self-fertilization. Out of the 55 cross-fertilized seeds, 26 resulted from reproduction between trees separated by 1 km or more of pasture (range, 128–3200 m) (table 3 and figure 4). The mean pollination distance for the Colosso pasture and gallery forest trees, 1288 m ($n = 45$ seeds), was much greater than the mean distance to nearest neighbour (DNN) of 235 m ($p < 0.001$, $n = 18$ trees). The mean pollination distance in the 10 ha fragment, 417 m ($n = 10$ seeds), also greatly exceeded the mean DNN of 50.5 m ($n = 12$ trees). Phenological observations confirmed that nearest neighbours flowered in synchrony and, thus, were potential mates.

The high genetic diversity of seeds was maintained across habitats, and even isolated trees sampled most of

the local adult alleles in their seed arrays (table 2). For example, the '1995' seeds from pasture tree Col.06 ($n = 35$) had at least six sires. This tree was separated from its two nearest neighbours by 600 m and 1300 m. In contrast, forest tree Km 41.13, with at least five sires for its 25 sampled seeds, was surrounded by 18 potential mates within a 600 m radius. The seed array from Col.13 ($n = 25$) contained 11 out of 18 alleles found in the adult population for locus DE48.

4. DISCUSSION

Honeybees have a long and global association with humans. However, their appearance in the neotropics is relatively recent. African honeybees (*A. mellifera scutellata*) escaped from research apiaries in southern Brazil in 1956 and spread rapidly, hybridizing with European honeybees while retaining behavioural and morphological traits of the African race. There were no *Apis* colonies in the Amazon basin prior to the invasion of African honeybees in the 1970s (Roubik 1989). Now there are an estimated 50–100 million African honeybee colonies throughout the neotropics (Winston 1992). Globally, feral honeybees visit roughly one-third of the plant species in local floras, but their effect on the genetic structure of native plants is largely unknown (Butz-Huryn 1997). Honeybees are expected to follow an optimal foraging strategy; in rare flights between densely flowering trees they are expected to visit only nearest neighbours (Butz-Huryn 1997; T. Seeley, personal communication).

The genetic data presented here suggest that inter-tree foraging is not necessarily rare or limited to nearest flowering neighbours. For example, pasture tree Col.06 was visited almost exclusively by honeybees and set *ca.* 8000 outcrossed seeds. The nearest flowering neighbours, located 600 m (Col.13) and 1000 m (Col.10) away, did not fertilize any of the seeds assayed ($n = 55$), although Col.06 did receive pollen from a stand of densely flowering trees in the 10 ha fragment (Col.26) (figure 4). Similar patterns of pollen flow have been reported among pasture trees in Costa Rica (Chase *et al.* 1996), although the insect pollinators in that study were not known. The gene flow between Col.07 and Col.03 represents the most distant pollination precisely measured in any plant species (Chase *et al.* 1996).

African honeybees may increase the neighbourhood area of fragmented *D. excelsa* above natural levels, since they have a vast foraging area (212 km²) compared with native stingless bees (12.5 km²) (Roubik 1989). Moreover, the large number of unassigned fathers in the Colosso progeny indicates that pollinator movements were not confined to the disturbed habitats. This result has practical conservation implications, since one guideline for reforestation is to avoid using seeds from isolated trees because of their presumed low genetic quality.

African honeybees can be considered to be at least a partial cause of the increased fecundity of trees in the degraded habitat. Fecundity is affected by a host of physical and biotic factors, such as competition for light and soil nutrients. Since the emergent canopy of *D. excelsa* is fully exposed to light in all habitats, the most important physical factor should be nutrient release. However, there was no significant difference in fecundity between popula-

tions in the 10 ha forest fragment and those in open pasture. African honeybees, which were generally abundant in disturbed sites, have been shown to increase seed set above natural levels in other tropical plant species (Aizen & Feinsinger 1994; Roubik 2001). Moreover, increased seed production may contribute to the demographic growth of *D. excelsa* in degraded habitats, since it does regenerate in pasture.

In conjunction with their new pollinators, isolated trees perform two conservation roles. First, they act as stepping-stones for gene flow between fragmented populations, and thereby help to preserve genetic diversity within the species. This is consistent with genetic studies of isolated tropical trees with unknown pollinators (Chase *et al.* 1996; Nason & Hamrick 1997; White *et al.* 1999). Second, because of their high output of genetically diverse seeds, isolated trees serve as foci for forest regeneration in abandoned pasture. In this dynamic agricultural landscape, an alien pollinator has helped to bring *D. excelsa* back from the ranks of the living dead.

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