

# Diversity and Genetic Structure of Natural Fragmented Populations of *Tapirira guianensis* Aubl. in Northeastern Brazil

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## ABSTRACT

Forest fragmentation causes strong impacts on ecosystems and biodiversity, including changes on the genetic structure of populations. We employed isozyme polymorphism to assess the impact of fragmentation on the genetic structure of *Tapirira guianensis* populations in four Atlantic Rainforest remnants in Pernambuco, Brazil, two small and two large, each population being represented by 31 plants. Isozyme diversity was analyzed at 10 loci that had bands with clean resolution. The isozymes polymorphism ( $P$ ) was 100% and the average of alleles per locus ( $A$ ) ranged from 2.7 to 3.0. Average observed ( $H_o$ ) and expected heterozygosity ( $H_e$  = gene diversity) revealed high genetic diversity. Average inbreeding indices were high among populations ( $F_{IT}=0.264$ ) and within populations ( $F_{IS}=0.215$ ). Individually, inbreeding rates were very high in smaller populations. The differentiation between populations was low ( $F_{ST}=0.071$ ) and the estimated average of gene flow ( $N_m$ ) among populations was 2.45. Analyzing populations by couple, gene flow was observed to be insufficient to avoid long-term differentiation between some fragment pairs. Results indicate that fragmentation has altered the genetic structure of small populations of *T. guianensis* from the effect of inbreeding and restricted gene flow. Therefore, these populations require special attention in order to avoid significant changes in their genetic structure.

**Keywords:** Atlantic Forest, conservation, fragmentation effects, gene flow estimation, inbreeding, isozyme

## INTRODUCTION

The process of fragmentation transforms large sections of an ecosystem into numerous smaller and isolated patches embedded within a matrix (Young *et al.* 1996; Fahrig 2003). This fragmentation generally has a negative effect on the local biodiversity due to a loss and reduction of habitat (Pinto and Brito 2003; Jacquemyn *et al.* 2003; Pinto *et al.* 2004; Torezan *et al.* 2005). The genetic consequences of fragmentation are mainly related to the loss of genetic diversity, with the effects of allele fixation normally being noted some generations after the fragmentation (Murcia 1995; Young *et al.* 1996; Jordán *et al.* 2003) including a reduction of the adaptive potential of many species.

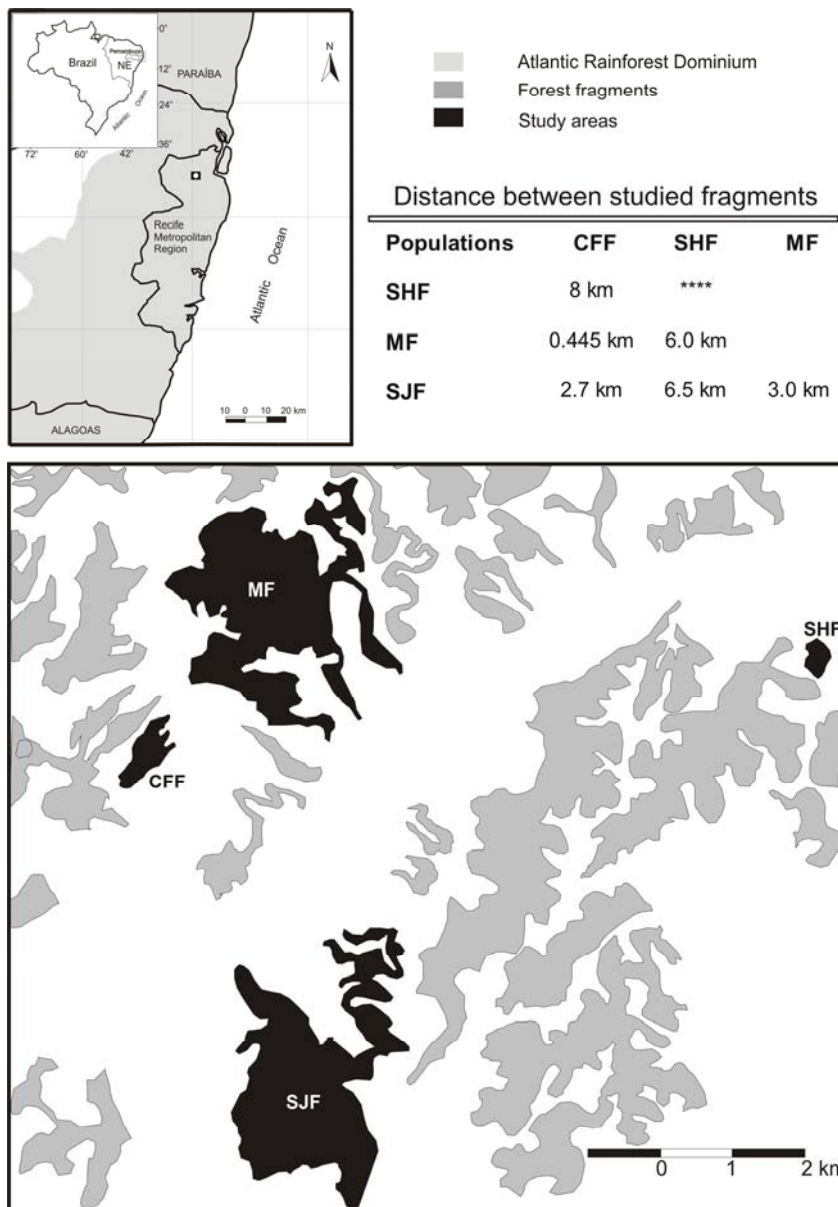
Forest fragments are generally vegetation patches that were not originally cut, but they may also be composed of areas that have undergone regeneration. These patches can constitute important refuges of biodiversity, although most are quite small and are often ignored in terms of their conservation value (Viana *et al.* 1998). Fragmentation tends to have different effects on different species and can be compounded by interactions with other species that were likewise impacted in the fragmentation process (Viana *et al.* 1998; Ribas and Kageyama 2004). Nonetheless, established parks and reserves account for less than 10% of the natural vegetation cover in tropical ecosystems (Gradwohl and Greenberg 1991), and studies investigating the genetic population structure of the species in remnant areas will be fundamental to viable conservation strategies, sustainable development, and preservation (Kageyama *et al.* 1998; Sebbenn *et al.* 2003).

