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ORIGINAL ARTICLE

Genetic structure of disjunct Argentinean populations of the subtropical tree *Anadenanthera colubrina* var. *cebil* (Fabaceae)

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Abstract *Anadenanthera colubrina* var. *cebil* is a native South American tree species inhabiting seasonally dry tropical forests (SDTFs). Its current disjunct distribution presumably represents fragments of a historical much larger area of this forest type, which has also been highly impacted by human activities. In this way the hypothesis of this study is that the natural populations of *A. colubrina* var. *cebil* from Northern Argentina represent vestiges of ancient fragmentation, but they are additionally influenced by a certain degree of gene flow among them. We aimed to analyze the genetic structure of both nuclear and chloroplast DNA to evaluate the relative role of ancient and recent fragmentation on intraspecific diversity patterns. Sixty-nine individuals of four natural populations were analyzed using eight nuclear microsatellites (ncSSR) and four chloroplast microsatellite loci (cpSSR). The level and distribution of genetic variation were estimated by standard population genetic parameters and Neighbor Joining as well as Bayesian analyses. The eight ncSSR loci were highly polymorphic, while genetic diversity of cpSSRs was low. Nuclear SSRs displayed lower genetic differentiation among populations than cpSSR haplotypes (F_{ST} 0.11 and 0.95, respectively). However, high differentiation between phytogeographic provinces was observed in both genomes. The high genetic differentiation detected emphasizes the role of ancient fragmentation. However, the Paranaense province also shows the effects of recent fragmentation on genetic structure, whereas gene flow by pollen preserves the effects of genetic drift in the Yungas province.

Keywords Curupay · Chloroplast haplotypes · Genetic structure · Microsatellites · Seasonally dry tropical forests (SDTFs)

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Introduction

Population genetic structure is defined by the amount of variability and its distribution within and among local populations and individuals within species (Templeton 2006). Historical events can have profound effects on the distribution of genetic variation. For example, climate changes during the Quaternary ages have caused fluctuations in species ranges and population sizes (Hewitt 2000). In South America, climatic changes during the Pleistocene have driven changes between vegetation types that influenced species demography (Naciri et al. 2006).

Anadenanthera colubrina (Vell.) Brenan var. *cebil* (Fabaceae—Mimosoideae) is a canopy, long-lived and semi-deciduous tree species that can reach up to 35 m in height. It has hermaphroditic flowers and long legume fruits (von Altschul 1964; Justiniano and Fredericksen 1998; Cialdella 2000). Little is known about the mating system of *A. colubrina* var. *cebil*. However, it is suggested that it behaves as predominantly outcrossing (Cialdella 2000). Bees are the main pollinators, and seeds are dispersed by autochory or anemochory after pod dehiscence (Justiniano and Fredericksen 1998; Abraham de Noir et al. 2002). Geographical distribution of this species is located in South America, inhabiting the Seasonally Dry Tropical Forests (SDTFs). At present, *A. colubrina* var. *cebil* is a dominant species of such forests, and it has been proposed by Prado and Gibbs (1993) as a paradigm species likely involved in the cyclic expansion-retreat migrations of the Pleistocene. Three nuclei of distribution for this species have been proposed by Prado and Gibbs (1993): (1) the Caatingas nucleus of Northeastern Brazil, (2) the Misiones nucleus occurring along the Paraguay–Paraná river system of Northeastern Argentina, Eastern Paraguay and Southeastern Brazil, and (3) the Sub-Andean Piedmont nucleus of Southwestern Bolivia and Northwestern Argentina. In Argentina, SDTFs are distributed in the Paranaense and Yungas phytogeographic provinces in Northeast and Northwest Argentina. Both phytogeographic provinces hold the highest biodiversity in the country (Brown et al. 2001; Di Bitetti et al. 2003; Inza et al. 2012).

The demographic structure may be stable as local populations persist in each area for long times or unstable as large-scale demographic changes occur frequently in the evolutionary history of a species (Slatkin 1987). Demographic instability can result from large-scale changes in geographic ranges, e.g., during major climatic changes (Slatkin 1987). Close floristic relationships exist between the distinct areas of SDTFs suggesting that they represent fragments of a much larger Pleistocene extension of this forest type (Prado and Gibbs 1993). The disjunct areas of the present-day distribution of neotropical SDTFs may represent current refugia for SDTF species (Pennington et al. 2004). Pennington et al. (2009) further argued that fragments of SDTFs have persisted over long evolutionary timescales with corresponding consequences on the evolution and biogeography of species. In addition, Werneck et al. (2011) proposed an alternative scenario of a lower Pleistocene or earlier Tertiary SDTF expansion, followed by fragmentation during the Pleistocene and secondary expansion in the Holocene. Four areas of long-term stability of SDTFs were identified, which possibly acted as current and historical refugial areas. Two of these potential refugia are located in south-central South America, one corresponding to the Misiones nucleus and another one to the Chiquitano region of eastern Bolivia (Werneck et al. 2011). Modeled ranges of SDTFs showed reductions in these forests in Northwestern Argentina during the Pleistocene. Hence, the area could be considered as temporally unstable in this sense. In addition, a more recent colonization of this area is expected (Werneck et al. 2011). Range expansions during Pleistocene glacial times were verified for *Astronium urundeuva* and suggest that the present distribution is a climatic relict of an ancient, widely distributed population (Caetano et al. 2008). However, the response of SDTFs to the Quaternary climate changes is highly complex and may differ among SDTF species (Collevatti et al. 2012).

Fragmentation of once continuous tree populations potentially disrupts natural ecological and evolutionary processes and could adversely modify their genetic composition (Hamrick 2004). The short-term effects of fragmentation depend on the genetic structure of the population prior to fragmentation. If gene flow between the fragments is limited, genetic drift causes an increase in genetic variability between fragments, but genetic variability decreases within them (Hamrick 2004). Hence, the effects of gene flow are the opposite of those of genetic drift, and the balance between them is a primary determinant of the population genetic structure (Templeton 2006).

In a previous study, Barrandeguy et al. (2011) detected high levels of polymorphism using four chloroplast microsatellite loci (cpSSR) in 24 individuals from Paranaense province. This study motivated an exhaustive analysis using more cpSSRs plus nuclear SSRs loci.

Suitable multivariate markers are required to analyze the genetic structure of populations. For vascular plants, comparative analyses of nuclear and chloroplast microsatellites can provide complementary information on the genetic diversity, genetic differentiation and gene flow (pollen and seed mediated) within and among their populations (Ennos 1994; Pakkad et al. 2008). The nuclear genome is biparentally inherited and reflects both seed and pollen gene flow (Petit et al. 2005), while the chloroplast genome is maternally inherited in most angiosperms (Corriveau and Coleman 1988) and disseminated by seeds (Petit et al. 2005). The chloroplast genome is haploid and exhibits a relative slow rate of sequence evolution as a consequence of the absence of recombination and low mutation rates. These characteristics make it suitable to assess the impact of historical factors (Petit et al. 2005, Ravi et al. 2008). The different inheritance patterns of organelle and nuclear genes can be used to unravel the complexity of gene flow in plants, as they are predicted to result in a very different distribution of genetic variability within and among populations (Petit et al. 2005). Even though these molecular techniques have been widely used in tree species, only few studies have combined markers from both genomes to study patterns of genetic diversity in subtropical tree species from South America (Caetano et al. 2008; Quiroga et al. 2012).

