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ORIGINAL PAPER

# Genetic structure of an insect-pollinated and bird-dispersed tropical tree in vegetation fragments and corridors: implications for conservation

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**Abstract** In the vegetation corridors that connect small remnants of undisturbed primary forest in the Lavras landscape (Brazil), *Protium spruceanum* is a representative of a mass-flowering insect-pollinated and bird-dispersed tree. Allozyme variation was quantified from five forest remnants ($N = 150$) from secondary vegetation corridors linking them ($N = 80$) to generate information for genetic conservation. The species adhered to H-W equilibrium in all fragments in most of the loci. The results indicated high gene diversity in the fragments ($\hat{H}_e = 0.381-0.507$) and corridors ($\hat{H}_e = 0.336-0.470$), positively correlated with the plant density ($r = 0.742$, $R^2 = 0.551$, d.f. $= 4$). We did not find evidence of inbreeding within fragments ($\hat{f} = -0.188, P<0.05$) nor overall ($\hat{F} = -0.101, P<0.05$). The genetic differentiation among remnants was low ($\hat{\theta}_p = 2.8\%$). Evidence of recent bottlenecks by anthropogenic disturbance was detected in fragments ($P < 0.05$, Wilcoxon sign-rank test). The minimal viable population was estimated for conservation in situ, indicating fragments with possibilities of maintaining genetic equilibrium diversity in the short term (except F3) and in the long term (only F5). The $\hat{N}_e/N$ ratios was also calculated to contribute to vegetation enrichment, area recovery or creation of new vegetation corridors. We found high levels of gene diversity in the vegetation corridors, genetic identity with the fragments and absence of inbreeding. Thus, our results suggest that landscape management strategies should therefore consider both the creation of new vegetation corridors and the protection of extant ones.

**Keywords** Allozymes · Conservation genetics · Habitat fragmentation · Landscape structure · Minimum viable populations · *Protium spruceanum* · Vegetation corridors

## Introduction

Tropical forests present very diverse terrestrial ecosystems, but much of this diversity is threatened by habitat destruction and extensive fragmentation of natural populations

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(Myers 1986). Forest loss and fragmentation alter the composition, structure and connectivity of the landscape (Taylor et al. 1993). Studies have shown that forest fragmentation negatively affects plant reproductive success by reducing pollinator activity (Aizen and Feinsinger 1994; Quesada et al. 2004), pollen deposition (Cunningham 2000; Cascante et al. 2002), fruit and seed set (Ghazoul et al. 1998; Fuchs et al. 2003; Quesada et al. 2004) and the regeneration of many species (Cascante et al. 2002; Benitez-Malvido and Martínez Ramos 2003). On the other hand, some studies have shown a positive effect of forest fragmentation on pollen flow (White et al. 2002; Dick et al. 2003), no effect on fruit and seed production (Cascante et al. 2002), and an effect on long distance dispersal (Bacles et al. 2006). The impacts of forest fragmentation on genetic resources have also been widely discussed (Lowe et al. 2005). These results indicated that tropical tree species have different responses to forest fragmentation and suggest that, for some species, trees isolated in fragmented landscapes may have considerable importance in conservation (Lowe et al. 2005).

Creation or preservation of landscape structures, such as vegetation corridors, has been indicated to minimize the effects of habitat fragmentation. The proposal that corridors may increase organism migration among the isolated remnants has been much discussed in conservation biology, and is always mentioned in management plans as an important factor for biological conservation in fragmented landscapes (Simberloff et al. 1992). However, there are few scientific studies that justify the use of corridors for conservation (Rosenberg et al. 1997). Some have shown that, in some cases, corridors may increase the migration rates of species among the isolated remnants (Andreassen et al. 1998; Haddad 1999; Mech and Hallet 2001). However, most deal only with corridor importance for fauna conservation and little is known about corridor importance for plant species (but see Johansson et al. 1996; Kirchner et al. 2003). Also, previous work on this topic was not focused on population genetic processes, especially in the Neotropical region.

The conservation of the habitat biodiversity within the fragments depends on the particular capacity of the species to survive genetic bottlenecks and stochastic events. Thus genetic conservation studies play an important role as they interpret the survival mechanisms of populations threatened by ecosystem fragmentation (Templeton et al. 1990; Young and Clarke 2000). For this, it is necessary to access the genetic diversity and structure in the natural populations and test for associations with various characteristics of the environment or the species. The genetic diversity levels may depend on factors including population size, environmental heterogeneity, plant density and landscape structure (Ellstram and Elam 1993; Franceschinelli and Bawa 2000; Manel et al. 2003). For example, rare species with large populations can show high levels of genetic diversity (Ellstram and Elam 1993), but can become more susceptible to founder effects and selective pressures because of local selection associated with differences in the microhabitat (Loveless and Hamrick 1984). Combining landscape ecology and population genetics, through the spatial mapping of allele frequencies from one or more populations and, subsequently the correlation of such patterns with the current landscape, may provide greater insight into how landscape characteristics structure populations (Manel et al. 2003).