Numerous studies investigating the genetic structure of arboreal species in tropical areas have been undertaken using isozyme polymorphism techniques (Loveless 1992; Liengsiri *et al.* 1995; Lepsc-Cunha *et al.* 1999; Oliveira *et al.* 2002; Moraes *et al.* 2002; Sebbenn *et al.* 2003). This methodology has been employed in many studies to ex-

amine the consequences of fragmentation on tropical species (Seoane *et al.* 2000; Souza *et al.* 2004; Pinto *et al.* 2004; Ribeiro *et al.* 2005). Studies of isozyme polymorphism allow investigators to examine not only the genetic structure and variability of a population, but also to estimate gene flow between populations and make inferences concerning reproductive processes (Hamrick 1983). Gene flow ( $N_m$ ) estimated from measures of genetic differentiation among populations (for example  $F_{ST}$ ) is classified as an indirect investigative method (Slatkin 1985), generating data can determine if genetic drift alone is sufficient to produce the genetic differentiation observed among populations (Slatkin and Barton 1989).

*Tapirira guianensis* Aubl. is an arboreal species within the family Anacardiaceae, being widely distributed on humid lowland areas in Central and South America (Guariguata 1999). This species is perennial, typically found on flatlands and secondary formations with humid soils such as drainage basins and river banks, and produces fruits that are much sought after by the general fauna (Oliveira Filho and Ratter 1995; Silva Júnior *et al.* 1998; Lorenzi 2002). The tree is heliophilous, demonstrating rapid growth and development, and a good capacity for regeneration, making it widely used in heterogeneous reforestation projects (Joly *et al.* 2001; Lorenzi 2002).

According to Oliveira-Filho and Ratter (2001), *T. guianensis* is widely distributed throughout Brazil, often being one of the most important species in the arboreal and regenerative layers of forest formations and riparian forests in the southeastern part of that country (Rodrigues and Nave 2001; Pinto *et al.* 2005) as well as in the Cerrado (Brazilian savannah) and riparian forests of the central plains (Antunes and Ribeiro 1999). In northeastern Brazil, this species is an important component of the upper canopy in the lowland forests of Pernambuco State and adjacent areas (Siqueira *et al.* 2001; Ferraz *et al.* 2004) and is greatly used by the local human populations living near remnant forests (Cunha and



**Fig. 1** Location of the study area in Northeastern Brazil, and forest fragments in which populations of *Tapirira guianensis* Aubl. were studied.

Albuquerque 2006).

*T. guianensis* is dioecious and is pollinated principally by insects (Oliveira Filho and Ratter 1995; Lenza and Oliveira Filho 2005). Panmixia is very common in dioecious plants and as self-fertilization is not possible in these plants, endogamy can only occur by crossing between closely related individuals in a given population. Due to the peculiarities of its biology, its wide distribution and great abundance on the South American continent, and as a result of its being panmictic, this species can serve as a model for studies of fragmentation on the structure of native plant populations. The present work was therefore designed to examine the effects of fragmentation on the diversity and genetic structure of populations of *T. guianensis* in the Atlantic Coastal Forest of Pernambuco State, Brazil, through the analysis of isozyme polymorphism.

## MATERIALS AND METHODS

The populations of *T. guianensis* examined were growing in rainforest fragments at the Usina São José sugarcane plantation. This property covers approximately 247 km<sup>2</sup>, and most of it is located within the county of Igarassu, Pernambuco State (PE), Brazil (Fig. 1), between the geographical coordinates 07°41'04.9"–07°54'41.6"S and 34°05'17.6"–35°05'07.2"W. Geologically, the area predominately occupies the Barriers Group, of plio-pleistocene age, composed of non-consolidated sandy-clay sediments of continental origin. The landscape is dominated by "tabuleiros", which are

generally flat plains occasionally cut by deep and narrow water courses with steep slopes (usually greater than 30%), and the region is intensively used in cultivating sugar cane (CPRH 2003).

The genetic material used in this study consisted of samples taken from four populations (forest fragments), these being: two small areas, the Santa Helena Forest (SHF) and the Pezinho/ Córrego da Foice Forest (CFF) with areas of 11.85 and 27.12 ha, respectively; and two larger forest fragments, the Macacos Forest (MF) and the Piedade/São José Forest (SJF), with areas of 356.92 and 305.78 ha, respectively. The distances between the forests, based on measurements derived from satellite images, vary from 8 km between SHF and CFF to 0.445 km between CFF and MF (Fig. 1).

Thirty-one adult individuals (with diameter at breast height greater than 5 cm) were sampled in each of the four areas, for a total of 124 plants sampled. Collections were undertaken along the border of the forests as well as in their interior to assure an even representation of the populations within the fragments. For each plants, young leaves were collected stored in plastic sacks and kept in ice coolers for transport to the Genetics Laboratory of the Department of Biology of the Federal Rural University of Pernambuco, where they were then stored at -80°C. Enzymes were extracted from all samples in 1 ml of extraction buffer № 1, according to the methodology developed by Alfenas *et al.* (1998).

The isozymes were separated by horizontal electrophoresis on 13% starch gel (Penetrose 30). The gel/electrode buffer systems used were TC (Tris Citrate, pH 7.5), TCB (Tris Citrate Borate, pH 7.5), and LB (Lithium Borate, pH 8.5). After running, the electro-

phoresis plates were removed and sliced, the slices being subjected to staining for the specific enzymes examined according to the methodology established by Alfenas *et al.* (1998).

