Fine-scale spatial genetic structure of eight tropical tree species as analysed by RAPDs

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The fine-scale spatial genetic structure of eight tropical tree species (*Chrysophyllum sanguinolentum*, *Carapa procera*, *Dicorynia guianensis*, *Eperua grandiflora*, *Moronobea coccinea*, *Symphonia globulifera*, *Virola michelii*, *Vouacapoua americana*) was studied in populations that were part of a silvicultural trial in French Guiana. The species analysed have different spatial distribution, sexual system, pollen and seed dispersal agents, flowering phenology and environmental demands. The spatial position of trees and a RAPD data set for each species were combined using a multivariate genetic distance method to estimate spatial genetic structure. A significant spatial genetic structure was found for four of the eight species. In contrast to most observations in temperate forests, where spatial structure is not usually detected at distances greater than 50 m, significant genetic structure was found at distances up to 300 m. The relationships between spatial genetic structure and life history characteristics are discussed.

Keywords: genetic distance, permutation test, RAPD, spatial genetic structure, tropical trees.

Introduction

Information about the spatial genetic structure of tree populations helps to elucidate the forces driving genetic dynamics. It has important implications for the management and conservation of forest genetic resources (Epperson, 1992), as well as for assessment of the expected impacts of forest exploitation and fragmentation (Young & Merriam, 1994).

Simulation studies have shown that restricted gene flow via pollen and seeds, the spatial distribution pattern of trees and microhabitat selection can all influence the spatial genetic structure of tree populations (Sokal & Wartenberg, 1983; Epperson, 1990; Epperson & Li, 1997; Doligez *et al.*, 1998). However, in contrast to theoretical expectations, experimentally observed spatial structure in temperate tree populations is generally weak, in most cases restricted to a scale of 5–80 m, and rarely consistent across loci (Schnabel *et al.*, 1991; Geburek, 1993; Sork *et al.*, 1993; Merzeau *et al.*, 1994; Young & Merriam, 1994; Shapcott, 1995; Leonardi *et al.*, 1996). Studies of tropical tree populations have

generally featured trees that occur at low densities in natural populations and for which no strong pattern of spatial structure has been shown (Hamrick *et al.*, 1993; Boshier *et al.*, 1995; Loiselle *et al.*, 1995; Doligez & Joly, 1997; Caron *et al.*, 1998).

Smouse & Peakall (1999) explain these weak patterns by a lack of sensitivity in the statistical procedures. They illustrate the advantages of undertaking multilocus analysis, using new polymerase chain reaction (PCR)-based genetic markers (e.g. RAPD), in combination with a multivariate approach to autocorrelation analysis. Following the suggestions of Smouse & Peakall (1999), we applied a genetic distance method to analyse the fine-scale spatial genetic structure detected by RAPD in a silviculture trial in French Guiana of eight tropical tree species: *Carapa procera* Aubl. (Meliaceae), *Chrysophyllum sanguinolentum* L. (Sapotaceae), *Dicorynia guianensis* Amshoff (Caesalpiniaceae), *Eperua grandiflora* Aubl. (Caesalpiniaceae), *Moronobea coccinea* Aubl. (Clusiaceae), *Symphonia globulifera* L. f. (Clusiaceae), *Virola michelii* Aubl. (Myristicaceae), *Vouacapoua americana* Aubl. (Caesalpiniaceae). Owing to low tree densities and the relatively undisturbed history of the study site, an *a priori* expectation was for a stronger spatial structure in

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the analysed tropical tree species than has been found previously in temperate trees. A further aim of the study was to search for relationships between spatial genetic pattern and different life history characteristics of the species, e.g. pollen and seed dispersal agents and tree density.

