

Research Article

Landscape-Level and Fine-Scale Genetic Structure of the Neotropical Tree *Protium spruceanum* (Burseraceae)

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Knowledge of genetic structure at different scales and correlation with the current landscape is fundamental for evaluating the importance of evolutionary processes and identifying conservation units. Here, we used allozyme loci to examine the spatial genetic structure (SGS) of 230 individuals of *Protium spruceanum*, a native canopy-emergent in five fragments of Brazilian Atlantic forest (1 to 11.8 ha), and four ecological corridors (460 to 1 000 m length). Wright's F_{ST} statistic and Mantel tests revealed little evidence of significant genetic structure at the landscape-scale ($F_{ST} = 0.027$; $rM = -0.051$, $P = .539$). At fine-scale SGS, low levels of relatedness within fragments and corridors ($Sp = 0.008$, $P > .05$) were observed. Differences in the levels and distribution of the SGS at both spatial scales are discussed in relation to biological and conservation strategies of corridors and forest fragments.

1. Introduction

Forest loss and spatial isolation of natural populations affect negatively the reproductive success of many tropical plants [1, 2]. In contrast, some studies have shown that the habitat fragmentation had resulted in facilitated pollen movement [3, 4], whilst gene flow measured among populations of several insect-pollinated woody plant species [5, 6] and in a wind-pollinated tree species [7] was not reduced, facilitating too the long distance dispersal and high genetic diversity of remnant populations [8]. However, when comparing continuous and naturally fragmented populations in a tropical tree species, fragmented populations exhibited slightly lower genetic diversity [9]. Landscape structures, such as ecological corridors, are then an important alternative in the demographic and genetic connection of isolated forest fragments, thus minimizing the negative effects of habitat fragmentation [10]. Nonetheless, most studies deal only with the use of corridor's importance for fauna conservation [11];

little is known about corridor importance for plant species mainly on genetic processes [12].

The genetic differentiation in fragment/corridor may be quantified with indirect methods which are based on the structure of neutral genetic diversity amongst populations, contributing to the knowledge of how landscape characteristics structure populations [13]. Moreover, studies of spatial genetic structure (SGS) provide insight into micro-evolutionary patterns by elucidating the movement of genes at a range of spatial scales [14]. At landscape-level scales between natural populations, genetic structure has been attributed to historical factors and isolation by distance [15]. At fine-scale SGS, seed dispersal and demographic processes acting at specific temporal and spatial scales probably have the greatest influence on the presence or absence of significant fine-scale genetic structuring [16].

The forest remnants in southern Minas Gerais state are characterized by a hilly topography covered by Atlantic seasonal forests. These plant formations were seriously