A total of 12 enzyme systems were initially tested and, of these, ten were selected for detailed examination because they presented loci and alleles with resolutions that facilitated interpretation: acid phosphatase (ACP), peroxidase-1 (PO-1), peroxidase-2 (PO-2), glutamate oxaloacetate transaminase (GOT), catalase-1 (CAT-1), catalase-2 (CAT-2), alcohol dehydrogenase (ADH), alkaline phosphatase (ALP), alpha-esterase ( $\alpha$ -EST) and glucose dehydrogenase (GLUDH). The interpretations of each enzymatic system were performed according to techniques described in detail in the literature (Alfenas *et al.* 1998; Oliveira *et al.* 2006).

The genetic diversity was quantified for allele frequencies and diversity indexes as expected ( $H_e$ ) and observed ( $H_o$ ) heterozygosity, percentage polymorphic loci ( $P$ ) and average number of alleles per locus ( $A$ ). The fixation index was estimated according to  $F$ -statistics (Wright 1965). A Chi-square test was used for to test the deviations of Hardy-Weinberg for each studied loci in each population. All these estimates were obtained using BIOSYS 1 software program (Swofford and Selander 1989).

In order to measure the historic gene flow ( $N_m$ ), the model proposed by Crow and Aoki (1984) was used according to the following equation:

$$Nm = \left(\frac{1}{4\alpha}\right) \left[ \left(\frac{1}{\hat{F}_{ST}}\right) - 1 \right]$$

$$\text{where: } \alpha = \left[ \frac{n}{(n-1)} \right]^2$$

and where,  $N_m$  is the number of migrants per generation,  $n$  is the numbers of populations,  $F_{ST}$  is the genetic differentiation among populations, which was calculated by the combinations of pairs of populations (using the BIOSYS-2 software program). If the value of  $N_m$  is larger than 1.0, it is considered that gene flow is high enough to avoid genetic differentiation due to drift (Slatkin and Barton 1989).

## RESULTS

Ten loci of eight enzymatic systems were analyzed: Got-1 locus, Po-2 loci, Acp-1 locus, Cat-2 loci, Adh-1 locus, Alp-1 locus, Est-1 locus, and Gludh-1 locus. The allelic frequencies ranged from high, as in allele B of the Got locus in the CFF population (0.846), very low, as in allele C of the Po-1 locus of the same population (0.017), to the complete absence of an allele, as in the allele C at the Po-2, Cat-1 and Cat-2 loci in the CFF population and the Acp locus in the MF population (Table 1). The majority of the loci were not in Hardy-Weinberg Equilibrium (HWE). The CFF population demonstrated the largest number of loci in non-equilibrium (eight) while the other populations each demonstrated five (Table 2).

It was observed 100% of polymorphic loci in all populations and the average numbers of alleles per locus varied from 2.7 to 3.0 (Table 3). In relation to average heterozygosity observed and expected among the four populations examined, the  $H_o$  varied from 0.331 to 0.584, while the  $H_e$  varied from 0.503 to 0.642.  $H_o$  was lower in the SHF and CFF populations – these being the populations occupying the smallest land areas.

Fixation indices varied from 0.090 to 0.112 within large populations MF and SJF and from 0.340 to 0.310 in the smaller populations SHF and CFF, respectively (Table 3). The allelic fixation of individual loci demonstrated the same tendency as the entire set of alleles considered together. The populations SHF and CFF had, respectively, two and three fixed loci (value = 1.00), and only three and two without fixation (negative value), while MF and SJF populations demonstrated each only one fixed locus (Got), and six loci without fixation (Table 2).

Inbreeding averages within ( $F_{IS}$ ) and among ( $F_{IT}$ ) populations were 0.215 and 0.264, respectively, and they were

**Table 1** Estimated allele frequencies at ten polymorphic loci in four fragmented populations of *Tapirira guianensis* in NE Brazil.

Locus/Allele	Populations			
	SHF	CFF	MF	SJF
<b>Acp</b>				
(N)	23	27	19	27
A	0.609	0.426	0.632	0.481
B	0.326	0.370	0.368	0.444
C	0.065	0.204	0.000	0.074
<b>Po – 1</b>				
(N)	30	30	28	30
A	0.383	0.500	0.268	0.433
B	0.367	0.483	0.554	0.333
C	0.250	0.017	0.179	0.233
<b>Po – 2</b>				
(N)	9	25	25	17
A	0.222	0.660	0.580	0.500
B	0.444	0.340	0.160	0.235
C	0.333	0.000	0.260	0.265
<b>Got</b>				
(N)	22	26	25	28
A	0.227	0.115	0.360	0.357
B	0.727	0.846	0.560	0.357
C	0.045	0.038	0.080	0.286
<b>Cat – 1</b>				
(N)	31	27	31	30
A	0.403	0.185	0.145	0.383
B	0.403	0.815	0.468	0.450
C	0.194	0.000	0.387	0.167
<b>Cat – 2</b>				
(N)	25	17	29	23
A	0.360	0.471	0.155	0.391
B	0.380	0.529	0.569	0.435
C	0.260	0.000	0.276	0.174
<b>Adh</b>				
(N)	28	28	31	31
A	0.143	0.393	0.242	0.403
B	0.429	0.429	0.355	0.323
C	0.429	0.179	0.403	0.274
<b>Alp</b>				
(N)	24	26	26	27
A	0.500	0.615	0.308	0.407
B	0.229	0.327	0.673	0.444
C	0.271	0.058	0.019	0.148
<b>Est</b>				
(N)	27	26	26	29
A	0.685	0.365	0.769	0.345
B	0.167	0.538	0.115	0.414
C	0.148	0.096	0.115	0.241
<b>Gludh</b>				
(N)	26	28	31	31
A	0.442	0.054	0.403	0.484
B	0.346	0.500	0.306	0.194
C	0.212	0.446	0.290	0.323

(N) = Number of individuals

significantly different from zero for the majority of loci (Table 4). The average of genetic differentiation among populations ( $F_{ST}$ ) was 0.071, but not significantly different in average and for majority of locus.

