

Demographic spatial genetic structure of the Neotropical tree, *Jacaranda copaia*

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Abstract

We used genotypes from six microsatellite loci and demographic data from a large mapped forest plot to study changes in spatial genetic structure across demographic stages, from seed rain to seedlings, juveniles, and adult diameter classes in the Neotropical tree, *Jacaranda copaia*. In pairwise comparisons of genetic differentiation among demographic classes, only seedlings were significantly differentiated from the other diameter classes; F_{ST} values ranged from 0.006 to 0.009. Furthermore, only seedlings showed homozygote excess suggesting biparental inbreeding in the large diameter reproductive adults. We found very low levels of relatedness in the first distance class of trees, 1–26 cm diameter ($F_{ij} = 0.011$). However, there was a 5- to 10-fold rise in relatedness in the smallest distance class, from the smallest to the largest tree diameter classes ($F_{ij} = 0.110$ for individuals > 56 cm diameter). A variety of non-mutually exclusive mechanisms have been invoked perviously to explain such a pattern, including natural selection, history, or nonequilibrium population dynamics. The long-term demographic data available for this species allow us to evaluate these mechanisms. *Jacaranda* is a fast-growing, light-demanding species with low recruitment rates and high mortality rates in the smaller diameter classes. It successfully regenerates only in large light gaps, which occur infrequently and stochastically in space and time. These factors contribute to the nonequilibrium population dynamics and observed low genetic structure in the small size classes. We conclude that the pattern of spatial genetic transitions in *Jacaranda* is consistent with overlapping related generations and strong but infrequent periods of high recruitment, followed by long periods of population decline.

Keywords: Barro Colorado Island, Bignoniaceae, biparental inbreeding, nonequilibrium dynamics, seed rain, S_p statistic, wind dispersal

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Introduction

Plant populations can exhibit changes in their spatial genetic structure among different life stages and across generations. Such demographic transitions in genetic structure may provide insights into the relative roles that gene dispersal, mating system, drift, and natural selection play in shaping spatial and genetic patterns within natural plant populations (Hamrick *et al.* 1993; Tonsor *et al.* 1993; Alvarez Buylla *et al.* 1996; Jorde & Ryman 1996; Aldrich &

Hamrick 1998; Kalisz *et al.* 2001; Murren 2003). There can be multiple causes of such changes. Recent colonization events, limited seed and pollen dispersal, low density of maternal seed sources, and differential survival due to adaptation to local edaphic conditions or microbial symbionts, can act singly or in concert to create high levels of genetic structure within populations (Kittelson & Maron 2001; Jones *et al.* 2005). Conversely, widespread dispersal, high density of seed sources, a generalist life history, and random thinning of individuals are expected to give rise to lower levels of spatial genetic structure.

Evolutionary and ecological forces interact within populations to determine spatial genetic structure across life stages. The relatedness of proximate reproductive

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adults, adult density and fecundity, seed dispersal distances, and the species' mating system, collectively determine whatever genetic structure may be present in the earliest stage — the seed rain. Because the seed rain forms the template upon which subsequent spatial and genetic patterns of relatedness are shaped, we can use any spatial genetic structure observed in the seed rain and early seedling stages as a basis for evaluating genetic changes in the seed to seedling transition, and subsequent demographic stages (Alvarez Buylla *et al.* 1996). Changes in the observed spatial scale of structure and levels of relatedness between seed, seedling, juveniles, and adults that are incongruent with patterns generated by dispersal and random mortality and thinning (drift) in early establishment stages can be the consequence of nonequilibrium conditions, demographic history (i.e. founder effects or bottlenecks), or the operation of selection. Determining the relative role of stochastic and deterministic ecological and evolutionary forces in shaping population dynamics and evolution is a fundamental problem in ecology and evolution.

Studies that have examined transitions among different life stages in plants have revealed two diametrically opposed sets of results: an increase in spatial genetic structure and relatedness among neighbouring individuals from seedling to adult stages (Tonsor *et al.* 1993; Kalisz *et al.* 2001; Latouche-Halle *et al.* 2003), and decreases in structure and relatedness from juveniles to large reproductive adults (Hamrick *et al.* 1993; Epperson & Alvarez Buylla 1997; Chung *et al.* 2003; Asuka *et al.* 2004; Cruse-Sanders & Hamrick 2004; Kelly *et al.* 2004; Ng *et al.* 2004; Hardesty *et al.* 2005). Historical factors (i.e. bottlenecks or founder effects) and local adaptation due to microhabitat selection can lead to an increase in spatial genetic structure in cohorts of older, larger individuals relative to cohorts of younger, smaller individuals. In addition, increasing spatial genetic structure in older cohorts can arise under limited dispersal, and also if older and larger plants in a given neighbourhood represent several overlapping generations, more than do the younger plants. Limited dispersal near the parent plant followed by random thinning and ultimately low density of adults can explain the decay of genetic structure across size classes (Hamrick *et al.* 1993; Epperson & Alvarez Buylla 1997; Hardesty *et al.* 2005). Identifying the mechanisms responsible for observed spatial genetic structure within populations is challenging because it requires genetic data on all life history stages of the plant, as well as information on the demographic history of the population (changes in the population spatially through time).

Here, we analyse patterns of spatial genetic structure (SGS) based on six microsatellite loci across six life stages in the common pioneer tropical tree *Jacaranda copaia* (hereafter *Jacaranda*), within the 50 ha mapped forest dynamics plot (FDP) on Barro Colorado Island (BCI), Panama. We

examined: SGS present at four diameter classes in established trees; the maternal structure of the seed rain (maternal tissue genotyped from seeds caught within seed traps); and seedlings grown from seeds caught in seed traps. We demonstrate SGS in the seed rain and seedling stages that are congruent with the level of relatedness found in large, reproductive *Jacaranda* trees. However, this robust structure in the seed rain and seedlings disappears in the earliest juvenile stages of established trees. After the earliest stages, however, we then observe a large increase in relatedness among neighbouring individuals as diameter class increases. The long-term spatially explicit demographic data available for the BCI plot allows us to explore the influence of demographic and environmental processes in shaping these patterns. We therefore asked the following questions: (i) what are the levels and spatial scales of relatedness in *Jacaranda* across life stages, from seed rain, seedlings, and juveniles to reproductive adults? (ii) how does the strength of SGS change across life stages? (iii) what potential ecological and evolutionary mechanisms are responsible for the observed changes in SGS across different demographic classes? and (iv) how can knowledge of the demographic history of the population shed light on these changes?

Materials and methods

Study site

We conducted our study within the 50-ha forest dynamics plot (FDP) on BCI, Panama. Croat (1978) and Leigh *et al.* (1990) describe the flora, fauna, and climate of BCI. The BCI FDP was established in 1982 and consists of approximately 220 000 mapped stems ≥ 1 cm diameter at breast height (dbh) of approximately 310 species. The entire plot is recensused every five years (Hubbell & Foster 1986). For each tree, status is recorded (alive or dead), diameters at breast height (d.b.h. *c.* 1.3 cm) are measured, and the location, diameter, and species identity of all new recruits ≥ 1 cm d.b.h. are recorded. Prior to this study the most recent census was completed in 2000.