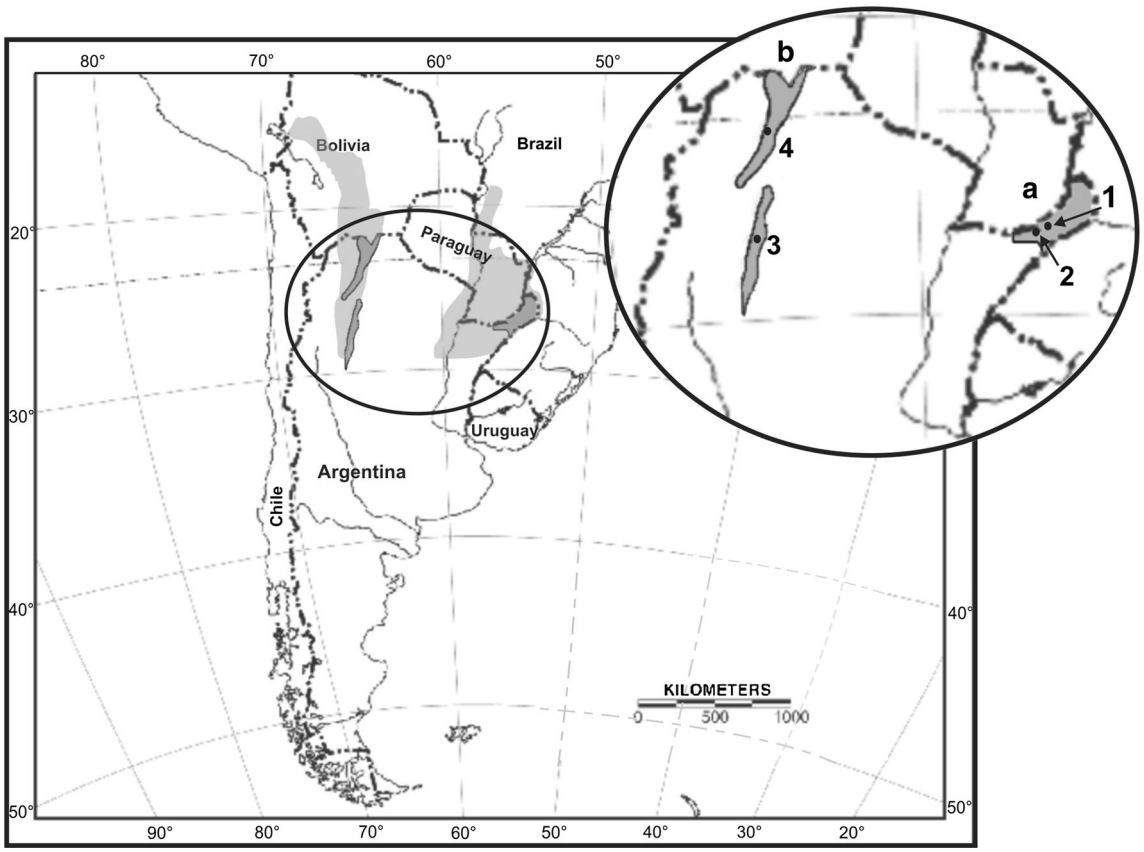

Fig. 1 Geographic origin of the populations studied in **a** the Paranaense and **b** the Yungas phytogeographic provinces: (1) Candelaria, (2) Santa Ana, (3) Tucumán and (4) Jujuy. Sub-Andean Piedmont and Misiones nuclei are indicated in *light gray*

A. colubrina var. *cebil* is an important hardwood species considering both its good wood quality and for nature conservation, since it can be used in reforestation and restoration of degraded forests (Justiniano and Fredericksen 1998). Combining the impacts on SDTFs in Argentina, we hypothesize that the natural populations of *A. colubrina* var. *cebil* from Northern Argentina represent vestiges of ancient fragmentation, but they are additionally influenced by a certain degree of gene flow among them. We, therefore, analyzed the genetic structure of natural populations of *A. colubrina* var. *cebil* using nuclear and chloroplast SSRs to observe the level and distribution of genetic variation in both genomes. Differences in genetic structure between nuclear and chloroplast markers of the same populations allow discussion of the relative role of ancient fragmentation as a consequence of historical events and recent fragmentation as result of human impact on natural populations of *A. colubrina* var. *cebil*.

Materials and methods

Population sites and sample collection

Young leaves from 69 adult trees were collected from four natural populations of *A. colubrina* var. *cebil* in North Argentina: (1) Candelaria (27°26′58.200″S, 55°44′20.184″W), (2) Santa Ana (27°25′55.920″S, 55°34′16.680″W), (3) Tucumán (26°47′26.100″S, 65°18′58.140″W), and (4) Jujuy (23°45′15.012″S, 64°51′12.996″W). These populations are located in the Paranaense (1–2) and Yungas (3–4) phytogeographic provinces representing the natural distribution of the species in Argentina (Fig. 1). Trees were georeferenced by GPS (Geographic Position System) and identified by an individual code. Trees were spatially distributed around 10 m apart in each population for avoiding the collection of relatives individuals. Geographic coordinates of the studied populations of *A. colubrina* var. *cebil* are presented in Appendix 1. Leaves were dried with silica gel and stored at room temperature until DNA extraction. Five dried leaves per individual are kept in the laboratory of Departamento de Genética (Facultad de Ciencias Exactas, Químicas y Naturales, Universidad Nacional de Misiones).

DNA extraction and microsatellite genotyping

Total genomic DNA was extracted from dry leaves using the DNeasy®-Plant Mini Kit (QIAGEN, Hilden, Germany). Eight specific nuclear microsatellite markers for

Table 1 Characterization of eight nuclear and four chloroplast microsatellite markers used for the study of *Anadenanthera colubrina* var. *cebil* populations

	Locus	T_a (°C)	Size range (bp)	N_A	H_o	H_e/h	Gene bank accession number
Nuclear genome	*Ac34.3*	TD 60–50 °C	171–203	16	0.853	0.914	JQ086537
	Ac48.1	TD 60–50 °C	129–171	20	0.651	0.875	JQ086538
	Ac11.2	TD 60–50 °C	110–124	08	0.478	0.772	JQ086539
	Ac28.3	65 °C	207–277	26	0.613	0.931	JQ086540
	Ac157.1	65 °C	106–201	28	0.868	0.883	JQ086541
	Ac41.1	TD 60–50 °C	125–158	19	0.779	0.810	JQ086542
	Ac172.1	TD 60–50 °C	087–122	15	0.667	0.870	JQ086543
	Ac162.1	TD 60–50 °C	114–170	20	0.875	0.864	JQ086544
Chloroplast genome	*Ccmp2*	50 °C	478–491	03	–	0.611	–
	Ccmp4	50 °C	109–112	02	–	0.491	–
	Ccmp5	50 °C	133–140	04	–	0.649	–
	Ccmp7	50 °C	150–155	02	–	0.499	–

T_a annealing temperature; *TD* touchdown; N_A number of alleles; H_O observed heterozygosity; H_E expected heterozygosity; *h* Nei's gene diversity index

A. colubrina var. *cebil* were used to genotype all individuals (Barrandeguy et al. 2012; Table 1). Additionally, ten universal angiosperm chloroplast microsatellite primers (*Ccmp*) were tested to analyze intraspecific chloroplast microsatellite variability (Weising and Gardner 1999). Out of ten, four were polymorphic: *Ccmp2*, *Ccmp4*, *Ccmp5* and *Ccmp7*, showing differences in mononucleotide repeats (Table 1).