The non-biased genetic identity of Nei (1978) of the populations that were compared in pairs is in agreement with the geographic distance that separates those pairs – with the lesser distances between pairs (CFF  $\times$  MF, CFF  $\times$  SJF and MF  $\times$  SJF) demonstrating genetic identities varying from 0.929 to 0.932, while the greater distances between pairs (SHF  $\times$  CFF, SHF  $\times$  MF and SHF  $\times$  SJF) demonstrated smaller genetic identities, varying from 0.843 to 0.894 (Table 5).

The estimates of historic gene flow ( $N_m$ ), obtained from the  $F_{ST}$ -statistic for the set of populations and for pairwise populations ranged from 0.77 to 1.95 (Table 5). The estimated value of the gene flow between all studied populations was 2.45.

**Table 2** Chi-square results ( $\chi^2$ ) and degrees of freedom (DF) for Hardy-Weinberg equilibrium, and fixation index ( $f$ ) for ten analyzed loci in four fragmented populations of *Tapirira guianensis* in NE Brazil.

Locus	Populations											
	SHF			CFF			MF			SJF		
	DF	$\chi^2$	$f$	DF	$\chi^2$	$f$	DF	$\chi^2$	$f$	DF	$\chi^2$	$f$
Acp	3	0.484 <sup>ns</sup>	-0.005	3	8.86 <sup>**</sup>	0.132	1	0.05 <sup>ns</sup>	-0.131	3	2.557 <sup>ns</sup>	0.083
Po-1	3	5.940 <sup>ns</sup>	-0.372	3	10.62 <sup>**</sup>	-0.615	3	7.82 <sup>**</sup>	-0.029	3	14.240 <sup>**</sup>	-0.433
Po-2	3	9.625 <sup>**</sup>	1.000	1	17.72 <sup>**</sup>	0.911	3	9.62 <sup>**</sup>	0.369	3	20.215 <sup>**</sup>	0.906
Got	3	22.718 <sup>**</sup>	1.000	3	24.35 <sup>**</sup>	1.000	3	34.91 <sup>**</sup>	1.000	3	47.272 <sup>**</sup>	1.000
Cat-1	3	9.900 <sup>**</sup>	0.342	1	21.68 <sup>**</sup>	1.000	3	7.09 <sup>ns</sup>	0.313	3	6.931 <sup>ns</sup>	0.358
Cat-2	3	5.544 <sup>ns</sup>	0.089	1	14.13 <sup>**</sup>	1.000	3	5.67 <sup>ns</sup>	0.401	3	12.361 <sup>**</sup>	-0.108
Adh	3	18.399 <sup>**</sup>	0.650	3	17.05 <sup>**</sup>	-0.247	3	11.37 <sup>**</sup>	-0.433	3	4.358 <sup>ns</sup>	-0.323
Alp	3	14.954 <sup>**</sup>	0.533	3	2.41 <sup>ns</sup>	0.247	3	0.06 <sup>ns</sup>	-0.106	3	5.351 <sup>ns</sup>	-0.085
Est	3	1.404 <sup>ns</sup>	0.076	3	4.09 <sup>ns</sup>	0.186	3	0.29 <sup>ns</sup>	-0.008	3	3.186 <sup>ns</sup>	-0.111
Gludh	3	0.995 <sup>ns</sup>	-0.142	3	9.62 <sup>**</sup>	0.544	3	14.20 <sup>**</sup>	-0.370	3	16.406 <sup>**</sup>	-0.550

P < 0.05, ns = non significant

**Table 3** Estimates of genetic diversity at ten loci in four fragmented populations (Pop.) of *Tapirira guianensis* (standard errors in parentheses) in NE Brazil and fixation indices ( $f$ ).

Pop.	Average № of alleles per locus	% of polymorphic loci	Average heterozygosity		Fixation indices ( $f$ )
			Observed ( $H_o$ )	Expected ( $H_e$ )	
SHF	2.7 (0.2)	100.0	0.331 (0.103)	0.503 (0.040)	0.3419
CFF	3.0 (0.0)	100.0	0.412 (0.093)	0.603 (0.028)	0.3167
MF	2.9 (0.1)	100.0	0.498 (0.087)	0.561 (0.029)	0.1122
SJF	3.0 (0.0)	100.0	0.584 (0.108)	0.642 (0.009)	0.0903

**Table 4** F-statistical calculated for ten loci in four fragmented populations of *Tapirira guianensis* in NE Brazil.

Locus	$F_{IS}$	$F_{IT}$	$F_{ST}$
Acp	0.031 <sup>ns</sup>	0.056 <sup>ns</sup>	0.026 <sup>ns</sup>
Po-1	-0.359 <sup>ns</sup>	-0.308 <sup>*</sup>	0.038 <sup>ns</sup>
Po-2	0.799 <sup>*</sup>	0.817 <sup>*</sup>	0.088 <sup>ns</sup>
Got	1.000 <sup>*</sup>	1.000 <sup>*</sup>	0.103 <sup>*</sup>
Cat-1	0.430 <sup>*</sup>	0.486 <sup>*</sup>	0.098 <sup>*</sup>
Cat-2	0.305 <sup>ns</sup>	0.340 <sup>*</sup>	0.050 <sup>ns</sup>
Adh	-0.099 <sup>ns</sup>	-0.059 <sup>ns</sup>	0.036 <sup>ns</sup>
Alp	0.163 <sup>ns</sup>	0.232 <sup>*</sup>	0.083 <sup>*</sup>
Est	0.032 <sup>ns</sup>	0.145 <sup>ns</sup>	0.117 <sup>*</sup>
Gludh	-0.154 <sup>ns</sup>	-0.070 <sup>ns</sup>	0.073 <sup>*</sup>
Average	0.215	0.264	0.071
	[-0.091 to 0.521] <sup>*</sup>	[-0.026 to 0.505]	[0.053 to 0.089]

\* P<0.05; <sup>ns</sup> non significant, [ ] confidence interval with p<0.05.

