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ORIGINAL PAPER

Fine-scale genetic dynamics of a dominant neotropical tree in the threatened Brazilian Atlantic Rainforest

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Abstract We present a case study of the relationship between spatial genetic structure (SGS) and age structure in *Protium spruceanum* (Burseraceae), an insect-pollinated, mass-fruiting, and secondary bird-dispersed tree, as determined through variation in allozyme loci. Using ten polymorphic loci, we investigated spatial and temporal patterns of a genetic structure within a 40 m×60 m plot in a small (1.0 ha) fragment of Atlantic Rainforest to investigate the processes shaping the distribution of genetic diversity. Individuals (n=345) from seedlings to adults were grouped and analyzed in four diameter classes. The results showed a high average level of genetic diversity (H_e=0.438), but genetic diversity parameters did not vary significantly among cohorts. The spatial distribution pattern analysis of individuals showed significant levels of aggregation among small- and medium-diameter classes and random distribution among the highest diameter class, likely due to processes of competitive thinning. There was an association between demographic and SGS at short distances (less than 10 m) which is likely the consequence of restricted seed dispersal. The degree of SGS decreased across small- to large-diameter classes. We inferred that limited seed dispersal and subsequent density-dependent mortality from the family clusters are responsible for the observed changes in fine-scale SGS across different demographic classes.

Keywords Cohorts · Genetic diversity · *Protium spruceanum* · Random thinning · Seedling bank · Spatial patterning

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Introduction

In the analysis of genetic structure, demography provides critical information about the mechanisms responsible for the observed genetic structure of forests (Hardesty et al. 2005; Ng et al. 2004; Ueno et al. 2002). The patterns of spatial genetic structure (SGS) within local plant populations are the result of genetic patterns (e.g., gene dispersal, mating system, and drift) combined with demographic processes (e.g., density of seed sources, adaptation to edaphic conditions, and microbial symbionts) acting at different temporal and spatial scales across the life stages (Jones

et al. 2006; Jones and Hubbell 2006; Vieira et al. 2010a). SGS is influenced by biological aspects of a species and is strongly dependent on life history, particularly in species with long reproductive cycles such as trees (Sagnard et al. 2011). Intraspecific life history variation in plants can arise under phenotypic plasticity shaped by environmental variation (Kittelson and Maron 2001), providing genetically different subpopulations as a result of spatial heterogeneity (Jones et al. 2005).

The analysis of spatial dynamics among cohorts have revealed either an increase in relatedness among neighboring plants from seed bank to adult stages (Kalisz et al. 2001; Latouche-Halle et al. 2003) or a decrease in relatedness from juveniles to adult stages (Hardesty et al. 2005; Ng et al. 2004). Normally, it is speculated that historical factors, including local adaptation (Kittelson and Maron 2001), nonequilibrium population dynamics and overlapping generations (Jones and Hubbell 2006), competitive thinning (Ng et al. 2004), limited dispersal near the parent plant (Dev et al. 2011), and low density of reproductive adults (Vieira et al. 2010a) determine the differences in relatedness among cohorts. Seed dispersal and demographic processes have the greatest influence on the presence or absence of significant SGS (Jones et al. 2005; Jordano et al. 2007). In fact, studies on SGS in fragmented landscapes in Brazilian tropical forests suggest that the spatial isolation of populations can restrict seed and pollen gene flow, increase SGS for both adults and seedlings, and affect the genetic diversity of future generations (Gonçalves et al. 2010; Sebbenn et al. 2011).

In Brazil, there is a lack of data on the genetics of tree species, in relation to both demographic distribution and fine-scale SGS for the Amazon basin (Latouche-Halle et al. 2003; Silva et al. 2008), the Caatinga Domain (Moreira et al. 2009), and the Cerrado Domain (Collevatti et al. 2010; Martins et al. 2006). However, some studies have examined the changes that occur in fine-scale SGS patterning throughout the life history of trees in the threatened Brazilian Atlantic Rainforest (Bittencourt and Sebbenn 2007; Conte et al. 2003; Gaino et al. 2010; Sebbenn et al. 2011). The inclusion of spatial dynamic analysis among neighboring plants can also provide compelling interpretations about the temporal relationships of relatedness. For instance, previous research within fragments of Atlantic Rainforest and corridors of secondary forest revealed high levels of genetic diversity with most of the diversity partitioned within populations (Vieira and Carvalho 2008). Moreover, this diversity was not found to be structured within populations, due to the absence of SGS between large trees within fragments (Vieira et al. 2010b). However, given the longevity of most tree species, the study of subsequent generations (i.e., younger cohorts) is required to provide a clearer picture of the spatial and temporal patterns of the genetic structure within the studied populations.