Material and methods

Study site

All samples for the study were collected at the Paracou experimental trial (5°18'N, 52°53'W) near Sinnamary in French Guiana. The forest at Paracou is classified as lowland moist forest on ferrallitic soil. The mean annual

rainfall in this area is 3076 mm with peaks in April–June and December. There are two dry seasons; a short one in March and a longer one from August to November. The experimental trial consists of 16 natural rainforest plots, 15 are 6.25 ha in size and one covers an area of 25 ha (Fig. 1). Since 1984, all trees with d.b.h. > 10 cm have been measured by CIRAD-Forêt. The trees have been mapped and, for many, families, genera and species identified. Three silvicultural treatments of different intensities were applied to different plots from 1986 to 1988, but some were left as unlogged controls. Since then, effective recruitment and strong microenvironmental variation have caused only slight differences in adult densities between logged and unlogged plots. A detailed description of the experimental trail and

Fig. 1 Position of all trees of *Moronobea coccinea* [d.b.h. > 10 cm] in the Paracou experimental site and distribution of the sampled trees of *M. coccinea*.

treatments can be found elsewhere (Forget *et al.*, 1999; Schmitt & Bariteau, 1988).

Species description

Distribution and tree aggregation. An overview of the characteristics of the eight canopy tree species studied is given in Table 1. Two species have a broad distribution; *Carapa procera* occurs across the Guyana plateau and also in West and Central Africa, and *Symphonia globulifera* is distributed across Central and South America and can be found in West Africa. The other six species are more or less restricted to the Guyana plateau and the neighbouring territories of Brazil and Venezuela.

Clark and Evans' index (R) (Ripley, 1981) was used as an indicator of fine-scale spatial distribution of the species within all 16 plots of the study site (Table 1):

$$0 < R = \frac{\bar{r}_{\text{observed}}}{\bar{r}_{\text{expected}}}; \bar{r}_{\text{observed}} = \frac{\sum_{i=1}^N r_i}{N}; \bar{r}_{\text{expected}} = \frac{1}{2 \cdot \sqrt{\frac{N}{F}}}$$

where r_i is the distance of tree i to the next neighbour, N is the total number of trees and F is the size of the plot (m^2).

Values of index (R) < 1 indicate a clumped and aggregated distribution, whereas an index value of 1 indicates a random distribution. A regular distribution is described by values (R) > 1 . According to mean (R) values, *Dicorynia guianensis*, *Vouacapoua americana* and *Eperua grandiflora* all have a clumped spatial distribution. The diameter of the aggregates is relatively small for *Dicorynia guianensis* (50–100 m) but relatively large for *Vouacapoua americana* and *Eperua grandiflora* (100–600 m). The spatial distribution of the other five species is random within the plots analysed. A detailed study of the spatial distribution of *Carapa procera* and *Vouacapoua americana* is given by Forget *et al.* (1999).

The density of trees (d.b.h. > 10 cm) varied from 11.66 individuals per ha (*Symphonia globulifera*) to 2.44 individuals per ha (*Virola michelii*). Some species had large standard deviations for density between plots (*Chrysophyllum sanguinolentum*, *Eperua grandiflora*, *Moronobea coccinea*, *Vouacapoua americana*). These species tended to occur in large aggregates (up to several 100 m) and were often correlated with specific environmental conditions (Forget *et al.*, 1999).

Dispersal, breeding systems, and niche. Two of the species are pollinated by birds: *Moronobea coccinea* (hummingbirds) and *Symphonia globulifera* (hummingbirds and perching birds). *Chrysophyllum sanguinolentum* and *Symphonia globulifera* are pollinated by bats. Depending on behaviour, both bird and bat pollination can lead to long-distance pollen transport. The other six

species are pollinated by insects, which is also a feature of the pollination systems of *Chrysophyllum sanguinolentum* and *Symphonia globulifera*. Seed dispersal for *Carapa procera*, *Dicorynia guianensis*, *Eperua grandiflora*, *Moronobea coccinea* and *Vouacapoua americana* (< 50 m) is primarily by gravity, and secondarily by rodents (Forget *et al.*, 1999) and is therefore considered restricted. For *Chrysophyllum sanguinolentum*, seeds are distributed by monkeys and those of *Virola michelii* are dispersed by birds, monkeys and kinkajous (*Potos flavus*). Bats have been observed distributing the seeds of *Symphonia globulifera*. Birds, bats and monkeys are known to disperse seeds up to several hundred metres (Howe, 1990; Julliot, 1994).