**Table 5** Genetic identity ( $I$ ), estimated genetic differentiation ( $F_{ST}$ ), average Nei's (1978), and gene flow ( $Nm$ ) in four fragmented populations of *Tapirira guianensis* in NE Brazil.

Combinations	$I$	$F_{ST}$	$Nm$
SHF × CFF	0.864	0.065	0.90
SHF × MF	0.843	0.036	1.67
SHF × SJF	0.894	0.031	1.95
CFF × MF	0.930	0.075	0.77
CFF × SJF	0.932	0.052	1.14
MF × SJF	0.929	0.034	1.78

**DISCUSSION**

**Genetic diversity**

All studied isozyme loci in *T. guianensis* were polymorphic ( $P=100\%$ ). Similar results were observed in other tropical tree species. For example, Melo Júnior *et al.* (2004) reported a  $P$  value of 100% for *Caryocar brasiliense* Camb. in northern Minas Gerais State, Brazil. In contrast, small percent of polymorphic loci has been detected in other tree species. Hamrick and Loveless (1986) reported a  $P$  value of 89.3% for *Alseis blackiana* Hemsl. in Central America; and Oliveira *et al.* (2006) obtained a  $P$  value of 83.3% for *Caesalpinia echinata* Lam. in Pernambuco State, Brazil. Still, the average numbers of allele per locus in this study (ranging from 2.7 to 3.0) are amongst the observed values in natural populations of tropical trees (Hamrick 1989), such as *C. brasiliense* (2.6 to 3.0; Melo Júnior *et al.* 2004).

High genetic diversity ( $H_e$ ) was observed in all the po-

pulations, ranging from 0.503 in SJF to 0.642 in SHF populations. In spite of the differences among them, these values are similar to those encountered in natural populations of other species, and are considered common in terms of genetic diversity. Oliveira *et al.* (2006) found genetic diversity ranging from 0.286 to 0.468 for *C. echinata* in their examination of two natural populations and a reforested area in Pernambuco State, Brazil; Melo Júnior *et al.* (2004) reported  $H_e$  values ranging from 0.450 to 0.530 in natural populations of *C. brasiliense* in the savannah of Minas Gerais; Gusson *et al.* (2005) reported  $H_e$  values ranging from 0.400 to 0.431 in natural populations of *Eschweilera ovata* (Cambess. - Miers) in the state of Bahia, Brazil; and Pinto *et al.* (2004) reported  $H_e$  values ranging from 0.396 to 0.282 in populations of *Copaifera langsdorffii* Desf. in two fragments of riparian forest in the state of Minas Gerais, Brazil. Thus, in comparison to these studies, it is possible to conclude that, in despite of forest fragmentation, the studied *T. guianensis* populations present high levels of genetic diversity.

**Effects of forest fragmentation**

The loss of alleles in the CFF and MF populations may be associated with genetic drift caused by fragmentation as each of these alleles were present in three fragment populations but absent in only one. This situation is most pronounced in the CFF population, which demonstrated absence of allele C for three loci (Po-2, Cat-1 and Cat-2). Additionally, the CFF population demonstrated the lowest frequency of C allele for these loci (Po-1, Got Alp and Est) (Table 1). According to Isagi *et al.* (2007), the effective number of alleles is a more sensitive approach for detecting the immediate loss following a population bottleneck. The intensity of the effects of genetic drift is inversely proportional to population size, making smaller populations more vulnerable, and reducing genetic diversity, which can result in the fixation of alleles due to inbreeding and/or loss of alleles from selection (Young *et al.* 1996). The present results agree with this statement. Still, Souza *et al.* (2004) observed that the greatest probability for allelic loss is associated with rare or low frequency alleles, while common alleles have a greater probability of being fixed in these populations. In agreement, it was observed in the present study that many of the lost alleles had low-frequency (Table 1). Still, the loss of low-frequency and rare alleles are reported in other studies about the effects of forest fragmentation on tropical tree species. For example, Ribeiro *et al.* (2005) working

with the tropical tree *Dalbergia nigra* (Vell.) Allemao ex. Benth. in three forest fragments observed a decrease in allele richness, alteration in allele frequencies, and allelic loss due to fragmentation in four of the five analyzed isozyme loci. Allelic loss was also observed by White *et al.* (1999) in their study of fragmented populations of *Swietenia humilis* Zucc. in Central America and Mexico.

The observed deviations from Hardy-Weinberg Equilibrium (HWE) in all populations suggest that they are experiencing alterations in gene frequencies and genotypic as a result of genetic drift, selection, or biparental inbreeding (deviation of random mating), with the CFF population demonstrating these effects more intensely.

As already commented, forest fragmentation is expected to cause the loss of rare and low-frequent alleles, decrease the heterozygosity and increase the coefficient of inbreeding. Although high levels of heterozygosity were observed in the examined populations, in the smaller populations SHF and CFF, in general, heterozygosities were smaller than detected in the larger populations (MF and SJF). In these two small populations, alleles were also lost and high fixation indexes (0.342 and 0.317, respectively) were also observed. The high fixation index most clearly demonstrated the effects of fragmentation on population structure. Fragmentation is probably affecting these smaller populations through the reduction in reproductive population sizes and increase in the spatial genetic structure due the seed dispersal near to the seed-tree, resulting in an increase in rates of mating among relatives, in agreement with the observations of Young *et al.* (1996) and Hartl and Clark (1997).