For this study, we used allozyme loci from a small fragment of Atlantic Rainforest in order to generate dynamic SGS data sets for the dominant neotropical tree *Protium spruceanum* and to investigate our hypotheses regarding factors influencing a fine-scale genetic structure. *P. spruceanum* is a large, dioecious canopy tree (up to 20 m tall), which is insect-pollinated and shade-tolerant (allowing for seedling recruitment beneath large trees). This species is characterized by a high population density ($232 N ha^{-1}$, Vieira and Carvalho 2008), with no crown competition between trees and a high rate of fruit production, with seeds dispersed mainly by gravity and secondary agents such as birds (Vieira et al. 2010c). Thus, if seed dispersal by secondary agents is restricted, our hypothesis is that there will be greater genetic relatedness and spatial aggregation among young individuals in close proximity than among adults (see Latouche-Halle et al. 2003). In addition, if the distribution of the number of individuals per diameter class follows the pattern of an inverted "J" curve (sensu Meyer 1952), it is expected that both demographic and SGSs disappear among neighboring individuals throughout life history stages, suggesting competitive thinning processes (Ng et al. 2004). Specifically, we inferred from SGS the role of seed dispersal in spatial patterning and density of each cohort in a small forest plot. Previous studies have reported a lack of SGS with spatial aggregation of seedlings (Sagnard et al. 2011), although patterns can vary with the unique biological characteristics of each species (Vekemans and Hardy 2004). Thus, to evaluate our hypotheses, the aims of this study are to assess the levels of spatial aggregation and SGS of the cohorts at a fine scale and correlate these results with genetic diversity parameters across the life stages. Further, we examine factors that may modify genetic diversity during the lifetime of the tree populations.

Materials and methods

Sample site

The fragment of Atlantic Rainforest used in this study (ca. 1.0 ha, Fragment number 1 from Vieira and Carvalho 2008) is located in the region of Lavras, Southern Minas Gerais State, Brazil, at 21°17′52″S and 44°59′13″W and an altitude of 973 m. The region currently consists of five forest remnants, a matrix of coffee plantations and planted pastures (*Brachiaria* spp.) for livestock rearing, and corridors of secondary forest. At the study site, the absolute density of adult individuals (diameter at breast height >16 cm) of *P. spruceanum* within the fragment is $195.8 N ha^{-1}$. The approximate density of the species across the region is $232 N ha^{-1}$ (Vieira and Carvalho 2008). Over the last 200 years, the population of *P.*

spruceanum in this region has declined rapidly because of habitat fragmentation caused by anthropogenic disturbance leading to recent genetic bottlenecks (Vieira and Carvalho 2008). The study plot was established in the center of the fragment where all individuals found within a 40 m×60 m plot were mapped with *x*–*y* coordinates (Electronic Supplementary Material 1).

Cohort definition and genotyping

The plot included 792 individuals (*N*) classified into four diameter classes according to diameter at breast height (d.b.h. at 130 cm above ground level) or at soil height (d.s.h.) (Fig. 1). The size classes of the cohorts, MED1, MED2, and BIG, were defined using the d.b.h. and increasing amplitudes (0.8–8, 8–16, 16–32, and 32–64 cm) to compensate for the fewer number of individuals in the larger diameter classes (e.g., BIG cohort), typical of the negative exponential distribution, known as "J" curve (Meyer 1952). These intervals allow better representation of demographic thresholds in the studied plot as the larger diameter classes have a lower density. The BIG cohort was defined by the combination of classes 16–32 and 32–64, since the largest class (32–64) included only three individuals. The SMA cohort (smallest individuals) was defined according to d.s.h., since individuals were shorter than 130 cm. Therefore, plant frequency distributions by diameter class for *P. spruceanum* census (*N*=792) were prepared using class intervals with exponentially increasing ranges to make up for the normally steep decrease in tree density correlated to larger diameters (Fig. 1). Although the allometric relationship between age and size is not available for *P. spruceanum*, the defined cohorts were used to minimize overlapping generations.

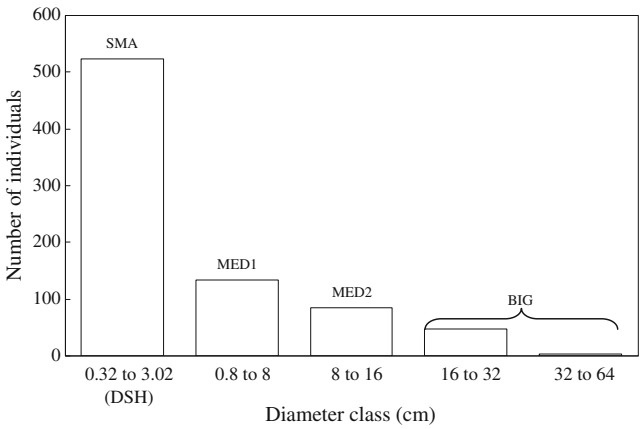

Fig. 1 Distribution of *Protium spruceanum* individuals by diameter class in the 2,400 m^2 forest fragment. Samples collected were classified according to diameter at breast height (d.b.h., 130 cm) in MED1, MED2, and BIG cohorts and diameter at soil height (d.s.h.) for the SMA cohort

The sample (*n*) for genetic analysis included 345 individuals across all size cohorts, which represents a mean of 68.4 % of the total species census (Table 1, Fig. 2). Tissue from the SMA cohort was collected only from healthy plants where there was sufficient leaf material, so that tissue removal was unlikely to cause mortality. Although SMA individuals represent 22.9 % of all sampled individuals, the samples reflect the real skewed spatial distribution in the plot (Fig. 2 and Electronic Supplementary Material 1). Some samples of the MED1, MED2, and BIG cohorts were excluded from analysis because banding patterns could not be reliably scored. The BIG cohort likely represents reproductive individuals; however, phenological observations and sex ratio were not part of this study. Live tissue was transported on ice to the laboratory and stored at −80°C until enzyme extraction.

