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Distribution of the Genetic Diversity of *Pinus ayacahuite* (Ehrenberg) at the Communal Forest of Totonicapán, Guatemala

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ABSTRACT

The genetic diversity of *Pinus ayacahuite* from six stands at the Communal Forest of Totonicapan (Guatemala) was evaluated with chloroplast microsatellite markers. The analysis along an altitudinal cline showed a diverse pattern in both genetic diversity and genetic differentiation estimates. Three areas were established: 1) a lower area, located near human establishments that includes a forest nursery, is characterized by the highest genetic diversity (He: 0.7-0.8) and differentiation (D^2: 0.22-0.44) detected. Human impacts are frequent and the introduction of foreign plant material could have caused alterations in the genetic composition, 2) an intermediate area is characterized by a moderate anthropogenic perturbation (familiar-small agricultural farms). It has experienced a recent reduction of their effective population size and exhibit a reduction of haplotype richness and 3) a higher area showed elevated genetic diversity and differentiation values. The latter, has been revealed as a genetic diversity reservoir thanks to a low human impact. This study shows several important data that can contribute to the management of this forest area in the future. The low-perturbated stands with high genetic diversity indexes are critical to ensure effective sampling with afforestation purposes.

Keywords: afforestation, chloroplast microsatellites, human impact
Abbreviations: cpSSR, chloroplast simple sequence repeats; **D^2**, within-population genetic distance between haplotypes; **H**, unbiased haplotypic diversity; **IAM**, infinite alleles model; **PCR**, polymerase chain reaction; **SMM**, stepwise mutation model

INTRODUCTION

Forests are a principal natural resource and major land cover type in Latin America and the Caribbean. This region has the highest deforestation rates of the developing world on a per unit of time and unit of land basis. Latin American forest areas are threatened by conversion to other land uses and by overexploitation. Most of this deforestation is the result of converting natural forest with a variety of productive and environmental functions into marginal agriculture land with little economic or environmental value. The excessive forest clearing and consequent decline in the productivity of the land causes accelerated erosion and watershed deterioration in the highland regions from Mexico to Andes (Veblen 1976).

The forest of western highlands of Mexico and Guatemala posses more species of the genus *Pinus* than any other region of comparable size (Mirov and Larsen 1958). The western highlands of Guatemala houses nine *Pinus* species. Two of them, *Pinus strobus* var. Chiapensis Martínez (= *Pinus chiapensis* (Mart.) Andresen) and *Pinus ayacahuite* (Ehren.) represent the soft pine group (Aguilar 1961). *P. ayacahuite* is one of the most highly valued pines in Guatemala because of its ecological role and because its wood is easily processed.

The forest genetic resources of highland Guatemala are under serious threat. Some factors such as land competition due to the growth of human populations have produced a drastic reduction of *P. ayacahuite* natural resources.

Efforts that ensure conservation and sustainable use of forest genetic resources that actually represent economic, social and environmental value (Laarman and Sedjo 1992) are more relevant in a community where the forest plays a major role in the conservation of regional water resources. This is the case of the Communal Forest of Totonicapán, where conservation of water resources directly is related to two species: the endangered *P. ayacahuite* and *Abies guatemalensis*, which is in danger of extinction. The preservation of this gene pool is the available option to future forest management and afforestation programs.

The Communal Forest of Totonicapán is located at an altitude of about 3000 m at the Mountain Chain of Sierra María Tecún in the western highlands of Guatemala. Mean annual temperatures range from 15-20°C, with freezing temperatures (-4°C) during night time from December to March. The annual precipitation is between 900-1000 mm. The main species is the *P. ayacahuite*, which is directly related with the accumulation of water. Fifty villages that constitute a Water Committee called "Land, Water and Forest" depend directly on the forest for their water supply.

Efforts on genetic conservation of *P. ayacahuite* have been made by the Central America and Mexico Coniferous Resources Cooperative CAMCORE (Donahue *et al*. 1991), and have included seed collection for ex situ conservation and generating information on soil requirements, wood properties and guidelines for the handling, management of germplasm and nursery stock for reforestation programs. But regardless its importance for the survival of the communities around the Communal Forest of Totonicapán, there is no plan for the conservation, management and sustainable use of its genetic resources.

