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RAPD and ISSR markers indicate diminished gene flow due to recent fragmentation of *Polylepis australis* woodlands in central Argentina

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Abstract

Two *Polylepis australis* BITT. populations differing in size were examined with the aim of determining future sampling strategies; assessing levels of genetic diversity and checking whether trees of different ages might vary in their genetic structure due to the effects of fragmentation. RADP and ISSR gave similar values of diversity. A re-sampling technique showed that for *P. australis*, 10 trees and 20 markers were enough to produce an unbiased estimator of heterozygosity. AMOVA suggested differences in allele frequencies between young and old trees in the small population ($p = 0.052$), but not in a large population ($p = 0.864$); suggesting that gene flow between the areas diminished in relatively recent times. This assumption is supported by the fact that allele frequencies among both woodlands were significantly different between the young ($p < 0.0001$), but not the older trees ($p = 0.87$).

Keywords: Genetic diversity; Sample size; Comparison of techniques; Connectivity

1. Introduction

Woodlands of the genus *Polylepis* (Rosaceae) are distributed along the South American tropical Andes and in the mountains of central Argentina. The genus comprises about 28 wind-pollinated species of short to tall trees and shrubs that are distributed from southern Venezuela to central Argentina (Kessler and Schmidt-Lebuhn, 2006). Some species grow in the area of the tropical upper mountain forest, and others in isolated stands far above the closed tree line. This peculiar distribution exemplifies the distribution of many mountain woodlands and is mainly

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the result of a reduction of former extensive forests (Ellenberg, 1979; Hensen, 1995, 2002; Kessler and Driesch, 1993; Kessler, 2000). Today, *Polylepis* woodlands belong to the most endangered ecosystems of the world (Walter and Gillett, 1998; UNEP-WCMC, 2004).

The most southern *Polylepis* woodlands are situated in the mountains of central Argentina, where they form woodlands whose canopy is dominated almost exclusively by *Polylepis australis* BITT. The original distribution of these woodlands has been drastically reduced and fragmented due to logging, domestic livestock browsing, and fires to produce grass re-growth (Cabido and Acosta, 1985; Teich et al., 2005; Renison et al., 2006; Cingolani et al., in press). This fragmentation might result in the reproductive isolation of woodland patches with a reduced gene flow between patches and an increase in genetic drift and inbreeding. In order to plan effective long-term conservation strategies, it is important to know the genetic structure of the species and whether woodland fragments are genetically isolated or not (Frankham et al., 2002).

Recent molecular studies on *Polylepis* employed amplified fragment length polymorphism (AFLP) (Schmidt-Lebuhn et al., 2006a,b) and restriction fragment length polymorphism (RFLP) (Lizarzaburu and Narváez-Trujillo, 2002) to reconstruct the phylogeny of *Polylepis* species. Here we use random amplified polymorphic DNA (RAPD) (Williams et al., 1990) and inter-simple sequence repeat (ISSR) (Zietkiewicz et al., 1994). Both methods are based on the polymerase chain reaction (PCR), are adequate for describing population genetic diversity and effects of fragmentation, and are easy to implement when little or no molecular genetic research has been conducted for the species (Tsumura et al., 1996; Bartish et al., 2000; Frankham et al., 2002; Nybom, 2004). Although, RAPD and ISSR differ mainly in what kind of DNA fragment they amplify, the data interpretation is identical and can be combined for statistical analysis (Souframanien and Gopalakrishna, 2004).

Our objectives were to: (1) identify a set of molecular markers suitable for the genetic analysis of *P. australis* populations; (2) estimate the number of trees and markers needed to reliably estimate genetic variability; (3) determine genetic diversity within and among two populations of differing size; and (4) assess whether trees of different ages might vary in their genetic structure due to effects of fragmentation.

This study will be useful as a basis for future analysis describing the effects of fragmentation on *P. australis* and to contribute to the development of adequate management strategies.