Enzyme extraction and electrophoresis

Small pieces of leaf tissue were crushed in 1 mL of the extraction buffer as described in Vieira and Carvalho (2008). Discontinuous vertical electrophoresis was performed in a polyacrylamide gel (10 %) and carried out at 4°C over 3 h (constant current of 80 mA and voltage of 300 V). Nine enzymatic systems were used: acid phosphatase (E.C.3.1.3.2, locus *Acp*), alcohol dehydrogenase (E.C.1.1.1.1, locus *Adh*), glucose dehydrogenase (E.C.1.1.1.47, locus *Gdh*), β-galactose dehydrogenase (E.C.1.1.1.48, locus *Gldh*), glutamate dehydrogenase (E.C.1.4.1.3, locus *Gtdh*), malate dehydrogenase (E.C.1.1.1.37, locus *Mdh2*), peroxidase (E.C.1.11.1.7, loci *Per1* and *Per2*), sorbitol dehydrogenase (E.C.1.1.1.14, locus *Sdh*), and shikimate dehydrogenase (E.C.1.1.1.25, locus *Skdh*). Staining protocols and the genetic basis of allozyme banding patterns were inferred from segregation patterns with reference to typical subunit structure and conceptual methods described in Wendel and Weeden (1989). Putative loci and alleles were designated sequentially. The locus with the most anodally migrating allozyme or alleles was designated as 1 and the next as 2.

Genetic diversity and comparisons among cohorts

Within each cohort, intrapopulation genetic diversity was analyzed by the percent of polymorphic loci (P_L; 0.95 criterion), mean number of alleles per locus (*A*), observed heterozygosity (H_o), Nei's gene diversity (H_e), and fixation index (*f*). Standard errors (SE) for H_o and H_e were calculated across loci. Significant negative *f* values were tested using 1,000 bootstraps across loci. Departures from Hardy–Weinberg (H–W) equilibrium at each locus were tested in each cohort using the Fisher exact tests, using BIOSYS-2 (Swofford and Selander 1989). A *G*-based exact test (Goudet et al. 1996), based on

Table 1 Population genetic statistics for four diameter classes from a *P. spruceanum* population in a 2,400-m^2 forest fragment sampled in Lavras, southeastern Brazil. Shown are the cohort codes, number of individuals according to the census, density of cohorts, and number of genotyped individuals

Cohort	N	Nha^{-1}	Genotyped (% census)	H_o (SE)	H_e (SE)	f (95 % CI)	b_{log} (SE)	Sp
BIG	50	195.8	45 (90.0)	0.518 (0.012)	0.454 (0.004)	−0.143* (−0.185, −0.102)	−0.011 (0.004)	0.011
MED2	85	354.2	60 (70.6)	0.467 (0.017)	0.434 (0.011)	−0.077* (−0.150, −0.023)	−0.0004 (0.003)	0.0004
MED1	133	554.2	120 (90.2)	0.519 (0.017)	0.429 (0.013)	−0.211* (−0.287, −0.135)	−0.013* (0.006)	0.013
SMA	524	2,183.3	120 (22.9)	0.504 (0.026)	0.437 (0.007)	−0.153* (−0.240, −0.050)	−0.009* (0.003)	0.009
Total	792	3,287.5	345 (mean=68.4)	0.504 (0.013)	0.438 (0.005)	−0.159* (−0.204, −0.097)		

N census, H_o observed heterozygosity, H_e genetic diversity, f fixation index, b_{log} regression slope, *Sp* statistic

*Significant at the 5 % level

6,000 permutations of genotypes among samples, was performed to test for cohort differentiations at allozyme loci, using the program FSTAT 2.9.3.2 (Goudet 2002). Comparisons among cohorts according to the parameters H_e, f, and F_{ST} were performed using the *G*-statistic (Goudet et al. 1996) by randomization of multilocus genotypes among cohorts. The tests implemented in FSTAT under "Comp. among groups of samples" aim at testing differences in diversity and differentiation among groups of populations. Thus, we defined subplots within the plots by randomization of samples. The significance level was adjusted for multiple pairwise comparisons using a sequential Bonferroni correction in FSTAT.

Demographic data analysis

The spatial distribution of individuals in the census (N) was tested for clumping using a univariate second-order spatial pattern analysis, based on Ripley's (1976) K function. This method considers the number of events within a circular radius sequentially larger t from each focal event and deviation from expectation at each t under CSR (complete spatial randomness). We used the modified L function, defined by Besag and Diggle (1977) as:

$$L(t) = \sqrt{k(t)/\pi} - t.$$

This L function has a more stable variance than the K function and is easier to interpret: $L(t)=0$ under CSR; $L(t)<0$ indicates inhibition, i.e., there are fewer neighbors within a distance t from an arbitrary point than expected under CSR, so that the pattern tends to be regular; and $L(t)>0$ indicates aggregation, i.e., there are more neighbors within a distance t from an arbitrary point than expected under CSR, so that the pattern tends to be clustered. Weighted edge corrections, based on those of Goreaud and Pelissier (1999) were calculated. We used t values of 1 to 25, as recommended by Wiegand and Moloney (2004), as they should not exceed approximately half the length of the shortest dimension of the plot. The 99 % confidence intervals (CIs) for the statistic under the null hypothesis of CSR were estimated by Monte Carlo simulations of the null models with 499 replicates for $\alpha=0.01$ (Besag and Diggle 1977). The