Study species

Jacaranda copaia (Bignoniaceae) is a large canopy tree (up to 45 m tall) and is a characteristic species of Neotropical moist forests ranging from Belize to Brazil and Bolivia (Croat 1978). The small wind-dispersed seeds (< 2 mg) are produced in large woody capsules in the canopy of adult trees (≥ 20 cm d.b.h.) and, on BCI, are dispersed August through November, with a peak in September (S. J. Wright, unpublished). *Jacaranda* is a light-demanding pioneer tree that requires large tree-fall gaps to regenerate (Brokaw 1985). Such large gaps are rare in the BCI forest.

In a separate study, we directly calculated the dispersal distances of seeds within the BCI population for the years 2000 and 2002 by genotyping maternal tissue from dispersed seeds caught in a large array of seed traps present in the plot (Jones *et al.* 2005). We found that seeds moved a mean of 40.11 m (SE \pm 4.30 m) in 2000, and 58.82 m (\pm 5.24 m) in 2002. Furthermore, we found clear evidence for a mixture distribution, having two separate kernels – presumably representing different dispersal processes – for seed dispersal near and far from the maternal parent tree.

Tissue sampling

We collected leaf tissue from the canopies of *Jacaranda* trees \geq 1 cm present on the FDP in 2000 and 2001. We mapped and collected tissue from an additional 95 trees \geq 20 cm d.b.h. from a 100 m wide strip surrounding the FDP (Jones *et al.* 2005; Fig. 1). We collected all *Jacaranda* seeds weekly from seed traps across the FDP in 2000. Two hundred of these seed traps are part of a long-term study of seed rain in the plot that has continued uninterrupted since 1986 (Wright *et al.* 1999, 2005; Harms *et al.* 2000; Dalling *et al.* 2002). However, because tree fall gaps were poorly sampled by this 200-trap network, we placed an additional 98 traps within new tree fall gaps of varying size across the FDP in 2000, with up to three seed traps placed within the largest gaps. Maternal tissue of seeds was removed, packaged and labelled individually, lyophilized, and stored on Drierite for transport to the University of Georgia (UGA). We randomly chose seeds for genotyping from collected samples (Jones *et al.* 2005). We genotyped all potential parents, and then performed an analysis to determine the distance that each genotyped seed moved from its maternal parent (Jones *et al.* 2005). After the maternal tissue was removed from seeds, we planted seeds and collected and genotyped the seedlings.

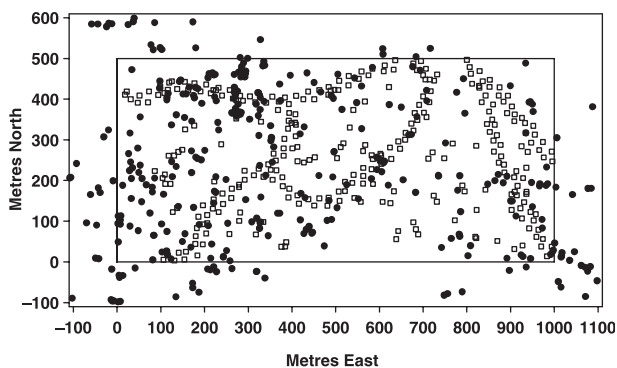


Fig. 1 A map showing the distribution of *Jacaranda copaia* individuals \geq 1 cm diameter at breast height (closed circles) and the location of 1/2 m² seed traps (open squares) within the 50 ha Forest Dynamics Plot on Barro Colorado Island, Panama.

DNA extraction and genotyping

Details of microsatellite library construction and development, primer sequences, genotyping reactions including PCR recipe, thermocycling conditions, and optimal primer annealing temperatures are in Jones & Hubbell (2003). DNA extraction and genotyping for adult and seed tissue are described in Jones *et al.* (2005). We used a modified CTAB extraction protocol for both adult and seed wing tissue (Doyle & Doyle 1990). We genotyped established *Jacaranda* individuals larger than 1 cm d.b.h. and seedlings grown from captured seeds at six microsatellite loci. We genotyped maternal tissue from 750 seed wings from seeds that were captured in 297 seed traps from across the plot (Fig. 1) at four loci (Jones *et al.* 2005). Individual reactions were multiloaded in a single 96-well plate and run on an ABI 3700 capillary electrophoresis automated sequencer, using ROX 400HD size standard (Applied Biosystems). We scored electropherograms using GENESCAN 3.1 and GENOTYPER 3.7 (Applied Biosystems).

Genetic diversity and spatial genetic structure

We analysed patterns of genetic diversity and spatial structure from established trees \geq 1 cm d.b.h. that are included in the main FDP census, and from maternal seed tissue removed from dispersed seeds caught in seeds traps, and seedlings grown from seeds captured in seed traps. We divided all established *Jacaranda copaia* trees into four equal-sample sized diameter classes based upon their d.b.h. in the 2000 census. We classified these as subadults 1–26.1 cm (hereafter, SUBA), mid-sized adults 26.3–42.5 cm (MID1) and 42.8–56.1 cm (MID2), and large adults 56.1–138.2 cm (BIGA) ($N = 86$ for each category). Although these diameter classes are arbitrary, the lower threshold for reproduction in the species is 20 cm d.b.h., and trees are not consistently reproductive until at least 30 cm d.b.h. (Wright *et al.* 2005). The smallest diameter class, SUBA, is therefore largely composed of nonreproductive individuals, and the three largest diameter classes MID1, MID2, and BIGA are composed of reproductive-sized individuals. We analysed seed maternal genotypes from the seed rain (hereafter, RAIN; $N = 750$) and seedling genotypes grown from seed captured within the traps (SEED; $N = 119$) using the coordinates of the seed trap where the seed was collected as the location of the genotype in the spatial analysis of genetic structure described below.

We used four loci in an analysis of spatial genetic structure in *Jacaranda* for seed rain, seedlings, and established plants within the 50-ha plot: JACC9, JACC21, JACC18, JACC31. For seedlings and established plants of all diameter classes, we also included genotypes from JACC 2 and JACC 22 (Jones & Hubbell 2003; Jones *et al.* 2005). We calculated genetic diversity statistics for each of the diameter

Table 1 Population genetic statistics for four diameter classes and seedlings germinated from seed rain from the population of *Jacaranda copaia* on the 50 ha Forest Dynamics Plot, Barro Colorado Island, Panama. Shown are the locus names, number of alleles per locus, number of genotyped individuals at each locus (*N*). *A*, alleles per locus; *H_O*, observed heterozygosity; *H_E*, expected heterozygosity; *F*, inbreeding coefficient.