Virola michelii is dioecious and the other seven species have a bisexual breeding system (hermaphroditic or monoecious). *Carapa procera*, *Chrysophyllum sanguinolentum*, *Symphonia globulifera* and *Virola michelii* flower once a year. *Dicorynia guianensis* has a three-year flowering cycle; after two years of flowering there is a barren year (Caron *et al.*, 1998). The flowering of *Moronobea coccinea* follows a supra-annual cycle. With an irregular number of years without any flowers, the flowering of *Eperua grandiflora* and *Vouacapoua americana* is also irregular and hard to predict. According to shade tolerance and light demands *Carapa procera*, *Symphonia globulifera* and *Virola michelii* are classified as heliophilic species which favour gaps for regeneration (Favrichon, 1994). The other species are hemitolerant or shade-tolerant. *Chrysophyllum sanguinolentum* and *Virola michelii* are mostly distributed in dry areas with good water drainage. The other species can tolerate wet soil conditions; *Symphonia globulifera* often occurs in wet areas at the Paracou site.

Sampling

The analysed data sets were the result of different projects with variable objectives. Beside the estimation of spatial structure, trees were sampled for gene diversity and mating system estimation. Each of these objectives has its own sampling criteria. An optimal strategy for the analysis of spatial structure would have been to sample trees in each plot proportional to their density. However, the realized sampling strategy did not fit this approach in all cases. A chi-squared test was used to check whether the sampling was proportional to the density of trees in the plots. No significant deviation was found for *Chrysophyllum sanguinolentum*, *Carapa procera* and *Moronobea coccinea*. An example of the location of sampled trees is illustrated for *Moronobea coccinea* in Fig. 1. For the other species, the sampling was significantly different from a proportional approach.

Table 1 Geographical distribution, mean and standard deviation of aggregation index (R) of Clark and Evans (Ripley, 1981) for spatial distribution within 16 plots of the experimental trial at Paracou ($R < 1$ indicates clumped distribution, $R = 1$ indicates random distribution and $R > 1$ show regular distribution of the tree species), mean and standard deviation of individuals per ha in 16 plots at Paracou, agents for pollen and seed dispersal, sexual system, flowering phenology, shade tolerancy and water drainage demands of the eight studied tree species

Scientific name	Family	Geographical distribution	R (Paracou)	$N \text{ ha}^{-1}$ > 10 cm d.b.h.	Pollinator	Seed dispersal agents	Sexual system	Flowering phenology	Shade tolerance	Water drainage
<i>Carapa procera</i>	Meliaceae	Guyana plateau, Amazonia, West and Central Africa	1.02 ± 0.12	6.43 ± 1.56	Insects	Rodents < 30 m	Bisexual	Regular, spread	Heliophilic	Intermediate
<i>Chrysophyllum sanguinolentum</i>	Sapotaceae	Guyana plateau, Amapa, South Venezuela	1.03 ± 0.19	3.17 ± 2.02	Insects, bats	Monkeys > 50 m	Bisexual	Regular, spread	Tolerant	Dry
<i>Dicorynia guianensis</i>	Caesalpinhiaceae	Guyana plateau, Amazonia	0.73 ± 0.09	6.16 ± 2.60	Insects	Gravity, rodents < 50 m	Bisexual	Regular biannual, synchrony dry	Hemitolerant	Intermediate
<i>Eperua grandiflora</i>	Caesalpinhiaceae	French Guiana, Surinam, Amapa	0.83 ± 0.28	6.80 ± 6.20	Insects	Gravity < 30 m	Bisexual	Irregular synchrony	Hemitolerant	Intermediate dry
<i>Moronobea coccinea</i>	Clusiaceae	Guyana plateau	1.01 ± 0.15	3.37 ± 3.17	Humming birds	Gravity, rodents < 50 m	Bisexual	Supra-annual	Hemitolerant	?
<i>Symphonia globulifera</i>	Clusiaceae	Central and South America, West Africa	1.00 ± 0.06	11.66 ± 3.87	Humming birds, perching birds, bats	Bats, gravity > 30 m	Bisexual	Regular, spread	Heliophilic	Wet
<i>Virola michelii</i>	Myristicaceae	Guyana plateau, Para, Amapa	1.06 ± 0.40	2.44 ± 1.39	Insects	Birds, kinkajou > 100 m	Dioecious	Regular, spread	Heliophilic	Dry
<i>Youacapoua americana</i>	Caesalpinhiaceae	French Guiana, Surinam, Para	0.82 ± 0.18	8.38 ± 6.38	Insects	Rodents < 30 m	Bisexual	Irregular synchrony	Tolerant	Intermediate

An overview of sample size and gene marker system used for each species is given in Table 2 and Table 3.