The forest remnants in the state of Minas Gerais, Southeastern Brazil, are characterized by a hilly relief covered by vegetation mosaics formed by the contact between Atlantic seasonal forests, *cerrado* (woody savanna) and montane grasslands (Pereira et al. 2006). Despite the forest characteristics, such as species richness and diversity, species composition, tree density, biomass and, consequently, international importance of ecosystems for conservation of biodiversity, most of the remnants of these forests are in either small fragments or larger areas sheltered on steep mountain slopes (Oliveira Filho and Fontes

2000). These plant formations were seriously plundered since European occupation goes back to colonial times (two centuries ago). The vegetation corridors (~6 m-wide) that connect small remnants of undisturbed primary forest are the result of colonization by native tree species in the ditches constructed by slaves to divide rural properties in the colonial period. These corridors may be considered essential for conservation, because of their considerable floristic diversity and the occurrence of species unique to this environment (Castro 2004).

The ecological and genetic consequences of anthropogenic fragmentation are being investigated in a range of species, with different life history characteristics, to inform conservation management of remnant native vegetation in the area (Bacles et al. 2006; Jump and Peñuelas 2006). *Protium spruceanum* has been selected for this research as representative of a mass-flowering insect-pollinated and bird-dispersed tropical tree that occur in the fragments and corridor systems studied. The species is widely distributed and normally occurs in relatively large populations in association with water courses. Where fragments and vegetation corridor areas coexist, the historic fragmentation of these plant formations provides the opportunity to assess the effects of chronic habitat fragmentation, the influence of vegetation corridors and to test theoretical predictions. Specifically, the objective of the present study was to characterize the genetic structure of *P. spruceanum*, quantify the genetic variability in the populations in the fragmented and corridor landscape and estimate the effective size of the populations, aiming to provide genetic information for in situ conservation strategies.

## Materials and methods

### Study species

*Protium spruceanum* (Burseraceae) is a widely distributed Neotropical tree species, found in the Amazon and the Atlantic rain forests and on the savannas inside riverbank woods (Oliveira-Filho and Ratter 1995). The species produces a fragrant resin used in popular medicine, the varnish industry, to caulk boats and as a perfume (Machado et al. 2003). In the study region, the species is a shade tolerant climax species and prefers wet environments (Castro 2004). *Protium spruceanum* produces flowers with greater intensity between September and October (F. A. V., unpublished data). The flowers are functionally unisexual in dense inflorescences and the individuals are dioecious. Staminate flowers supply a large quantity of viable pollen (>88%, stained by acetic carmine) coinciding with the reproductive phase of the stigma of the pistillate flowers (peroxidase activity method) that manifests itself immediately after flowering and lasts throughout the day (F. A. V., unpublished data). Staminate and pistillate flowers produce relatively abundant nectar (~4 μl), with an average of 30% concentration in sucrose equivalents. The effective pollinators are *Apis mellifera* and *Trigona* sp. (Hymenoptera, Apidae) (F.A.V, unpublished data). Fructification occurs in September. Fruits are sub-globoid, reddish berries, containing 1–2 seeds surrounded by a sweet aril that are dispersed by birds.

### Study site and populations

The fragment-corridor system studied is located in the city of Lavras, South of Minas Gerais state, in Brazil (Fig. 1). The following can be observed in the current landscape:

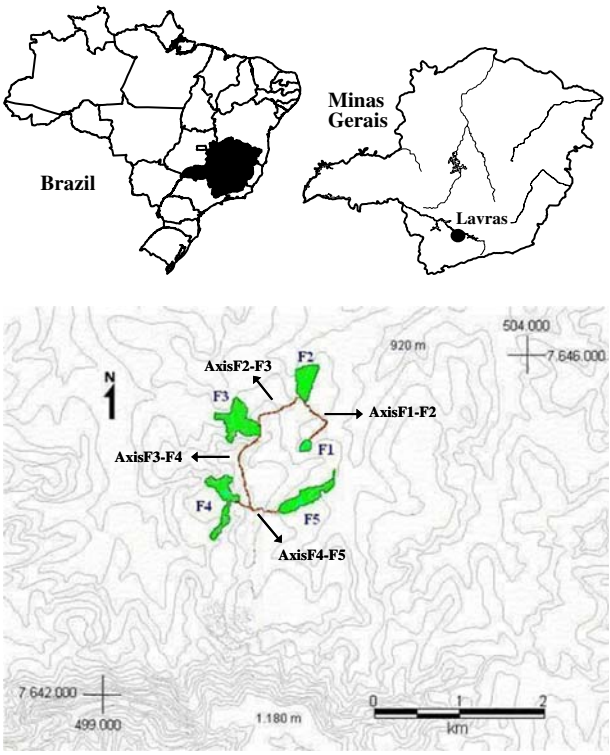


**Fig. 1** Location of the study system with primary forest remnants and secondary vegetation corridors in Minas Gerais state, Brazil. F1–F5 (fragments) and Axis F1-F2 to F4-F5 (vegetation corridors). The coordinates are according to the Universal Transverse Mercator (UTM) system

(a) few and small forest remnants of primary forest, (b) a matrix consisting of coffee plantations and planted pastures (*Brachiaria* spp.) for livestock rearing and (c) vegetation corridors of secondary forest. The fragments studied showed evidence of localized impacts, caused by cattle entering the forest, damaging regeneration in several stretches. Fragments and vegetation corridors are pretty similar in terms of floristic composition, but corridors are denser, have larger basal area and trees are concentrated in the upper diameter classes and lower height classes.

