

# Genetic diversity of the tropical tree *Terminalia amazonia* (Combretaceae) in naturally fragmented populations

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The effect of long-term fragmentation on the genetic diversity of populations of the neotropical tree species, *Terminalia amazonia*, was studied using random amplified polymorphic DNA (RAPD) analysis. Leaf material from 104 trees was collected from three naturally fragmented gallery forest patches and three plots in nearby continuous forest in the Mountain Pine Ridge, Belize. In total, 30 RAPD bands generated by five decamer primers were used to compare the genetic diversity of the six populations in the two groups. Genetic variation within the populations ( $H_0$ ), as estimated by the Shannon diversity index, ranged from 0.32 to 0.38, with an overall diversity of 0.38 ( $H_{\text{species}}$ ). Analysis of molecular variation revealed that most (94.4%,  $P < 0.001$ ) of the

variation was attributable to differences among individuals within populations. Population differentiation was significantly ( $P = 0.038$ ) lower among the fragmented populations than among continuous forest populations. On average, the fragmented populations also had slightly, but statistically significant ( $P = 0.046$ ) lower levels of genetic diversity. However, one gallery forest site had a higher level of genetic diversity than two of the continuous forest sites. We suggest that the long-term effect of fragmentation on the genetic diversity of tropical trees will depend upon the amount of local forest cover in proximity to the fragmented populations. *Heredity* (2003) 91, 307–313. doi:10.1038/sj.hdy.6800299

**Keywords:** conservation genetics; forest fragmentation; gallery forest; genetic diversity; RAPD; tropical trees; gene flow

## Introduction

Tropical rainforests are the most biologically diverse terrestrial ecosystems, but much of this diversity is under threat from extensive forest loss and fragmentation (Myers, 1986). The threat lies not only in the outright loss of species, but also in the potential loss of genetic diversity and fitness of remnant populations. The maintenance of genetic variation is considered essential for the long-term survival of a species since genetic diversity provides a species' evolutionary potential (Frankel and Soule, 1981). Furthermore, a reduction in diversity through the loss of alleles reduces a population's ability to respond to biotic challenges (eg, pathogens) and to changes in the abiotic environment.

Forest loss and fragmentation alters the composition, configuration, and connectivity of the landscape (Taylor *et al.*, 1993). These changes can affect genetic diversity in several ways: through the direct loss of genotypes as habitats and its residents are extinguished, through processes related to reduced population sizes, and through the isolation of remnant populations, which can modify and limit gene flow (White *et al.*, 2002). Such impacts can lead to genetic bottlenecks, increased random genetic drift, and inbreeding depression, which can ultimately result in a loss of genetic variation

and increased genetic differentiation between remnant populations (Young *et al.*, 1996).

Thus far there have been only a few studies that have assessed the impact of forest fragmentation on the genetic diversity of tropical tree species. For example, in Costa Rica, allozyme analysis revealed that small, isolated populations of the canopy tree *Pithecellobium elegans* in remnant forests had suffered genetic erosion (Hall *et al.*, 1996). In the same region, fragmented populations of *Carapa guianensis* had lower allelic diversity as a result of restricted gene flow, although there was no evidence of inbreeding in saplings (Dayanadan *et al.*, 1999). Evidence of significant inbreeding was found in seedlings of *Symphonia globulifera*, resulting in significant genetic differentiation among fragmented forest populations (Aldrich *et al.*, 1998). However, the low density, canopy emergent *Dinizia excelsa* was found to thrive in forest fragments and as isolated individuals in pastures in Amazonia, thanks to pollination by the non-native African honeybee (Dick, 2001). And although fragmentation of a tropical dry forest in Honduras led to the loss of some low-frequency microsatellite alleles in *Swietenia humilis*, White *et al.* (2002) observed increased pollen flow among fragmented populations, which they hypothesized could eventually restore and augment levels of genetic variation.