2. Material and methods

2.1. Sample collection

Samples were collected from two *P. australis* woodlands differing in fragment size. The small fragment consisted of 30 scattered trees in a highly fragmented area (31°25′02″ S; 64°48′09″ W; 2200 m asl). The large fragment had an extension of several hectares comprising thousands of trees in a fairly well preserved area (31°58′12″ S; 64°56′36″ W; 1900 m asl) 34 km south of the small fragment. Leaf samples were obtained from a total of 20 trees from each of the two woodlands. In each woodland we randomly selected 10 trees with a basal diameter greater than 6.4 cm (hereafter old trees) and 10 trees with a basal diameter of less than 6.4 cm (hereafter young trees). Trees were separated by a minimum distance of 100 m to minimize the chance of sampling closely related or genetically identical individuals. Leaf tissue was stored at −80 °C within 10 h of collection.

2.2. Genomic DNA extraction

DNA was isolated from leaf material using columns of the ‘‘QIAGEN DNeasy Plant Minikits’’. The determination and adjustment of the DNA concentration was performed with a spectrophotometer at 260 nm.

2.3. RAPD analysis

Forty arbitrary primers were tested for PCR reaction in order to select the best profiles for polymorphism, readability and reproducibility. Two series (Kit A and B) of 10 arbitrary primers (Biodynamics S.R.L. Buenos Aires, Argentina and 20 primers (set D) (Roth & Co., Germany) were screened. The three chosen primers were A06 5′–GAGTCT CAGG–3′; A10 5′–ACGGCGTATG–3′ and D16 5′–AGGGCGTAAG–3′. Reactions were carried out in 25 μl reaction volumes containing 10 mM Tris–HCl buffer, pH 9, 2.5 mM of $MgCl_2$, 0.2 mM of each dNTP (dATP,

dCTP, dGTP and dTTP), 15 ng of primer, 0.75 U of *Taq* polymerase (Amersham) and 10 ng of *P. australis* genomic DNA. The mixture was amplified in a programmable thermal cycler (Biometra Uno II). The steps of temperature cycling were as follows: 92 °C for 5 min followed by 45 steps of 1 min at 92 °C, 1 min at 35 °C and 2 min at 72 °C with a final extension step of 5 min at 72 °C (modified after Kambhampati et al., 1992).

PCR products were loaded into agarose gels prepared with 1% agarose with TBE buffer system at 3 V/cm for 6 h and stained with 0.005 μg/ml ethidium bromide and photographed over UV light.

2.4. ISSR analysis

Sixteen arbitrary ISSR primers were tested; three of them were chosen for the analyses because they produced readable and highly reproducible bands: Pa1 (GA)8 C; Pa3 (GTT)5 ST and Pa6 (CCA). The reaction mix was prepared in accordance with Bornet and Branchard (2001) as follows: buffer 1× (50 mM KCl, 10 mM Tris–HCl, pH 9), 2.5 mM $MgCl_2$, 0.2 mM of each four dNTPs, 10 pmol of primer, 0.1 U of *Taq* polymerase (Amersham) and 10 ng/μl of DNA, in a final volume of 25 μl. An Eppendorf Mastercycler was used for PCR amplifications. The steps of temperature cycling were as follows: 94 °C for 3 min, followed by 35 cycles of 30 s at 93 °C, 1 min at 50 °C, 1 min 30 s at 72 °C and 10 min final extension step at 72 °C. PCR products were separated by electrophoresis in 1% agarose gels with a TBE buffer system at 6 V/cm for 3 h and stained with 0.005 μg/ml ethidium bromide. Fragments were observed and photographed over UV light.

2.5. Band profile reproducibility

Three replicate DNA extractions from leaves of both *P. australis* woodlands were used to assess the consistency of the band profiles. RAPD and ISSR amplifications were repeated at least three times and only the reproducible PCR products were scored.

2.6. Data analysis

2.6.1. Polymorphism analysis

Amplified RAPD and ISSR markers bands were scored as present (1) or absent (0) and entered into a binary data matrix. Allele frequencies were calculated from RAPD and ISSR band frequencies following the methods and corrections employed by Lynch and Milligan (1994).

2.6.2. Sampling distribution of the unbiased mean heterozygosity estimator

The effect of sample size (number of trees) on the median value and variability of the sampling distribution of the unbiased mean heterozygosity estimator (H) was studied via a re-sampling technique using the InfoGen program (Balzarini and Di Rienzo, 2004). For this purpose the empirical association between the median and the amplitude of the 95% prediction interval of the sampling distribution of H was found for different sample sizes (number of trees) and the number of loci considered.