PCR amplifications were performed in a final volume of 15 µL containing 0.5 ng/µl of genomic DNA, 1× Hot Start Buffer (0.8 M Tris–HCl pH 9.0, 0.2 M $(NH_4)_2SO_4$, 0.2 % w/v Tween-20; Solis BioDyne, Estonia), 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 1 U Hot Start DNA Polymerase (5 U/µL Hot FirePol, Solis BioDyne, Tartu, Estonia) and 0.33 pmol of each primer. Forward primers had a fluorescent tag (FAM = blue or HEX = green) on the 5′-end. PCR was performed in a gradient cycler (Biometra, Göttingen, Germany) using a touchdown program or a fixed annealing temperature depending on the locus considered (Table 1). For six loci, we used a touchdown program (TD) with the following conditions: 94 °C for 15 min, 10 cycles of 1 min at 94 °C, 1 min annealing with corresponding temperature regimes and 1 min at 72 °C followed by 29 similar cycles using 50 °C for annealing and a final elongation at 72 °C for 20 min. For the remaining loci, we applied the following conditions: 94 °C for 15 min, 30 cycles of 1 min at 94 °C, 1 min annealing at specific temperatures, 1 min at 72 °C, and a final elongation at 72 °C for 20 min. Electrophoresis was carried out in an ABI Prism® 3100 (Applied Biosystems, Foster City, CA), and fragment sizes were scored with GENESCAN™ analysis software using GS 500 ROX™ (Applied Biosystems) as internal size standard.

Data analysis

Genetic diversity

Nuclear genetic diversity was defined by the number of alleles (N_{An}), effective number of alleles (N_{En}), number of private alleles (N_{Pan}), observed heterozygosity (H_{On}), expected heterozygosity (H_{En}), allelic richness per population (R_n) and private allelic richness (R_{PAn}) to compare the expected gene multiplicity among populations in the case that all samples were equally sized (Gillet et al. 2005). The rarefaction method was used for this estimation using the ADZE version 1.0 software (Szpiech et al. 2008). Significant differences in allelic richness (R_n), and observed and expected heterozygosity (H_{On} and H_{En}, respectively) among populations were tested using a permutation procedure (10,000 iterations) with the FSTAT version 2.9.3.2 software (Goudet 1995).

Departures from Hardy–Weinberg equilibrium (HWE) at each population were tested, and a global test of heterozygote deficit across loci and populations was applied. Analyses were performed using the GENEPOP version 4.0.10 software (Raymond and Rousset 1995). The Markov chain method was employed to estimate the probability of significant deviation from HWE using the GENEPOP software with the following parameters: dememorization = 1000, batches = 100, and iterations = 1,000.

The presence of null alleles and genotyping errors were estimated in all loci within each population using MICRO-CHECKER (van Oosterhout et al. 2004). The overall frequency of null alleles (*r*) in each population was estimated using the method of Brookfield (1996), and we reestimated H_E from allele frequencies corrected for null alleles ($H_{E\text{-}cor}$). We also used the Individual Inbreeding Model

(IIM) approach to partition out the influence of null alleles on F_{IS} values using the INEST software (Chybicki and Burczyk 2009). In addition, we estimated global population F_{ST} values using the FreeNA software (Chapuis and Estoup 2007) to obtain unbiased F_{ST} values in the presence of null alleles and confidence intervals (CI) were reported.

Chloroplast genetic diversity was defined by the number of alleles (N_{Ac}), effective number of alleles (N_{Ec}), number of private alleles (N_{APc}) and Nei's gene diversity index (h_c) (Nei, 1978). Haplotypes were identified based on the simultaneous observation of all cpDNA polymorphisms due to the non-recombinant nature of the chloroplast genome. Nei's haplotypic diversity index (H_{Ec}) was calculated (Nei 1987).

Except for allelic richness and Nei's haplotypic diversity index, genetic diversity estimators were calculated using the GenAlEx 6.5 software (Peakall and Smouse 2012).

Genetic structure

Genetic relationships between nuclear multilocus genotypes or chloroplast haplotypes were analyzed by the distance-based unweighted Neighbor-joining method (Gascuel 1997). Based on the distance matrix an unrooted tree was constructed using the Darwin 5.0.84 software (Perrier and Jacquemoud-Collet 2006). The robustness of each node was assessed by bootstrapping with 1,000 replications. Data matrices are available from the corresponding author.

An Analysis of Molecular Variance (AMOVA) was performed for both markers to examine the hierarchical genetic structure (Excoffier et al. 1992). Populations used for this analysis were defined a priori based on their geographic distribution without a genetic criterion. The global F_{ST} index over all loci was calculated from AMOVA. Statistical significance was calculated using 1,023 permutations. Genetic differentiation between populations was estimated by pairwise F_{ST} comparisons within and among provinces. Analyses were performed using the Arlequin 3.5 software (Excoffier and Lischer 2010).

Population genetic structure was described using the Bayesian theory (Pritchard et al. 2000). Within a given data set the proportion of individuals correctly assigned to each population can provide useful insights regarding the relative patterns of population genetic structure (Manel et al. 2005). This model-based method estimated the number of genetic clusters (K) and assigned the total number of individuals to these clusters (Pritchard et al. 2000). Bayesian analysis was performed for ncSSR data using the admixture model with independent allele frequencies between populations. The number of genetically different clusters (K) ranged from 1 to 6. The model was run with ten independent simulations for each K using a burn-in length of 500,000 and a run length of 750,000 MCMC iterations. Other parameters were set to default values. Similarly, each phytogeographic province was analyzed independently to detect the genetic substructure. Analyses were carried out with the Structure 2.3.3 software (Pritchard et al. 2000). The real number of clusters was determined using the ad hoc ΔK statistics (Evanno et al. 2005), based on the second order of change in the log likelihood of data (ΔK) as a function of K calculated over ten replicates (Evanno et al. 2005). This estimation was calculated using the STRUCTURE HARVESTER web application version 0.6.92 (Earl and von Holdt 2011).

Bayesian analysis was also performed for cpSSR data using the mixture model for linked loci due to the non-recombinant nature of cpDNA. Simulations were run using similar settings to those used for the ncSSR data. The size of ten best visited partitions with their log marginal likelihood [log (ml)] values is used to estimate the "correct" number of clusters (Corander et al. 2006). The partition with the highest log (ml) value was considered as the optimal cluster number. Relationships among clusters identified by Bayesian analysis were determined by a dendrogram built using the Neighbor-Joining clustering technique based on Kullback–Leibler (KL) genetic distance matrix (Kullback and Leibler 1951). KL index can be used as a measure of relative dissimilarity between two data sets (Kasturi et al. 2003). This distance measure was used as an alternative to Nei's genetic distance index since Tucumán samples presented a private haplotype that resulted in a zero value for Nei's similarity index and an infinite value of genetic distance. Instead, KL estimated the distance among all clusters and allowed their representation in a dendrogram. All of these analyses were performed using the BAPS version 5.3 software (Corander et al. 2006). BAPS provides estimates of posterior probabilities for specific partitions of given sampling units, as well as a hierarchical tree representation of the closeness of the sampling units, from which a Bayesian model-averaged partition can be extracted (Corander et al. 2006).