Most of the studies mentioned were conducted in landscapes in which forest fragmentation was a relatively recent event. Consequently, the adult trees within the forest remnants would predate fragmentation and

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ecological processes would not have had sufficient time to adjust to the modified environment. Even when seedlings are analysed rather than adults, one cannot be sure whether the genetic diversity of the seedling cohort can be used to predict the future diversity of the remnant population (Hamrick *et al*, 1993; Aldrich *et al*, 1998; Condit *et al*, 1998). Thus, the conditions in relatively young forest fragments may be a poor indicator of the long-term genetic consequences of fragmentation. Instead, an alternative approach would be to use natural fragments, such as gallery forests within savannas (Pither and Kellman, 2002). These types of forests may be many centuries old, which means sufficient time would have passed to allow for the turnover of at least a few generations of trees and for landscape processes to have adjusted to the altered landscape (Kellman, 1996).

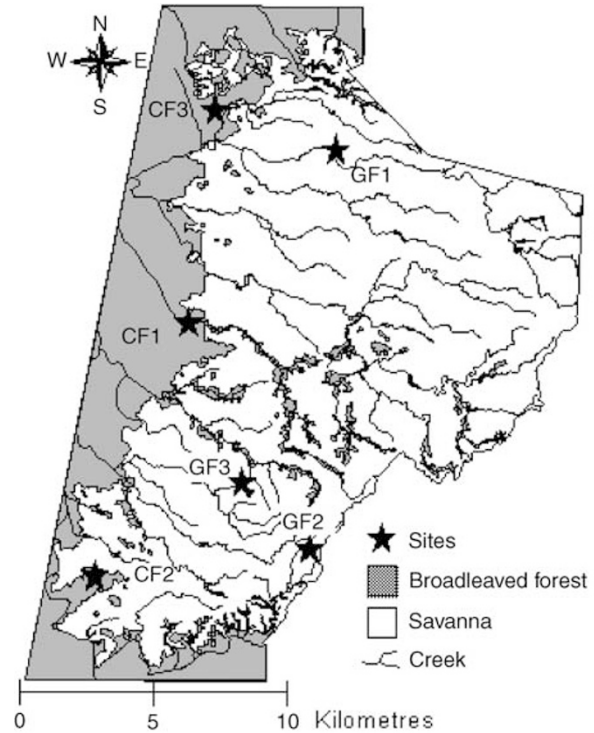
This paper presents results from a study on the genetic diversity of the tropical tree *Terminalia amazonia* (Combretaceae) in gallery forests of the Mountain Pine Ridge (MPR) savanna, Belize. In the continuous forests surrounding the MPR, *T. amazonia* is very common but in the naturally fragmented gallery forests within savanna, it is present at much lower densities. The objectives of the study were to determine whether long-term fragmentation had led to a loss of genetic variation within gallery forests and to increased genetic differentiation among populations. Random amplified polymorphic DNA (RAPD) analysis was used to compare populations from three gallery forests to populations from three plots within nearby continuous forests. This study is a component of a larger project whose purpose is to determine whether a network of very small forest fragments can contribute to the maintenance of regional diversity in human-dominated landscapes (Pither and Kellman, 2002).

## Materials and methods

### Study area

Research was conducted in the Mountain Pine Ridge in Belize, Central America (17°N, 88°W). Although surrounded by continuous broad-leaved forest, the MPR itself is dominated by a pine savanna. Within the savanna is a naturally fragmented network of broad-leaved gallery forests (Figure 1). Previous work has determined that the gallery forests contain levels of plant diversity comparable to that found in regional continuous forests at local scales (Meave and Kellman, 1994). High levels of tree diversity were also found within the smaller micro-forests (less than 1 ha in size) and tree thickets (linear riparian forests less than 20 m in width) that can be found scattered along creeks throughout the MPR (Pither and Kellman, 2002).