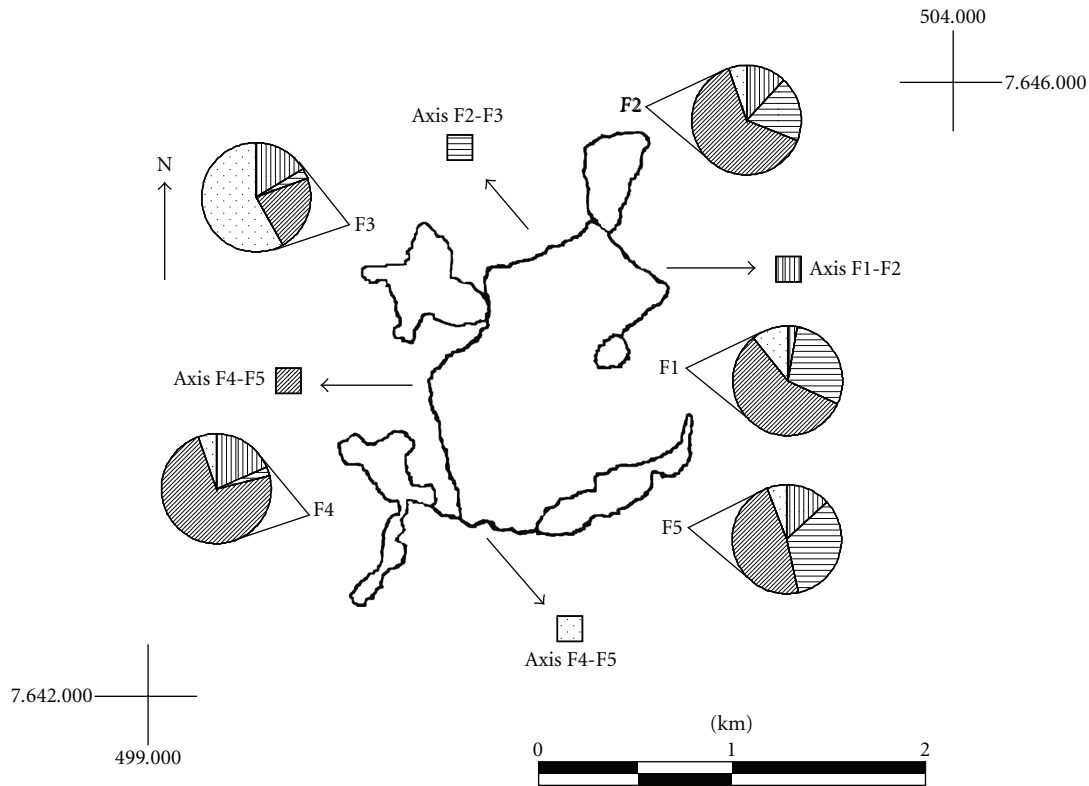


FIGURE 1: Location of the study system with forest fragments and ecological corridors in Minas Gerais state, Brazil. F1 to F5 (fragments) and Axis F1-F2 to F4-F5 (ecological corridors). Distances between fragments varied from 0.8 to 2 km. The circles represent the contribution to total genetic differentiation of each fragment with the corridors, using allozymes.

plundered since European occupation goes back to colonial times ca. two centuries ago, resulting in the fragmentation and isolation of these populations at a particularly rapid pace. At the same time, ditches to divide rural properties (~6 m-wide) were constructed by slaves, resulting in the ecological corridors (colonization by native tree species) that connect small fragments [17]. *Protium spruceanum* is a large canopy tree (up to 25 m tall) found in those fragment and ecological corridors, selected for this research as representative of a mass-flowering insect-pollinated and bird-dispersed tropical tree. The small pale yellowish flowers are functionally unisexual and organized in dense inflorescences, the individuals are dioecious. The effective pollinators are *Apis mellifera* and *Trigona* sp. [17].

We examined the neutral genetic differentiation between populations that can help define important strategies for conservation genetics of remaining populations and ecological corridors. We therefore asked the following questions: (1) what are the levels and spatial scales of relatedness in *P. spruceanum* across landscape-level and within population? (2) is there any correlation between the fragment-corridor connection and genetic differentiation? And if so, are genotypes randomly or distributed clusters in corridors? (3) what are the potential ecological mechanisms likely responsible for the observed fine-scale SGS?

2. Materials and Methods

2.1. Sampled Sites. The fragment-corridor system studied is located in the city of Lavras, South of Minas Gerais state, in Brazil (Figure 1). The populations studied have rapidly declined because of habitat fragmentation caused by anthropogenic disturbance over the last 200 years, corroborated by detection of recent bottlenecks [17]. Five interlinking fragments and an ecological corridor were analyzed. *Protium spruceanum* presence in fragments F2, F3, and F4 coincides with the presence of water courses. In fragments F1 and F5 the species occur in a large part of the fragment, which may be determined by the almost permanently flooded soil (Figure 4). All samples along the fragments came from trees with diameter at breast height (d.b.h.) >20 cm and ~16 m height, that is, representing a larger diameter class. The sampling in each corridor axis was along the length of each corridor axis. Individuals were mapped by their compass direction and distance from the last individual was sampled. Live tissue was transported on ice and stored at -80°C until enzyme extraction.