Inbreeding was estimated using the Bayesian approach implemented in the Hickory version 1.1 software (Holsinger and Lewis 2003). Hickory estimates the Bayesian analog of Wright's F_{IS} (1951) designated in the program as f (Holsinger and Lewis 2003) and the confidence interval for this value (CI). Bayesian analyses of the ncSSR data set in Hickory were performed with default parameter settings (burn-in = 50,000, sampling = 250,000, thinning = 50) under the full model analysis.

Results

Genetic diversity

The eight nuclear microsatellite loci were highly polymorphic, with numbers of detected alleles for each locus

Table 2 Diversity parameters assessed by nuclear and chloroplast microsatellite markers in *Anadenanthera colubrina* var. *cebil* populations

Phytogeographic province		Paranaense		Yungas		Total
Population		Candelaria	Santa Ana	Tucumán	Jujuy	
ncSSRs	N	20	16	14	19	69
	N_{An}	9.875	9.250	8.625	**11.125**	9.719#
	N_{En}	5.166	4.479	5.548	**7.692**	5.721#
	N_{Pan}	2.500	1.125	1.625	**3.125**	2.094#
	H_{On}	0.698	0.703	0.748	**0.752**	0.725#
	H_{En}	0.780	0.756	0.785	**0.841**	0.791#
	$H_{E\text{-}cor}$	0.796	0.779	0.789	**0.851**	0.804#
	R_n	6.388	6.353	6.573	**7.698**	6.753
	R_{Pan}	1.829	1.255	1.607	**2.559**	1.813
	p	<0.001 (0.000)	0.002 (0.001)	0.001 (0.004)	<0.001 (0.000)	<0.001
	r	0.071†	0.060†	0.027†	0.070†	0.057#
	F_{IS} (IIM)	0.020–0.021	0.027–0.029	0.033 -0.035	0.023–0.025	0.026–0.028#
cpSSRs	N_{Ac}	**1.250**	**1.250**	1.000	**1.250**	2.750
	N_{Ec}	1.055	**1.221**	1.000	1.028	1.076#
	N_{Pac}	1.000	0.000	1.000	**2.000**	0.250#
	h_c	0.045	**0.117**	0.000	0.025	0.047#
	H_{Ec}	0.400	**0.500**	0.000	0.094	0.249#

The highest values for each parameter are indicated in bold

N number of individuals; N_{An} number of alleles (ncSSR); N_{En} effective number of alleles (ncSSR); N_{Pan} number of private alleles (ncSSR); H_{On} observed heterozygosity (ncSSR); H_{En} expected heterozygosity (ncSSR); $H_{E\text{-}cor}$ expected heterozygosity corrected for null alleles (ncSSR); R_n allelic richness (ncSSR); R_{Pan} private allelic richness (ncSSR); p probability of significant deviation from HWE (Markov chain procedure, $p < 0.05$), numbers in parentheses indicate the probability (p) of significant heterozygote deficiency; r estimated frequency of null alleles (ncSSR); F_{IS} (IIM) inbreeding coefficient for loci with null alleles; N_{Ac} number of alleles (cpSSR); N_{Ec} effective number of alleles (cpSSR); N_{PAc} number of private alleles (cpSSR); h_c Ne's gene diversity index; H_{Ec} Ne's haplotypic diversity index

Average value; †denotes null alleles detected in at least one locus using MICROCHECKER (van Oosterhout et al. 2004)

ranging from 8 to 28 (Table 1). Levels of heterozygosity were high for all loci (Table 1). A total of 152 alleles were identified among 69 individuals across four natural populations from Argentina. Forty-one out of those 152 alleles (27 %) were present only in the Paranaense province, 46 alleles (30 %) were present only in the Yungas province, whereas 65 (43 %) were shared between provinces. The overall mean number of alleles was 9.719 whereas population values ranged from 8.625 to 11.125 in Tucumán and Jujuy, respectively (Table 2). The effective number of alleles (N_{En}) in each population was lower than the mean number of alleles (N_{An}) showing the presence of alleles at low frequencies in all populations (Table 2). Among genetic diversity estimators, number of alleles, effective number of alleles and number of private alleles depend on sample sizes, whereas allelic richness is independent of this value (Gillet et al. 2005). In this regard, the highest allelic richness was found in Jujuy followed by Tucumán, Candelaria, and Santa Ana. The highest private allelic richness was also found in Jujuy populations followed by Candelaria, Tucumán and Santa Ana (Table 2). Allelic richness and expected heterozygosity differences were statistically significant when Jujuy was compared with the other populations (FSTAT comparison among groups: $p = 0.029$ and $p = 0.038$, respectively), whereas observed heterozygosity showed no significant differences for all comparisons made.

Both global tests across loci within and among populations showed departures from HWE ($p < 0.05$). All populations exhibited heterozygote deficiencies (Table 2).

No evidence of genotyping errors was found using MICROCHECKER. Null alleles were detected in at least one locus in each population, but no locus showed null alleles in all populations. The overall frequency of null alleles (r) ranged from 0.071 to 0.027 in the populations examined (Table 2). Deviations from HWE were primarily due to the presence of null alleles, since F_{IS} values were reduced to essentially zero in all populations analyzed (IIM F_{IS} average range: 0.026–0.028) (Table 2). Simulation and empirical studies have shown that null alleles lead to underestimation of allelic diversity, and observed and expected heterozygosity, but this bias is particularly low for expected heterozygosity (Chapuis et al. 2008; Shama et al. 2011). In that respect, expected heterozygosity from

Table 3 Number of trees per chloroplast haplotype in each *Anadenanthera colubrina* var. *cebil* analyzed population

Haplotype	Population				Total
	Candelaria	Santa Ana	Tucumán	Jujuy	
HA	18	–	–	–	18
HB	02	10	–	–	12
HC	–	06	–	–	06
HD	–	–	14	–	14
HE	–	–	–	18	18
HF	–	–	–	01	01
Total	20	16	14	19	69

allele frequencies corrected for null alleles ($H_{E\text{-}cor}$) was similar to expected heterozygosity. Global F_{ST} estimated from uncorrected genotypes was 0.078 (CI: 0.053–0.11) while F_{ST} estimated using the ENA method in FReeNA was 0.076 (CI: 0.054–0.107) (Chapuis and Estoup 2007) indicating that null alleles had only a small effect on F_{ST} estimates. The overlapping confidence intervals indicated that null alleles were evenly distributed among populations.

A total of 11 alleles were identified at the four chloroplast microsatellite loci among 69 individuals. Three out of four populations had 25 % of polymorphic loci whereas Tucumán is monomorphic for all loci tested (Table 2). The combination of alleles from four cpSSR loci resulted in a total of six haplotypes (Table 3). Five out of six haplotypes were private while haplotype HB was found in both populations of the Paranaense province (Table 3). Santa Ana showed the highest population diversity ($h_c = 0.12$, $H_{Ec} = 0.50$) and Jujuy showed the highest number of private alleles ($N_{APc} = 2.00$) (Table 2).

Genetic structure

Approximately 90 % of the nuclear genetic variation was detected within populations. The remaining variation was equally distributed among populations and provinces (~5 %) (Table 4). Nearly 70 % of the chloroplast genetic variation was found between provinces, while 25 % of variation was observed among populations (Table 4).