The average fixation index for the total populations ( $F_{IT}$  = 0.264) indicates an homozygosity excess in relation to the expected in a panmictic species, confirming once again a tendency for inbreeding and genetic drift within these populations. The average fixation index within populations ( $F_{IS}$  = 0.215) was also very high (Table 4), indicating that deviation of random mating, due to mating among relatives, has a stronger effect on genetic structure than the effect of genetic drift.  $F_{IS}$  has the value of zero in a random-mating population and increases towards one when inbreeding becomes predominant.  $F_{IT}$  has the value of zero when the population is under HWE and does not experience genetic drift, increasing towards one as inbreeding becomes higher and genetic drift occurs. The average  $F_{ST}$  (0.071) demonstrated that only 7% of genetic diversity was distributed among population and 93% was within populations. According to Loveless and Hamrick (1984), outcrossing species typically demonstrate high levels of genetic diversity within populations and low genetic differentiation among populations. Moreover, when gene flow is high, differentiation between populations decreases.

### Historic gene flow

According to Slatkin and Barton (1989), Pinto and Carvalho (2004), and Souza *et al.* (2004), values of estimated historic gene flow greater than 1.0 are sufficient to eliminate differentiation due to genetic drift, and the populations are not genetically isolated. In this study, the value obtained considering all populations was high ( $N_m=2.45$ ), suggesting absence of isolation. However, considering the populations in pairs, the estimated gene flow ranged from 0.77 to 1.95 (Table 5). Population pairs MF × CFF ( $N_m=0.77$ ) and SHF × CFF ( $N_m=0.90$ ) have  $N_m$  values lower than 1.0. As  $N_m$  is estimated from the differentiation among populations, these results indicate that the alleles are being exchanged at low levels between the two populations. In studying the genetic and reproductive consequences of forest fragmentation on *Magnolia obovata* Thunb in Japan, Isagi *et al.* (2007) found a larger gene flow into the conserved sites than into the fragmented populations. Results presented here also reflect the effects of genetic drift observed in the CFF population – with a loss of alleles and a high degree of homozygosity. Still, the CFF population demonstrated a gene flow lower than 1.0 with SHF and with MF, even though CFF is less

than 1 km apart from MF.

The existence of other small populations of *T. guianensis* (not sampled) adjacent to the four sampling areas may have contributed to the high general levels of gene flow in those cases where the gene flow was reasonably high. According to Chase *et al.* (1996) and Aldrich and Hamrick (1998), even remnant trees in open pasture lands can influence gene flow among nearby fragmented populations. Seoane *et al.* (2000) stated that gene flow favors homogeneity of genetic diversity among populations and reduces the consequences of selection and genetic drift, thus reducing the genetic differentiation among populations. The mechanisms involved in gene flow between these fragmented populations have not yet been closely examined, but various species of insects were identified in the fragments that could potentially facilitate pollen flow between those populations (Schessl *et al.* 2005) as the distances between these fragments are relatively short. Additionally, birds may be dispersing the seeds of these trees, as Guimarães (2003) has reported that the fruits of *T. guianensis* are very sought after by these animals.

### Consequence of forest fragmentation

Considering that the populations of *T. guianensis* examined in this work were fragmented between 25 and 40 years ago, and the reproductive cycle of this species average about five years, sufficient time has passed for the potential establishment of five to eight generations of trees. This number of generations should have been sufficient to allow for the appearance of alterations in the genetic structure of the populations due to genetic drift following the fragmentation event – and this was in fact observed in the high levels of fixation in the CFF and SHF populations, and make them both more vulnerable to the risks of genetic differentiation. For minimizing the effects of forest fragmentation on genetic diversity of populations, it is possible to suggest some options as: 1) artificial exchange of seeds or propagules from nearby fragments with the fragments demonstrating genetic erosion; 2) increasing the size of fragments, using seeds adequately collected in terms of effective population size ( $N_e$ ) and, 3) creation of genetic corridors to facilitate genetic connectivity between fragments with small populations.

In conclusion, the fragmented populations of *T. guianensis* examined here demonstrated genetic diversity comparable to that seen in other tropical species, and this diversity is considered satisfactory even though the populations have suffered from the effects of genetic drift and inbreeding, as demonstrated by the Hardy-Weinberg test. Smaller forested areas showed smaller genetic diversity and are more vulnerable to the effects of fragmentation, which was demonstrated by the loss of alleles and by their higher indices of fixation. There is more diversity within the four populations examined than among them, indicating that genetic differentiation between them has not been strongly affected by forest fragmentation (based on the Nei's genetic distance). This is probably due to the short time since the fragmentation event. The estimated gene flow demonstrated a medium value that was sufficient to avoid differentiation between populations by genetic drift. However, considering the populations in pairs, gene flow was observed to be insufficient to avoid long-term differentiation between some fragment pairs. Finally, the results presented in this study indicate that the preservation of naturally forested areas in the Usina São José has been effective in maintaining the genetic diversity of the species *T. guianensis*. However, the smaller populations will require special attention in order to avoid significant alterations in their genetic structure, with resulting loss of variability.