2.2. Enzyme Extraction and Electrophoresis. Enzymes were extracted from 200 mg of frozen leaf tissue in 1 mL of phosphate extraction buffer: 0.2 mM sucrose, 2.5% polyvinylpyrrolidone (PVP), 1 mM ethylenediaminetetraacetic acid

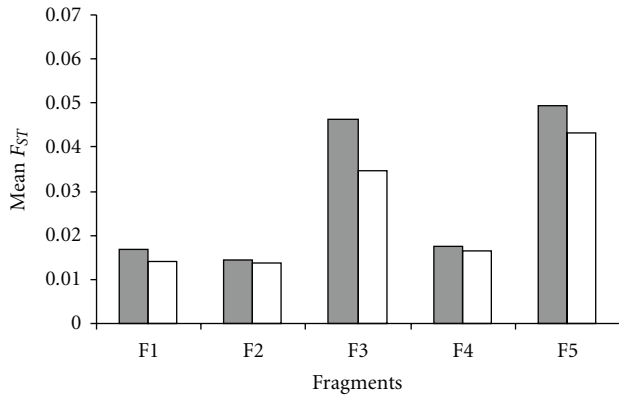


FIGURE 2: Degree of mean F_{ST} values of each fragment in comparison with those of the remaining fragments. Solid and open bars represent degree of mean F_{ST} values for fragments group without ecological corridors and considering both fragments and corridors, respectively.

(EDTA), 5.7 mM ascorbic acid, 5.8 mM sodium diethyl carbamate (DIECA), 2.6 mM sodium bisulphite, 2.5 mM Borax, and 0.2% β -mercaptoethanol [18]. Discontinuous vertical electrophoresis in a polyacrylamide gel was performed using 10% gels and carried out at 4°C over 3 hours (constant current of 80 mA, and voltage of 300 V). Eight enzymatic systems were used: alcohol dehydrogenase (*Adh*, E.C.1.1.1.1), glucose dehydrogenase (*Gdh*, E.C.1.1.1.47), β -galactose dehydrogenase (*Gldh*, E.C.1.1.1.48), glutamate dehydrogenase (*Gtdh*, E.C.1.4.1.3), malate dehydrogenase (*Mdh*, E.C.1.1.1.37), peroxidase (*Per*, E.C.1.11.1.7), sorbitol dehydrogenase (*Sdh*, E.C.1.1.1.14), and shikimate dehydrogenase (*Skdh*, E.C.1.1.1.25). Stain recipes were taken from Wendel and Weeden [19]. Allozymes had banding patterns consistent with expected quaternary structures and putative loci, and alleles were designated sequentially. The one with the most anodally migrating allozyme or alleles was designated as 1 and the next as 2 (Figure 5). The *Mdh* and *Per* enzyme patterns showed two polymorphic loci; so we used ten polymorphic loci to genotype the individuals.

2.3. Genetic Differentiation Analysis at Landscape Level. A *G*-test, based on 3 600 permutations of genotypes among samples, was performed to test for population differentiation F_{ST} at allozymes loci [20]. We adjusted the significance level for multiple pairwise comparisons using a sequential Bonferroni correction. The overall F_{ST} was calculated based on allele frequencies of the population of each at allozyme loci [21], using the program FSTAT 2.9.3.2 [22]. The degree of mean F_{ST} values of each fragment in comparison with those of the remaining fragments was estimated (Figure 2). To test for isolation by distance (*rM* Mantel tests), pairwise $F_{ST}/1 - F_{ST}$ matrices [23] were related to geographical distances between fragments, using 1 000 permutations. These calculations were performed using the program FSTAT. Additionally, the Pearson correlation coefficient (*r*) was used to test if gene diversity (H_E) varies according to fragment size.

2.4. Fine-Scale SGS Analysis. Population structure was further analyzed using Nason's kinship coefficients (F_{ij}) between pairs of individuals *i* and *j* [24]. 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 [25]. Distance class intervals between individuals were determined by testing to get rule of thumb for each distance interval as suggested by Hardy and Vekemans [26]. To test for significant deviations from random SGS, observed values for each distance class were compared to the 95% confidence interval derived from 1 000 permutations. The extent of SGS was estimated using the *Sp* statistic following Vekemans and Hardy [14]. The *Sp* statistic here is used as a simple measure to allow for comparisons among fragments, not as an estimate of the variance in gene dispersal distances. *Sp* was quantified by $Sp = -b_{log}/(1 - F_{(20,m)})$, where b_{log} is the regression slope and $F_{(20,m)}$ is the mean kinship coefficient between individuals belonging to the first distance interval (0–20 m). The b_{log} standard errors were obtained by jack-knifing over loci. These calculations were performed using the program SPAGeDi 1.2 g [26].