Global F_{ST}s were statistically significant and indicated genetic differentiation in both genomes. According to Wright's qualitative guidelines for the interpretation of F_{ST} (Hartl and Clark 2007) these populations are moderately structured at the nuclear genome ($F_{ST} = 0.11$), but are highly structured at the chloroplast genome ($F_{ST} = 0.95$) (Table 4). The highest values of pairwise F_{ST} based on ncSSRs were observed between populations of the different phytogeographic provinces (Table 5). Populations of the Paranaense province are more differentiated than those of the Yungas. Pairwise F_{ST} values based on cpSSRs were high between all populations (Table 5).

Two nuclear clusters were defined by the Bayesian model. Individuals were assigned to these clusters according to their phytogeographic province of origin (Fig. 2a). Province-specific analyses found differentiation among populations of the Paranaense province, but no differentiation among those of the Yungas province (Fig. 2b).

Six chloroplast clusters were defined by the Bayesian model [log (ml) = 0.957]. Individuals were assigned to each cluster according to their chloroplast haplotypes (Fig. 3a). The dendrogram showed two main groups of haplotypes corresponding to the phytogeographic origin of individuals (Fig. 3b). One group was formed by clusters 3, 4 and 6, which included all the individuals from the Yungas province, while the second group was formed by clusters 1, 2 and 5, and comprises all the individuals from the Paranaense province.

The unweighted Neighbor-Joining trees showed two main groups in correspondence with the phytogeographic province in both genomes (Fig. 4).

The global inbreeding coefficient estimated by a Bayesian approach reached a low positive value, $F_{IS} = 0.13$ (CI: 0.093–0.135).

Table 4 Analyses of molecular variance (AMOVA) in *Anadenanthera colubrina* var. *cebil* populations

Source	Source of variation	*df*	Sum of squares	Variance components	Percentage of variation	Fixation index
ncSSR	Among provinces	1	14.600	0.123 *Va*	5.59	$F_{ST} = 0.11$*
	Among populations within provinces	2	12.048	0.120 *Vb*	5.48	
	Within populations	134	261.403	1.951 *Vc*	88.94	
	Total	137	288.051	2.193		
cpSSR	Among provinces	1	54.593	1.337 *Va*	69.690	$F_{ST} = 0.95$*
	Among populations within provinces	2	16.533	0.482 *Vb*	25.110	
	Within populations	65	6.497	0.010 *Vc*	5.200	
	Total	68	77.623	1.919		

* Statistically significant. Significance of variance components using 1,023 permutations

Table 5 Genetic differentiation at nuclear (above diagonal) and chloroplast SSRs (below diagonal) estimated between pairs of populations in *Anadenanthera colubrina* var. *cebil*

Population	Candelaria	Santa Ana	Tucumán	Jujuy
Candelaria		0.741*	0.972*	0.963*
Santa Ana	0.075*		0.926*	0.921*
Tucumán	0.122*	0.123*		0.942
Jujuy	0.112*	0.088*	0.039*	

Comparison of populations from different (dark gray) and identical (light gray) phytogeographic provinces

* Statistically significant at 95

Discussion

High nuclear genetic diversity was observed within the four populations considered (Table 2). This finding is expected in forest tree species as a consequence of high population sizes, longevity of individuals, high levels of cross fertilization and/or high levels of gene flow between populations (White et al. 2007). Nuclear genetic diversity in *A. colubrina* var. *cebil* populations ($H_{En} = 0.756–0.841$; Table 2) is similar to *Dalbergia monticola*, an insect-pollinated tree species ($H_{En} = 0.64–0.81$) (Andrianoelina et al. 2009), while most tropical tree species are characterized by lower genetic diversity ranging from 0.4 to 0.7 (e.g., Dutech et al. 2002; Born et al. 2008; Muller et al. 2009; Ndiade-Bourobou et al. 2010; Debout et al. 2011). Studied populations from the Yungas province are located in protected areas. The lowest number of alleles was observed in the Tucumán population while allelic richness in this population was similar to observed values in the Paranaense province populations. These results deserve a conservative analysis because the Tucuman population contains the most restricted sampling size. The highest nuclear genetic diversity was observed in the Jujuy population, which also contains the highest number of private alleles. In contrast, the lower number of private alleles found in the other populations may indicate a reduction in their population sizes, which is a strongly increasing problem of the SDTFs. It has been suggested that the loss of alleles is a primary consequence of population size reduction (Leimu et al. 2006).

Analyses of molecular variance and F_{ST} indices showed that although most of the nuclear genetic diversity was contained within populations, they could be considered as genetically distinct in accordance with their moderate genetic structure ($F_{ST} = 0.11$). The F_{ST} value for this species agrees with the level of genetic differentiation expected for tropical tree species dispersing their seeds mostly by abiotic vectors such as gravity or wind (Finkeldey and Hattemer 2007).

Pairwise F_{ST}s revealed that the highest differentiations occur between the populations from different provinces, and also that the populations from the Paranaense province are more differentiated than that from the Yungas province

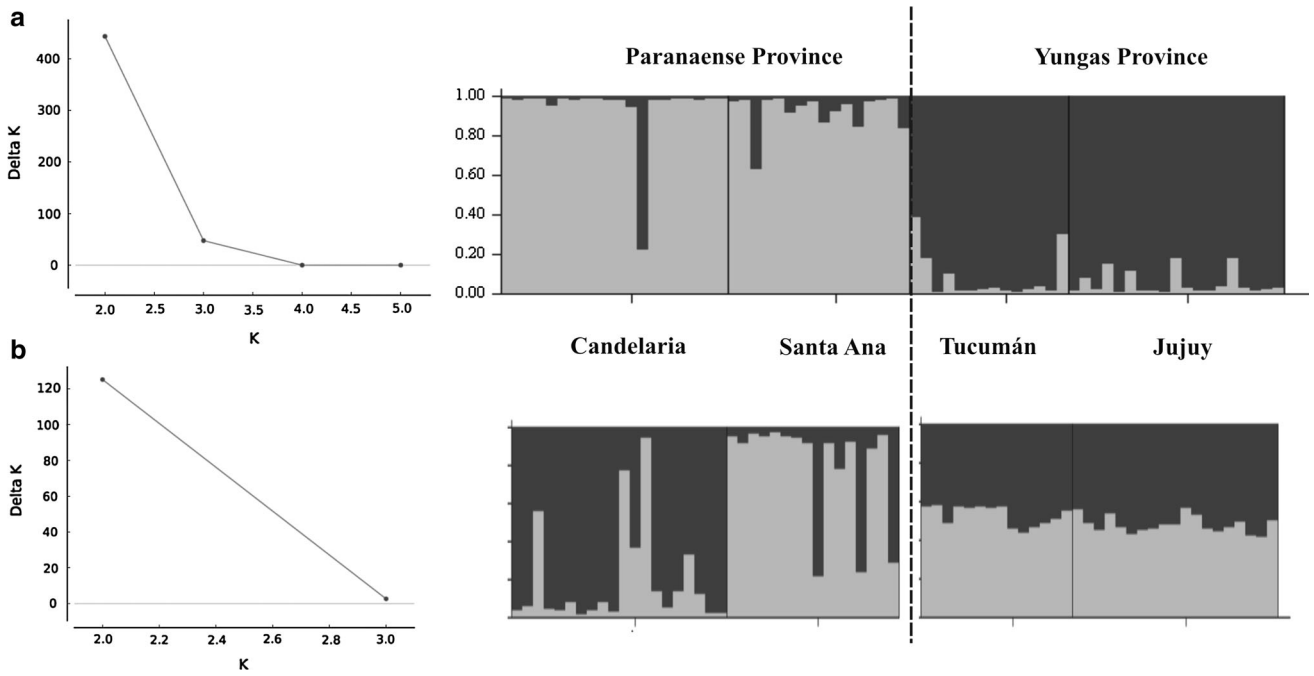

Fig. 2 Inference of *K*, the most probable number of clusters, based on the second order of change in the log likelihood of data (ΔK) as a function of *K* calculated over ten replicates. Bayesian clustering of individuals using ncSSR genetic data in an admixture model: **a** global genetic structure ($K = 2$) and **b** substructure of each province