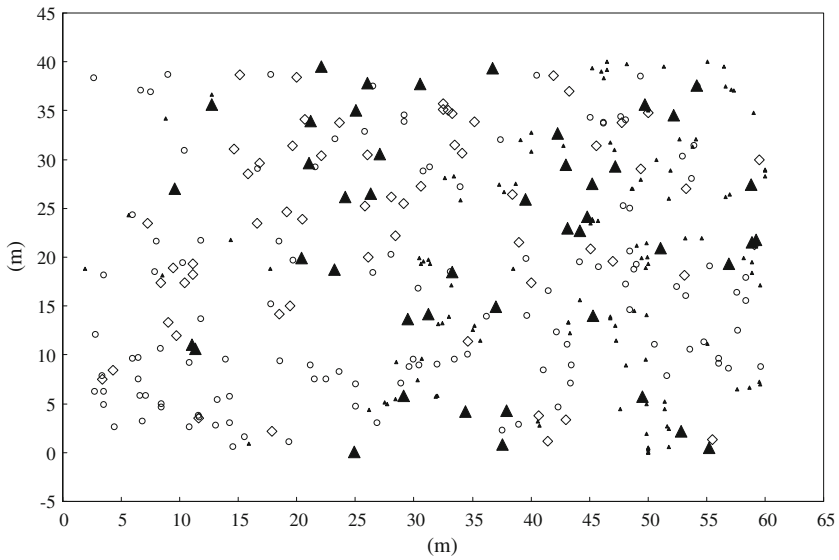

Fig. 2 Distribution of the sampled *Protium spruceanum* trees by cohort for the spatial genetic structure analysis. *Filled triangle* BIG, *unfilled diamond* MED2, *unfilled circle* MED1, *filled triangle* SMA

sample statistic was compared with this probability. These calculations were analyzed with SpPack version 1.38 (Perry 2004).

Spatial genetic structure (SGS)

SGS for each cohort was further analyzed using Nason's kinship coefficient F_{ij}, or coancestry (Loiselle et al. 1995). This coefficient can estimate between pairs of mapped individuals (x and y) a ratio of differences of probabilities of identity-in-state between homologous genes (Rousset 2002). For fine-scale SGS analysis, distance class intervals between individuals were determined as suggested by Hardy and Vekemans in the SPAGeDi user's manual (p. 16). We used all genotyped individuals in calculating the reference allele frequencies for the analysis in SPaGeDi. To test for significant deviations from random SGS, observed values for each distance class were compared to the 95 % CI derived from 1,000 jackknife replicates across loci. The extent of SGS was estimated using the Sp statistic following Vekemans and Hardy (2004). The Sp statistic here is used as a simple measure to allow for comparisons among cohorts, not as an estimate of the variance in gene dispersal distances. Sp was quantified by $\mathrm{Sp} = -b_{\log}/\left(1 - F_{(10,\mathrm{m})}\right)$ where $b_{\log}$ is the regression slope of the F_{ij} on log(distance) and $F_{(10,\mathrm{m})}$ is the mean kinship coefficient between individuals belonging to the first distance class (0–10 m). The $b_{\log}$ standard errors were obtained by jackknife replicates across loci. These calculations were performed using the program SPAGeDi 1.2 g (Hardy and Vekemans 2002).

Experimental random thinning

We used a simulation method to investigate whether random mortality could be responsible for a reduction of SGS across age classes. For this, we forecast future SGS for adult trees performing Monte Carlo randomizations (Manly 1991) to create "pseudo-cohorts", corresponding to the BIG cohort. Subsequently, the patterns of randomized cohorts were statistically compared with the real SGS data sets of the BIG cohort. Given the 240 genotyped individuals in SMA and MED1 size classes (young cohorts, see Table 1), the experimental random thinning was calculated by following the rarefaction method to include replicates of 45 individuals, equivalent to the sample size of BIG cohort (oldest cohort). We randomly designated 45 individuals from the two smallest size classes (SMA and MED1) and calculated the average F_{ij} within each distance class with 1,000 replicates using a jackknife procedure in the SPAGeDi software. The average F_{ij} within each distance class was compared to the empirical results (i.e., BIG cohort) to determine if the CI around the F_{ij} overlaps, particularly at the shortest distance class (0–10 m). Three expectations were hypothesized: (1) random thinning of "pseudo-cohorts" would result in an nonsignificant departure from empirical results at that stage; (2) if the SGS observed in those size classes is preserved, then there is evidence of other genetic processes impacting the BIG cohort, i.e., significant deviations from random SGS; and (3) random thinning would not be expected to change the regression slope of the F_{ij} on log(distance), i.e., nonsignificant $b_{\log}$ at first distance interval (0–10 m).