Powerful insights have been gained into the knowledge of forest tree genetics from the analysis of microsatellite markers. Microsatellite markers provide a powerful tool for addressing genetic questions related to genotyping (DNA fingerprinting) i.e. Lopez-Arjona *et al*. (2007), monitoring breeding programs, genetic linkage mapping and characterisation of QTLs (quantitative trait loci), genetic resources

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Table 1 Code, location, number, and elevation at the six stands where individuals of *Pinus ayacahuite* (Ehren) were sampled.

Code	Location	No. of individuals sampled	Latitude range (N)	Longitude range (W)	Elevation range (m)
S1	Salvachán	10	14°54′	91°18′	3000-3100
S2	Pacajá	9	14°54′	91°17′ - 91°18′	3150-3250
S3	Chuijolom	10	14°54′	91°18′ - 91°19′	3166-3250
S4	Chojolom	11	14°54′ - 14°55′	91°19′	3075-3200
L2	Chuipachec	29	14°54′ - 14°55′	91°19′ - 91°20′	2815-2900
L3	Trojales	12	14°53′ - 14°54′	91°16′ - 91°17′	3187-3300
Total		81			

conservation assessment and monitoring the effects of forest management practices and forest fragmentation (see Butcher *et al*. 1999). Chloroplasts DNA in *Pinus* does not recombine due to its paternal inheritance in conifers (e.g. Vendramin and Ziegenhagen 1997; Sperisen *et al*. 1998). Thus, chloroplast molecular markers as microsatellite markers (cpSSRs, chloroplast simple sequence repeats) represent a useful and informative approach for studying population history, for monitoring gene flow and hybridisation and for identifying areas harbouring high levels of genetic diversity in conifer species (Anzidei *et al*. 1999). Also, genetic diversity studies constitute a valuable tool in the strategy planning for the conservation of a species (Moritz 1994; FAO 2000, 2001, Gómez *et al*. 2002; Korol *et al*. 2002; Gómez *et al*. 2003a).

The objective of the present study is to analyse, through chloroplast microsatellites, the distribution of the genetic diversity of the *P. ayacahuite* at the Communal Forest of Totonicapán. These results can translate the general principles of the genetic structure of this forest into an operational national program where the selection of the seeds for breeding, reforestation, conservation and use of the genetic resources of the *P. ayacahuite* in Guatemala, can be sustained under scientific evidence.

MATERIALS AND METHODS

Plant material

Six different stands of *P. ayacahuite* were sampled in the Communal Forest of Totonicapán, Guatemala (**Table 1; Fig. 1**): Salvachán (S1), Pacajá (S2), Chuijolom (S3), Chojolom (S4), Chuipachec (L2) and Trojales (L3). At each stand, we sampled needle tissue from 10-200 years-old trees. Needles from 81 individual trees were collected. The number of samples from each stand is proportional to the stand density. More than five individuals are sampled in order to obtain an optimal sample size to minimize the variance of gene differentiation among subpopulations (Pons and Petit 1995). All tissue was conserved at –80°C at the Universidad del Valle de Guatemala until use.

DNA extraction

DNA was extracted from 81 individuals using a modification of the procedure described by Doyle and Doyle (1990). We used 0.079 g of needle tissue from each individual for DNA extraction, cut them in very small pieces and let it soaked in 1 ml CTAB extraction buffer for 15-20 min (5 M NaCl, 1 M Tris-HCl (pH 8), 0.5 M EDTA (pH 8), CTAB, 2% PVP-40). Samples were then grounded using a mortar and pestle. After homogenization 5% *N*-lauryl-sarcosine was added. Mixture was heated at 55°C for 1 h. Extraction with chloroform (24: 1) followed by centrifugation at 13,000 rpm (4°C) for 10 min. Supernatant was removed and isopropanol was added and let cool at –20°C for 1 h. Another centrifugation at same conditions followed and DNA was left to dry for 15 min. One ml 76% ethanol was added and centrifugated at 13,000 rpm (4°C), left to dry for 1 h. The DNA was re-suspended in 100 µl water and stored at –20°C, since it was immediately used.