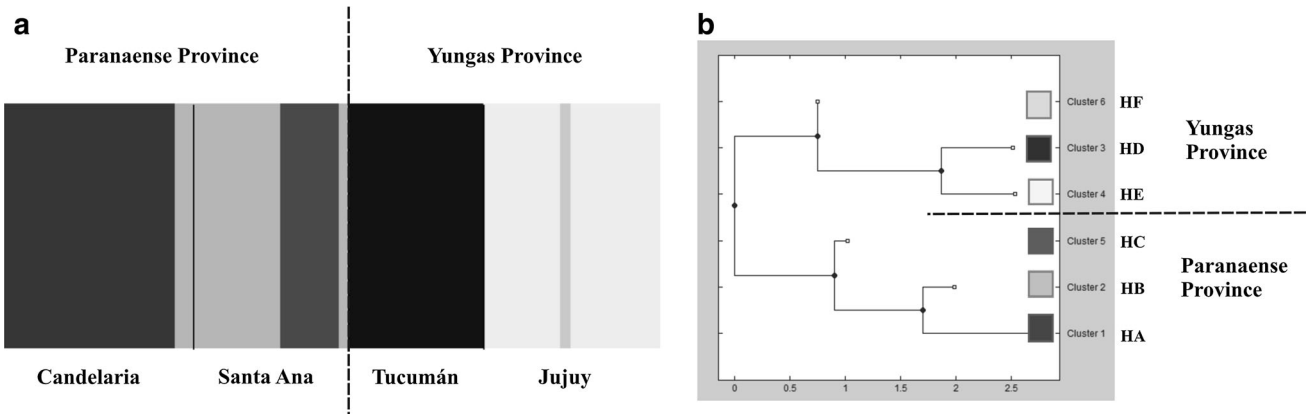

Fig. 3 **a** Bayesian clustering of individuals using cpSSR genetic data in a mixture model for linked loci. **b** Relationships among clusters defined by the Bayesian analysis of genetic structure. HA to HF represent the different haplotypes. Phytogeographic provinces of origin were included in the figure

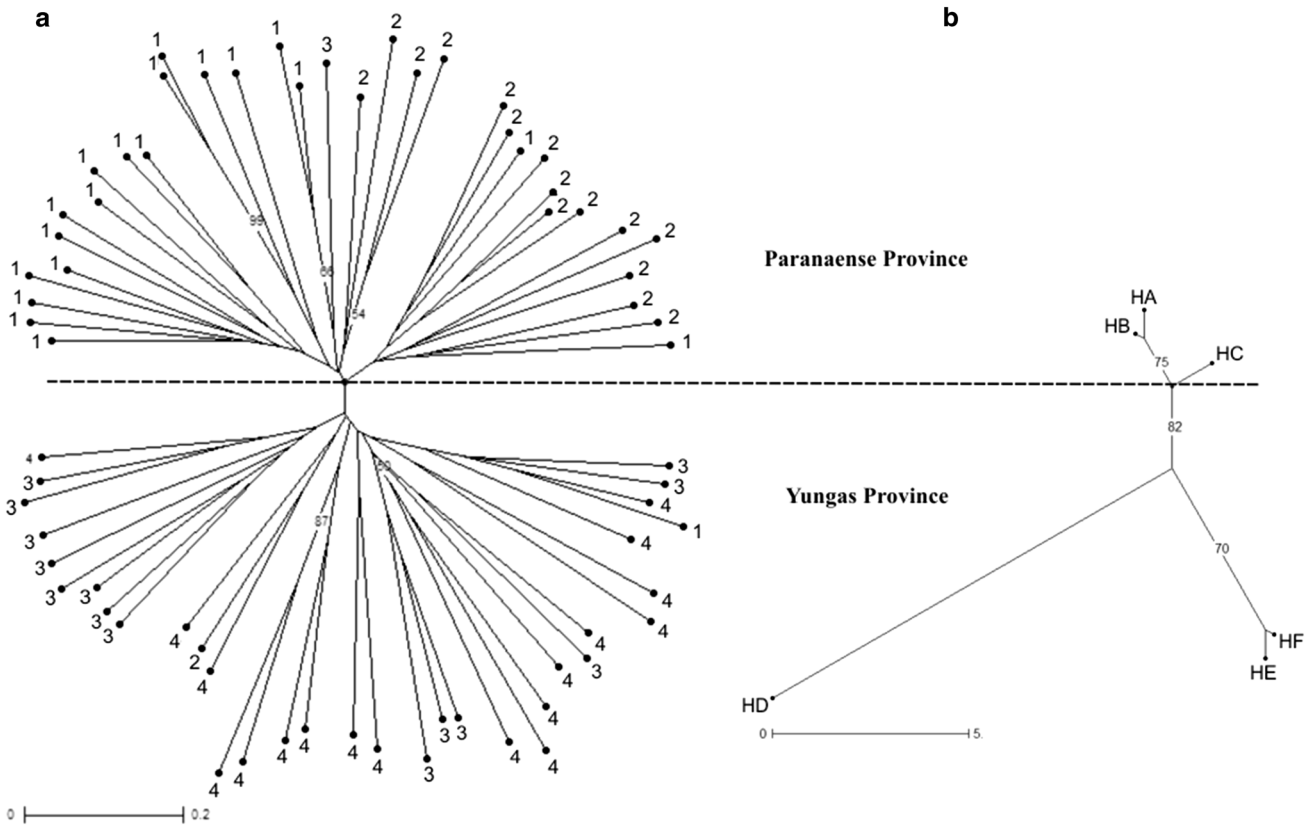

Fig. 4 Genetic relationships between **a** nuclear multilocus genotypes and **b** chloroplast haplotypes presented as unweighted Neighbor-Joining trees. Numbers in branches are bootstrap values after 1,000 replications (only bootstrap values higher than 50 % are shown). Populations: (1) Candelaria, (2) Santa Ana, (3) Tucumán and (4) Jujuy

despite the close geographic relationship between the former populations. Correspondingly, the Neighbor-Joining and Bayesian analyses grouped individuals according to their phytogeographic provinces of origin. The strong genetic substructure of the Paranaense province was also evident from the province-specific Bayesian analysis. High genetic differentiation of populations from this province may be a consequence of recent fragmentation caused by human impact. Recent fragmentation increases the isolation of remaining populations due to the elimination of possible intermediate populations, and thus limiting gene flow (Young et al. 1996). Populations from the Yungas

province showed less differentiation despite comparatively high geographic distances among them. As these populations are located in protected areas, it is likely that regular gene flow counterbalanced genetic drift (Templeton 2006). Natural populations are usually part of spatial networks and they are interconnected through gene flow (Chikhi et al. 2010). SDTFs in this region are well connected, and *A. colubrina* var. *cebil* is distributed along the pedemontane forest whereby pollen gene flow occurs along a network of interconnected local populations maintaining high levels of genetic diversity.