3. Results

3.1. Genetic Differentiation and Spatial Genetic Structure. Wright's F_{ST} revealed little evidence of significant genetic structure between fragments. The average genetic differentiation (F_{ST}) in each forest fragment pair was generally low ($F_{ST} = 0.027$). Only the genetic differentiation between fragment F3 and F5 was significant ($F_{ST} = 0.111$, $P < .01$). Indeed, the mean F_{ST} results showed the greater genetically distinct fragments, F3 and F5 (Figure 2). At landscape-level scales, the Mantel test suggests that the populations' structure appears not to be due to differences in gene flow produced by an isolation by distance model ($rM = -0.051$, $P = .539$). The degree of mean F_{ST} values was also calculated considering both fragments and corridors, indicating the decrease of the level of divergence (Figure 2). All fragments have generally lower genetic differentiation with corridors to which they are connected (Figure 1). The highest and most significant genetic differentiation occurred between fragment F5 and F2-F3 ($F_{ST} = 10.5\%$, $P < .05$) and F3-F4 ($F_{ST} = 14.8\%$, $P < .01$) axis. Negative correlation was observed between gene diversity and fragment size (Pearson $r = -0.626$, $R^2 = 0.392$, d.f. = 4).

3.2. Fine Scale SGS. At fine-scale, in all fragments, coancestry values were within the range of 95% confidence limits in all of the distance classes (Figure 3). Random genotype distribution was observed also when estimating the coancestry for individuals within ecological corridors, in all distance classes. Coancestry values were close to zero or negative, and nonsignificant at 95% probability. *Sp* values revealed weak patterns SGS (average $Sp = 0.008$) and the slope of the regression of F_{ij} was not significant.