Charcoal and pollen evidence suggest that the savanna has been in existence for at least 11 000 years and is likely to be a remnant of a system of larger savannas that were present throughout Central America during the last Glacial Maximum (Kellman, 1989). Given that the MPR savanna has existed for thousands of years, it is likely that gallery forests have been present throughout the same time period (Meave and Kellman, 1994). It is also probable that the amount of forested area is dynamic, increasing or decreasing depending on fluctuations in climate and fire frequencies. The three gallery forest (GF)



**Figure 1** Gallery and continuous forest sites in the western section of the Mountain Pine Ridge, Belize.

sites used in this study all possessed characteristics that we assume are indicative of very old forests: well-developed canopies, very large trees, and the presence of tree species not normally found in recently established forests. All GF sites were smaller than 1 ha in size (Table 1) and approximately 4 km from continuous forest but within 2 km of networks of larger GF (Figure 1). Three control plots were located along creeks in the nearby continuous forest (CF).

### Species description

*T. amazonia* (Combretaceae) is a common large tree found throughout much of the neotropics (Standley, 1962). Little is known about its reproductive biology but most species in the genus are outcrossing despite being hermaphroditic (Srivastav *et al*, 1993). *T. amazonia* is pollinated by insects and its seeds are dispersed by wind (Gentry, 1993). It grows in lowlands and uplands and is both a commercial timber species, and a native tree favoured for reforestation projects. Although reports indicate that the species can apparently grow in savanna, this is not the case in the MPR, where extensive reconnaissance determined that it is restricted to forests with closed canopies. In fact, *Terminalia* seeds failed to germinate during a seed planting experiment in the MPR savanna (Kellman and Miyanishi, 1982). Within the continuous forest surrounding the MPR, *T. amazonia* is a very common tree species (approximately 29 adults/ha, Table 1) but within GF it exists in much lower densities (approximately 11 adults/ha). Like several other hardwood species, *T. amazonia* was selectively logged many years ago in the continuous forests of the MPR but it is highly unlikely that the relatively remote

**Table 1** Summary of site information: size of plot or forest, density of adults ( $\geq 5$  cm dbh) and number of individuals (adults+juveniles) of *Terminalia amazonia* analysed from study sites in the Mountain Pine Ridge, Belize

Forest type	Site	Size of plot or forest (ha)	Density (adults/ha)	No. of trees analysed
Continuous forest	CF1	1	28.1	17
	CF2	1	35.7	17
	CF3	1	23.3	18
Gallery forest	GF1	0.75	12.3	15
	GF2	0.35	11.6	17
	GF3	0.89	9.0	20
Total				104

gallery forest sites used in this study were subjected to logging. Ohri (1996) reported that there is considerable variation in chromosome size and polyploidy level among species of the pantropical genus *Terminalia*. However, we did not find any published reports with data on the genetics of the species *T. amazonia*.

### Sampling

Field work was conducted over a 3-week period in August, 2000. Leaf material was collected from all adults ( $\geq 5$  cm dbh) and some juveniles ( $> 1$  m in height) within the three GF sites. Collection of leaf material in continuous forest was restricted to plots of approximately 1 ha (to correspond to the small size of the GF) and to individuals at least 10 m apart. Young leaves were collected when possible. All leaves were stored in individual containers with labels and at the end of the day deposited in liquid nitrogen. Upon return to Toronto, Canada, the collection was stored in a deep freezer at  $-80^{\circ}\text{C}$ .

### Laboratory procedures

DNA was extracted from frozen leaf material using the CTAB technique (Doyle and Doyle, 1987) and further purified using the Wizard® DNA Clean-up kit (Promega). PCR amplification solutions (25  $\mu\text{l}$ ) included approximately 5–10 ng of genomic DNA, 1.5 mM  $\text{MgCl}_2$ , 200  $\mu\text{M}$  of each dNTP, 1 U of *Tsg* DNA Polymerase and reaction buffer (BioBasic Inc), and 1  $\mu\text{M}$  of primer (Operon Technologies). Eighteen 10bp primers were tested for the process (Operon Technology E-4, 5; I-1, 2, 4, 5, 6, 7, 8, 9, 16, 17, 18, 19, 20; J-4, 5, 16). Only those primers that produced high intensity and reproducible bands were used for the remainder of the analyses: I-5, 8, 9, 18, and 19.