Locus	11–26.1 cm d.b.h.					26.3–42.5 cm d.b.h.					42.8–56.1 cm d.b.h.					56.1–138.3 cm d.b.h.					Seedlings				
	<i>N</i>	<i>A</i>	<i>H_O</i>	<i>H_E</i>	<i>F</i>	<i>N</i>	<i>A</i>	<i>H_O</i>	<i>H_E</i>	<i>F</i>	<i>N</i>	<i>A</i>	<i>H_O</i>	<i>H_E</i>	<i>F</i>	<i>N</i>	<i>A</i>	<i>H_O</i>	<i>H_E</i>	<i>F</i>	<i>N</i>	<i>A</i>	<i>H_O</i>	<i>H_E</i>	<i>F</i>
JACC 2	79	19	0.90	0.87	-0.03	82	20	0.87	0.87	0.01	82	20	0.83	0.87	0.05	81	22	0.90	0.88	-0.03	107	19	0.75	0.87	0.15
JACC 9	85	10	0.81	0.84	0.04	86	11	0.90	0.85	-0.05	86	10	0.87	0.85	-0.02	85	10	0.86	0.86	0.00	117	9	0.81	0.82	0.01
JACC 18	84	14	0.86	0.86	0.00	85	13	0.85	0.86	0.02	86	12	0.88	0.85	-0.04	84	12	0.95	0.86	-0.10	117	12	0.82	0.86	0.04
JACC 21	85	12	0.69	0.68	-0.03	86	9	0.71	0.69	-0.03	86	10	0.63	0.64	0.01	86	8	0.80	0.67	-0.19	94	10	0.64	0.64	0.00
JACC 22	67	12	0.82	0.87	0.06	79	14	0.90	0.87	-0.03	76	14	0.80	0.85	0.06	76	14	0.83	0.87	0.05	111	15	0.74	0.89	0.17
JACC 31	79	9	0.87	0.82	-0.07	85	11	0.89	0.83	-0.08	86	9	0.81	0.81	-0.01	85	10	0.84	0.81	-0.03	115	11	0.77	0.80	0.04
Total	86	57	0.83	0.82	-0.01	86	58	0.85	0.83	-0.03	86	55	0.80	0.81	0.01	86	54	0.86	0.83	-0.05	119	57	0.75	0.81	0.07

classes of trees and seedlings using GENEPOP software (Table 1). We did not calculate these statistics for the RAIN category, because this represents genotyped maternal tissue from reproductive adults and not established plant genotypes. We calculated pairwise differentiation between individuals within different diameter classes using F_{ST} statistics. We conducted tests to evaluate the significance of differences in pairwise F_{ST} using an analysis of molecular variance (AMOVA) with 999 permutations and calculating 95% confidence intervals in the program GENALEX version 5.1 (Peakall & Smouse 2001), which follows the methods of Excoffier *et al.* (1992) and Michalakis & Excoffier (1996). We adjusted the significance level for multiple pairwise comparisons using a sequential Bonferroni correction (Rice 1989).

Analyses of spatial genetic structure

We performed the analyses of SGS on the genotypes and spatial coordinates of plants, seedlings, and seeds using the SPAGEDI program (Hardy & Vekemans 2002), which calculates the pairwise metric F_{ij} (Loiselle *et al.* 1995). We used 50 m distance intervals to a maximum distance of 500 m in each analysis. The F_{ij} metric is analogous to a correlation coefficient between allelic states (Loiselle *et al.* 1995). A variety of individual pairwise relatedness and kinship metrics are available for use in SPAGEDI and elsewhere. These statistics differ mostly in how variance among alleles is treated in the calculation. Stochasticity in allele frequencies reduces the ability to detect spatial genetic structure above that expected at random. We found strong structure regardless of the metric we used. SPAGEDI performs pairwise relatedness between individuals within the same class and between individuals within different classes (Hardy & Vekemans 2002). The program determines standard errors around the F_{ij} estimates, and 95% confidence limits around the null expectation of no genetic

structure ($F_{ij} = 0.00$). SPAGEDI obtains the confidence envelopes by permuting multilocus genotypes and spatial coordinates (1000 iterations) under the null hypothesis of no genetic structure. Values of $F_{ij} = 0.25$ and 0.125 refer to individuals that have a pairwise relatedness coefficient expected from that of full and half-siblings, respectively. Values of F_{ij} above or below the 95% confidence envelopes indicate the presence of significantly more or less genetic structure (two-tailed test, $P < 0.05$), respectively, than that expected at random.

We also computed the Sp statistic for pairwise comparisons, within and among diameter classes (Vekemans & Hardy 2004). The Sp statistic is a synthetic way of calculating the strength of spatial genetic structure and enables simple comparisons to be made within diameter classes and among species. $Sp = -b/(1 - F_1)$, where $-b$ is the slope of the regression of F_{ij} on $\ln(\text{distance})$, and F_1 is the mean F_{ij} between individuals in the first distance class (here, 0–50 m). In SPAGEDI, Sp can be calculated directly, or through simulation. Because the variance of gene movement and therefore the neighbourhood size of *Jacaranda* is large (Jones *et al.* 2005; Hardy *et al.* 2006) relative to the plot size (84 ha), simulations of Sp failed to converge across this population. Therefore, we present Sp calculated directly from the slopes of the regression of pairwise relatedness across the distance classes and the level of relatedness, F_1 , in the first distance class of each size category. The Sp statistic here is used as a simple measure to allow for comparisons among demographic classes, not as an estimate of the variance in gene dispersal distances. The slope of the regression of pairwise F_{ij} is done across all pairs of individuals, not across average values of F_{ij} within different size classes; therefore the choice of size classes only determines the value in F_1 . Our selection of 50 m distance classes was done to include as many neighbouring individuals as possible (97%) within the first distance class, as well as to allow for ease of comparison among different size classes.

Demographic rates

In addition to testing for possible differences among diameter classes in genetic structure, we also tested whether the populations within these diameter categories were in demographic equilibrium. Knowing whether populations are growing or in decline helps to interpret the results of the spatial genetic analyses across demographic classes. We assessed the rates of *Jacaranda* population growth, recruitment, and mortality from five censuses of the FDP conducted in 1982, 1985, 1990, 1995 and 2000. We examined demographic rates across the entire population, as well as within each d.b.h. size category in the population in each year. We calculated the intrinsic rate of increase for each interval and for the entire 18 years census interval as:

$$r = \frac{\ln(N_t) - \ln(N_{t-1})}{t}$$

where N_t is the population size at time t . Time t was determined by taking the arithmetic mean of the time in days between censuses of the individual 20×20 m quadrats in which *Jacaranda* trees were located. These rates were annualized to 365-day years. Mortality rate was calculated as:

$$m = \frac{\ln(N_{t-1}) - \ln(S_t)}{t}$$

where S_t is the number of survivors to time t from $t - 1$. Recruitment rate as calculated as:

$$g = \frac{\ln(N_t) - \ln(S_t)}{t}$$

The log transformation in these calculations converts rates to a per capita basis. We calculated these rates for the entire population and for each of the four diameter categories across all census intervals from 1982–3 to 2000.