Genetic analysis

DNA extraction. Owing to the high density of trees in tropical forest, and the inherent difficulties of reaching the canopy to collect leaves, DNA was extracted from cambial tissue. Leaves were, however, used for a small number of individuals of *Dicorynia guianensis*. A thin piece of cambium (diameter 2 cm) was stamped out of the tree trunk using a leather punch and stored in a mixture of absolute ethanol and 2% CTAB in the ratio 70:30 vv/vv in small tubes. In the field the tubes were

kept at ambient temperature and in the laboratory each sample was dried at 50°C overnight (E. Bandou, unpubl. method). The extraction method followed a modified protocol of Doyle & Doyle (1987). DNA extracts were quantified before use by agarose gel electrophoresis against a concentration standard but were sufficient for RAPD analysis in all cases.

RAPD conditions. PCR reactions (20 µL) contained 5–10 ng of genomic DNA; 250 µM of each of dATP, dCTP, dGTP, and dTTP (Pharmacia); 2.5 mM of MgCl₂, 2 µM of 10-mer primer (Operon); and 0.8 unit of *Taq* DNA polymerase (Gibco BRL). Between 6 and 15 10-mer primers were used for each species (Table 2,

Table 2 Number of sampled individuals, number of polymorphic fragments and code of the primers used to analyse the eight species in the Paracou experimental trial. See Table 3 for primer sequences

Species	No. individuals	No. of polymorphic fragments	Primers
<i>Carapa procera</i>	65	46	B-11, E-04, M-05, M-12, N-12, N-15, R-12, W-05, W-12, Y-04, Y-05, Y15
<i>Chrysophyllum sanguinolentum</i>	68	48	E-02, E-05, E0-7, Y-04, Y-06, Y-07, Y-10, Y-13, Y-14, Y-15, Y-16
<i>Dicorynia guianensis</i>	70	52	Y-09, Y-15, Y-16, Y-17, Y-18, Y-19, Y-20
<i>Eperua grandiflora</i>	53	31	A-01, J-05, K-09, K-15, K-16, K-17, K-18, K-19, Q-14, U-01
<i>Moronobea coccinea</i>	68	40	E-16, E-20, K-09, K-16, K-17, K-19, K-20, Y-04, Y-09, Y-10, Y-11, Y-17, Y-20
<i>Symphonia globulifera</i>	46	39	B-12, E-06, H-14, I-13, I-14, I-20, O-04, P-03, P-14, R-09, S-10, U-19, Y-14, Y-20
<i>Virola michelii</i>	80	30	Y-05, Y-09, Y-10, Y-14, Y-16, Y-18
<i>Vouacapoua americana</i>	59	40	E-02, E-06, E-19, Y-11, Y-13, Y-14, Y-17