Five interlinking fragments and a vegetation corridor were analyzed (Fig. 1, Table 1). The fragmentation and isolation of these populations has occurred for over two centuries and at a particularly rapid pace in the colonial period. Thus, the estimated age of the trees sampled is of 5–7 generations before present, assuming a generation time of 30 years. All the fragments contained a water course in their interior. *P. spruceanum* is the most abundant tree species in the fragments. The presence of *P. spruceanum* in fragments F2 and F4 coincides with the presence of water courses. In fragments F1 and F5 the species occurs in a large proportion of the fragment, which may be determined by the almost permanently flooded soil. Fragment F3 is the result of a recent colonization in a small canyon. *P. spruceanum* is one of the most abundant in the vegetation corridors.

We sampled adult individuals in the fragments randomly, with diameter at breast height >20 cm and ~16 m-height. The sampling in each corridor axis was along the length of

**Table 1** Fragments and corridor codes, altitude and area of the fragments, sample size (*N*) and density of the species (*DA*) sampled in this study

| Code | Fragments | | | | | Corridors | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F1-F2 | F2-F3 | F3-F4 | F4-F5 |
| Altitude (m) | 973.0 | 969.8 | 984.3 | 985.0 | 986.0 | | | | |
| Area (ha) | 1.0 | 7.2 | 11.8 | 7.4 | 7.8 | 380[a] | 450 | 510 | 590 |
| *N* | 30 | 30 | 30 | 30 | 30 | 20 | 20 | 20 | 20 |
| *DA* (ind ha$^{-1}$) | 850.0 | 50.0 | 8.3 | 175.0 | 175.0 | | | | |

In the vegetation corridor the absolute density of the species is 135.19 ind ha$^{-1}$ (Castro 2004)

[a] Extension (m) of the vegetation corridors

the each corridor axis (Table 1). All sampled leaf material was kept on ice until transportation to the laboratory, where it was stored at −80°C until enzyme extraction.

## Electrophoresis procedures

For enzyme extraction 200 mg leaf tissue were used per 1 ml of the extraction solution (buffer no. 1 of Alfenas et al. 1998). The extracts obtained were centrifuged at 12,000 rpm at 4°C for 10 min. After centrifuging, 20µl of the supernatant was placed in the gel wells to proceed to the electrophoretic runs. Discontinuous vertical electrophoresis in a polyacrylamide gel was used, with the concentration and separation gels at 4 and 10.0%, respectively, and run at 4°C for 3 h (constant current of 80 mA, and voltage of 300 V) (Alfenas et al. 1998). Eight enzyme 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). The *Mdh* and *Per* enzyme patterns showed two polymorphic loci; so we used 10 polymorphic loci to genotype the individuals. 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 Alfenas et al. (1998).

## Data analyses

### *Genetic diversity*

The following genetic diversity parameters were estimated using the program BIOSYS 2 (Swofford and Selander 1997): proportion of polymorphic loci ($\hat{P}$), mean number of alleles per locus ($\hat{A}$), observed heterozygosity ($\hat{H}_o$) and Nei's gene diversity ($\hat{H}_e$) (Berg and Hamrick 1997). A locus was considered polymorphic if the frequency of the most common allele does not exceed 0.95 (Nei 1987). The percentage of loci in genetic equity (low variation amplitude) was calculated, with allele frequencies among 0.350 and 0.650 (Frankel et al. 1995. After analyzing the homogeneity of the variances and normality of distribution by the Kolmogorov–Smirnov test, the gene diversity ($\hat{H}_e$) was submitted to analysis of variance by the *F*-test (ANOVA) and the means of the fragments and corridors were compared. We adjusted the significance level for multiple pairwise comparisons

using a sequential Bonferroni correction ($\alpha = 0.005$) (Zar 1999). In addition, the Pearson correlation coefficient ($r$) was calculated between $\hat{H}_e$ and plant density.

*F*-statistics

The parameters estimated were the coancestry coefficient among populations ($\hat{\theta}_p$), the over all inbreeding coefficient ($\hat{F}$) and within population coefficient of inbreeding ($\hat{f}$) (Weir and Cockerham 1984). Confidence intervals at 95% probability were established for each population using the bootstrap procedure with 10,000 repetitions (Weir 1996). The analyses of variance and the bootstraps were carried using the GDA (Lewis and Zaykin 2000) and FSTAT 2.9.3.2 programs (Goudet 2002). The Wright fixation index ($\hat{f}$) was obtained using the GENETIX 4.05.2 program (Belkhir et al. 2004). The genotypic frequency deviations obtained compared to the expected frequencies by the Hardy–Weinberg (H-W) proportions were estimated and tested using the Fisher exact test and the BIOSYS 2 program.