PCR-cpSSR markers

DNA was amplified by PCR (Polymerase Chain Reaction) using a Perkin Elmer 9600 thermocycler with the following PCR profile: 5 min denaturation at 95°C, followed by 25 cycles of 1 min denaturation at 94°C, 1 min annealing at 55°C and 1 min extension at 72°C, with a final extension of 20 min at 72°C. With each sample eight cpSSR (Vendramin *et al*. 1996) were amplified: PT 1520 (A), PT 3655 (B), PT 7195 (C), PT 3025 (D), PT 8730 (E), PT 9400 (F), PT 1100 (G), Pt 2610 (H). Each 20 µl amplification reaction contained: 20 ng of total DNA, 0.2 µM of fluorescently labeled forward primer and unlabeled reverse primer (Progenetic), 200 µM each dNTPs, 50 mM KCl, 10 mM Tris-HCl (pH 9), 2.5 mM $MgCl_2$ and 0.5 U of *Taq*-DNA polymerase (Ecogen). Standards were used for length determination of alleles. Amplified products were checked by 1.8% agar electrophoresis in 1X TAE buffer and ethidium bromide dye were visualized in UV light. Fragment size of fluorescently labeled PCR products were determined in an ABI 3100 automated sequencer from Applied Biosystems.

Fig. 1 Location of sampling area for *Pinus ayacahuite* at the Communal Forest of Totonicapan in the western highlands of Guatemala.

Data analysis

1. Genetic diversity estimates

Since the chloroplast genome does not undergo recombination, it can be considered as a single locus, and the size scores for the eight polymorphic fragments analyzed were combined in order to derive the chloroplast haplotype of each individual. Nevertheless, we will use the term locus to refer to a cpSSR region, and allele to refer to a size variant at a given cpSSR region. Haplotype frequencies were estimated in each stand and Network 4.5 software (available at fluxus-engineering.com) was used to represent the genetic relationships among haplotypes. The following population genetic parameters were computed for each population: number of haplotypes (nh), number of private haplotypes (nhp), haplotype richness (Petit *et al*. 1998) for specified sample sizes as based on the rare-

faction method developed in ecology by Hurlbert (1971), and Nei's unbiased haplotypic diversity (H; Nei 1987). Within-population genetic distance between haplotypes, D^2, as defined by Goldstein *et al*. (1995) was also computed. This distance is based on the differences among the number of repeat units at the microsatellite regions (stepwise mutation model, SMM) considering the chloroplast DNA as a single locus.

2. Population genetic structure

Between stands differentiation and stands genetic structure has been analyzed. Genetic differentiation and gene flow using microsatellite data was calculated by RSTCALC software (Goodman 1997). R_{ST}, a measure of genetic differentiation analogous to F_{ST}, which are unbiased with respect to differences in sample size between populations and differences in variance between *loci,* was calculated. R_{ST} explicitly accounts for mutation rates at microsatellite *loci* (Slatkin 1995):

$$R_{ST} = (S_T - S_W)/S_T$$

- R_{ST} is the fraction of the total variance of allele size between populations
- S_T and S_W are respectively, total and within stand average sum of squares of difference in allele sizes.

The number of migrants between locations was estimated as:

$$Nm = [(1/ R_{ST}) -1]/2$$

Mantel tests 2.0 (Mantel 1967; Liedloff 1999) were used to test the specific hypotheses about spatial patterns in the distribution of chloroplast gene diversity at the stand level. Comparisons were made computing the product-moment correlation between a matrix of pairwise genetic distances based on allele size differences (Goldstein *et al*. 1995) and a matrix of diverse distances: the geographic matrix, calculated using the program "Surface distance between two points of latitude and longitude" (available at: www.wcrl.ars.usda.gov/cec/java/latlong.htm); and the altitude matrix, based on altitudinal measurements at each locality, using data obtained from a GPS. Original values of the different matrices were transformed to Euclidian distances.