Amplifications were conducted in an Eppendorf Mastercycler with a heated lid. Amplifications were initiated for 2 min at  $94^{\circ}\text{C}$ , followed by 35 cycles of the following: denaturation at  $94^{\circ}\text{C}$  for 2 min, annealing at  $37^{\circ}\text{C}$  for 1 min, and elongation at  $72^{\circ}\text{C}$  for 2 min. An additional 5 min at  $72^{\circ}\text{C}$  was used to ensure that all amplified products completed their elongation. Amplification products were resolved electrophoretically on a 1.5% agarose gel and bands were visualized with ethidium bromide fluorescence. Several gels included a PCR control (without DNA) to test for artefactual bands. Samples were assigned randomly to lanes and all gels

**Table 2** Oligonucleotide primers and sizes of bands used in analyses

Primer	Nucleotide sequence	Band sizes (base pairs)
OPI-05	5'-TGTTCCACGG-3'	416, 819, 1300, 1790, 2270
OPI-08	5'-TTTGCCCGGT-3'	654, 825, 895, 1110, 1310
OPI-09	5'-TGGAGAGCAG-3'	600, 1000, 1250, 1620, 2100
OPI-18	5'-TGCCCAGCCT-3'	600, 660, 750, 840, 1000, 1300, 1400, 1540, 1614, 1726
OPI-19	5'-AATGCGGGAG-3'	693, 841, 983, 1249, 1684

included lanes containing DNA ladders to facilitate standardization. Gels were digitally photographed and the images of multiple gels were standardized using GelCompar software.

### Data analysis

The presence/absence of RAPD bands was recorded in binary format for statistical analysis. The study used only band locations where the presence or absence of the band was clearly identifiable and reproducible in subsequent amplifications: a total of 30 bands from five primers (Table 2). Only samples that were successfully scored for all 30 band locations were used in the statistical analyses.

Genetic variation was estimated using the Shannon Diversity Index (Lewontin, 1972):

$$\text{Shannon Index: } H = - \sum P_i \times \log_2(P_i)$$

where  $P_i$  = frequency of the  $i$ th band.

Loss of genetic variation manifested by the reduction of rare alleles and the fixation of common ones would be reflected by changes in overall allele frequencies and hence Shannon Index values. Unlike some other studies (eg, Martin and Bermejo, 2000), we did not treat primers as loci and their bands as alleles but instead considered each band location as a locus at which there were two possible alleles: one encoding the presence of the band and the other, its absence. Allele frequencies were obtained by assuming that all RAPD loci exhibit dominance (for the presence of a RAPD band) and follow Hardy-Weinberg equilibrium frequencies. A diversity index was calculated for each locus based on the allele frequencies among all individuals within a given population. The diversity of that population was then calculated by averaging the indices for all 30 loci. Following the convention outlined by Lewontin (1972), genetic variation can be measured at several levels:

$H_0$  = genetic variation among individuals within a population.

$H_{\text{pop}}$  = average of all populations' variations ( $H_0$ ) within a group.

$H_{\text{groups}}$  = genetic variation among all individuals within a group.

$H_{\text{species}}$  = genetic variation among all individuals within the species.

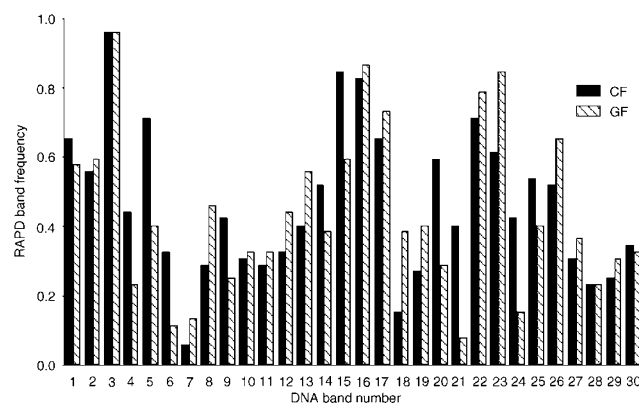
In this study there were only two groups into which populations were assigned on the basis of forest type: GF or CF.