UPGMA

As a measure of genetic identity among the pairs of fragments, the genetic identity of Nei (1978) was used and then dendrograms were constructed using the UPGMA (Unweighted pair group method arithmetic average) method. The clustering obtained was assessed by the cophenetic correlation coefficient by comparing the identity similarity matrix with cophenetic similarity. The multivariate genetic identity analysis (UPGMA) and the cophenetic correlation were obtained using the NTSYS 1.5 package (Rohlf 1989).

Minimum viable populations

The effective population size ($\hat{N}_e$) was estimated using the components of variance method (Crow and Kimura 1970; Vencovsky 1997). The minimum viable populations (PMV) calculated corresponds to the number of necessary individuals for the maintenance of the genetic equilibrium diversity of the population. The difference ($\hat{D}$) between the population size estimated for each fragment ($\hat{N}$) and the PMV was calculated ($\hat{D} = \hat{N} - \text{PMV}$), that is, $\hat{D} = DA \cdot A - (\hat{N}_{e(\text{reference})}/(\hat{N}_e/N))$, where $DA$ is the density of the species (ind ha$^{-1}$), $A$ is the area of fragment (ha), $\hat{N}_{e(\text{reference})}$ is the effective size of reference (150 or 1,500, for conservation in the short or long term, respectively) and $N$ is the sample size of each fragment. The effective size of reference adopted was according to Nunney and Campbell (1993).

Departure from random mating

We used the BOTTLENECK 1.2.02 program (Cornuet and Luikart 1996) to test for significant recent decreases in $\hat{N}_e$. These tests are based on the principle that populations that have gone through a severe and recent genetic bottleneck show a faster reduction in the number of alleles than in the $\hat{H}_e$. Luikart et al. (1998) demonstrated that populations that have undergone a recent bottleneck show a transient excess of heterozygotes. This means that $\hat{H}_e$ becomes greater than the expected heterozygosity on balance between mutation and drift ($\hat{H}_{eq}$), because this is calculated from the number of alleles (Cornuet and Luikart 1996). Consequently, in a population that suffered a recent bottleneck, $\hat{H}_e$ will

be higher than $\hat{H}_{eq}(\hat{H}_e > \hat{H}_{eq})$. The analysis was carried out only on the loci in H–W equilibrium (as suggested by Lee et al. 2002), and all enzyme loci are assumed to fit an infinite allele model of mutation (IAM) (Kimura and Crow 1964). The significance was assessed using the Wilcoxon signed rank test, based on 5,000 replications.

## Results

### Genetic structure

The eight enzyme systems used showed 10 loci that could be interpreted and 20 alleles. The phenotypic expression of the enzymes was compatible with the typical monomeric loci pattern and segregating two alleles of the locus, following the Mendelian segregation model. No exclusive alleles were detected by the analyses of the allele frequencies of the 10 polymorphic loci in the five fragments and the vegetation corridor studied, because both alleles were present in all populations (Table 2). No major allele frequency difference was detected among fragments F1, F2 and F5 and the corridors axis F1-F2 and F4-F5, resulting in genetic equity (EG) for more than 50% of loci.

A positive correlation was observed between genetic equity and genetic diversity for the fragments ($r$ de Pearson = 0.957, $R^2 = 0.915$; d.f. = 4) and corridors ($r$ de Pearson = 0.997, $R^2 = 0.995$; d.f. = 3). The relationship between the observed $(\hat{H}_o)$ and expected $(\hat{H}_e)$ mean heterozygosities resulted in a negative fixation index $(\hat{f})$ in all the fragments analyzed, indicating a greater proportion of heterozygotes. This index was significant for fragments F3 and F5, indicating the H–W deviations. The mean estimated $\hat{H}_e$ values on the loci were significant for the fragments ($F_{ANOVA} = 20.79$; $P < 0.05$) and the corridors axis ($F_{ANOVA} = 8.65$; $P < 0.05$). All comparisons with fragment F3 and the comparisons F1 × F4 and F4 × F5 were significant after Bonferroni correction ($\alpha = 0.005$). Significant differences were observed in the corridor axis among the F1-F2 × F3-F4, F2-F3 × F4-F5 and F3-F4 × F4-F5. A positive correlation was observed between gene diversity and plant density in the fragments ($r$ de Pearson = 0.742; $R^2 = 0.551$; d.f. = 4).

The mean estimates obtained for the coancestry coefficient (Cockerham 1969) were negative and significant, showing excess heterozygosity compared to that expected under H–W proportions (Table 3). The estimates indicated the absence of inbreeding for the set of the fragments $(\hat{F})$ and a tendency to present a greater number of heterozygotes within of the fragments $(\hat{f})$, suggesting that, on average, the populations in the fragments and corridors were not inbreeding. The population differentiation for the fragment-corridor system was low $(\hat{\theta}_p = 0.028)$. This meant that approximately 2.8% of the genetic variability was found among the fragments and that 97.2% of this variability occurred within the fragments. Generally, the species presented loci adhering to H–W proportions in some fragments. Significant deviations from the equilibrium model were detected only in the *Mdh-1* ($P = 0.012$) locus of fragment F2 and the *Adh* ($P = 0.021$) and *Mdh-2* ($P = 0.025$) locus on fragment F5. The significant deviations resulted from the excess heterozygosity verified in these loci.