Stand genetic structure was analyzed using kinship coefficients between pairs of individuals. This coefficient is the probability that two genes drawn randomly from each two individuals are identical by descendant (IBD). In natural populations, we have only access to the identity in state (IIS). However, at a small spatial scale, mutation can be neglected relative to migration-genetic drift and IIS is similar to IDB (Rousset 1996). The kinship coefficient for each pair of individuals and each allele k was estimated following Loiselle *et al.* (1995) as:

$$r_j = [(p_i-p_k)(p_j-p_k)/p_k(1-p_k)] + [1/(2n-1)]$$

where pk is the mean frequency of allele k in the population sampled, p_i and p_j are the frequencies of k in individuals i and j, respectively (taking the values 0, 0.5 and 1) and n is the sample size. The second term of the equation corrects for finite sampling. When estimating the multiallelic coefficient, r_{ij} was weighted by its respective polymorphism $p_k(1-p_k)$. The multilocus estimate of r_{ij} was the sum of multiallelic coefficients divided by the sum over all loci and alleles of $p_k(1-p_k)$.

Global and hierarchical genetic structure were evaluated by analysis of molecular variance (AMOVA; Excoffier *et al*. 1992) under two mutations models, infinite alleles model (IAM) and stepwise mutation model (SMM). The IAM model assumes that genetic drift is the driving force behind population divergence (Michalakis and Excoffier 1996; Weir and Cockerham 1984). The analysis under the SMM model was performed by using a genetic distance approach based on the sum of the squared number of repeat differences between two haplotypes (Slatkin 1995). Statistical significance was tested for pairwise population comparisons and covariance components using permutation tests (1,000 permutations) Only P-values lower than 0.05 were considered significant. The AMOVA analysis and significance tests were performed with Arlequin software (v. 2.0; Schneider *et al*. 2000).

RESULTS

Genetic diversity estimates

Between one and six alleles (size variants) were identified at each polymorphic site, and the distribution of allele sizes was largely unimodal with the mode near the middle (**Fig. 2**). Locus D was the most variable with six alleles while loci B, F and G were monophormic. The combination of 18 variants found in eight regions resulted in 14 haplotypes (**Table 2**). Three haplotypes were shared between locations (**Fig. 3**).

Genetic diversity parameters based on haplotypes are shown in **Table 3**. Estimates of haplotype richness were based in rarefaction methods with an average of 3.98. The highest values have been obtained in L2 (3.8) and S4 (3.7). Unique haplotypes (57%) were found in eight individuals and at least one occurred in each stand (private haplotypes) with exception for stands S1 and S3 where only the most frequent haplotype was detected (**Fig. 3**). This haplotype No. three (130/145/165/150/84/86/106/145) was the only one present in all stands. This most common haplotype occurred in 48 (59%) samples followed by haplotype two which occurred in seven (9%) samples and haplotype 14 which was present in five samples (6%) all from stand S4. The genetic relationships among haplotypes (**Fig. 4**) showed a clear pattern for SMM for haplotypes from the same stand, thus it is possible to establish the phylogenetic pattern from the most common haplotype (No. three). Unbiased haplotypic diversity *sensu* Nei (1987) was remarkably lower for stands S1, S2 and S3, while it was larger than the grand mean for the L2 and S4 stands. Furthermore, stand L2 showed a double squared allelic distance, D^2 than L3 and S4 (**Fig. 5**).

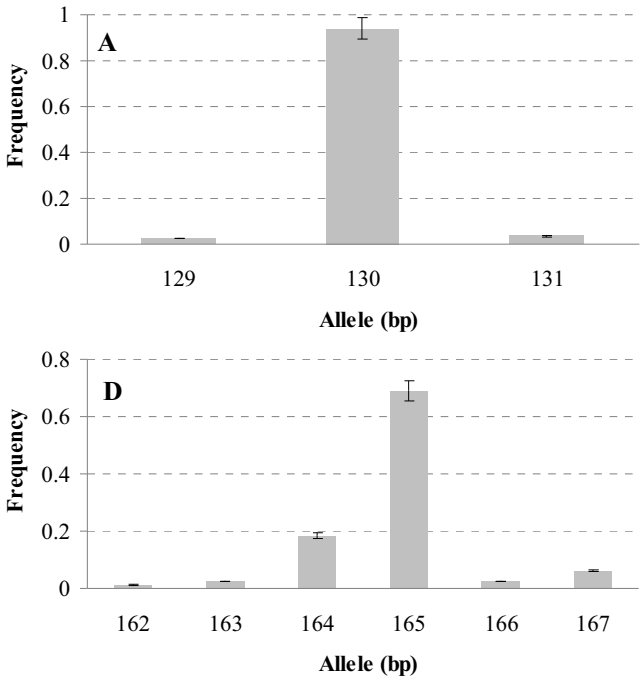

Fig. 2 Frequency distribution of polymorphic alleles at two cpSSR *loci* (A and D) for *Pinus ayacahuite* from the Communal Forest of Totonicapan (Guatemala). Columns in each frame indicate the frequency of each allele.