Results

Genetic diversity

P. spruceanum shows a high level of genetic diversity across all analyzed diameter classes (Table 1). The nine enzyme systems utilized revealed ten interpretable loci with a total of 20 alleles (Electronic Supplementary Material 2). No cohort-specific alleles were detected from the analysis of the allele frequencies for the ten polymorphic loci. The percentage of polymorphic loci within local cohorts was 100.0 % with two alleles segregating per locus. The relationship between the observed (H_o) and expected (H_e) mean heterozygosities resulted in a negative fixation index (f) for all analyzed cohorts, indicating a high proportion of heterozygotes and an absence of inbreeding (Table 1).

The mean values of genetic diversity and fixation index were not significantly different between the cohorts (Table 2). Less than 1.4 % of the genetic variation was partitioned among different diameter classes; the majority of genetic variation was within diameter classes. Based on Goudet's G test, differentiation between cohorts was not significant at the 5 % level (F_{ST}=0.005; CI=−0.001 to 0.016). However, considering pairwise estimates, only young cohorts (MED1 vs.

Table 2 Differences in levels of genetic diversity, fixation indices, and genetic differentiation among cohorts as measured by H_e, f and F_{ST} based on Goudet's G test. Levels of significance were obtained after 6,000 permutations using FSTAT (Goudet 2002)

Comparisons	Mean (H_e)	Mean (f)	F_{ST}
BIG vs. MED2	0.444 ns	−0.110 ns	0.004 ns
BIG vs. MED1	0.442 ns	−0.177 ns	0.003 ns
BIG vs. SMA	0.446 ns	−0.148 ns	0.004 ns
MED2 vs. MED1	0.432 ns	−0.144 ns	0.014 ns
MED2 vs. SMA	0.436 ns	−0.115 ns	0.001 ns
MED1 vs. SMA	0.430 ns	−0.190 ns	0.010*

ns not significant

*P<0.01

SMA) were significantly different ($P<0.01$), with a multilocus estimate of F_{ST} of 0.01 (Table 2).

Spatial distribution

Our study plot contained 792 *P. spruceanum* individuals (Table 1), covering ca. 24 % of the area. *P. spruceanum* showed a skewed size–class distribution with many small individuals and few large individuals (Fig. 1). The spatial distribution of plants was significantly different from that expected for random distribution for the cohorts SMA (t=0–25 m) and MED1 (t=2–11 m). For both cohorts, the L function curves exhibited a divergence from the null hypothesis of a random distribution, i.e., the observed $L(t)$ values exceeded the upper bound CIs (Fig. 3). Only MED1 cohort showed a spatial structure with both clumped and random patterns. The univariate analysis of the individuals in the MED2 and BIG cohorts showed no evidence of nonrandom spatial distribution. The magnitude of spatial aggregation showed a decrease from a high aggregation to random distribution with an increase in diameter class (SMA>MED1>MED2/BIG).

Spatial genetic structure (SGS)

Considering a 95 % CI, the spatial autocorrelation analysis detected a significant SGS up to a radius of approximately 10 m in SMA and MED1 cohorts, based on kinship coefficients (Fig. 4). This same pattern was observed in the SGS analysis of the SMA-MED1 cohorts together (n=240, Electronic Supplementary Material 3). A continuous decrease in the autocorrelation values was detected with increasing distances in MED1 cohort and from 65 m onward. The analysis showed significant negative values, suggesting that nearby trees are more related and distant trees are less related than the average (Fig. 4). In the MED2 and BIG cohorts, coancestry values were within the range of 95 % CIs in all of the distance classes as well as in the MED2-BIG cohorts together (n=105, Electronic Supplementary Material 4). The overall SGS across all cohorts (n=345) showed no spatial genetic structuring (Electronic Supplementary Material 5). Slopes (b_{log}) of correlograms of SMA and MED1 cohorts were significantly different ($P<0.025$) from the null hypothesis of no SGS ($b_{log}=0$) (Table 1). The overall slope of the correlogram was not significantly negative in the analysis of the MED2 and BIG cohorts.