The lowest genetic identity was observed among the individuals sampled in fragment F5 and those from axis F3, F2-F3 and F3-F4, with significant values (Fig. 2). The cophenetic correlation coefficient, which assesses the existence of the clustering obtained, was de $r_C = 0.70$.

**Table 2** Levels and distribution of genetic diversity of a mass-flowering insect-pollinated and bird-dispersed tree (*P. spruceanum*) in fragment-corridor system

| | Fragments | | | | | Corridor axis | | | |
|---|---|---|---|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 | F1-F2 | F2-F3 | F3-F4 | F4-F5 |
| EG (%) | 90 | 80 | 20 | 30 | 100 | 60 | 30 | 20 | 70 |
| $\hat{A}$ | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| $\hat{P}$ | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| $\hat{H}_e$(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) |
| $\hat{f}$ | −0.170 | −0.182 | −0.250* | −0.093 | −0.248* | 0.078 | −0.023 | −0.123 | −0.029 |
| Loci | Allele /*N* | | | | | | | | |
| *Adh* | 1 | 0.617 | 0.667 | 0.783 | 0.700 | 0.482 | 0.725 | 0.825 | 0.825 | 0.600 |
| | 2 | 0.383 | 0.333 | 0.217 | 0.300 | 0.518 | 0.275 | 0.175 | 0.175 | 0.400 |
| | *N* | 30 | 30 | 30 | 30 | 28 | 20 | 20 | 20 | 20 |
| *Gdh* | 1 | 0.603 | 0.650 | 0.767 | 0.717 | 0.533 | 0.684 | 0.775 | 0.816 | 0.579 |
| | 2 | 0.397 | 0.350 | 0.233 | 0.283 | 0.467 | 0.316 | 0.225 | 0.184 | 0.421 |
| | *N* | 29 | 30 | 30 | 30 | 30 | 19 | 20 | 19 | 19 |
| *Gldh* | 1 | 0.603 | 0.643 | 0.810 | 0.683 | 0.500 | 0.650 | 0.775 | 0.825 | 0.575 |
| | 2 | 0.397 | 0.357 | 0.190 | 0.317 | 0.500 | 0.350 | 0.225 | 0.175 | 0.425 |
| | *N* | 29 | 28 | 29 | 30 | 29 | 20 | 20 | 20 | 20 |
| *Gtdh* | 1 | 0.617 | 0.650 | 0.776 | 0.650 | 0.517 | 0.725 | 0.800 | 0.850 | 0.600 |
| | 2 | 0.383 | 0.350 | 0.224 | 0.350 | 0.483 | 0.275 | 0.200 | 0.150 | 0.400 |
| | *N* | 30 | 30 | 29 | 30 | 30 | 20 | 20 | 20 | 20 |
| *Mdh-1* | 1 | 0.650 | 0.567 | 0.517 | 0.600 | 0.533 | 0.500 | 0.625 | 0.550 | 0.750 |
| | 2 | 0.350 | 0.433 | 0.483 | 0.400 | 0.467 | 0.500 | 0.375 | 0.450 | 0.250 |
| | *N* | 30 | 30 | 29 | 30 | 30 | 20 | 20 | 20 | 20 |

**Table 2** continued

| Loci | Allele /*N* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| *Mdh-2* | 1 | 0.586 | 0.650 | 0.783 | 0.717 | 0.467 | 0.778 | 0.750 | 0.825 | 0.625 |
| | 2 | 0.414 | 0.350 | 0.217 | 0.283 | 0.533 | 0.222 | 0.250 | 0.175 | 0.375 |
| | *N* | 29 | 30 | 30 | 30 | 30 | 18 | 20 | 20 | 20 |
| *Per-1* | 1 | 0.683 | 0.600 | 0.583 | 0.683 | 0.550 | 0.625 | 0.650 | 0.816 | 0.675 |
| | 2 | 0.317 | 0.400 | 0.417 | 0.317 | 0.450 | 0.375 | 0.350 | 0.184 | 0.325 |
| | *N* | 30 | 30 | 30 | 30 | 30 | 20 | 20 | 19 | 20 |
| *Per-2* | 1 | 0.617 | 0.650 | 0.783 | 0.650 | 0.533 | 0.525 | 0.600 | 0.550 | 0.725 |
| | 2 | 0.383 | 0.350 | 0.217 | 0.350 | 0.467 | 0.475 | 0.400 | 0.450 | 0.275 |
| | *N* | 30 | 30 | 30 | 30 | 30 | 20 | 20 | 20 | 20 |
| *Sdh* | 1 | 0.600 | 0.667 | 0.767 | 0.733 | 0.466 | 0.650 | 0.800 | 0.800 | 0.600 |
| | 2 | 0.400 | 0.333 | 0.233 | 0.267 | 0.534 | 0.350 | 0.200 | 0.200 | 0.400 |
| | *N* | 30 | 30 | 30 | 30 | 29 | 20 | 20 | 20 | 20 |
| *Skdh* | 1 | 0.583 | 0.621 | 0.750 | 0.655 | 0.536 | 0.625 | 0.789 | 0.861 | 0.600 |
| | 2 | 0.417 | 0.379 | 0.250 | 0.345 | 0.464 | 0.375 | 0.211 | 0.139 | 0.400 |
| | *N* | 30 | 29 | 30 | 29 | 28 | 20 | 19 | 18 | 20 |