Table 3 Code and sequence of the Operon primers used

code	Sequence 5' to 3'	code	Sequence 5' to 3'	code	Sequence 5' to 3'
A-01	CAGGCCCTTC	K-16	CTCCTGCCAA	W-05	GGCGGATAAG
B-11	GTAGACCCGT	K-17	GAGCGTCGAA	W-12	TGGGCAGAAG
B-12	CCTTGACGCA	K-18	CCTAGTCGAG	Y-04	GGCTCCAATG
E-02	GGTGCGGGAA	K-19	CACAGGCGGA	Y-05	GGCTGCGACA
E-04	GTGACATGCC	K-20	GTGTCCGCGAG	Y-06	AAGGCTCACC
E-05	TCAGGCAGGT	M-05	GGGAACGTGT	Y-07	AGAGCCGTCA
E-06	AAGACCCCTC	M-12	GGGACGTTGG	Y-09	AGCAGCGCAC
E-07	AGATGCAGCC	N-12	CACAGACACC	Y-10	CAAACGTGGG
E-16	GGTGACTGTG	N-15	CAGCGACTGT	Y-11	AGACGATGGG
E-19	ACGGCGTATG	O-04	AAGTCCGCTC	Y-13	GGGTCTCGGT
E-20	AACGGTGACC	P-03	CTGATACGCC	Y-14	GGTCGATCTG
H-14	ACCAGGTTGG	P-14	CCAGCCGAAC	Y-15	AGTCGCCCTT
I-13	CTGGGGCTGA	Q-14	GGACGCTTCA	Y-16	GGGCCAATGT
I-14	TGACGGCGGT	R-09	TGAGCACGAG	Y-17	GACGTGGTGA
I-20	TCATCCGAGG	R-12	ACAGGTGCGT	Y-18	GTGGAGTCAG
J-05	CTCCATGGGG	S-10	ACCGTTCCAG	Y-19	TGAGGGTCCC
K-09	CCCTACCGAC	U-01	ACGGACGTCA	Y-20	AGCCGTGGAA
K-15	CTCCTGCCAA	U-19	GTCAGTGCGG		

Table 3). A thermal cycler (GeneAmp PCR system 9600, Perkin Elmer) was programmed for one preamplification step of 1 min at 94°C, followed by 20 cycles, with a denaturation step of 30 s at 94°C, annealing step of 50 s, progressively decreasing by 1 s per cycle, at 37°C, and extension step of 1 min at 72°C. Finally 30 more cycles, with a denaturation step of 30 s at 93°C, annealing step of 30 s at 37°C, and extension step of 1 min at 72°C, were used. The final 15 extension steps were progressively extended by 5 s per cycle and the last cycle was followed by 2 min at 72°C. After amplification, 5 µL of the samples was loaded and fragments separated by electrophoresis in 1.8% agarose gels using a 0.5 × TBE running buffer.

Data analysis

The RAPD data for each individual were recorded as a matrix where a value of 1 indicated fragment presence and 0 coded fragment absence. Only those fragments that were amplified reproducibly and facilitated a clear band identification were used in the study. As suggested by Smouse & Peakall (1999), a genetic distance method was used to analyse the spatial genetic structure of multilocus RAPD profiles. Using the program SGS (Degen, 2000), spatial genetic structure was analysed as mean genetic distances (D_k) between all pairs of trees belonging to a given spatial distance class (k). In the case of spatial genetic structure, the genetic distance, D_k , increased with spatial distances (Degen & Scholz, 1998; Vendramin *et al.*, 1999).

The genetic dissimilarity (D_{ij}) between the binary profile of two individuals, i and j , was computed using Tanimoto's distance (Deichsel & Trampisch, 1985):

$$0 \leq D_{ij} = 1 - \frac{w_{ij}}{w_{ij} + y_i + y_j} \leq 1,$$

where w_{ij} represents the number of fragments shared between individuals i and j , the number of fragments which were only recorded in either individual i or j is indicated by y_i and y_j , respectively. For random spatial structure, the probability that two genotypes are selected for a pairwise distance calculation is the same for all distance classes, it only depends on the frequency of genotypes. Hence, the overall mean of genetic distances (D_r) between all trees serves as a reference for random spatial structure. Distances below this reference indicate a spatial structure where genotypes are more similar than expected for a random distribution and values above this reference indicate greater genetic differences than expected for a random spatial genetic structure. The plot of spatial distance classes

against mean genetic distance (D_k) is called a distogram.

In a pre-analysis of the data it was shown that the method of calculating mean genetic distances is more sensitive to detecting spatial genetic structures than using coefficients of correlation between genetic and spatial distances (Smouse & Peakall, 1999).