In order to evaluate whether fragmentation led to a loss of genetic variation, we subtracted the mean variation of the three GF populations from the mean of the CF populations. The significance of the difference was tested using a nonparametric (Monte Carlo) approach in which we randomly reassigned individuals among populations, after which the difference was recomputed. Repeating the process 1000 times provided a null distribution of values that would occur by chance alone, against which the observed value was compared (Manly, 1998; Schneider *et al*, 2000).

Genetic differentiation was assessed using a pairwise similarity index within an ANOVA framework to partition diversity within and among populations. Pairwise Euclidean genetic distance measures were calculated and analysed using the analysis of molecular variation (AMOVA) module within Version 2.0 of Arlequin (Schneider *et al*, 2000). Using our own macro, the equivalent of a *posthoc* comparison was conducted in order to obtain average among population/within forest type genetic differentiation measures. The similarities of all possible pairs of individuals from different populations within GF sites and within CF sites were calculated using the Sørensen Index (Brower *et al*, 1998). The mean of the three possible among-site similarities within GF was compared to the mean for those for sites within CF. Spatial autocorrelation was tested by calculating the Pearson's correlation between intersite Sørensen similarities and Euclidean geographical distances. As before, significance values for all relationships were obtained using Monte-Carlo procedures.

## Results

A total of 104 individuals of *T. amazonia* were successfully scored for all 30 band locations produced by the five primers. A visual assessment of the raw frequencies of the 30 bands indicated that although most bands were present in similar frequencies in the two forest types, some were much less common in GF than in CF sites (eg, bands 4, 5, 6, 20, 21; Figure 2). Conversely, other bands showed minor increases in frequencies in GF populations (eg, bands 8, 18, 23 and 26). No bands were unique to any of the six populations but two GF sites had two



**Figure 2** Frequencies of 30 RAPD bands among continuous and gallery forest populations of *Terminalia amazonia*, in the Mountain Pine Ridge, Belize, amplified using five primers (Operon I-5, 8, 9, 18, 19). Bands were numbered in order of increasing primer number and fragment size (Table 2).

**Table 3** Genetic variation of populations of *Terminalia amazonia* in the Mountain Pine Ridge, Belize, as measured using the Shannon Diversity Index

Site	$H_0$	$H_{pop}$	$H_{groups}$	$H_{species}$
CF1	0.383			
CF2	0.353	0.367	0.383	
CF3	0.365			0.379
GF1	0.346			
GF2	0.322	0.349	0.364	
GF3	0.378			

bands each that were found in juveniles only and not in adults (GF2, bands 6 and 30; GF3, bands 7 and 21). In addition, four bands (6, 7, 21 and 28) were absent from at least one population.

Estimations using the Shannon Index (Lewontin, 1972) indicated that the mean within-population genetic variation of the GF ( $H_{pop}=0.349$ , Table 3) was slightly but significantly (difference = 0.018,  $P=0.046$ ) lower than the mean of the CF populations ( $H_{pop}=0.367$ ). A similar loss of variation was apparent when individuals, rather than populations, were grouped by forest type ( $H_{groups}$ , difference = 0.019,  $P=0.038$ ). Continuous forest site #1 had the highest within-population diversity ( $H_0=0.383$ ), while GF site #2 had the lowest ( $H_0=0.322$ ). The overall genetic diversity was 0.379 ( $H_{species}$ ).

Analysis of molecular variation showed that the vast majority of the genetic variation of *T. amazonia* was attributable to differences among individuals within populations ( $P<0.001$ , Table 4). Approximately 4% could be credited to differences among GF and CF populations but the Monte-Carlo procedure revealed that this figure could be obtained by random placement of individuals among populations ( $P=0.100$ ). An even smaller proportion (1.4%) of variation was produced by differences among populations within the two forest types. Even so, there was a significant ( $P<0.001$ ) difference in the level of genetic differentiation in the CF and naturally fragmented populations.