The re-sampling procedure consisted of taking repeated samples of sizes $n = 2$, $n = 3$, $n = 4$, and so on from the original sample of n trees. For each sample size, 100 samples with replacement were taken and an estimation of H calculated for every one. These medians, 2.5th and 97.5th percentiles, were plotted against the corresponding sample size from which they were obtained and the resulting scattergram was smoothed using the Lowess algorithm (Cleveland, 1979). The set of smoothed medians were then used to draw the graph that described the empirical association of the sampling median value of H and the sample size. Likewise, the set of smoothed 2.5th and 97.5th percentiles were used to draw the lower and upper limits of a prediction band for the sampling distribution of H. An analogous procedure was used to study the relationship between the median and the 95% prediction interval limits of the sampling distribution of H, when different numbers of loci were considered.

2.6.3. Within population diversity

Expected heterozygosities (H) and percentage of polymorphic loci (P) of each woodland were calculated for RAPD and ISSR loci as well as for combined data (RAPD + ISSR) using the TFPGA program (Miller, 1997). Significance of the differences in H between woodlands was evaluated via a Friedman test. To compare H and P values among

woodlands, confidence intervals were obtained by 'bootstrap' using the InfoGen program (Balzarini and Di Rienzo, 2004). The pair-wise distances between trees were calculated as $1 - S$, where S is the Jaccard′s similarity coefficient from the binary RAPD and ISSR data. These distances were used to generate, by means of a metric principal coordinate analysis, a set of principal coordinates for both primers. These sets of coordinates were further subjected to Procrustes analysis to assess the degree of consensus between the ordinations produced by different markers. Principal coordinate and Procrustes analyses were carried out with the InfoGen program.

2.6.4. Diversity among populations

Genetic differentiation between woodlands was analysed through θ values with the methods proposed by Weir and Cockerham (1984) and Lynch and Milligan (1994) using the RAPDFST program (Black, 1997), which can be used without information on heterozygote frequencies. The significance of θ was obtained using a Jackknife procedure and the significance of F_{ST} value was evaluated with a Chi Square test. To determine whether the genetic differentiation among woodlands is a result of the ancient variability, we performed an analysis of molecular variance (AMOVA) (Schneider et al., 1997) comparing trees of the two different age classes within and among woodlands using the program InfoGen.

3. Results

3.1. Polymorphism analysis

The 40 RAPD primers tested amplified a variable and high number of fragments, showing high levels of polymorphism among individuals. The ISSR primers produced a varying number of DNA fragments regardless of their SSR motif. All of the 16 primers tested showed polymorphism. RAPD and ISSR data gave similar values of P and H in both populations (Table 1). Furthermore, in both populations, the Procrustes analysis suggested a high consensus between tree ordinations obtained from ISSR and RAPD data (92% and 93% for the small and large woodlands, respectively).

3.2. Sampling distribution of the unbiased mean heterozygosity estimator

When considering 57 markers, the median value of the unbiased mean heterozygosity estimator (H) is a growing function of the number of trees, reaching a plateau when sample size is approximately 10 (Fig. 1a). This means that under these conditions, sample sizes higher than 10 trees will not provide more information on the level of heterozygosity. The prediction interval is a representation of the sampling variability of H and is expected to be narrower for increasing sample size. Although, it becomes narrower after sample size 10, it does not tighten much more. Given the sample of 40 trees included in this work, H does not change very much when considering different numbers of markers. However, its variability was very high when the number of markers was lower than 20 and remained quite high for the maximum number of markers (Fig. 1b).

3.3. Within and among population diversity

Differences in expected heterozygosity values and polymorphism levels (P and H) among populations were not significant (Table 1). Genetic structure on the other hand, differed among populations. In the small woodland the

Table 1
Measures of genetic variability estimated from RAPD, ISSR and pooled data in a small and large *Polylepis australis* woodland fragment ($n = 20$ from each of the two woodland fragments)

	RAPD		ISSR		RAPD + ISSR	
	Small	Large	Small	Large	Small	Large
H	0.2541 (s.d.: 0.1714)	0.2906 (s.d.: 0.1606)	0.2395 (s.d.: 0.1721)	0.2613 (s.d.: 0.1676)	0.2446 (s.d.: 0.1705)	0.2716 (s.d.: 0.1643)
P	80 (l.l.: 60; u.l.: 95)	90 (l.l.: 75; u.l.: 100)	89 (l.l.: 78; u.l.: 97)	92 (l.l.: 81; u.l.: 100)	86 (l.l.: 77; u.l.: 95)	91 (l.l.: 84; u.l.:98)

H: mean heterozygosity per locus; P: percentage of polymorphic loci; s.d.: standard deviation; l.l.: lower limit of confidence interval; u.l.: upper limit of confidence interval.