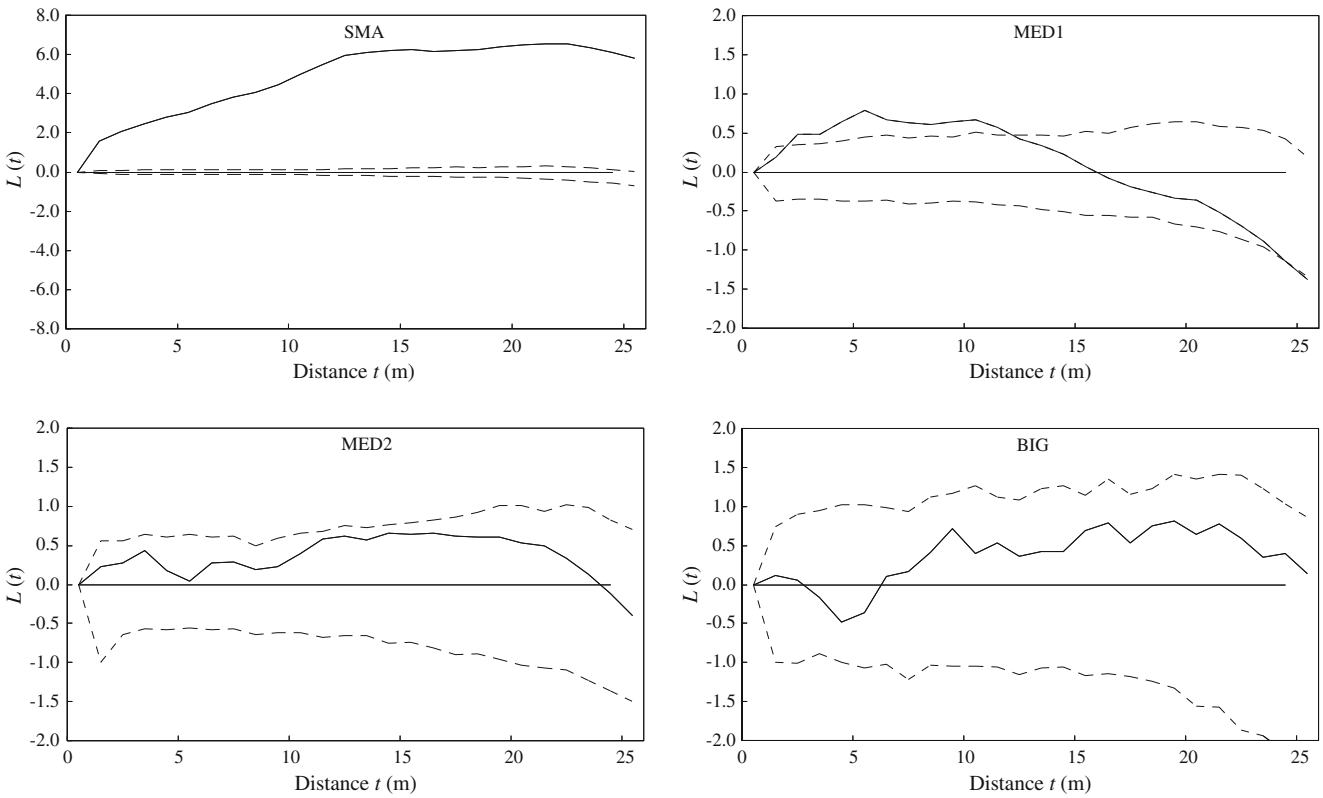

Fig. 3 Variation of the $L(t)$ function according to distance (t) in four diameter classes of *Protium spruceanum*. The *lines* correspond to observed data. *Broken lines* correspond to 99 % confidence intervals computed by Monte Carlo simulations under the hypothesis of complete spatial randomness

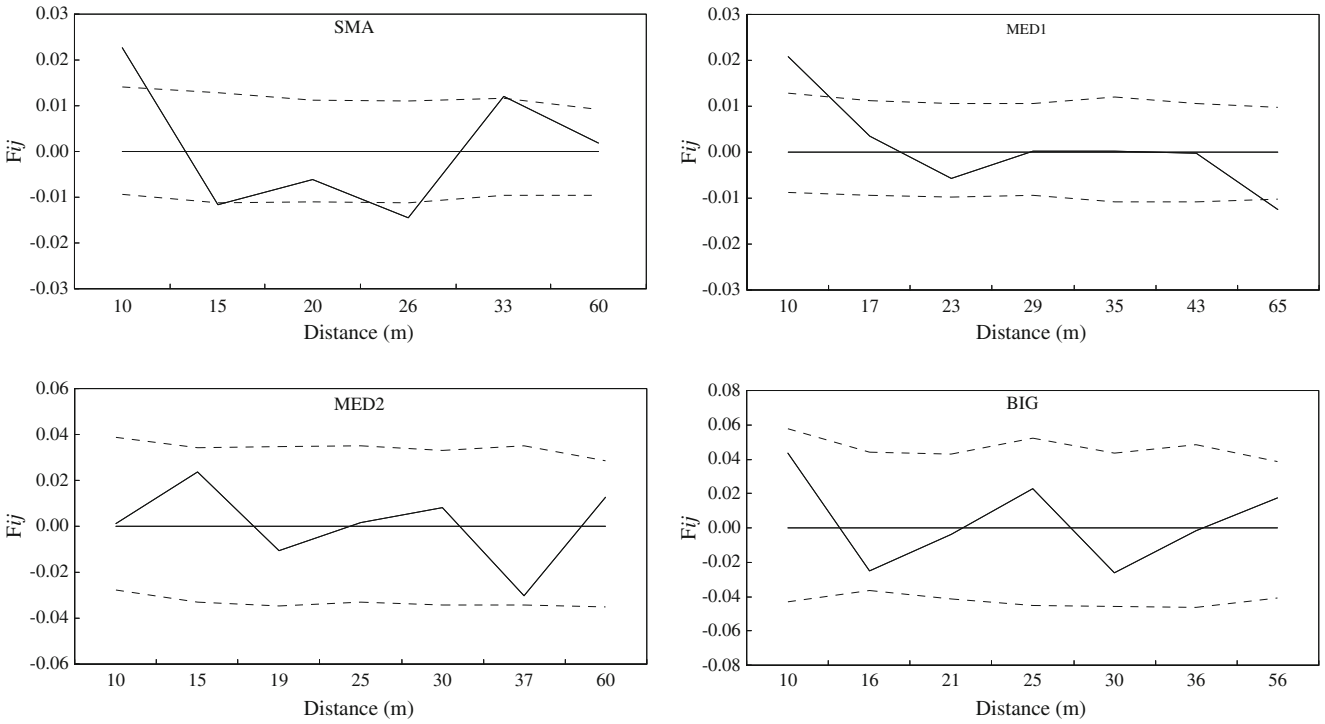

Fig. 4 Spatial genetic structure according to distance in four diameter classes of *Protium spruceanum*. *Dotted lines* represent upper and lower 95 % CIs around zero relatedness