4. Discussion

4.1. Genetic Diversity. Allozyme study showed that the species exhibited high levels of gene diversity (H_E) in

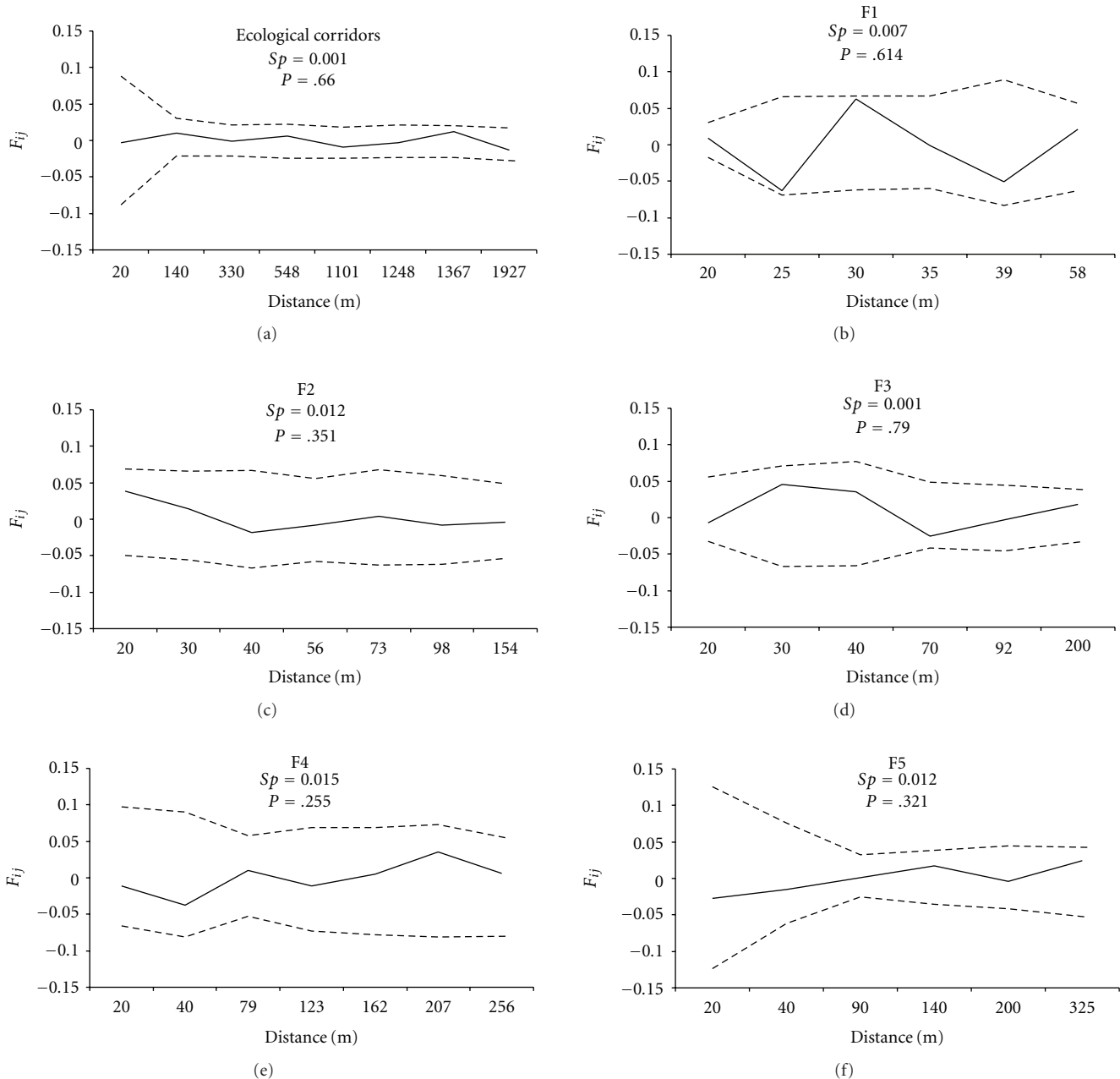


FIGURE 3: Correlograms of coefficient of coancestry (F_{ij}) per distance classes in fragments (F1 to F5) and ecological corridors. Confidence intervals around each F_{ij} -value were obtained through a jackknife procedure over loci.

fragments and in corridors (Table 1). In fact, forest trees are generally highly genetically diverse and characterized by high levels of gene diversity within populations and comparatively low gene diversity between them [27]. In addition, the highlights importance of small-sized fragments was demonstrated by negative correlation between fragment size and gene diversity. Some studies have shown a tendency of lower genetic diversity within small fragments [28], but little or no genetic diversity difference has been found between populations of large and small fragments [29]. However, our findings show that small-sized fragments may be important units within fragmented landscape and may

play a fundamental role to connect larger fragments and contribute to gene flow between populations.

4.2. Landscape-Level and Fine-Scale Genetic Structure. At both spatial scales, the average relatedness estimates indicated essentially random spatial distributions of alleles. At the landscape-scale (fragments), local populations of *P. spruceanum* exhibit little variation in their internal genetic structures. This suggests that the causal mechanisms generating genetic structure (e.g., seed dispersal, recruitment processes, etc.) are relatively homogeneous at this scale. Considering that for an insect-pollinated, bird dispersed