A low F_{IS} value indicates that populations present minor degrees of inbreeding. This is in agreement with the usual outcrossing matting system of *A. colubrina* var. *cebil* (Cialdella 2000) and its predominant pollination performed by insects (von Altschul 1964).

Out of ten cpSSR loci tested four showed polymorphism in the analyzed samples. Notwithstanding two of these universal primers, *Ccmp3* and *Ccmp5*, were previously used by Barrandeguy et al. (2011), primer *Ccmp3* did not show polymorphism in the current study. This result could be explained by differences in the methodologies used for genotyping the individuals. In the previous study, individuals were genotyped using polyacrylamide gels and visualized by silver staining while the allele size was determined by comparison to a DNA ladder, whereas in the current study individuals were genotyped by a capillary electrophoresis system on an automated sequencer. Low chloroplast genetic diversity was revealed by four cpSSR loci, but variation detected in the data set was effective to distinguish populations and provinces. High genetic differentiation was indicated by a very high global F_{ST} index ($F_{ST} = 0.95$) (Table 4). These results suggest low levels of seed-mediated gene flow between populations, and a long-lasting isolation regarding the distribution of haplotypes in each population. The different chloroplast haplotypes were grouped according to their province of origin. Bayesian analysis identified six clusters of individuals reflecting the haplotypes and dendrogram of clusters which mirror differentiation between provinces (Fig. 3).

According to the SDTF predictive distribution map based on paleodistribution modeling (Werneck et al. 2011), the Paranaense province corresponds to a historically stable area whereas the Yungas to an unstable area. From a genetic standpoint, temporally unstable areas are expected to display lower levels of intra species genetic diversity when compared with stable areas. On the other hand, stable areas probably had higher persistence and are expected to retain higher genetic diversity than those in recently colonized unstable areas (Hewitt 2004). Thus, populations from the Paranaense province shared one haplotype that could represent their historical diversity. On the other hand, populations from the Yungas province showed high genetic distance and private haplotypes that could be a consequence of subsequent colonization events.

Hamrick (2004) has proposed post-colonization pollen flow between sites to explain the low structure for biparentally inherited traits. Both genomes showed the effects of SDTF ancient fragmentation. Recent fragmentation could be invoked to explain the nuclear differentiation of populations from the Paranaense province while historical stability might be indicated by higher genetic diversity and lower genetic structure in the chloroplast genome of the Paranaense populations compared to those from the Yungas. Populations from the Yungas province show subsequent colonization patterns and a consequential increase in genetic differentiation for the chloroplast genome but relatively low differentiation for biparentally inherited traits.

In this study, ncSSR markers displayed lower genetic differentiation among populations than cpSSR markers. This is not surprising since similar results have been reported for other forest species (Naciri et al. 2006; Pakkad et al. 2008; Andrianoelina et al. 2009; Muller et al. 2009). The causes for the discrepancy between the two marker systems are different inheritances, effective population sizes and mutation rates. In angiosperms, gene flow of ncDNA occurs via both, seeds and pollen, while gene flow of cpDNA occurs solely via seed (McCauley 1995). The uniparental inheritance of cpDNA reduces effective population sizes, and thus the extent of genetic differentiation increases in this genome (McCauley 1995; Templeton 2006). Finally, higher mutation rates in ncSSRs determine higher levels of genetic diversity and low genetic differentiation (Provan et al. 1999).

The assessment of intraspecific genetic variation is essential to understand the current genetic status and to design appropriate conservation strategies. The highest number of populations prioritizing those more diverse and divergent from different localities should be preserved (Inza et al. 2012).

Ancient fragmentation was detected at the genetic level in both genomes. In this way, natural populations of *A. colubrina* var. *cebil* from Northern Argentina represent vestiges of ancient fragmentation. Therefore, demographic instability resulting from ancient fragmentation played the main role in determining genetic structure for these populations. At a lower scale, i.e., within phytogeographic provinces, we draw different conclusions. In the Paranaense province, we highlight the effects of recent fragmentation on genetic structure, based on the fragmented landscape where these populations are located, and the high human utilization of these forests. In contrast, in the Yungas province, we highlight the importance of gene flow by pollen compensation of the effects of genetic drift. Petit et al. (1998) suggested that populations with higher priority

for conservation efforts can be determined by considering allelic richness. In this regard, the Jujuy population is considered as a maximum priority population for conservation since it has the highest nuclear diversity. Fortunately, this population is located in the Calilegua National Park, a natural protected area. Populations from the Paranaense province, considered as a historically stable area, displayed the highest chloroplast diversity and high nuclear genetic differentiation. Thus, promoting the protection status of Santa Ana area is advisable, and sustainable forest management of Candelaria is urgent since it is located on private property and, therefore, highly endangered by logging activities.

The effects of historical fragmentation were identified in both genomes, in natural populations of *A. colubrina* var. *cebil* from Northern Argentina. This result was unexpected for the nuclear genome considering the high mutation and recombination rates of ncSSR, as well as the high levels of gene flow by pollen that can erase these differences. Chloroplast genome keeps valuable information about historical population changes in this species that can be used, i.e., to contrast different hypothesis about historical distribution of SDTFs by phylogeographic studies. Therefore, this study is valuable to further assess the current genetic status of this valuable tree and to highlight the importance of historical events in shaping the genetic structure in a neotropical forest tree species. Despite low number of populations analyzed this study has been useful for generating new questions that may drive future genetic studies on natural populations of neotropical forest species.

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References

Abraham de Noir F, Bravo S, Abdala R (2002) Mecanismos de dispersión de algunas especies leñosas nativas del chaco occidental y serrano. Quebracho 9:140–150

Andrianoelina O, Favreau B, Ramamonjisoa L, Bouvet JM (2009) Small effect of fragmentation on the genetic diversity of *Dalbergia monticola*, an endangered tree species of the eastern forest of Madagascar, detected by chloroplast and nuclear microsatellites. Ann Bot 104(6):1231–1242

Barrandeguy ME, García MV, Argüelles CF, Cervigni GD (2011) Genetic diversity of *Anadenanthera colubrina* Vell. (Brenan) var *cebil*, a tree species from the South American subtropical forest as revealed by cpSSR markers. Silvae Genet 60:123–132

Barrandeguy ME, Prinz K, García MV, Finkeldey R (2012) Development of microsatellite markers for *Anadenanthera colubrina* var. *cebil* (Fabaceae), a native tree from South America. Am J Bot 99(9):e372–e374

Born C, Kjellberg F, Chevallier M, Vignes H, Dikangadissi J, Sanguié J, Wickings EJ, Hossaert-Mckey M (2008) Colonization processes and the maintenance of genetic diversity: insights from a pioneer rainforest tree *Aucoumea klaineana*. Proc R Soc 275:2171–2179

Brookfield JFY (1996) A simple new method for estimating null allele frequency from heterozygote deficiency. Mol Ecol 5:453–455