Simulation of the random thinning

At the first distance interval for "pseudo BIG cohort", all tested parameters were not significant ($F_{(10,m)}$=0.025; b_{log}(SE)=−0.016 (0.010); Sp=0.016; P>0.05). The experimental random thinning results were similar to the empirical results for the BIG cohort, since $F_{(10,m)}$=0.043, b_{log}(SE)=−0.011(0.004) and Sp=0.011 (also not significant at deviations from random SGS; P>0.05). Thus, the experimental random thinning indicated no significant departure from empirical results at older stages (Fig. 5). Following our expectations (see "Experimental random thinning"), the random mortality (thinning) could be responsible for a reduction in SGS across age classes, since hypothesis (1) and (3) were corroborated.

Discussion

Genetic diversity

Our findings show that cohorts of *P. spruceanum* in a small forest fragment maintain high levels of allozyme diversity. In general, estimates of genetic diversity (H_e, f, and F_{ST}) within the study population were not significantly different among cohorts. This is similar to other studied tropical tree

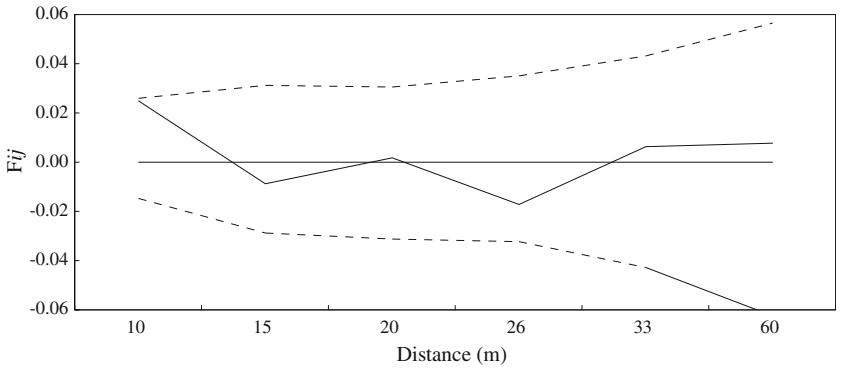

Fig. 5 Spatial genetic structure of 240 random individuals from the two smallest size classes (SMA and MED1). F_{ij} within each distance class was obtained by Monte Carlo simulations (1,000 times for randomly selected 45 individuals)

species (Conte et al. 2003; Hall et al. 1994), suggesting extensive gene flow (Kelly et al. 2004). The minor differences among cohorts suggest that gene flow was greater in the older generation; in fact, SGS was not significant for MED2 and BIG cohorts. These results support two expectations, since older trees are (1) offspring from local individuals that are no longer present in the population (e.g., through random mortality or logging) or (2) the product of long distance dispersal from trees that may have disappeared as the result of increasing human impact in the surrounding landscape.

Furthermore, our results suggest that the fragmentation event and recent bottlenecks (ca. 200 years ago), previously reported for this species and study site (Vieira and Carvalho 2008), did not change the level of genetic diversity between the old and young cohorts. This supports the conclusion that the impact of the recent episode of forest fragmentation for *P. spruceanum* populations may be insignificant to detect impacts on genetic diversity because preframentation trees continue to contribute to the gene pool (Vieira and Carvalho 2008). The BIG cohort trees sampled for this study may have been present prior to anthropogenic disturbances and their consistent contributions to the gene pool may be the cause of the low current rates of differentiation between old individuals prefragmentation and young individuals after fragmentation. Thus, it is likely that the young cohorts (SMA and MED1) come from local mating involving older individuals after anthropogenic impacts in the neighborhood area. In this context, few studies have examined the differences in the genetic diversity between cohorts pre- and post-fragmentation in the Brazilian Atlantic Rainforest. Torezan et al. (2005) used random amplified polymorphic DNA (RAPDs) to quantify genetic diversity within populations of *Aspisdosperma polyneuron*, a long-lived, late-reproducing tropical tree, from adults (prefragmentation, >300 years old) and seedlings (after-fragmentation, <50 years old). Their results showed a decrease in genetic variation among post-fragmentation cohorts in small fragments of the Atlantic Rainforest. Lowe et al. (2005) reviewed studies of fragmented habitats, and the results were consistent with the prevailing theory that inbreeding is often observed immediately following the impact of the event, but genetic diversity is lost slowly over subsequent generations, which for trees may take several hundred years or longer.

Fixation indices were negative and significantly different from zero for all cohorts (Table 1). Although the mean value of f across cohorts (−0.159) may indicate a general excess of heterozygotes above that expected for panmictic populations, the range of fixation indices from other neighboring forest fragments varies from −0.250 to 0.078 (Vieira and Carvalho 2008). This could be due to random variation, positive assortative mating or phenological differences within and among trees (El-Kassaby et al. 1984; Shaw and Allard 1982). Future studies should focus on multilocus and single locus outcrossing rates to provide a clearer picture of the factors influencing the negative fixation indices.