Population genetic structure

The genetic differentiation and gene flow between stands were calculated based on the inference of a relationship between allele length difference and divergence time (**Table 4**). Thus the highest R_{ST} value was obtained between L2 and L3 stands, the most distant stands. S2 stand showed the greatest number of migrants (Nm) with all other stands.

A negative and not significant relationship (r = -0.760) was found when the Euclidean distances among sampling

Table 2 Haplotypes of *P. ayacahuite* from the Communal Forest of Totonicapán (Guatemala) defined by the combination of eight cpSSR markers. (Allele sizes are in base-pairs, bolds indicate shared haplotypes between stands).

HAPLOTYPE	Pt 1520	Pt 3655	Pt 3025	Pt 8730	Pt 9400	Pt 1100	Pt 2610	Pt 7195
1	130	145	163	150	84	86	106	145
2	**130**	**145**	**164**	**150**	**84**	**86**	**106**	**145**
3	**130**	**145**	**165**	**150**	**84**	**86**	**106**	**145**
4	131	145	165	150	84	86	106	145
5	130	145	164	150	84	86	106	144
6	130	145	166	150	84	86	106	144
7	**130**	**145**	**166**	**150**	**84**	**86**	**106**	**145**
8	129	145	162	150	84	86	106	145
9	130	145	165	150	84	86	106	144
10	130	145	163	150	84	86	106	144
11	129	145	165	150	84	86	106	145
12	130	145	165	151	84	86	106	145
13	130	145	165	150	84	86	105	145
14	130	145	167	150	84	86	106	145

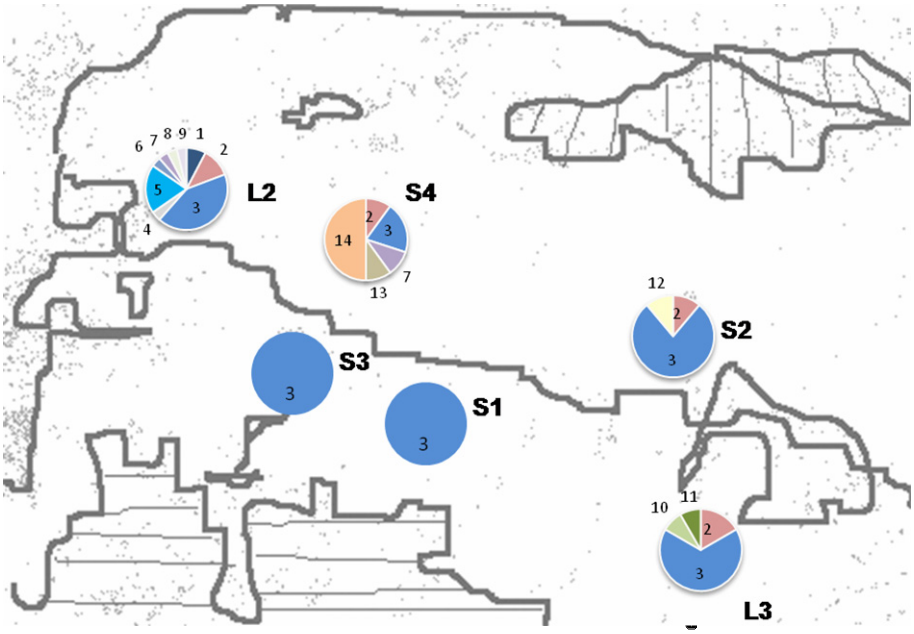

Fig. 3 Distribution of *Pinus ayacahuite* cpSSR haplotypes in six stands at the Communal Forest of Totonicapan (Guatemala). See **Table 1** for location-code identifications. Numbers 1-14 are haplotypes as described in **Table 2**.

Table 3 Genetic diversity parameters based on haplotype frequencies for six stands of *P. ayacahuite* of the Communal Forest of Totonicapán (Guatemala). Sample size (*n*), number of haplotypes (*Nh*), number of private haplotypes (Nh_p), Nei´s genetic diversity (*H*), within-population genetic distance between haplotypes (D^2) and haplotype richness (A_9) standardized to a common sample size of 9 individuals.