Results

Genetic diversity and inbreeding coefficients

Jacaranda shows a high level of genetic diversity across all stages and diameter classes observed (Table 1; see also Jones & Hubbell 2003; Jones *et al.* 2005). Observed heterozygosity (H_O) was 0.75, 0.83, 0.85, 0.80, and 0.86, and expected heterozygosity (H_E) was 0.81, 0.82, 0.83, 0.81, and 0.83 for SEED, SUBA, MID1, MID2, and BIGA, respectively (Table 1). The total number of alleles within each diameter class ranged from 54 in the BIGA diameter class to 58 in the SUBA diameter class. Positive values of the inbreeding coefficient, F , indicate a lower frequency of heterozygotes than expected — a potential effect of selfing or biparental inbreeding. However, F values showed a general decrease

with increasing diameter classes: F was 0.079, 0.001, -0.021 , 0.013, and -0.042 , within SEED, SUBA, MID1, MID2, and BIGA respectively, indicating a trend towards fewer homozygotes than expected within diameter classes as diameter class increases (Table 1).

We found no genetic differentiation among the four diameter classes of established plants (Table 3). However, seedlings (SEED) showed low, but statistically significant differentiation from all diameter categories of established plants (Table 3). SEED was most strongly differentiated from the MID1 diameter class according to F_{ST} (0.009) measures. In these analyses, less than 1% of the genetic variation was partitioned among different diameter classes; the large majority of genetic variation was within diameter classes.

Spatial genetic structure

We analysed SGS within SEED and RAIN (Fig. 2) and within and among four diameter classes of established trees (Fig. 3). The diameter classes show different levels of relatedness in the smallest distance category, which includes all nearest neighbours. SEED averaged $F_{ij} = 0.049$ (SE = 0.013) and RAIN averaged $F_{ij} = 0.081$ (SE = 0.012) in the first 50 m distance class (Fig. 2). The smallest diameter

Table 2 Demographic rates for different diameter classes in the *Jacaranda copaia* population on Barro Colorado Island, Panama. r is the intrinsic rate of increase, recruitment is the recruitment rate, and mortality is the mortality rate during each census interval and averaged across all four census intervals.

	82–85 Interval	85–90 Interval	90–95 Interval	95–00 Interval	82–00 Average
All					
r	−0.023	−0.013	−0.015	−0.010	−0.015
Mortality	0.033	0.021	0.024	0.021	0.024
Recruitment	0.010	0.008	0.008	0.010	0.009
1.0–26.1 cm					
r	−0.075	−0.033	−0.048	−0.062	−0.054
Mortality	0.047	0.025	0.039	0.033	0.036
Recruitment	−0.028	−0.008	−0.009	−0.029	−0.018
26.1–42.5 cm					
r	0.084	0.000	−0.018	0.000	0.017
Mortality	0.007	0.006	0.011	0.004	0.007
Recruitment	0.091	0.006	−0.007	0.004	0.023
42.5–56.1 cm					
r	0.027	0.033	0.027	0.019	0.027
Mortality	0.000	0.028	0.005	0.012	0.011
Recruitment	0.027	0.061	0.031	0.031	0.038
56.1–138.3 cm					
r	0.020	−0.004	0.028	0.020	0.016
Mortality	0.021	0.020	0.013	0.019	0.018
Recruitment	0.041	0.016	0.041	0.039	0.034

Table 3 Pairwise F_{ST} (lower diagonal) and P values for the population of *Jacaranda copaia* within different diameter classes and with seedlings within the 50 forest dynamics plot on Barro Colorado Island, Panama. Numbers in bold indicate that pairwise population estimates are significantly different from zero at $P < 0.05$ level using a sequential Bonferroni correction.

	SEED	SUBA	MID1	MID2	BIGA
SEED	~	0.002	0.001	0.001	0.001
SUBA	0.006	~	0.439	0.429	0.467
MID1	0.009	0.001	~	0.287	0.280
MID2	0.008	0.001	0.001	~	0.211
BIGA	0.008	0	0	0	~

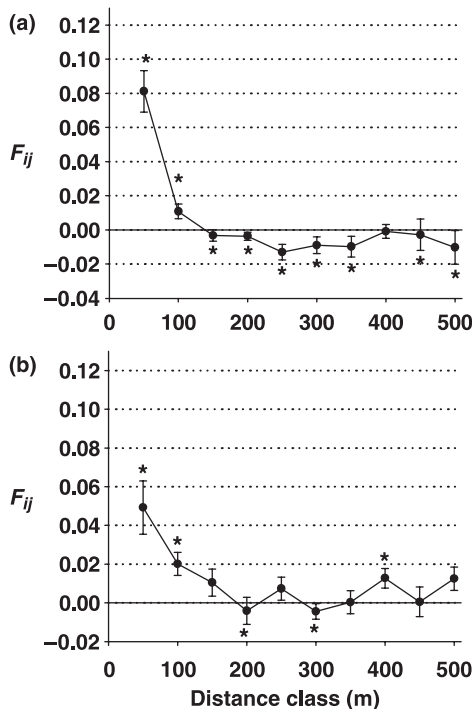


Fig. 2 Spatial genetic structure present in (a) the seed rain (RAIN) based upon maternal genotypes of seed wing tissue of seeds caught in seed traps, and (b) seedlings (SEED) grown from seeds caught in seed traps. Asterisks indicate significantly greater or lesser relatedness within a distance class than expected at random (two-tailed test, $P < 0.05$).

category for established trees included in the FDP census, SUBA, showed the lowest level of relatedness in the first distance class, $F_{ij} = 0.011$ (SE = 0.014), of all demographic classes examined. In the reproductive diameter classes the two intermediate diameter classes show equivalent levels of structure in the first distance class. MID1 averaged $F_{ij} = 0.053$ (SE = 0.017), MID2 averaged $F_{ij} = 0.053$ (SE = 0.017). The largest diameter class showed the highest level of relatedness in the first distance class: BIGA $F_{ij} = 0.11$ (SE = 0.014) (Fig. 3 on the diagonal). Moreover, the

spatial scale at which significantly greater spatial structure exists also increases with the diameter class. In SUBA, significant spatial structure is found only at the smallest distance category, 0–50 m. In the MID1 category, this spatial structure exists out to 150 m; in BIGA it is significantly greater than expected at random at scales of 200 m (Fig. 3).

We illustrate the patterns of within and between diameter class comparisons for three diameter classes, SUBA, MID1, and BIGA (Fig. 3 on the subdiagonals). In pairwise comparisons among individuals within and between diameter classes, several patterns emerge. First, all SUBA individuals irrespective of distance class show very little genetic structure, and what little genetic structure occurs is among neighbouring SUBA individuals. Nevertheless, there is less genetic structure among neighbouring individuals in the SUBA class than among neighbouring trees in the larger diameter classes (MID1 $F_{ij} = 0.031$, SE = 0.010; MID2 $F_{ij} = 0.047$, SE = 0.014; BIGA $F_{ij} = 0.066$, SE = 0.012; Fig. 2).

This general pattern is also shown with the S_p statistic for within- and between-class comparisons (Table 4). The greatest structure exists in the largest diameter class, BIGA, where $S_p = 0.0216$. The least structure exists in the smallest diameter class, SUBA, where $S_p = 0.0070$. In general, we found stronger genetic structure in between-diameter class comparisons than within-diameter class comparisons. For example, the smallest diameter class, SUBA, shows stronger structure when individuals are compared to larger diameter classes (S_p ranges from 0.104 to 0.153) than when they are compared to other individuals within the SUBA diameter class ($S_p = 0.007$). The exception to this is the BIGA diameter class, which shows the greatest structure within the diameter class ($S_p = 0.0216$), but still shows strong structure between diameter classes where S_p ranges from 0.0093 to 0.0198 (Table 4).