Spatial demographic and genetic structures

In general, spatial distribution pattern analyses have shown that the majority of tropical tree species exhibit varied aggregation across diameter classes (Condit et al. 2000; He et al. 1997; Hubbell 1979). Normally, this aggregation decreases with an increase in age, as reported for *Cecropia obtusifolia* (Epperson and Alvarez-Buylla 1997), *Alsesis blackiana* and *Platypodium elegans* (Hamrick et al. 1993), and *Shorea leprosula* (Ng et al. 2004). In this study, we also found significant spatial aggregation and clustering in small- and medium-diameter classes (SMA and MED1) than for medium- and large-diameter class trees (MED2 and BIG). The probable mechanisms for clustering in tropical trees have been discussed from the viewpoint of seed dispersal, gap recruitment, distance-dependent mortality, and herbivore predation (Condit et al. 2000; Itoh et al. 1997; Ng et al. 2004; Plotkin et al. 2000). Adult densities (195.8N ha^{-1}) and seed dispersal by gravity from the largest diameter class probably have the greatest influence on the occurrence of clustering in the small- and medium-diameter classes, suggesting dispersion from individuals and a radial distribution of the density.

Likely, seed shadow due to limited seed dispersal primarily determines the clustering in small-diameter classes. Thus, if seeds fall beneath the maternal tree, individuals in younger age classes should exhibit a more grouped spatial distribution and genetic familial structure. In addition, the distribution of *P. spruceanum* plants across the diameter classes followed the pattern of an inverted "J" curve, suggesting seedling bank behavior in association with SGS and subsequent density-dependent mortality. The level of relatedness detected here for SMA and MED1 cohorts at 0 to 10 m reflects the aggregation of these cohorts, as suggested by the SGS analysis (Fig. 4) and illustrated by the spatial distribution of individuals (Fig. 3). Hence, there is a correlation between demographic and fine-scale SGS in small distance classes. This is likely the consequence of restricted seed dispersal as indicated by Latouche-Halle et al. (2003), meaning that the limited area of aggregates corresponds to the dispersion distance from the maternal tree.

We found SGS in the young plants (SMA and MED1 cohorts), but this structure almost completely disappeared among the older plants (MED2 and BIG cohorts). The reduction of SGS with age could occur when juveniles show genetically random mortality that is density-dependent (Epperson and Alvarez-Buylla 1997). Skabo et al. (1998) argued that there is a tendency for this to happen because the family grouping caused by limited seed dispersal would be reinforced in each generation by short-distance pollen dispersal (Barbour et al. 2005) and biparental inbreeding;

however, the development of SGS in the juvenile cohort would be counteracted by selection against the effects of inbreeding as they mature (Hardner et al. 1998). Although no inbreeding was found in the SMA and MED1 cohorts, we found relatedness among the shortest distance class. Indeed, a fine-scale structure can develop when variance in the seed dispersal is smaller than variance in pollen dispersal (Kalisz et al. 2001). While this study focused on differences in SGS in contemporary cohorts, further investigations incorporating longitudinal data over generations is necessary.

The ecological and evolutionary processes (e.g., environmental heterogeneity, mating system, and colonization history) that affect spatial distribution patterns can also be contributing factors to the observed fine-scale SGS (Jones et al. 2006; Vekemans and Hardy 2004). In this study area, a large number of recruits (SMA class) was established in the east half of the plot, showing a skewed spatial distribution (Electronic Supplementary Material 1), while adult individuals are scattered randomly over the entire area. There are likely external factors (other than dispersal) determining the absence of recruitment in half of the plot such as soil and humidity or skewed spatial distribution of maternal plants (although this was not quantified for this study).

In the light of the Sp-based SGS review performed by Vekemans and Hardy (2004), SGS in *P. spruceanum* (Sp ~0.009) is consistent with other tree species (Sp=0.010), other plant species with animal-dispersed seeds (Sp=0.009) and *P. spruceanum* adult populations in four other forest fragments (Sp=0.008) (Vieira et al. 2010b). However, it is important to consider the sample size used in this study; we sampled 345 individuals inside of the plot and categorized them into four different diameter classes. Although the ability to detect SGS is associated with the sampling strategy and type of genetic marker (Cavers et al. 2005), our simulations of random thinning showed that the sample sizes (e.g., 45 individuals into BIG cohort) provided no sensitivity to SGS, given the rarefaction method of 240 genotyped individuals in young cohorts and the simulation method performed by Monte Carlo randomizations. In fact, the "pseudo BIG cohort" simulations showed no significant deviations from random SGS, similar to empirical data sets for the BIG cohort. According to Cavers et al. (2005), large sample sizes are required in cases where SGS is weaker than for the simulated population, mainly in species with effective seed dispersal mechanisms, which is not the case for *P. spruceanum*, because the studied population does not have effective seed dispersal.

Conclusions

The skewed size–class distribution of *P. spruceanum* indicates seedling bank behavior because seeds are mostly dispersed by gravity, producing natural regeneration of siblings in the neighborhood of the mother trees. Regeneration is likely the result of local seed production. Fine-scale SGS among seedlings and young individuals was detected to be significant and stronger than SGS among older individuals. As seedlings and younger individuals grow, competition among individuals within cohorts will induce thinning, shifting the cohorts from a clustered distribution to a random distribution as individuals age.

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