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## REFERENCES

- Aldrich PR, Hamrick JL** (1998) Reproductive dominance of pasture trees in a fragmented tropical mosaic. *Science* **281**, 103-105
- Alfenas CA, Peters I, Brune W, Passador GC** (1998) *Eletroforese de proteínas e isoenzimas afins: fundamentos e aplicações em plantas e microorganismos*, UFV, Viçosa, Brazil, 574 pp
- Chase MR, Moller C, Kesseli R, Bawa KS** (1996) Population genetics of *Cordia alliodora* (Boraginaceae), a neotropical tree: gene flow, neighborhood, and population substructure. *American Journal of Botany* **82**, 484-490
- CPRH (Companhia Pernambucana do Meio Ambiente)** (2003) *Diagnóstico Sócioambiental do Litoral Norte de Pernambuco*, CPRH, Recife, 211 pp
- Crow JF, Aoki K** (1984) Group selection for polygenic behavioral trait: estimating the degree of population subdivision. *Proceedings of the National Academy of Sciences USA* **8**, 6073-6077
- Cunha LVFC, Albuquerque UP** (2006) Quantitative ethnobotany in an Atlantic Forest fragment of Northeastern Brazil - implications to conservation. *Environmental Monitoring and Assessment* **114**, 1-25
- Fahrig L** (2003) Effects of habitat fragmentation on biodiversity. *Annual Review of Ecology and Systematics* **34**, 487-515
- Ferraz EMN, Araújo EL, Silva SI** (2004) Floristic similarities between lowland and montane areas of Atlantic Coastal Forest in Northeastern Brazil. *Plant Ecology* **174**, 59-70
- Gradwohl J, Greenberg M** (1991) Small forest reserves: making the best of a bad situation. *Climatic Change* **19**, 235-256
- Guariguata MR** (1999) Early response of selected tree species to liberation thinning in a young secondary forest in Northeastern Costa Rica. *Forest Ecology and Management* **124**, 255-261
- Guimarães MA** (2003) Frugivoria por aves em *Tapirira guianensis* (Anacardiaceae) na zona urbana do município de Araruama, Estado do Rio de Janeiro, sudeste brasileiro. *Atualidades Ornitológicas* **116**, 12-12
- Gusson E, Sebbenn AM, Kageyama PY** (2005) Diversidade e estrutura genética espacial em duas populações de *Eschweilera ovata*. *Scientia Forestalis* **67**, 123-135
- Hamrick JL** (1983) The distribution of genetic variation within and among natural plant populations In: Schonewald-Cox CM, Chambers SM, MacBryde B, Thomas L (Eds) *Genetics and Conservation*, Benjamin-Cummings, London, UK, pp 335-348
- Hamrick JL** (1989) Isozymes and the analysis of genetic structure in plant populations. In: Soltis DE, Soltis PS (Eds) *Isozymes in Plant Biology*, Chapman and Hall, London, UK, pp 87-105
- Hamrick JL, Loveless MD** (1986) Isozyme variation in tropical trees: procedures e preliminary results. *Biotropica* **18**, 201-207
- Hartl DL, Clark AG** (1997) *Principles of Population Genetics*, Sinauer Associates, Sunderland, USA, 542 pp
- Isagi Y, Tateno R, Matsuki Y, Hirao A, Watanabe S, Shibata M** (2007) Genetic and reproductive consequences of forest fragmentation for populations of *Magnolia obovata*. *Ecological Research* **22**, 382-389
- Jacquemyn H, Van Rossum F, Brys R, Endels P, Hermy M, Triest L, De Blust G** (2003) Effect of agricultural land use and fragmentation on genetic demography and population persistence of the rare *Primula vulgaris* and implications for conservation. *Belgium Journal of Botany* **136**, 5-22
- Joly CA, Spigolon J, Lieberg AS, Salis SM, Aidar MPM, Metzger JPW, Zickel CS, Lobo PC, Shimabukuro MT, Marques MCM, Salino A** (2001) Projeto Jacaré-Pepira: o desenvolvimento de um modelo de recomposição da mata ciliar com base na florística regional. In: Rodrigues RR, Leitão-Filho HF (Eds) *Matas Ciliares: Conservação e Recuperação*, EDUSP, São Paulo, Brazil, pp 271-287
- Jordán L, Báldi A, Orci KM, Rác I, Varga Z** (2003) Characterizing the importance of habitat patches and corridors in maintaining the landscape connectivity of a *Pholidoptera trassylvanica* (Orthoptera) metapopulation. *Landscape Ecology* **18**, 83-92
- Kageyama PY, Gandara FB, Souza LMI** (1998) Consequência genética da fragmentação sobre populações de espécies arbóreas. *Série Técnica IPEF* **12**, 65-79
- Lenza E, Oliveira PE** (2005) Biologia reprodutiva de *Tapirira guianensis* Aubl. (Anacardiaceae), uma espécie dióica em mata de galeria do Triângulo Mineiro, Brasil. *Revista Brasileira de Botânica* **2**, 179-190
- Lepsc-Cunha N, Kageyama PY, Vencovsky R** (1999) Genetic diversity of *Couratari multiflora* and *Couratari guianensis* (Lecythidaceae): consequences of two types of rarity in central Amazonia. *Biodiversity and Conservation* **8**, 1205-1218
- Liengsiri C, Yeh FC, Boyle TJB** (1995) Isozyme analysis of a tropical forest tree, *Pterocarpus macrocarpus* Kurtz in Thailand. *Forest Ecology and Management* **74**, 13-22
- Lorenzi H** (2002) *Brazilian Trees: a guide to the identification and cultivation native trees* (Vol I), Plantarum, São Paulo, Brazil, 384 pp
- Loveless MD** (1992) Isozyme variation in tropical trees: patterns of genetic organization. *New Forest* **6**, 165-175
- Melo Júnior AF, Carvalho D, Povoá JRS, Bearzoti E** (2004) Estrutura Genética em populações Naturais de Pequizeiro (*Caryocar brasiliense* Camb.). *Scientia Forestalis* **66**, 56-65