* $P < 0.05$; EG, genetic equity; $\hat{A}$, mean number of alleles per locus; $\hat{P}$, percentage of polymorphic loci; $\hat{H}_e$, Nei's gene diversity; SD, standard deviations; $\hat{f}$, mean fixation index

**Table 3** Estimates of Wright's *F*-statistics described for each polymorphic locus in *Protium spruceanum*

| Loci | $\hat{f}$ | $\hat{F}$ | $\hat{\theta}_p$ |
|---|---|---|---|
| *Adh* | −0.158 | −0.109 | 0.042 |
| *Gdh* | −0.121 | −0.094 | 0.024 |
| *Gldh* | −0.130 | −0.087 | 0.038 |
| *Gtdh* | −0.110 | −0.072 | 0.034 |
| *Mdh-1* | −0.174 | −0.166 | 0.006 |
| *Mdh-2* | −0.140 | −0.087 | 0.046 |
| *Per-1* | −0.035 | −0.029 | 0.006 |
| *Per-2* | −0.138 | −0.117 | 0.018 |
| *Sdh* | −0.177 | −0.129 | 0.041 |
| *Skdh* | −0.145 | −0.112 | 0.029 |
| Mean | −0.133* | −0.101* | 0.028* |
| | [−0.153 to −0.108] | [−0.122 to −0.079] | [0.019 to 0.037] |

* $P < 0.05$; $\hat{f}$, mean fixation index of individuals relative to their population; $\hat{F}$, mean overall inbreeding coefficient of an individual; $\hat{\theta}_p$, populations coancestry coefficient; [ ], confidence intervals

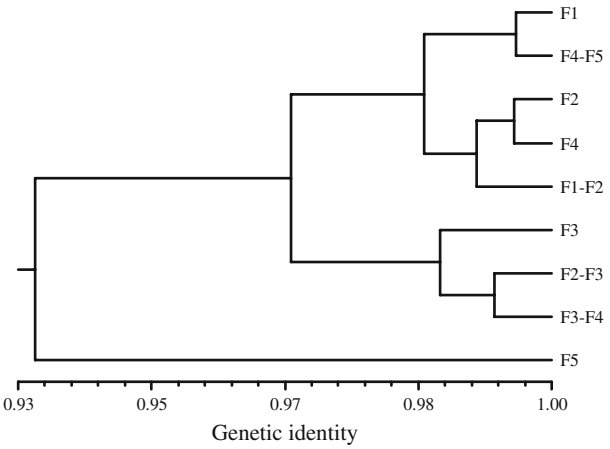


**Fig. 2** Dendrogram constructed according to values of Nei's genetic identity (UPGMA) found for *P. spruceanum* in five fragments and vegetation corridors axis

## Minimum viable populations and bottlenecks

To plan in situ conservation, the $\hat{N}_{e(reference)}/(\hat{N}_e/N)$ ratios permitted estimation of the minimum viable population size (PMV), corresponding to the minimum number of trees that should be maintained or that would be necessary to ensure the maintenance of the genetic variability levels in the fragments. Therefore, the $\hat{N}_{e(reference)}$ of 150 and 1,500, proposed by Nunney and Campbell (1993), for short and long-term conservation, respectively, was used as reference. For example, considering PMV calculated for F3 of 113 individuals for short term conservation and the estimates of 98 individuals in the fragment [(11.8 ha) (8.3 ind $ha^{-1}$)], the difference was $\hat{D} = -15$ individuals. Only fragment F5, because of the greater heterozygosity, did not present PMV deficit for conservation in the short or long term.

From the tests of fit to the infinite alleles model of mutation (Table 4), no population in the fragments was shown to be in equilibrium, indicating recent bottlenecks ($P < 0.05$,

**Table 4** Number of loci showing deficiency/excess of heterozygosity under IAM for bottleneck detection in *Protium spruceanum*

| | Fragments | | | | |
|---|---|---|---|---|---|
| | F1 | F2 | F3 | F4 | F5 |
| Expected number of loci showing excess of heterozygosity | 3.8 | 4.2 | 4.4 | 4.3 | 3.4 |
| Deficiency/excess of heterozygosity | 0/9* | 0/10* | 0/10* | 0/10* | 0/8* |
| $\hat{N}_e/N$ | 1.20 | 1.22 | 1.33 | 1.10 | 1.33 |
| $\hat{N}$ | 850 | 360 | 98 | 1,295 | 1,365 |
| PMV (150) | 125 | 123 | 113 | 136 | 113 |
| $\hat{D}(150)$ | 725 | 237 | −15 | 1,159 | 1,252 |
| PMV (1,500) | 1,245 | 1,227 | 1,125 | 1,361 | 1,128 |
| $\hat{D}(1,500)$ | −395 | −867 | −1027 | −66 | 237 |

* Significant as determined by Wilcoxon signed rank tests ($\alpha = 0.05$); $\hat{N}_e$, effective population size; $N$, sample size; $\hat{N}$, population size estimated; PMV, minimum viable populations; (150), conservation in the short term; (1,500), conservation in the long term; $\hat{D}$, difference

Wilcoxon sign-rank test). In all fragments, the populations showed a significant number of loci with excess heterozygosity, that is, the heterozygosity from the H–W proportions ($\hat{H}_e$) in the polymorphic loci was greater than the expected heterozygosity under equilibrium between mutation and drift ($\hat{H}_{eq}$).