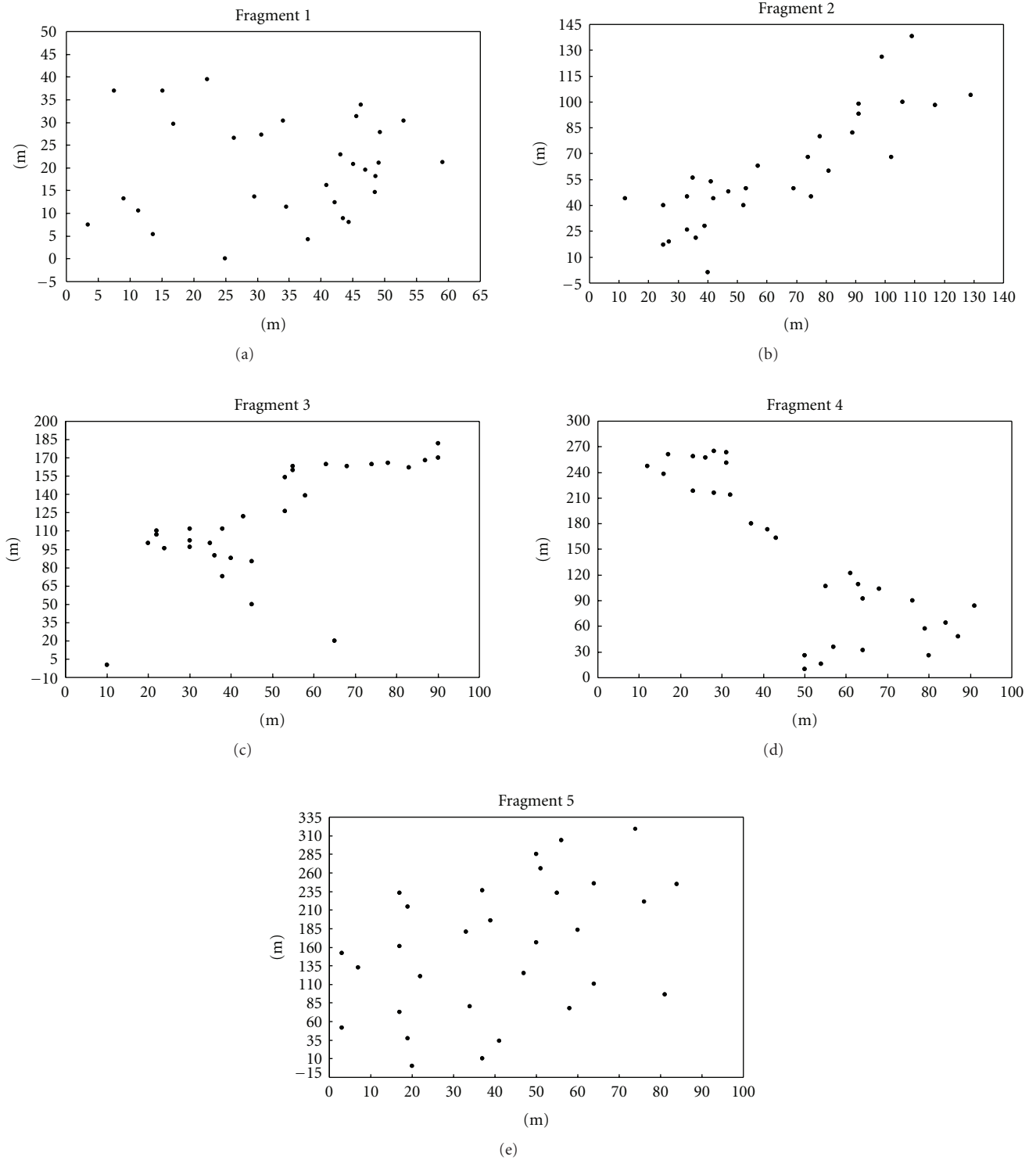


FIGURE 4: Distribution of the trees sampled in five fragments for the analysis of the fine-scale SGS in *Protium spruceanum*.

tree, connectivity by ecological corridors, low genetic differentiation among fragments was actually expected and observed ($F_{ST} = 0.027$). Results obtained for genetic differentiation between fragments and ecological corridors corroborate this hypothesis, because all fragments have generally lower genetic differentiation with corridors which

they are connected to. The level of divergence (mean F_{ST}) for each fragment against the rest of the fragments showed the greater genetically distinct fragments (F3 and F5) and could be included in a high-priority conservation programme.

At fine scale, many factors interact to determine genetic structure within populations including mating system and

TABLE 1: Fragments and corridors code sampled in this study.