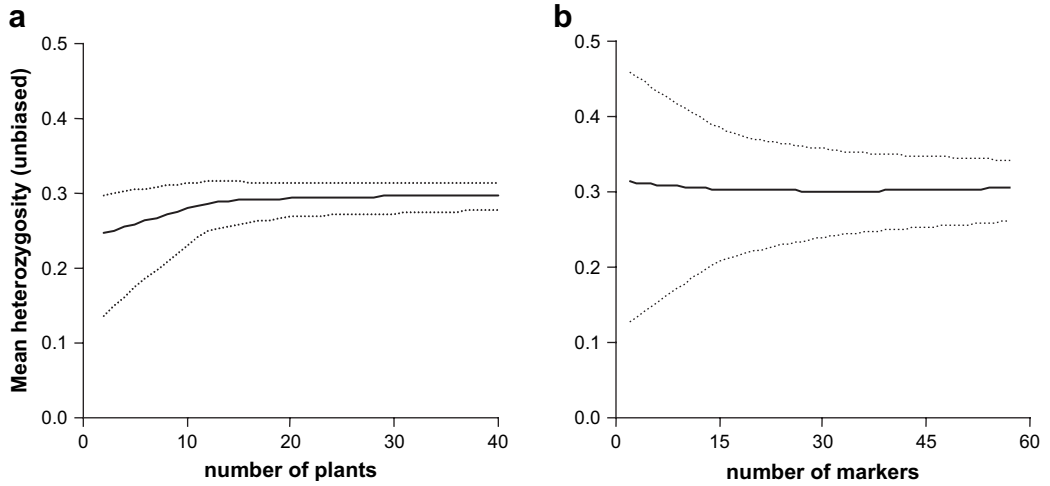

Fig. 1. Median of the unbiased mean heterozygosity estimator (H) (solid lines) and the upper and lower limits of the 95% prediction interval of the sampling distribution of H (dotted line) for different sample sizes (a) and different number of markers (b).

difference between old and young trees was marginally significant (AMOVA: $p = 0.052$; Table 2) while in the large woodland the differences were clearly not significant ($p = 0.864$). When only considering young trees, the large and small woodland showed significant differences in allele frequencies ($p < 0.0001$; Table 3), whereas no significant differences were found for old trees in the two localities ($p = 0.871$).

Values of F_{ST} were 0.028 using the θ estimator and 0.015 for Lynch and Milligan's (1994) estimation; both values were not statistically significant. The graphical representation of the Procrustes analysis did not show any grouping.

4. Discussion

RAPD and ISSR markers were applied to characterize and compare genetic diversity between two populations of *P. australis* with different population sizes. Both RAPD and ISSR showed similar results. Notwithstanding, the ISSR technique generated more bands per primer and revealed higher levels of polymorphism, so we recommend ISSR for future studies. The re-sampling technique suggests that at least 10 trees per population and 20 loci should be used to produce an unbiased estimate of the expected heterozygosity. This calculation is important to avoid unnecessary costs when a geographically extended sampling effort is performed.

The detected heterozygosity levels in *P. australis* (mean $H = 0.258$) are close to those previously reported for RAPD-derived estimates performed in plant species with similar life history characteristics, such as long-lived perennials ($H = 0.25$, mean for 37 studies), out-crossing species ($H = 0.27$, 38 studies), or species with pollen or seeds dispersed by wind and/or water ($H = 0.27$, 22 studies) (Nybom, 2004). Both θ and F_{ST} suggest high levels of between-population gene flow in *P. australis*, which could be determined by the breeding system (mainly out-crossing) and by the prevalence of wind pollination in the species (Seltmann et al., 2007). These results coincide with the studies of Schmidt-Lebuhn et al. (2006a,b), who found extensive gene flow in other *Polylepis* species of the high Andes. Long distance pollen dispersal and/or self-incompatibility mechanisms favoring cross-pollination are common in tree species inhabiting mountain or fragmented woodlands (White and Boshier, 2000; Rocha and Aguilar, 2001; Céspedes et al., 2003).