Test. A permutation procedure using Monte Carlo simulations was applied to test significant deviation from random spatial distribution of the mean Tanimoto distance D_k (Manly, 1997). Each permutation consisted of a random redistribution of the RAPD profiles over the spatial coordinates of the sampled trees. For each of the spatial distance classes h_k , observed values were compared with the distribution obtained after 5000 permutations. A 99% confidence interval for the parameters was constructed as the interval from the 25th to the 4975th ordered permutation estimates (Streiff *et al.*, 1998). Each distogram has, according to the number of spatial distance classes, several D_k -values that have to be tested. In order to control the overall probability of mistakenly declaring D_k -values as significant, the significance level used is set at 5%/m, where m is the number of values tested. The Bonferroni inequality then ensures that the probability of erroneously declaring any difference significant is 0.05 or less (Manly, 1997; p. 108). With eight distance classes, the significance level is 5/8 = 0.62%. To guarantee this level, we used the 99% confidence interval with 0.5% of the D_k -values outside the interval at each side of the distribution.

Results

For all eight species, the mean genetic distances were calculated for eight different spatial distance classes of 150 m each from 0 to 1200 m. The size of 150 m for each spatial distance class was chosen so that in all cases at least 30 pairs of trees belonged to each class. This critical limit was suggested for statistical reasons by Wartenberg (Doligez & Joly, 1997). All distograms and the 99% confidence intervals of the permutation tests are given in Fig. 2. For six out of eight species, the mean genetic distance, D_k , of the first two distance classes is below that of the reference value.

The distograms demonstrate that significant D_k -values are found for the first 150 m for *Carapa procera*, *Virola michelii* and *Vouacapoua americana* (Fig. 2). *Moronobea coccinea* has significant values for the first two distance classes (< 300 m). The remaining species displayed no significant values for any distance class, D_k -values of *Chrysophyllum sanguinolentum*,

Fig. 2 RAPD distogram of *Carapa procera*, *Chrysophyllum sanguinolentum*, *Dicorynia guianensis*, *Eperua grandiflora*, *Moronobea coccinea*, *Symphonia globulifera*, *Virola michellii* and *Vouacapoua americana*, P , probability for D_k values to be greater than the observed values estimated with 5000 permutations.

Dicorynia guianensis and *Eperua grandiflora* are close to the limit of confidence.

We also tested whether calculating distograms for single RAPD fragments was more or less sensitive than detecting spatial genetic structure using a multilocus approach. By using the same procedure that was applied to multilocus calculation for *Moronobea coccinea* and *Carapa procera*, distograms were computed using each RAPD fragment for eight spatial distance classes (each of 150 m in size). Again the significance of each genetic distance D_k was tested for 5000 permutations. The tests for single RAPD fragments for *Monorobea coccinea* revealed that 14% of all D_k -values were significant (6.24% for *Carapa procera*), whereas when using the multilocus approach, 25% of values were significant (12.5% for *Carapa procera*). Thus, the empirical results presented herein are consistent with the theoretically expected advantage of the multilocus approach (Smouse & Peakall, 1999).

Discussion

Application of RAPDs for analysis of spatial genetic structure

RAPDs are sometimes criticized owing to reproducibility problems (Ford-Lloyd & Painting, 1996) and their sensitivity to small alterations in PCR conditions. Such nonsystematic bias may mask spatial genetic structure, but it is highly unlikely that such variation could generate spatial structure as an artefact. Another issue in this context is the mode of inheritance of RAPDs. The general interpretation is that RAPDs are biparentally inherited dominant markers. According to the relationship between the genome sizes of the nuclear and cytoplasmic DNA there should only be a small proportion of fragments that are located within organelles, and which will be inherited uniparentally. Olmstead & Palmer (1994) indicate that 'the typical chloroplast genome size in angiosperms ranges from 135 to 160 kbp...'. Information on the nuclear genome comes from Arumuganathan & Earle (1991), who reported on the nuclear DNA content of some important plant species. For angiosperm trees, they listed a range of values from 396 Mbp (*Citrus*) to 796 Mbp (*Malus*), with some species exhibiting great intraspecific variation, e.g. *Prunus*, 300–800 Mbp. Thus, the relationship between the size of organelle and nuclear genomes of trees is in the range of 1:300–1:5000. This means that if RAPDs are distributed randomly over the whole genome, the proportion of uniparentally inherited fragments should be low. Lu *et al.* (1995) analysed the inheritance of RAPD fragments for *Pinus sylvestris*. In their study, segregating fragments were either present or absent