	Fragments					Corridor axis			
	F1	F2	F3	F4	F5	F1-F2	F2-F3	F3-F4	F4-F5
Area (ha)	1.0	7.2	11.8	7.4	7.8	650	460	1 000	540
Length (m)									
N	30	30	30	30	30	20	20	20	20
H_E (SD)	0.480 (0.016)	0.469 (0.015)	0.381 (0.065)	0.437 (0.027)	0.507 (0.002)	0.454 (0.050)	0.383 (0.071)	0.336 (0.094)	0.470 (0.041)
f	-0.170	-0.182	-0.250*	-0.093	-0.248*	0.078	-0.023	-0.123	-0.029

N : sample size; H_E , Nei's gene diversity; SD: standard deviations; f : mean fixation index. Detailed descriptions of genetic diversity parameters (H_E and f) may be found in de Almeida Vieira and de Carvalho [17].

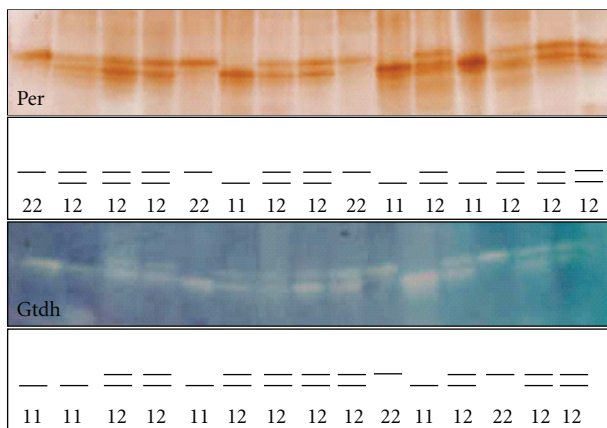


FIGURE 5: Zymograms of *Protium spruceanum* for the enzyme systems peroxidase (*Per*) and glutamate dehydrogenase (*Gtdh*) with schematic illustrations and genotype designation.

colonization history [14, 30]. The failure to detect genetic structure within populations may be due to the adult density (average = 232 individuals \cdot ha⁻¹), intraspecific competition or random mortality among samplings, resulting in extensive thinning within maternal half-sib groups. To determine whether this form of thinning occurred during recruitment in the study populations, investigations on contemporary patterns of genetic structure within populations and long-term demographic data are necessary to provide a clear picture of the genetic structure across demographic stages. In fact, our findings show that the magnitude of fine-scale SGS in *P. spruceanum* was observed to be decreasing from smaller- to larger-diameter classes (F.A. Vieira, unpublished data, 2010), within a small fragment (1.0 ha, fragment F1). Thus, we found SGS in the young plants, but this structure almost completely disappeared in the old plants, due to random thinning of individuals (F.A. Vieira, unpublished data, 2010). In general, other studies have also found within-population genetic structure to be greater in juveniles than adult trees [31, 32].

The Sp statistic is robust to different sampling schemes, being useful here to compare among species and sites [14]. The fine-scale SGS in *P. spruceanum* (average $Sp = 0.008$)

was similar to that observed for trees ($Sp = 0.010 \pm 0.0096$), outcrossing species ($Sp = 0.012 \pm 0.0101$) and animal-dispersed ($Sp = 0.008 \pm 0.0050$) species [14]. Spatial patterns of genetic variation within populations depend primarily on the seed dispersal and adult densities [16]. This way, the high density of seed sources and random thinning of individuals should favor the random divergence of the allelic frequency of *Protium spruceanum* in the fragments and corridors.

4.3. Implications for Conservation Genetics. Here, we studied a mass-flowering insect-pollinated and bird-dispersed tropical tree, as representative of a tree species with similar life history characteristics that occur in the fragments and corridor systems. Our findings show higher gene diversity and a low differentiation between the studied forest fragments. Moreover, those ecological corridors presented high gene diversity, absence of inbreeding, and little genetic differentiation with the fragments. Thus, the creation and the protection of ecological corridors should be an effective conservation strategy for tree species. That would be an important alternative in the demographic and genetic connection of isolated forest fragments, thus minimizing the negative effects of habitat fragmentation. Some studies have provided convincing evidence that, in some cases, corridors can enhance migration rates among fragments [10, 33]. In addition, the functional aspect of such an approach relates to the biological response of the species and landscape structure, thus new approaches of other species with different life history characteristics would be very important aspects to be considered.

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