Demographic rates

Demographic rates calculated across four census intervals from 1982 to 2000 indicate the *Jacaranda* population on BCI has a negative intrinsic rate of increase over this time period ($r = -0.015$, Table 2). Recruitment for the entire population is low, averaging $g = 0.009$ per year across all censuses. The intrinsic mortality rate per year was $m = 0.024$, averaged across the four census intervals. Mortality across the entire population was greatest in the 82–85 census interval. The greatest average mortality rates are within the SUBA diameter class, where $m = 0.036$. This rate is five times greater than the next largest diameter class, MID1, where $m = 0.007$, and mortality rate in the smallest diameter class is twice the largest diameter class, BIGA, where $m = 0.018$. The probability of survival greatly increases as trees leave the SUBA d.b.h. size class (Table 2). When we compare mortality rates among years, we find that they are almost always highest in the SUBA diameter

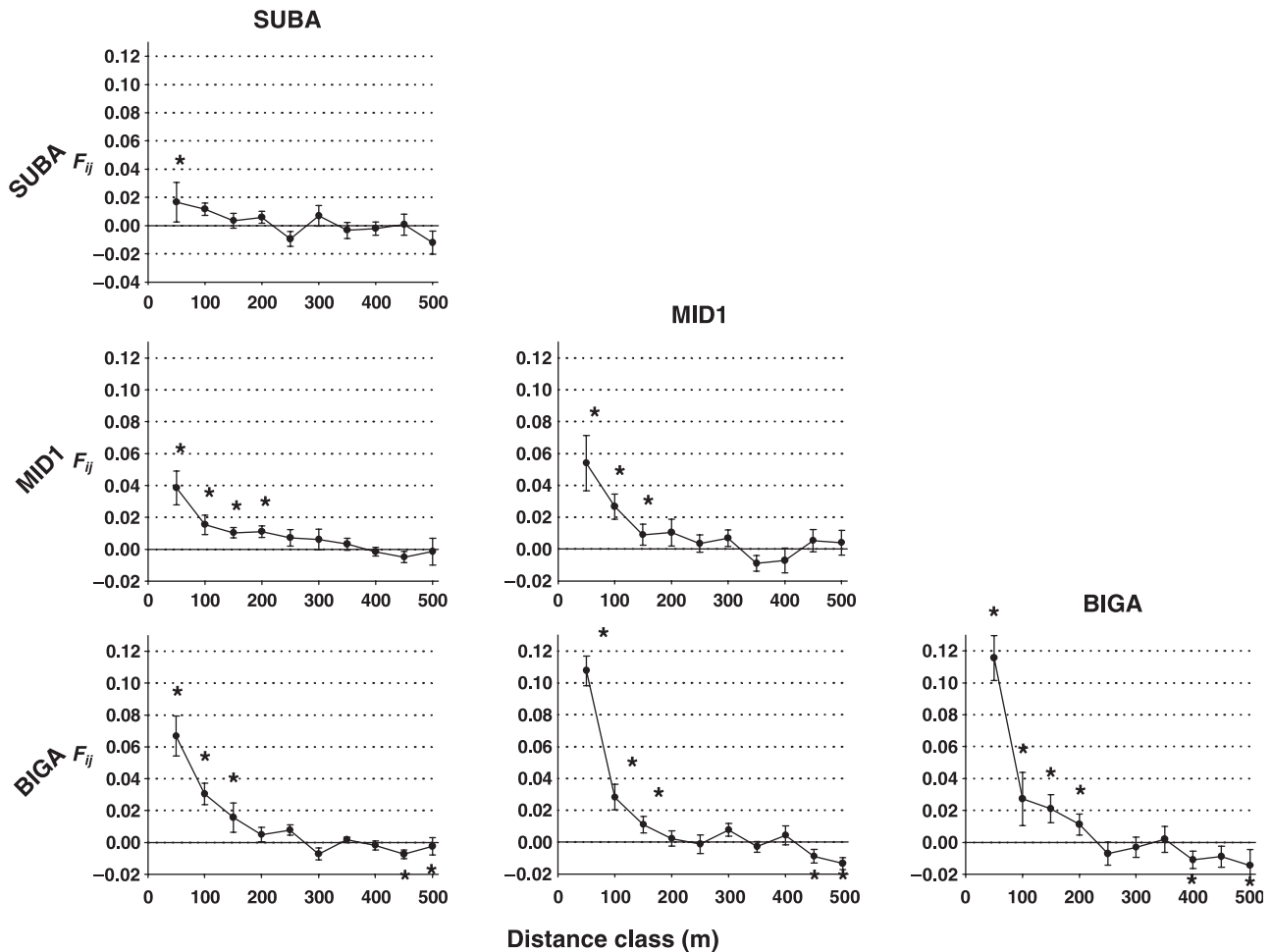


Fig. 3 Spatial genetic structure across distances classes in three diameter classes of *Jacaranda copaia*. Graphs on the diagonal are pairwise comparisons among individuals within a diameter class. Graphs on the subdiagonal are comparisons made among individuals between diameter classes. Asterisks indicate significantly greater or lesser relatedness within a distance class than expected at random (two-tailed test, $P < 0.05$).

Table 4 Pairwise matrix of the S_p statistic for five diameter classes (see text) of the tropical tree *Jacaranda copaia*. Values on the diagonal are within class comparisons, values on the subdiagonal are comparisons of genetic structure made between classes.

	SEED	SUBA	MID1	MID2	BIGA
SEED	0.0150	~	~	~	~
SUBA	0.0084	0.0070	~	~	~
MID1	0.0108	0.0122	0.0113	~	~
MID2	0.0162	0.0105	0.0150	0.0092	~
BIGA	0.0189	0.0153	0.0145	0.0093	0.0216

class, whereas recruitment rates are the lowest. Thus, new recruitment fails to compensate for individuals lost through mortality or by trees growing into the next larger size class. Overall, the population decline of *Jacaranda* is largely due to high mortality in the two smallest size

classes, coupled with low recruitment of saplings into the population over this 18-year period.

In addition to the negative rate of population growth for *Jacaranda*, the size distribution of the log of tree diameters is changing (Fig. 4). The average d.b.h. of the population has monotonically increased with each census, a pattern typical of declining tree populations. Average d.b.h. for individuals ≥ 1 cm diameter was 25.32 cm (SD = 22.52) in 1982, 28.48 (SD = 22.15) in 1985, 31.96 (SD = 22.79) in 1990, 35.48 (SD = 22.27) in 1995, and 38.83 (22.72) in 2000. Moreover, a larger proportion of the total *Jacaranda* population consisted small stems < 20 cm d.b.h. in the first census than in later censuses (Fig. 4).