Location	*n*	*Nh*	Nh_p	*H*	D^2	A_9
L2	28	9	6	0.8	0.44	3.81
L3	12	4	1	0.56	0.21	2.46
S1	10	1	0	0	0	0
S2	9	3	2	0.42	0.05	2
S3	10	1	0	0	0	0
S4	10	5	2	0.71	0.22	3.7
Average	13.5	3.8	1.8	0.57	0.25	3.97

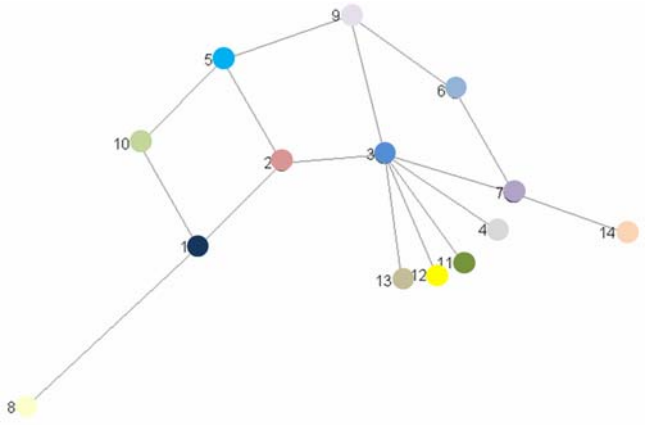

Fig. 4 Haplotype network for *P. ayacahuite* cpSSR markers. Branch lengths are drawn to scale of the total number of mutational steps in each clade. Colours correspond to frequencies in **Fig. 3**. No 3 is the most common haplotype, the ancestral haplotype.

stands and genetic distances were analysed by the Mantel tests.

Distributions of pair-wise cpSSR length differences among individuals are shown in **Fig. 6**. Only Trojales location (L3) showed a bimodal distribution.

Two stands whit similar D^2 values (L3 and S4) were analyzed by the multilocus estimate of the average kinship coefficient (**Fig. 7**). Both stands are located at diverse altitudes, approximately an equal number of samples were analyzed and showed similar genetic diversity index. In the first class (0-50 years of difference between trees) the multilocus estimate of the average kinship coefficient was 0.10 in L3 and -2.27 in S4, in the second class (50-100 years difference) values of 0.40 and 2.97 were obtained in L3 and S4, respectively. The correlation including all sam-

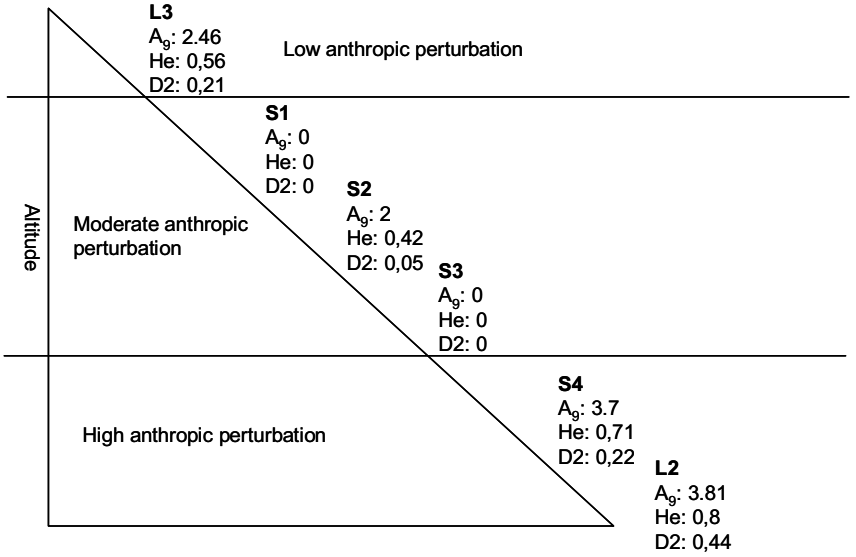

Fig. 5 Estimates of He, D^2 and Nhe for *P. ayacahuite* stands. See **Tables 1** and **3** for abbreviations.