## Discussion

### Genetic diversity

Our findings show that populations of *P. spruceanum* in vegetation fragments and corridors maintain high levels of allozyme diversity. The similarity in the allele frequencies in fragments F1, F2 and F5, together with the similar genetic equity proportions, suggested low divergence among these fragments. The genetic equity is the least variation in the allele frequencies in the species, and is therefore an indication of greater genetic diversity (Frankel et al. 1995). Moreover, this diversity is not structured within populations, because the genetic differentiation among fragments was low, suggesting that vegetation corridors have helped maintain genetic connectivity among remaining primary forest fragments. However, given the longevity of most tree species, the study of the next generations will be required to provide a clear picture of the genetic fate of the studied populations.

The high proportion of polymorphic loci and the number of alleles per locus detected here were similar to reports for other tree species in investigation about impacts of habitat degradation on genetic resources, using allozymic markers (Bacles et al. 2004; Hall et al. 1996; Fuchs et al. 2003). The gene diversity ($\hat{H}_e$) detected for the species in the fragments and corridors was greater than the value estimated for tree species in general (0.17, Hamrick and Godt 1989), and can be explained by the absence of rare alleles, allele frequencies in equity, functionally unisexual flowers and high population density. The positive correlation between gene diversity and plant density was probably due to the reproductive system of the species. The outcrossing rates in tropical tree species can have an impact on the genetic structure (Murawski and Hamrick 1991; Nason and Hamrick 1997) and can result from ecological factors, population size and density (Franceschinelli and Bawa 2000; Murawski and Hamrick

1991). The plant's floral neighborhood (Feinsinger et al. 1986) in one area has a great effect on the plant reproductive success, because individuals in larger aggregations can attract more or better pollinators (Calvo and Horvitz 1990; Corbet 1998). In this context, the species in the fragments studied presented high demographic density. Thus the greater number of reproductive individuals probably favored the increase in the levels of genetic diversity in function of the increases in the outcrossing rates, as discussed by van Treuren et al. (1993) and Franceschinelli and Bawa (2000).

The genetic differentiation detected in the study (2.8%) is in line with that reported for other tropical tree species, that is, a greater proportion of the genetic variability was found within the populations (Hall et al. 1996; Dayanandan et al. 1999; White et al. 1999). The typically outcrossing species presented high genetic variability within populations (Loveless and Hamrick 1984). Furthermore, the allele frequencies were generally equally distributed in the fragments and were thus an indication of gene flow acting as homogenizer of these frequencies, contributing to lower genetic differentiation observed among the fragments. The pattern of genetic identity found among the fragments and corridors may be associated to the gene flow among them. However, the genetic effects on tree species in anthropogenic forest fragments studied might be all less than 200 years old, a period, that may be insufficient for genetic changes to develop. Additional studies of direct methods (DNA-based) for estimating contemporary gene flow across the landscape are necessary to provide a clear picture of the contribution of seed and pollen to the overall contemporary gene immigration. Investigations on contemporary patterns of genetic structure within populations, e.g. at fine-scale spatial genetic structure, also is necessary. Nevertheless, considering the practically irreversible fragmentation of populations and the larger genetic diversity found in vegetation corridors, landscape management strategies should consider both the creation of new vegetation corridors and the protection of extant ones.

## Effective population size and conservation applications

The estimate of $\hat{N}_e$ was associated with the conditions of heterozygosity and, therefore, the values observed reaffirmed the existence of low inbreeding in the fragments studied. $\hat{N}_e/N$ ratios are a very important parameter in germplasm preservation activities, seed collection and in situ genetic conservation, helping in genetic management and conservation projects. Data on the genetic representativeness of the population mother trees ($\hat{N}_e/N$) are important to maximize seed collecting activities (Vencovsky 1997). According to the $\hat{N}_e/N$ ratios obtained, a larger sampling of mother plants in the fragments that present lower $\hat{N}_e/N$ ratios (e.g. F4) is recommended for seed collection to ensure the maintenance of the genetic variability and minimum inbreeding in the seeds. Furthermore, for seed collection and germplasm conservation, the sampling should be random, not of seeds but of the mother plants. In vegetation enrichment, area recovery or creation of new vegetation corridors, seed collection based on this principle will provide new genetic recombinations in the population and raise the evolutionary potential (Vencovsky 1997). The results of this will be important, because the species is indicated for use in the recovery of degraded areas. The species can be used especially in the recovery of riparian woods because of its abundant occurrence in the study region and its ecological adaptation to wet environments, along with fast growth and abundant seed production.