Closer examination of genetic variation using Sørensen similarity measures (Table 5) revealed that similarities among GF populations (mean = 0.604) were significantly ( $P=0.038$ ) higher than among continuous forest populations (mean = 0.584), reflecting a decrease in genetic differentiation in the naturally fragmented populations. Otherwise there seems to be little pattern to intersite similarities. The two sites with the most similar genotypes were GF sites 1 and 2 but the similarities among all other combinations of GF populations and CF populations were comparable. Analysis of spatial autocorrelation found a weak and nonsignificant ( $R^2=0.135$ ,  $P=0.112$ ) correlation between intersite genotype similarity and geographical distance.

## Discussion

RAPD analysis was used to estimate the genetic diversity of CF and naturally fragmented, GF populations of the tropical tree *T. amazonia* in Belize. The six populations had Shannon index values ranging from 0.32 to 0.38, with an overall diversity of 0.38 ( $H_{spp}$ ). These values are

**Table 4** Results from analysis of molecular variance for within and among populations sources of variation for *Terminalia amazonia* in the Mountain Pine Ridge, Belize

Source of variation	df	Sums of squares	Variance components	% of variation	P-values
Among forest types	1	21.635	0.270	4.24	0.100
Among populations within forest types	4	30.188	0.089	1.39	<0.001
Within populations	98	589.658	6.017	94.37	<0.001
Total	103	641.481	6.376	100.00	

**Table 5** Mean pairwise Sørensen similarity values of genotypes (above diagonal) and geographical distances (km) (below diagonal) between continuous forest and gallery forest populations of *Terminalia amazonia* in the Mountain Pine Ridge, Belize

	CF1	CF2	CF3	GF1	GF2	GF3
CF1	—	0.574	0.576	0.566	0.572	0.558
CF2	10.54	—	0.601	0.589	0.590	0.570
CF3	7.95	18.32	—	0.572	0.578	0.580
GF1	8.38	18.68	4.74	—	0.633	0.579
GF2	9.42	8.27	16.65	14.86	—	0.601
GF3	6.85	6.68	14.51	13.43	2.93	—

comparable to values published for populations of other tropical tree species, including 0.35 for *Swietenia macrophylla* (Gillies *et al.*, 1999), 0.45 for *Cedrela odorata* (Gillies *et al.*, 1997), and 0.59 for *Poulsenia armata* (Aide and Rivera, 1998). Care should be taken when comparing values across studies because they are influenced by the genetic markers employed and because of inconsistent methods of calculating diversity (eg, Gillies *et al.*, 1999; Martin and Bermejo, 2000).

The analysis of molecular variation revealed that the vast majority (94%) of the molecular variance recorded in *T. amazonia* in the MPR was attributable to differences among individuals within the populations. Low levels of population differentiation are common in tropical tree species at small spatial scales (Hamrick and Loveless, 1989). For example, a study in Costa Rica found that more than 96% of the genetic variation in *Dendropanax arboreus*, *Inga thibaudiana*, and *Protium glabrum* was attributable to differences within plots along a 9 km transect (Schierenbeck *et al.*, 1997). However, population differentiation does tend to rise with increasing distances between populations and may be exacerbated by forest fragmentation (Hall *et al.*, 1996; Aide and Rivera, 1998; Gillies *et al.*, 1999).

Most research investigating the effect of forest fragmentation on the genetic diversity of tropical trees has been conducted in recently disturbed landscapes in which ecological processes have had little time to adjust to the altered environment (eg, Hall *et al.*, 1996; Aldrich *et al.*, 1998; Dick, 2001). To the best of our knowledge, only one other researcher has utilized natural forest fragments to predict the long-term genetic consequences of fragmentation on tropical trees (Shapcott, 1998, 1999, 2000). That project concluded that isolated populations of *Carpentaria acuminata* were effectively inbred and had low genetic diversity, while *Syzygium nervosum* had maintained high levels of genetic diversity regardless of population size or isolation (Shapcott, 2000).