Discussion

The incorporation of demographic history into the analysis of genetic structure provides critical information about

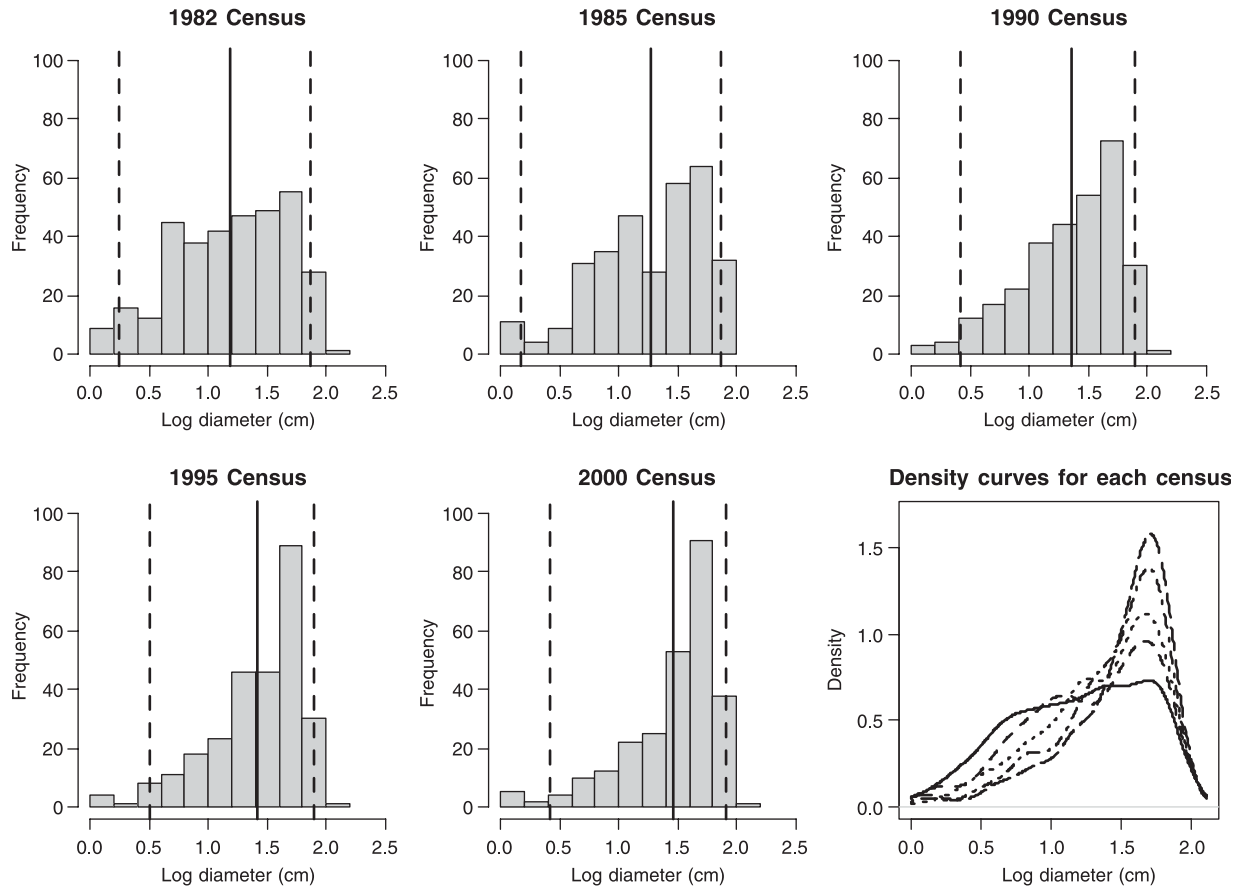


Fig. 4 Frequency histograms of diameters of all *Jacaranda copaia* > 1 cm diameter breast height across five censuses from 1982 to 2000. The mean and 95% confidence envelopes are shown in solid and dashed lines, respectively. The final panel shows the Gaussian density curves for each census 1982 (solid line), 1985 (short dashed line), 1990 (dotted line), 1995 (dash-dot line), 2000 (long dashed line), computed by fitting Gaussian curves to diameter frequencies.

the mechanisms responsible for the observed genetic structure. Without a demographic history of a population, it is more difficult to determine which mechanisms are responsible for generating the observed genetic structure. We found strong spatial genetic structure in the seed rain and seedling stage of *Jacaranda*, but this structure almost completely disappeared in the sapling/subadult size class. Furthermore, we found an approximately 10-fold increase in the F_{ij} in the first distance class from SUBA to BIGA diameter classes and a threefold increase in the Sp statistic. There is a parsimonious explanation of many of these patterns of genetic structure in the light of the nonequilibrium population dynamics of *Jacaranda* in the BCI FDP. We first discuss patterns of relatedness and inbreeding, and then consider the changes in genetic structure that occur over size classes.

Patterns of relatedness and inbreeding

The average level of relatedness in the smallest distance class of RAIN, $F_{ij} = 0.081$, is approximately the same as for

all reproductive sized individuals (MID1, MID2, and BIGA) in the smallest distance class (data not shown). The spatial scale of positive pairwise relatedness in RAIN (between 50 and 100 m) is also consistent with the scale at which significant genetic structure occurred in the three largest tree diameter classes, and it is consistent with a separate study of directly measured *Jacaranda* seed dispersal distances within the plot. Jones *et al.* (2005) found that about three-quarters (76%) of seeds dispersed < 100 m from the maternal parent, and about 5% dispersed > 300 m. Despite this, the clumped distribution of *Jacaranda* trees (Fig. 1), along with our relatedness in the RAIN and SEED stages, suggest that there is little overlap in the seed shadows of individual reproductive *Jacaranda*. Overlapping seed shadows decrease levels of spatial genetic structure in the seed rain and therefore should decrease the spatial scale of genetic structure in seedlings (Hamrick *et al.* 1993). However, our results may reflect the short time period over which we collected seeds and seed rain. If there is variation among trees in their phenological timing of seed fall, this could have reduced the overlap in seed

shadows observed among years, even among aggregated reproductive adults.

The SEED class shows a significant, positive inbreeding value, F , and reduced heterozygosity. *Jacaranda* is largely an outcrossing species (James *et al.* 1998). In the absence of selfing, homozygote excess in neutral alleles is caused by biparental inbreeding. Close proximity of related reproductive individuals is the main cause of biparental inbreeding in plants (Epperson 2003). SEED was the only class that differed significantly in pairwise F_{ST} comparisons with larger diameter classes (Table 3). The observed inbreeding coefficients are consistent with the hypothesis of mating among related individuals that are half-sibs ($F_{ij} = 0.125$) to cousins ($F_{ij} = 0.0625$), among near neighbours (< 50 m apart), in the MID1, MID2, and BIGA diameter classes. Some of the excess homozygosity in the SEED class is probably due to biparental inbreeding (Stacy *et al.* 1996; Epperson 2003), but we probably also sampled seed rain and seedlings across several neighbourhoods that were not completely panmictic (given the strong spatial structure found in all reproductive size classes). The observed heterozygote deficit could also be partly due to a Wahlund effect (Jones *et al.* 2005) because we sampled seedlings from a more limited area than we sampled established trees.