The genetic divergence between young trees of the small and large *P. australis* woodlands was significantly higher than that observed between the group of old trees from those populations, suggesting that levels of gene flow between

Table 2
Analysis of molecular variance between young and old trees at a small woodland fragment

Source of variation	Sum of squares	df	Variance component	Total variance (%)	p Value
Between populations	8.9	1	0.08	0.94	0.052
Within population	138.4	18	7.69	99.02	

Table 3
Analysis of molecular variance for young trees of a large and a small woodland fragment

Source of variation	Sum of squares	df	Variance component	Total variance (%)	*p* Value
Between populations	14.3	1	0.6	7.5	<0.0001
Within population	133.4	18	7.4	92.5	

the areas has been modified in relatively recent times, in agreement with the reports on woodland reduction and fragmentation occurring in the last 400 years (Cingolani et al., 2004, in press; Renison et al., 2006). This result points to the conservation of remaining *P. australis* woodlands and the restoration of their connectivity through natural or assisted woodland regeneration. More exhaustive studies will be needed for descriptions of the magnitude and direction of genetic exchange to better determine where woodland restoration is necessary.

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References

Balzarini, M., Di Rienzo, J., 2004. InfoGen. Available from: www.info-gen.com.ar.

Bartish, I.V., Garkava, L.P., Rumpunen, K., Nybom, H., 2000. Phylogenetic relationships and differentiation among and within populations of *Chaenomeles* Lindl. (Rosaceae), estimated with RAPD and isozymes. Theor. Appl. Genet. 101, 554–556.

Black IV, W.C., 1997. FORTRAN programs for analysis of RAPD-PCR data. http://lamar.colostate.edu/pub/wbc4.

Bornet, B., Branchard, M., 2001. Nonanchored inter-simple sequence repeat (ISSR) markers: reproducible and specific tools for genome fingerprinting. Plant Mol. Biol. Rep. 19, 209–221.

Cabido, M., Acosta, A., 1985. Estudio fitosociológico en bosques de *Polylepis australis* BIT. (tabaquillo) de las Sierras Grandes de Córdoba, Argentina. Doc. Phytosociol. 9, 385–400.

Céspedes, M., Gutierrez, M.V., Holbrook, N.M., Rocha, O.J., 2003. Restoration of genetic diversity in the dry forest tree *Swietenia macrophylla* (Meliaceae) after pasture abandonment in Costa Rica. Mol. Ecol. 12, 3201–3212.

Cingolani, A.M., Renison, D., Zak, M.R., Cabido, M.R., 2004. Mapping vegetation in a heterogeneous mountain rangeland using Landsat data: an alternative method to define and classify land-cover units. Remote Sens. Environ. 92, 84–97.

Cingolani, A., Renison, D., Tecco, P., Gurvich, D., Cabido, M. Predicting cover types in a mountain range with long evolutionary grazing history: a GIS approach. J. Biogeogr., in press. doi:10.1111/j.1365-2699.2007.01807.x.

Cleveland, W.S., 1979. Robust locally weighted regression and smoothing scatterplots. J. Am. Stat. Assoc. 74, 829–836.

Ellenberg, H., 1979. Man's influence on tropical mountain ecosystems in South America. J. Ecol. 67, 401–416.

Frankham, R., Ballou, J.D., Briscoe, D.A., 2002. Introduction to Conservation Genetics. University Press, Cambridge, 617 pp.

Hensen, I., 1995. Die Vegetation von *Polylepis*-Wäldern der Ostkordillere Boliviens. Phytocoenologia 25, 235–277.

Hensen, I., 2002. Impacts of anthropogenic activity on the vegetation of *Polylepis* woodlands in the region of Cochabamba, Bolivia. Ecotropica 8, 183–203.

Kambhampati, S., Black, W.C., Karamjit, S.R., 1992. Random amplified polymorphic DNA of mosquito species and populations (Diptera, Culicidae): technique, statistical analysis and applications. J. Med. Entomol. 29, 939–945.

Kessler, M., Driesch, P., 1993. Causas e historia de la destrucción de bosques Altoandinos en Bolivia. Ecol. Boliv. 21, 1–18.

Kessler, M., 2000. Observations on a human-induced fire event at a humid timberline in the Bolivian Andes. Ecotropica 6, 89–93.