In the MPR, we found that GF populations of *T. amazonia* had slightly, but statistically significant ( $P=0.046$ ) lower levels of genetic variation than those of nearby continuous forest populations, indicating that the long-term fragmentation has had a negative effect on genetic diversity. Theory suggests that random processes in fragmented and isolated populations could lead to a decline in the frequency of rare alleles and an increase in common ones (Young *et al.*, 1996; White *et al.*, 2002). However, evidence provided by the raw frequencies of the 30 DNA bands generated in this study does not reflect that expectation since the bands that experienced dramatic changes in frequencies were not rare nor common ones.

Interestingly, while two of three GF populations had the lowest variation of all six sites, the other fragmented population (GF3) actually had a higher level of diversity than two of the CF populations. This difference in genetic variance appears to be matched by a difference in local GF cover. Both genetically depauperate sites had very little local forest cover with less than 1 ha within a 1.5 km radius each. The relatively genetically diverse GF3, on the other hand, had more than 10 ha of gallery forest within the same radius. This limited data raises the possibility of a landscape effect in which local forest cover is affecting gene flow, most likely via impacts on pollinator movement. Although some seed and pollen dispersers are undeterred by nonforested habitat and continue to move among forest remnants (Estrada *et al.*, 1993; Dick, 2001), other dispersers appear to be reluctant to cross even small breaks between forest fragments (Powell and Powell, 1987). In the MPR, the limited forest cover surrounding the two GF sites with low genetic variation must have provided an insufficient degree of connectivity to facilitate visits from pollinators.

Landscape composition provides a plausible explanation for differences in genetic variation among the GF populations, but it cannot explain why site GF3 had a higher level of variation than two of the CF sites. The data suggest that somehow, of those three sites, the fragmented one had experienced the highest amount of gene flow. Other studies have found evidence of increased pollen flow following forest fragmentation, including for the tropical tree species *Spondias mombin* (Nason and Hamrick, 1997) and *Swietenia humilis* (White *et al.*, 2002), as well as for the temperate species *Acer saccharum* (Young *et al.*, 1993). White *et al.* (2002) went as far as to suggest that over time, increased gene flow arising from forest fragmentation could eventually augment levels of genetic variation. But given that two of the GF sites in the MPR had low levels of variation, there is likely a threshold of local forest cover required to facilitate increased gene flow after forest fragmentation, below which populations will experience

reduced gene flow and consequently the expected loss of genetic diversity.

Forest fragmentation is also expected to lead to an increase in interpopulation genetic differentiation as a result of random genetic drift (Young *et al.*, 1996; Dayanadan *et al.*, 1999). However, we found the opposite for *T. amazonia* in the MPR, with significantly ( $P = 0.038$ ) lower levels of differentiation (higher levels of similarity) among GF populations than among CF populations. Young *et al.* (1993) recorded a similar result for *A. saccharum*, which they attributed to an increase in gene flow among forest fragments. That explanation would not be consistent with our data since the two most isolated populations with the lowest genetic variation (GF1 and GF2) were also the most similar of all possible pairs of sites.

In summary, results from this study suggest that the long-term fragmentation of populations of *T. amazonia* in the MPR has, on average, had a negative effect on the genetic diversity of the species. However, variation in diversity among the sites indicates a potential landscape effect in which local forest cover may have determined the outcome. Although limited, our data suggest that sites with sufficient cover may acquire higher levels of genetic diversity, possibly as a result of increased gene flow, while populations in sites with forest cover below a certain threshold would experience the expected decline in genetic diversity. Further research is required to test this hypothesis. The results from this study complement other research attempting to evaluate the effect of forest loss and fragmentation on the genetic diversity of tropical trees (eg, Hall *et al.*, 1996; Aldrich *et al.*, 1998; Dayanandan *et al.*, 1999; Dick, 2001; White *et al.*, 2002). At this point it is difficult to identify generalities because results from the few studies conducted thus far have not been consistent, even from those utilizing natural and potentially ancient forest fragments (Shapcott, 1998, 2000). Ultimately, it is likely that the effect of fragmentation will be species specific and will depend on each species' reproductive biology and in particular the response of their pollen and seed dispersal vectors to the modified landscape.

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