- Moraes PLR, Monteiro R, Vencovsky R** (2002) Genetic differentiation and diversity of natural populations of *Cryptocarya* spp. (Lauraceae) from the Brazil Atlantic rain forest. *Lundiana* **3**, 99-109
- Murcia C** (1995) Edge effects in fragmented forests: implications for conservation. *Trends in Ecology and Evolution* **10**, 58-62
- Ney M** (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. *Genetics* **89**, 583-590
- Oliveira AF, Carvalho D, Rosado SCS** (2002) Taxa de cruzamento e sistema reprodutivo de uma população natural de *Copaifera langsdorffii* Desf. na região de Lavras (MG) por meio de isoenzimas. *Revista Brasileira de Botânica* **25**, 331-338
- Oliveira CAM, Silva EF, Molina SG, Ferreira RLC, Lira DAS, Barros Júnior, JAB** (2006) Diversidade e estrutura genética em populações de *Caesalpinia echinata* (Lam.) na Estação Ecológica do Tapacurá, PE. *Scientia Forestalis* **70**, 77-83
- Oliveira Filho AT, Ratter JA** (1995) A study of the origin of Central Brazilian forest by the analysis of plant species distributions patterns. *Edinburgh Journal of Botany* **52**, 141-194
- Oliveira Filho AT, Ratter JA** (2001) Padrão florístico das matas ciliares da região do Cerrado e a evolução das paisagens do Brasil Central durante o Quaternário Tardio. In: Rodrigues RR, Leitão Filho HF (Eds) *Matas Ciliares: Conservação e Recuperação*, EDUSP, São Paulo, Brazil, pp 73-89
- Pinto LP, Brito MCW** (2003) Dynamics of biodiversity loss in the Brazilian Atlantic Forest: an introduction. In: Galindo-Leal C, Câmara IG (Eds) *The Atlantic Forest of South America: Biodiversity Status, Threats and Outlook*, Conservation International, Washington, USA, pp 27-30
- Pinto LVA, Botelho AS, Oliveira-Filho AT, Davide AC** (2005) Estudo da vegetação como subsídios para propostas de recuperação das nascentes da bacia hidrográfica do Ribeirão Santa Cruz, Lavras, MG. *Revista Árvore* **29**, 775-793
- Pinto SIC, Souza AM, Carvalho D** (2004) Variabilidade genética por isoenzimas em populações de *Copaifera langsdorffii* Desf. em dois fragmentos de mata ciliar. *Scientia Forestalis* **65**, 40-48
- Ribas LA, Kageyama PY** (2004) Diversidade e estrutura genética em populações naturais de *Trema micratha* (L.). *Scientia Forestalis* **70**, 77-83
- Ribeiro RA, Simões Ramos AC, Lemos Filho JP, Lovato MB** (2005) Genetic variation in remnant populations of *Dalbergia nigra* (Papilionoideae), an endangered tree species from the Brazilian Atlantic Forest. *Annals of Botany* **95**, 1171-1177
- Rodrigues RR, Nave AG** (2001) Heterogeneidade florística das Matas Ciliares. In: Rodrigues RR, Leitão-Filho HF (Eds) *Matas Ciliares: Conservação e Recuperação*, EDUSP, São Paulo, Brazil, pp 45-71
- Schessl M, Krause L, Piechowski D, Gottsberger G** (2005) A fragmentação da Mata Atlântica em Pernambuco e suas consequências biológico-reprodutivas. In: Franke CB, Rocha PLB, Klein W, Gomes SL (Orgs) *Mata Atlântica e Biodiversidade*, UFBA, Salvador, Brazil, pp 43-164
- Sebbenn AM, Kageyama PY, Vencovsky R** (2003) Conservação genética *in situ* e número de matrizes para a coleta de sementes em população de *Genipa americana* L. *Scientia Forestalis* **63**, 13-22
- Seoane CES, Kageyama PY, Sebbenn AM** (2000) Efeitos da fragmentação florestal na estrutura genética de populações de *Esenbeckia leiocarpa* Engl. (Guarantã). *Scientia Forestalis* **57**, 123-139
- Silva Júnior MC, Felfili JM, Nogueira PE, Resende AV** (1998) Análise florística das matas de galeria do Distrito Federal. In: Ribeiro JF (Ed) *Cerrado - Matas de Galeria*, Embrapa-CPAC, Planaltina, Brazil, pp 53-93
- Siqueira DR, Rodal MJN, Lins-e-Silva ACB, Melo AL** (2001) Physiognomy, structure and floristics in an area of Atlantic Forest in Northeast Brazil. *Disseratações Botânica* **346**, 11-27
- Slatkin M, Barton NHA** (1989) Comparison of three indirect methods for estimating average levels of gene flow. *Evolution* **43**, 1349-1368
- Slatkin M** (1985) Rare alleles as indicators of gene flow. *Evolution* **39**, 53-65
- Souza LMFI, Kageyama PY, Sebbenn AM** (2004) Estrutura genética em populações fragmentadas de *Chorisia speciosa* St. Hil. (Bombacaceae). *Scientia Forestalis* **65**, 70-79
- Swofford DL, Selander RB** (1989) Biosys-1: A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. *Journal of Heredity* **72**, 282-283
- Torezan JMD, Souza RF, Ruas PM, Ruas CF, Camargo EH, Vanzela ALL** (2005) Genetic variability of pre and post-fragmentation cohorts of *Aspidosperma polyneuron* Muell. Arg. (Apocynaceae). *Brazilian Archives of Biology and Technology* **45**, 171-180
- Viana VM, Pinheiro LAFV** (1998) Conservação da biodiversidade em fragmentos florestais. *Série Técnica IPEF* **12**, 25-42
- White GM, Boshier DH, Powell W** (1999) Genetic variation within a fragmented population in a tropical dry forest: an example from *Swietenia humilis* Zucc. *Molecular Ecology* **8**, 1899-1909
- Wright S** (1965) The interpretation of population structure by F-statistics with special regard to system of mating. *Evolution* **19**, 395-420
- Young A, Boyle T, Brown T** (1996) The population genetic consequences of habitat fragmentation for plants. *Trends in Ecology and Evolution* **11**, 413-418