Table 4 Genetic differentiation, R_{ST} values, between *P. ayacahuite* stands in the Communal Forest of Totonicapán (Guatemala) below diagonal. Number of migrants between stands is shown above diagonal.

	L2	L3	S1	S2	S3	S4
L2	0	0.11	2.81	22.23	2.81	3.96
L3	0.819	0	2.88	14.65	2.88	3.32
S1	0.151	0.148	0	7.97	-	11.13
S2	0.022	0.033	0.059	0	7.97	5.75
S3	0.151	0.148	-	0.059	0	11.13
S4	0.112	0.131	0.043	0.08	0.043	0

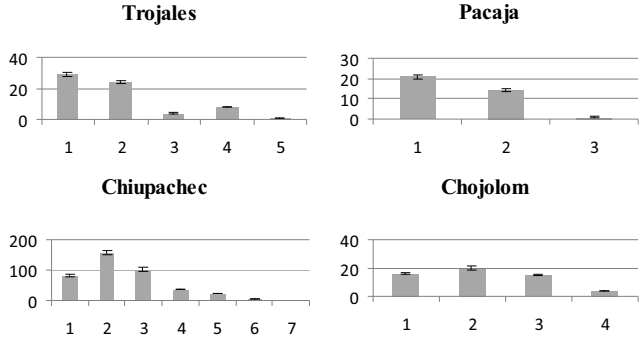

Fig. 6 Distributions of pair-wise cpSSR length differences among individuals in four stands showing variability of *Pinus ayacahuite* from Communal Forest of Totonicapan (Guatemala).

pled trees has a negative slope (-0.11) for L3 and a positive one (0.11) for S4.

AMOVA-IAM model, showed 11% of genetic variance among stands and 89% within them. Significant differences were found between stand L2 and stands S1, S2 and S3; between stand S1 and stands S3 and S4; and between stands S3 and S4. Two groups were established, the first one included stands L2 and S4 (from the lowest altitude) and the other stands were grouped together. The hierarchical analysis of variance (AMOVA-SMM model) showed that the majority of the genetic variance (85.93 %) was found within stands (**Table 5**). An important differentiation was found among groups near 13% while only 1% of variation was detected among stands within groups.

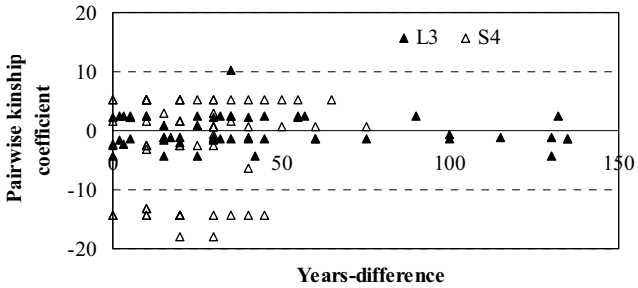

Fig. 7 Pairwise kinship coefficient (Y-axis) between individuals within stands L3 (■) and S4 (Δ) as a function of years-difference (X-axis).

DISCUSSION

The afforestation programs need a consistent ecological approach to be implemented for the natural ecosystem. Pine stands specially can introduce effects on the structure and diversity of the woody components of their area (Andres and Ojeda 2004). The combination of ecological and genetic analysis is particularly valuable. In this sense, DNA markers have a great impact for the analysis of genetic variation (Baker 2000; Gómez *et al*. 2001).

Chloroplast microsatellite markers have been used to evaluate the levels of genetic variation within and among 6 stands of *P. ayacahuite*. Three cpSSRs resulted in a monomorphic pattern while the other five were polymorphic. All detected variants are within the size range reported for the same markers in other species. Different patterns were detected for marker B, which was polymorphic for other *Pinus* species and for marker G, which was monophormic as reported in *P. pinea* (Gómez *et al.* 2004). Many studies based on chloroplast microsatellite *loci* have been reported in *Pinus* species (i.e. Vendramin *et al*. 1996; Bucci *et al*. 1998;

Table 5 AMOVA analysis under SMM for *P. ayacahuite* locations in the Communal Forest of Totonicapán (Guatemala).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among groups	1	2.625	0.05394 Va	12.92
Among populations within groups	5	1.657	0.00478 Vb	1.14
Within populations	75	26.902	0.35870 Vc	85.93
Total	81	31.185	0.41741	

Echt *et al*. 1998; Morgante *et al*. 1998; Provan *et al*. 1998; Gómez *et al*. 2003b). Results suggest a moderate genetic diversity (H = 0.57), similar to data obtained for other pine species (Gómez *et al.* 2004).