The determination of the PMV is, generally, performed without previous definition of when the conservation of the population under study will be carried out. Thus, the forest remnants studied gave important references for short and long-term conservation. The

suitable values of PMV to be adopted for genetic conservation were determined by mainly two criteria: prevention of inbreeding depression and maintaining the evolutionary potential (Nunney and Campbell 1993). Thus maintenance of the suggested PMV will decrease the likelihood of genetic oscillations and inbreeding (Ellstrand and Elam 1993; Nunney and Campbell 1993). Our results showed that except for fragment F3, the species presented possibilities of maintaining its genetic equilibrium diversity in the short term, because the difference $(\hat{D})$ between the estimated population size $(\hat{N})$ and PMV was positive. Only fragment F5 presented possibilities of maintaining its genetic equilibrium diversity in the long term, that is, not present individual deficit for PMV for conservation in the long term. Corridor maintenance and conservation would then be plausible figures to ensure the demographic dynamics and higher neighborhood rates (gene flow by insect-pollinated and bird-dispersed) in the fragments, increasing the effective population sizes.

## Recent bottlenecks

Recent decreases in $\hat{N}_e$ have been analyzed in the genetic study of populations (Bacles et al. 2004; Jump and Peñuelas 2006). Here, we also found a significant excess of H–W expected heterozygosity under both the infinite allele and stepwise mutation models, indicating the occurrence of recent population bottlenecks in all the fragments. Populations in mutation–drift equilibrium of the number of alleles present equal likelihood of a locus presenting an excess or a deficit of heterozygosity, considering an $\hat{N}_e$ that has remained constant in a recent past. In populations that have suffered recent drift, most of the loci will exhibit an excess of H–W expected heterozygosity (Luikart and Cornuet 1998), a situation that was presented in the fragments analyzed here. Thus, in a population recently reduced in population size, the genetic diversity observed will be greater than the genetic diversity equilibrium. The detection of recent bottlenecks also corroborates historical evidence that the forest fragments were once part of a much larger population, and can be interpreted as a consequence of the habitat fragmentation resulting from human disturbance—from the Brazilian colonization period, two centuries ago.

The detection of populations that have undergone recent bottlenecks is important mainly because it allows inference of the risk of local extinction in consequence of the reduced population size (Lee et al. 2002). After detecting a bottleneck, the likelihood of the deleterious effects being avoided or minimized is greater because mitigating management procedures or immigrant introduction can be carried out (Luikart et al. 1998). Furthermore, these practices can be effective if associated with knowledge of ecological and demographic factors of the species. Thus, considering the recent genetic bottleneck detected, the lower genetic diversity and population size in fragment F3, and the practically irreversible situation of the habitat fragmentation, conservation of the vegetation corridors (e.g. F2-F3 and F3-F4) is a plausible option to maintain the number of migrants.

## Management and conservation genetics

The understanding of the survival mechanisms of populations threatened by fragmentation of the ecosystems is the focus of the genetic conservation studies (Bacles et al. 2004; Cascante et al. 2002; Hall et al. 1996; Nason and Hamrick 1997; Templeton et al. 1990; White et al. 2002), and assessment of the consequences of reducing and isolating populations is fundamental for predicting the destiny of species in forest fragments for effective planning of management programs in small forest areas (Young and Clarke 2000). In population

conservation and management programs, it is important to note the variations in genetic diversity caused by ecological and anthropological factors (Dick et al. 2003; Bacles et al. 2004). In this context, it is important to understand the landscape structure and the species biology, and conservation strategies based on species models can contribute to the conservation of entire ecosystems (Bacles et al. 2006, Jump and Peñuelas 2006). Here, we studied a mass-flowering insect-pollinated and bird-dispersed tropical tree. In the same place, other tree species with the similar life history characteristics, such as *Tapirira guianensis*,*Copaifera langsdorffii* and *Ocotea pulchella* are the most important and abundant species (Castro 2004), besides the congeneric species *Protium widgrenii* and *Protium heptaphyllum*. *Tapirira guianensis* is the most abundant species and present massive annual flowering with quick and well synchronized blooming peaks of both male and female individuals (Lenza and Oliveira 2005), similar reproductive traits to the one of *P. spruceanum*. In conclusion, the high genetic diversity compared to the mean observed in other tropical tree species (Hamrick and Godt 1989) suggested a high potential for in situ genetic conservation and for seed collection destined to restore degraded areas, including for the creation of new vegetation corridors. Thus, our study suggests that creation and the protection of vegetation corridors should be an effective conservation strategy for this tree species. That would be an important alternative to the demographic and genetic connection of isolated forest remnants, thus minimizing the negative effects of habitat fragmentation (Andreassen et al. 1998; Mech and Hallet 2001). In this sense, the vegetation corridors studied presented high gene diversity, genetic identity with the fragments and the absence of inbreeding. Therefore, to reduce the likelihood of deleterious effects from the bottleneck detected, vegetation corridors conservation would be a plausible option to maintain the number of migrants. These results give important data for species conservation. Furthermore, data on the reproductive biology, regeneration and genetic distribution pattern of the different forest species can contribute to the conservation strategies of the small forest remnants.

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