Kessler, M., Schmidt-Lebuhn, A.N., 2006. Taxonomical and distributional notes on *Polylepis* (Rosaceae). Org. Divers. Evol. 6, 67–70.

Lizarzaburu, J., Narváez-Trujillo, A., 2002. Análisis Molecular mediante PCR-RFLP's del género *Polylepis* en el Ecuador. In: Libro de Resúmenes del Octavo Congreso Latinoamericano y Segundo Congreso Colombiano de Botánica. Ecuador. Available from: http://www.botanica-alb.org/publicaciones.php.

Lynch, M., Milligan, B.G., 1994. Analysis of population genetic structure with RAPD markers. Mol. Ecol. 3, 91–99.

Miller, M.P., 1997. Tools for population genetic analyses (TFPGA). Available from: www.herb.bio.nau.edu/~miller/tfpga.htm

Nybom, H., 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol. Ecol. 13, 1143–1155.

Renison, D., Hensen, I., Suarez, R., Cingolani, A., 2006. Cover and growth habit of *Polylepis* woodlands and shrublands in the mountains of central Argentina: human or environmental influence? J. Biogeogr. 33, 876–887.

Rocha, O.J., Aguilar, G., 2001. Reproductive biology of the dry forest tree *Enterolobium cyclocarpum* (Guanacaste) in Costa Rica: a comparison between trees left in pastures and trees in continuous forest. Am. J. Bot. 88, 1607–1614.

Schneider, S., Kueffer, J.M., Roessli, D., Excoffier, L., 1997. Arlequin Ver. 1.1: a Software for Population Genetic Data Analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Schmidt-Lebuhn, A.N., Kessler, M., Kumar, M., 2006a. Promiscuity in the Andes: species relationships in *Polylepis* (Rosaceae, Sanguisorbeae) based on AFLP and morphology. Syst. Bot. 31, 547–559.

Schmidt-Lebuhn, A.N., Kumar, M., Kessler, M., 2006b. An assessment of the genetic population structure of two species of *Polylepis* Ruiz and Pav. (Rosaceae) in the Chilean Andes. Flora 201, 317–325.

Seltmann, P., Renison, D., Cocucci, A., Hensen, I., Jung, K., 2007. Fragment size, pollination efficiency and reproductive success in natural populations of wind-pollinated *Polylepis australis* (Rosaceae) trees. Flora 202, 547–554.

Souframanien, J., Gopalakrishna, T., 2004. A comparative analysis of genetic diversity in blackgram genotypes using RAPD and ISSR markers. Theor. Appl. Genet. 109, 1687–1693.

Teich, I., Cingolani, A.M., Renison, D., Hensen, I., Giorgis, M., 2005. Do domestic herbivores retard *Polylepis australis* Bitt. woodland recovery in the mountains of Córdoba, Argentina? Forest Ecol. Manag. 219, 229–241.

Tsumura, Y.K., Ohba, K., Strauss, S.H., 1996. Diversity and inheritance of inter-simple sequence repeat polymorphisms in Douglas-fir (*Pseudotsuga menziessi*) and Sugi (*Cryptomeria japonica*). Theor. Appl. Genet. 92, 40–45.

UNEP-WCMC, 2004. United Nations Environment Programme, World Conservation Monitoring Center. Available from: www.unep-wcmc.org.

Walter, K.S., Gillett, H.J., 1998. IUCN Red List of Threatened Plants. Compiled by the World Conservation Monitoring Centre. IUCN – The World Conservation Union, Gland, Switzerland and Cambridge, UK, 862 pp.

Weir, B.S., Cockerham, C.C., 1984. Estimating *F*-statistics for the analysis of population structure. Evolution 33, 1358–1370.

White, G., Boshier, H., 2000. Fragmentation in Central American dry forest: genetic impact on *Swietenia humilis* (Meliaceae). In: Young, A.G., Clarke, G.M. (Eds.), Genetics, Demography and Viability of Fragmented Populations. Cambridge University Press, Cambridge, pp. 293–311.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, A., Tingey, S.V., 1990. DNA polymorphism amplified by arbitrary primers are useful as a genetic markers. Nucleic Acids Res. 18, 6531–6535.

Zietkiewicz, E., Rafalski, A., Labuda, D., 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20, 176–183.