The analysis along the altitudinal cline (**Fig. 5**) showed a diverse pattern in both genetic diversity (H) and genetic differentiation (D^2) estimates. Three areas were established. A negative relationship was found when altitudinal distances among sampling stands and genetic distances were analyzed by the Mantel test, probably due to the fact that in the intermediate part of the altitudinal range could exist a negative impact over the genetic diversity of the forest.

The lowest area is located near human establishments that include a forest nursery. Two stands L2 (Chuipachec) and S4 (Chojolom) are present in this area. Both stands are characterized by high genetic diversity and differentiation indexes. When both stands are grouped the hierarchical analysis of variance (from SMM model) explains 13% of variation between this area and the other stands. The SMM appears to be suitable given the stepwise pattern of microsatellite lengths (**Figs. 1, 4**), although it could not provide a more reliable estimator of differentiation than traditional approaches if the upper size limit for microsatellite allele was small (Nauta and Weising 1996).

The intermediate area comprises three stands: S1 (Salvachán), S2 (Pacajá) and S3 (Chuijolom). Two of them (S1 and S3) have the lowest genetic diversity and differentiations indeces, featuring the most common haplotype (No. three). The other stand, S2, showed the greatest number of migrants of all stands (**Table 5**). The reduction in the haplotype richness could be the result of a recent reduction of the effective population size in these three stands (Morgante *et al.* 1998). Samples from stand S2 are very similar among them as a low D^2 value (0.05) indicates. Furthermore, this stand is very similar to all the other stands as was reflected in the high number of migrants.

The highest area is represented by the Trojales (L3) stand. This stand showed elevated genetic diversity and differentiation values. In addition, our study detected a high genetic diversity and a bimodal distribution of pair-wise cpSSR length differences among individuals. This result indicates the presence of older populations that remained relatively constant in size (Gómez *et al.* 2005). The analysis based on the kinship coefficient between S4 and L3 (at the lower and the upper areas respectively), showed clear differences. Thus Trojales (L3) have the most ancient trees, and important differences among the DNA samples from this stand were detected. The Chojolom (S4) samples showed more uniformity in age (less differences among trees ages), while differences due to multilocus estimate are most important. The observed pattern of isolation by distance and isolation by age can be expected by a model of subdivided population (Rousset 2000). The stands studied might have experienced variation in size and composition. This agrees with the hypothesis that local communities could have introduced plants from other locations mainly as a consequence of the forest nursery.

It could be two possible effects derived from the status of *P. ayacahuite* at the Communal Forest of Totonicapán. First the loss of haplotype richness and an increasingly uniform population could more likely to suffer the effects of natural selection. Second, the introduction of individuals from diverse origins might result in the loss of fitness.

The examination of partitioning of the genetic variation within and among stands could be used as a guide to sampling strategies for afforestation programs. The less perturbed stands with high genetic diversity indexes are critical to ensure effective germplasm sampling. Furthermore, the number of alleles is an important criterion that has been widely used in genetic conservation, particularly in setting priorities for population conservation (Brown and Briggs 1991) and in the management of germplasm collections (Gómez *et al.* 2003). The haplotype distribution helps the identification of stands with unique suites of haplotypes (private haplotypes).

In conclusion, this genetic diversity analysis of six stands performed through chloroplast microsatellite markers of *P. ayacahuite* point out two especially interesting areas with regards to conservation and afforestation activities at the Communal Forest of Totonicapán. The Trojales stand, at the highest altitude range has been revealed as a local genetic diversity reservoir. The other area that needs a special attention is the lower one, where human impacts were more frequent and the introduction of foreign plant material can cause alterations in the genetic composition of the area. It can imply a decrease of fitness and alteration in the ecosystem equilibrium and water conservation regime which adds substantially to the food security in many ways. These results in addition to data based in nuclear markers will provide an important point for sustainable development in this forest area.

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