confirming the dominant character of RAPDs. None of the detected fragments appeared to originate from uniparentally inherited cytoplasmic DNA. In contrast to these findings, Aagaard *et al.* (1998) screened the inheritance of 237 RAPD fragments in Douglas fir and found that 19% of the fragments originated in the mitochondrial and chloroplast genome. Hence, in the absence of inheritance data for the RAPD fragments in the present study, we interpret them as a mixture of dominant biparentally and maternally inherited markers. The fragments are affected by both the pollen flow and the seed flow.

Spatial structure and seed dispersal

The seeds of *Carapa procera*, *Dicorynia guianensis*, *Eperua grandiflora*, *Moronobea coccinea* and *Vouacapoua americana* are dispersed primarily by gravity but are also distributed by rodents (secondary dispersal), not more than 50 m away from the mother tree (Forget, 1992; Forget *et al.*, 1999). With the exception of *C. procera*, trees (d.b.h. > 10 cm) of these species had an aggregated spatial distribution in the Paracou experimental site (see Clark and Evans' Index (R) in Table 1). These aggregates could also be caused by microenvironmental differences favouring different species in different patches (Forget *et al.*, 1999). Three of five species in this group displayed a significant spatial genetic structure for the first distance class (<150 m).

The seeds of *Chrysophyllum sanguinolentum*, *Symphonia globulifera* and *Virola michelii* are distributed primarily by animals and hence would be expected to be dispersed over large distances. *Symphonia globulifera* and *Chrysophyllum sanguinolentum* showed no spatial genetic structure, in contrast to *Virola michelii*. Hence, no simple causal link was found between seed dispersal and spatial genetic structure (short seed dispersal, strong genetic structure; long seed dispersal, weak genetic structure).

The fine-scale spatial structure for maternally inherited cpDNA markers have been studied for *Dicorynia guianensis* in one plot in Paracou (Caron *et al.*, 2000), where significant autocorrelation was detected for adult trees up to 130 m; this significant structure was interpreted to be primarily the result of limited seed dispersal.

Ecological investigations of frugivory and seed dispersal of the red howler monkeys in French Guiana, have shown that the monkeys regularly use a limited number of sleeping sites and exhibit a very long digestive time. Concentrations of faeces which contain large numbers of ingested seeds, occur under sleeping trees (Julliot, 1994). As a consequence of this behaviour, long-distance seed dispersal by monkeys does not

necessary imply a random distribution of seeds and may produce aggregates of saplings which consist of single tree progenies. For some tropical tree species the effect of restricted seed dispersal may be overshadowed by high mortality of seedlings in the neighbourhood of parent tree (Harms *et al.*, 2000). This phenomenon has been studied in the bird-dispersed *Virola surinamensis* in Panama (Howe, 1990). As part of the investigation, 54% of seeds were scattered randomly in the forest, while 46% were dropped under the parent trees. Owing to the high density of seed predators and pathogens, seed and seedling mortality under the parent trees exceeded 99.99%. Only seeds dispersed away from the parent tree had an appreciable chance of survival. This tendency could help explain why tree species with restricted seed dispersal strategies exhibit no or very weak spatial genetic autocorrelation in the most proximate distance classes.

Using allozymes, Hamrick *et al.* (1993) studied the influence of seed dispersal mechanism on the genetic structure of four tropical tree species at Barro Colorado Island in Panama. They found that *Platypodium elegans*, a low-density, wind-dispersed species, displayed the strongest spatial structure, while *Swartzia simplex*, a high-density, bird-dispersed species, had only weak spatial structure. They also compared the spatial genetic structure at different life history stages. For two species, *Platypodium elegans* and *Alseis blackiana*, spatial genetic structure present in the seedling cohort disappeared in later life history stages.

Significant spatial structure was shown in a distance class of size < 150 m for *Carapa procera* and *Vouacapoua americana* and < 300 m for *Moronobea coccinea* (Fig. 2). Because the dispersal of seeds is expected to be below this range, this can be seen either as spatial structure developed over a long period of time in a natural forest or as the combined effect of seed and pollen dispersal.