The change in pairwise relatedness between the maternally derived seed rain and the pre-establishment seedlings followed our expectations. Vekemans & Hardy (2004) and Epperson (2003) note that the scale of pollen movement largely determines the level of relatedness (F_{ij}) observed within the nearest distance class. We can immediately observe the effect of pollen dispersal on the level relatedness in the 50 m distance class when comparing RAIN to SEED in Fig. 2. The scale of positive genetic structure is approximately the same in RAIN and SEED: it can be detected out to 100 m. However, the average level of relatedness in the 50 m distance class in SEED ($F_{ij} = 0.049$) is more than one-half that for RAIN ($F_{ij} = 0.081$). Recall that RAIN is the nonrecombinant diploid maternal genotype of the seed, while SEED is composed of recombinant maternal and paternal haploid genotypes. Therefore, assuming panmixis, SEED would be expected to share one-half of its genes with the maternal tissue genotyped in RAIN (Kalisz *et al.* 2001). SEED F_{ij} has a value slightly greater than one-half the value observed in RAIN, an observation consistent with the positive inbreeding value for the SEED class (Table 1).

The positive and significant inbreeding values of F found in the SEED layer (Table 1) are not found in the smallest diameter class of established trees, or in the larger diameter classes. This suggests that inbred individuals have lower fitness relative to outcrossed individuals. The decrease in homozygosity in the SEED to SUBA transition could be due to inbreeding depression and viability selection

acting on inbred individuals at the seed-to-seedling, and seedling-to-sapling, transition. Evidence for viability selection acting at these early stages has been found within the BCI FDP in the wind dispersed tree *Platypodium elegans* (Hufford & Hamrick 2003). Increase in heterozygosity across increasing diameter classes has been observed for other Neotropical tree species on BCI (Hamrick *et al.* 1993) and elsewhere (Alvarez Buylla & Garay 1994; Latouche-Halle *et al.* 2003). On the other hand, if the heterozygote deficit in the SEED layer is due to a Wahlund effect, then random mortality across the plot at early stages could also reduce the genetic structure and heterozygote deficit, without selection against inbred individuals.

Genetic structure and population dynamics

We turn now to a discussion of the mechanisms responsible for the loss of genetic structure in the transition from seed rain and seedlings to SUBA individuals, the mechanisms responsible for high levels of genetic structure in the reproductive-sized individuals, and the decrease of the strength of genetic structure across successively larger diameter classes. We were led to consider population dynamics in *Jacaranda* after discovering unexpected patterns of increasing spatial genetic structure with increasing tree size class. We expected the reverse pattern because we anticipated gradual loss of the genetic structure initially established by the spatially autocorrelated dispersal of full- and half-sib seeds from individual maternal parents.

Some patterns were correctly anticipated. As expected, we did find *Jacaranda* seed rain and seedlings to be strongly genetically structured in space (Fig. 2). Moreover, there is concordance between the levels of relatedness and the scale of spatial structure in the adult reproductive-sized individuals (MID1, MID2, and BIGA), and between spatial structure in the maternally derived seed rain (RAIN) and the seedlings (SEED).

But what is responsible for the loss of genetic structure from the SEED to SUBA transition? We have shown that recruitment is low and mortality rates are high for individuals in the SUBA class (1–26 cm d.b.h.; Table 2). Mortality rates are likely to be even greater for individuals smaller than 1 cm. Although the numbers of *Jacaranda* seeds captured by seed traps are among the highest recorded seed rain of any species within the plot, seedling recruitment is extremely low. For example, 64 713 *Jacaranda* seeds were captured in 200 seed traps from 1995 to 2003, but only 116 seedlings successfully recruited to 600 1 m² seedling plots immediately adjacent to these traps, or approximately one seedling recruit for every 557 captured seeds (Wright *et al.* 2005).

Low recruitment in *Jacaranda* is partly a reflection of its life history. *Jacaranda* is among the most light-demanding species within the BCI FDP (Wright *et al.* 2003). Successful

germination and growth require high light environments found only within large tree fall gaps within the FDP – sites that are rare in the forest (Brokaw 1985; Hubbell & Foster 1986). Furthermore, negative density dependence is strong in *Jacaranda* and in other pioneer species at the seedling and juvenile stages (Harms *et al.* 2000; Hubbell *et al.* 2001; Wright *et al.* 2005), reducing the absolute number of individuals that survive once they establish within a gap. Since most seeds land in the vicinity of the maternal parent in this species (Jones *et al.* 2005), strong density-dependent mortality among the seedlings near the mother tree may also reduce relatedness in the SUBA size class.

In addition to the relatively rare occurrences of large gaps, there is high interannual variation in seed production in the *Jacaranda* population (Wright *et al.* 2005), as well as high reproductive variation within diameter classes within and among years (F. A. Jones, unpublished). Spatial and temporal variation in seed production increases dispersal limitation (Nathan & Muller-Landau 2000), which further increases the heterogeneity of mixing of the progeny of individual adults in the seed rain, and which in turn creates heterogeneity in the seedling pool across the FDP landscape (De Steven & Wright 2002). Each of these factors would reduce the number of individuals establishing within gaps, increase the spatial and genetic heterogeneity of the seed rain spatially and temporally, and increase spatial and genetic variation within and among cohorts that do establish, thereby reducing spatial genetic structure from SEED to SUBA.

From the reduction in relatedness from the SEED to SUBA stages, we expected to see a further decay of structure in larger diameter classes due to random thinning of individuals (Hamrick *et al.* 1993). Instead what happened is that relatedness in the 50 m distance class increased by a factor of five from SUBA to the next two diameter classes (MID1 and MID2), and by a factor of 10 to the largest diameter class (BIGA), a pattern also shown by the *Sp* statistic. There is also the problem of the strong genetic structure present in the reproductive size classes, structure which gets passed on to the seed rain and seedling stages, but is then lost and subsequently reconstituted in the reproductive size classes.

There are several possible explanations for these patterns, including overlapping generations, selection, historical factors, and nonequilibrium dynamics. Similar patterns of increases in local genetic structure from juveniles to reproductives have been shown in populations of *Trillium grandiflorum* (Kalisz *et al.* 2001) and *Plantago lanceolata* (Tonsor *et al.* 1993) both temperate herbs, and a tropical tree *Dicorynia guianensis* (Caron *et al.* 2000; Latouche-Halle *et al.* 2003), although none of these studies assessed the equilibrium status of the population. Each of these studies hypothesizes a different mechanism for the observed increase in genetic structure across life stages. Kalisz *et al.*

(2001) speculated that a historical bottleneck or micro-environmental selection may have been responsible for the observed pattern in *Trillium* and *Plantago*, and Latouche-Halle *et al.* (2003) attribute their results to overlapping generations in the larger diameter classes, potentially further shaped by selection. In *Jacaranda*, we believe the most likely explanation is nonequilibrium population dynamics and overlapping generations, but we will consider the hypotheses of selection and historical factors first.

Selection for related individuals (families) that are locally adapted to microhabitat conditions (moisture, soil pH, soil nutrients, locally adapted herbivores, etc.) is one potential explanation for high levels of genetic structure in the largest size classes. Linhart & Grant (1996) reviewed the evidence that selection can create small-scale differentiation within tree populations on scales of 100–300 m. For trees with limited dispersal, significant differentiation could be measured on scales to order of 10–50 m. Applied to *Jacaranda*, the idea would be that after dispersal and establishment of seedlings, SUBA individuals then undergo microhabitat selection, which results in increasingly greater levels of relatedness within increasing-diameter classes such as MID1 and BIGA. If this were the case, we would expect that the between-class comparisons of SUBA to MID1, MID2, and BIGA (subdiagonals in Fig. 3) would show little structure, similar to the lack of structure within the SUBA diameter class. However, SUBA individuals are more related to the larger diameter class individuals than they are to neighbours within their own diameter class, suggesting that as they grow into the larger diameter class, they grow beside relatives.

Historical factors could also explain both the observed demographic trends and the loss of spatial genetic structure with decreasing demographic classes. Non-steady-state source-sink dynamics (Pulliam 1988) or a founder effect could be responsible for both the negative population growth rates and observed spatial genetic patterns in observed *Jacaranda* across diameter size classes. Hubbell & Foster (1990) suggested that the FDP is losing pioneer species because a region of secondary forest just north of the plot, cleared 90 years prior to the first census, is undergoing secondary succession. They postulate that as pioneer species are replaced in the canopy by more shade tolerant species, this area is no longer a major source of immigrant seeds from pioneer species. According to this hypothesis, *Jacaranda* recruitment now is lower than in the past because of the loss of the propagule subsidy from secondary forest into old-growth forest. The original colonists or their descendants, now the largest individuals, would represent a founding generation with high levels of genetic structure (Sezen *et al.* 2005). This structure is then lost in the smaller size classes because of the homogenizing effect of pollen flow, the onset of reproduction of established individuals within the plot, and the increased overlap

of seed shadows as the founding individuals mature and reproduce over time (Jones *et al.* 2006).

However, there are several lines of evidence that do not support this hypothesis for *Jacaranda*. Although abundances of shade intolerant pioneer species are undergoing a decline within the plot (Condit *et al.* 1996), a 17-year study of seed rain in the plot shows no decrease in seed input by *Jacaranda* (Wright & Calderon 2006; S. J. Wright, unpublished). Moreover, the age of the secondary forest is less than 100 years, so late secondary heliophiles such as *Jacaranda* adjacent to the plot would be expected to be reaching their most productive stages, rather than declining in reproduction. Moreover, preliminary data from a 25-ha plot located just to the north of the FDP in secondary forest actually has a lower *Jacaranda* density than old-growth forest within the FDP (S. J. Wright, unpublished). A genetic analysis of seed dispersal distances and seed flow also show that more seed input comes from within the plot than from outside the plot (Jones *et al.* 2005). Finally, if the oldest diameter classes were a recently established founding population, then we would expect that the largest and oldest individuals should show a reduced number of alleles and greater potential differentiation from subsequent smaller diameter classes. This is not the case; although there are fewer alleles in the BIGA diameter class, there are only four fewer alleles (across six diverse microsatellite loci) in BIGA than in SEED, which has 58. Therefore, there is little evidence in support of a founder effect in explaining the observed spatial genetic transitions among size classes.

Demographic factors

The life history and low survival rate in the early stages of *Jacaranda* may best explain the observed pattern of increased relatedness with increasing diameter class. The smallest diameter classes represent a few cohorts or a single generation. These cohorts are dispersed randomly in space due to the strong light requirement and unpredictable nature of gap formation, hence low relatedness among nearby individuals. However, as the cohort grows and reaches the canopy, it joins the reproductive diameter classes (MID1, MID2, BIGA), and grows beside relatives. These relatives may be the parents or even grandparents of the new cohort. In this case, the increase in relatedness in the smallest distance classes with increases in diameter class reflects effects due to overlapping generations.

This pattern may be exacerbated by population cycles. Strong recruitment by *Jacaranda* occurred following the strongest El Niño southern oscillation (ENSO) on record on BCI in 1976 and 1977, six years before the first census of the FDP. Strong ENSO events result in large increases in fruit and seed production in *Jacaranda* and many other species, attributed to increased solar radiation during the normally cloudy wet season on BCI (Wright *et al.* 1999).

Strong droughts also increase tree death rates, causing more new gap creation (Condit *et al.* 1996). Therefore, under ENSO conditions, increases in seed production in pioneers, along with more gaps, increases recruitment rates and abundances of gap-dependent shade-intolerant species (Becker & Smith 1990).

Thus, *Jacaranda* exhibits population dynamics in which there are pulses of massive recruitment at irregular, multi-year intervals, followed by intervening, typically stronger periods of population decline. We believe that this explains the increasing spatial structure in the larger size classes in this population. Our hypothesis is that, at some time in the past century, a group of individuals reproduced and produced a massive cohort of even-aged individuals, the survivors of which now dominate the cohort of the largest reproductive adults in the population. Such a boom-bust cycle would produce a pattern of increasing relatedness and genetic structure with increasing size class. The loss of this genetic structure in the subadult classes (larger than seedlings) would then reflect smaller cohorts of offspring produced by overlapping generations. This hypothesis is the most parsimonious explanation we currently have, although definitive proof must wait until we have recruited a cohort of reproductive *Jacaranda* adults from genetically known seedling cohorts.

Conclusions

Studies of the spatial genetic structure of natural plant populations are foundational for understanding ecological and evolutionary processes in plant populations. Although there have been many such studies, to our knowledge, none has analysed changes in genetic structure through all life history stages, including seed rain and seedlings. Here we present such an analysis for the Neotropical pioneer tree, *Jacaranda copaia*. We found unexpected changes in spatial genetic structure from the seed rain and seedling stages to trees in the largest adult size classes. One such surprising finding was an increase in genetic relatedness in larger/older trees – a pattern that could be best explained by the hypothesis that the *Jacaranda* population is not in demographic equilibrium. In particular, *Jacaranda* is a species characterized by large temporal variation in reproduction, triggered especially by irregularly spaced ENSO events, in between which the population undergoes steady decline and decreases in SGS are found. Thus, the hypothesized reason for greater genetic structure in large adults is that they represent cohorts of descendents from one or a few such past recruitment events, and progeny of a similar cohort of related adults in the preceding generation. More such coupled studies of population ecology and population genetics are needed to evaluate how often such patterns occur in tropical tree species. We predict that such patterns will be common among

light-demanding species, especially those that are persistent in the canopy layer. However, we predict that shade-tolerant species, which generally have less episodic recruitment and greater survival, will typically show a different pattern, one in which spatial genetic structure decreases with each successively larger life history stage. How genetic structure changes across life history stages is important because it can potentially affect mating patterns, levels of biparental inbreeding, inbreeding depression, and the action of selective forces within natural populations. Future studies of the spatial genetic structure of natural plant populations should consider including not only demographic analyses, but also repeated sampling across several censuses to observe spatial population genetic dynamics directly.

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