As many of the species were noted as having seed dispersal distance < 50 m, it is possible that greater spatial structure may have been detected if it had been possible to further partition the 150 m classes. This was restricted by the sample sizes.

Spatial structure and pollen dispersal

Chrysophyllum sanguinolentum (bats and insects), *Moronobea coccinea* (hummingbirds) and *Symphonia globulifera* (hummingbirds, perching birds and bats) are ranked as species with high pollen dispersal capability. The pollen of the other species studied here are distributed by insects and dispersal is therefore expected to be more limited. A clear relationship between spatial genetic structure and this life history trait was, however, not evident. For example, *Symphonia globulifera* and

Chrysophyllum sanguinolentum, which are bat and/or bird pollinated, showed no genetic structure in the distograms (Fig. 2), whereas genetic structure was shown by *Moronobea coccinea*, a hummingbird pollinated species. One potential problem is that there are large variations in the potential flight distances of different insect pollinators (Roubik, 1989). Hence, a simple classification such as insect pollinated and noninsect pollinated species may be insufficient.

Spatial structure and density of individuals

The occurrence of trees of *Symphonia globulifera* at relatively high density (11.66 trees ha⁻¹) may explain the absence of any spatial genetic structure. In areas with high densities of individuals, overlapping seed shadows may produce a mixture of different single tree progenies. In such cases only weak spatial structure is expected (Hamrick *et al.*, 1993).

Spatial structure and sampling strategy

For all eight species, samples were taken from individuals located in different plots of the experimental site at Paracou. For *Chrysophyllum sanguinolentum*, *Carapa procera* and *Moronobea coccinea* the sampling was proportional to the density in each plot. For the other species, some plots were over-represented. Because the permutation test made a random redistribution of the RAPD profiles over the existing spatial co-ordinates of the trees, the chance that significant spatial structure could be detected as a result of the location of sampled trees is effectively eliminated.

The total sample is an amalgamation of data from different trial plots where intensity of sampling is high, but between which there has been no sampling (e.g. Fig. 1). This sampling design was determined during the original design of the experimental Paracou trial (Schmitt & Bariteau, 1988), which aimed primarily to investigate ecological and species distribution questions. How suitable then is this sampling strategy to detect 'real' spatial genetic structure within populations? The construction of distograms is based on mean genetic distances, D_k , computed for pairs of trees belonging to the same spatial distance classes. The precision of the estimated D_k value increases with increasing numbers of trees belonging to that distance class. Generally, our groupwise sampling design led to an over-representation of pairs of trees separated by short spatial distances and an under-representation of pairs of trees separated by longer distances. Hence, it may be that the spatial genetic structure present at the larger scale is not detected by the imposed sampling design but it is unlikely that the significant genetic structures detected in

this study within the proximate distance classes are an artefact.

Advantage of the new approach

The application of a multilocus approach using PCR-based genetic markers in combination with the genetic distance calculation method used have been shown to be more sensitive and efficient in detecting spatial genetic structure than former approaches using allozyme data and single allele statistics such as Moran's Index (Smouse & Peakall, 1999). The results of the present study fully support this observation. For four of the eight species studied here, significant spatial structure was revealed using RAPD analysis (Fig. 2). In a former study of spatial genetic structure of *Carapa procera* at the same experimental site, no spatial structure was found based on allozyme variation (Doligez & Joly, 1997). In contrast, the present RAPD study detected significant spatial structure up to 300 m for this species. Differences in sample sizes cannot explain these contrasting results, as for allozymes more samples were analysed.

Acknowledgement

This study was financially supported by two European Union projects (program INCO: TS3*CT94-0316, STD3 project) and two French grants: one from BRG (Bureau des Ressources Génétiques) and a grant from the French Ministry of Environment (SOFT-project 1995). We are thankful to Cirad-Forêt for giving us access to the Paracou experimental site and for providing data. We wish to thank Catherine Julliot and Damien Bonal for helpful information on species ecology and Andrew Lowe, Cyril Dutech and two anonymous reviewers for useful comments on the manuscript.

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