Brown AD, Grau HR, Malizia LR, Grau A (2001) Argentina. In: Kappelle M, Brown AD (eds) Bosques nublados del Neotrópico. Editorial IMBIO, Costa Rica, pp 623–658

Caetano S, Prado D, Pennington RT, Beck S, Oliveira Filho A, Spichiger R, Naciri Y (2008) The history of Seasonally Dry Tropical Forests in eastern South America: inferences from the genetic structure of the tree *Astronium urundeuva* (Anacardiaceae). Mol Ecol 17:3147–3159

Chapuis M, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24:621–631

Chapuis MP, Lecoq M, Michalakis Y, Loiseau A, Sword GA, Piry S, Estoup A (2008) Do outbreaks affect genetic population structure? A worldwide survey in *Locusta migratoria*, a pest plagued by microsatellite null alleles. Mol Ecol 17(16):3640–3653

Chikhi L, Sousa VC, Luisi P, Goossens B, Beaumont MA (2010) The confounding effects of population structure, genetic diversity and the sampling scheme on the detection and quantification of population size changes. Genetics 186:983–995

Chybicki IJ, Burczyk J (2009) Simultaneous estimation of null alleles and inbreeding coefficients. Heredity 100(1):106–113

Cialdella A.M (2000) Flora Fanerogámica Argentina. Proflora. Fascículo 67: Fabaceae Subfamilia Mimosoideae 1–10

Collevatti RG, Terribile LC, Lima-Ribeiro MS, Nabout JC, de Oliveira G, Rangel TF, Rabelo SG, Diniz-Filho JAF (2012) A coupled phylogeographical and species distribution modelling approach recovers the demographical history of a Neotropical seasonally dry forest tree species. Mol Ecol 21:5845–5863

Corander J, Marttinen P, Mäntyniemi S (2006) Bayesian identification of stock mixtures from molecular marker data. Fish B 104:550–558

Corriveau JL, Coleman AW (1988) Rapid screening method to detect potential biparental inheritance of plastid DNA and results for over 200 angiosperm species. Am J Bot 75(10):1443–1458

Debout GD, Doucet JL, Hardy OJ (2011) Population history and gene dispersal inferred from spatial genetic structure of a Central African timber tree, *Distemonanthus benthamianus* (Caesalpinioideae). Heredity 106:88–99

Di Bitetti MS, Placci G, Dietz LA (2003) Una visión de biodiversidad para la ecorregión del Bosque Atlántico del Alto Paraná: diseño de un paisaje para la conservación de la biodiversidad y prioridades para las acciones de conservación. World Wildlife Fund, Washington, D.C.

Dutech C, Seiter J, Petronelli P, Joly HI, Jarne P (2002) Evidence of low gene flow in a neotropical clustered tree species in two rainforest stands of French Guiana. Mol Ecol 11:725–738

Earl DA, von Holdt BM (2011) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4(2):359–361

Ennos RA (1994) Estimating the relative rates of pollen and seed migration among plant populations. Heredity 72:250–259

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Excoffier L, Lischer H (2010) Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol Ecol 10:564–567

Excoffier L, Smouse P, Quattro J (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Finkeldey R, Hattemer HH (2007) Tropical forest genetics. Springer, Heidelberg

Gascuel O (1997) Concerning the NJ algorithm and its unweighted version, UNJ. In: Boris M, McMorris FR, Roberts FS, Rzhetsky A (ed) Mathematical Hierarchies and Biology. DIMACS workshop, Series in discrete mathematics and theoretical computer science. Bull Amer Math Soc pp 149–170

Gillet E, Gömöry D, Paule L (2005) Measuring genetic variation within and among populations at marker loci. In: Geburek T, Turok J (eds) Conservation and Management of forest genetic resource in Europe. Arbora Publishers, Zvolen, pp 237–270

Goudet J (1995) FSTAT (vers.2.9.3.2): a computer program to calculate F statistics. Heredity 86:485–486

Hamrick JL (2004) Response of forest trees to global environmental changes. For Ecol Manag 197:323–335

Hartl DL, Clark AG (2007) Principles of population genetics. Sinauer Associates, Inc Publishers, Sunderland

Hewitt G (2000) The genetic legacy of the Quaternary ice ages. Nature 405:907–913

Hewitt G (2004) The structure of biodiversity-insights from molecular Phylogeography. Front Zool 1:4

Holsinger KE, Lewis PO (2003) Hickory: a package for analysis of population genetic data version 1.1. Department of Ecology and Evolutionary Biology, University of Connecticut, Storrs, Connecticut USA. http://darwin.eeb.uconn.edu/hickory/hickory.html

Inza MV, Zelener N, Fornes L, Gallo LA (2012) Effect of latitudinal gradient and impact of logging in genetic divertisy of *Cedrela lilloi* along the Argentina Yungas Rainforest. Ecol Evol 2(11):2722–2736

Justiniano MJ, Fredericksen TS (1998) Ecologia y silvicultura de especies menos conocidas Curupaú *Anadenanthera colubrina* (Vell.Conc.) Benth. Mimosoideae Proyecto de Manejo Forestal Sostenible (BOLFOR). Santa Cruz, Bolivia

Kasturi J, Acharya R, Ramanathan M (2003) An information theoretic approach for analyzing temporal patterns of gene expression. Bioinformatics 19(4):449–458

Kullback S, Leibler RA (1951) On information and sufficiency. Ann Math Stat 22:79–86

Leimu R, Mutikainen P, Koricheva J, Fischer M (2006) How general are positive relationships between plant population size, fitness and genetic variation? J Ecol 94:942–952

Manel S, Gaggiotti O, Waples R (2005) Assignment methods: which approaches best address which biological questions? Trends Ecol Evol 20:136–142

McCauley DE (1995) The use of chloroplast DNA polymorphism in studies of gene flow in plants. Trends Ecol Evol 10(5):198–202

Muller F, Voccia M, Ba A, Bouvet JM (2009) Genetic diversity and gene flow in a Caribbean tree *Pterocarpus officinalis* Jacq.: a study based on chloroplast and nuclear microsatellites. Genetica 135:185–198

Naciri Y, Caetano S, Pennington RT, Prado D, Spichiger R (2006) Population Genetics and inference of ecosystem history: an example using two neotropical seasonally dry forest species. In: Pennington P, Lewis GP, Ratter JA (eds) Neotropical Savannas and Seasonally dry forest: plant diversity, biogeography, and conservation. The Systematics Association Special, 69th edn. Taylor and Francis Group, London

Ndiade-Bourobou D, Hardy OJ, Favreau B, Moussavou H, Nzengue E, Mignots A, Bouvet JM (2010) Long distance seed and pollen dispersal inferred from spatial genetic structure in the very low-density rainforest tree, *Baillonella toxisperma* Pierre, in Central Africa. Mol Ecol 19:4949–4962

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590

Nei M (1987) Molecular evolutionary genetics. Columbia University Press, New York

Pakkad G, Ueno S, Yoshimaru H (2008) Genetic differentiation of *Quercus semiserrata* Roxb. In northern Thailand revealed by nuclear and chloroplast microsatellite markers. For Ecol Manag 255:1067–1077

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537–2539

Pennington RT, Lavin M, Prado DE, Pendry CA, Pell SK (2004) Historical climate change and speciation: neotropical seasonally dry forest plants show patterns of both Tertiary and Quaternary diversification. Philos Trans R Soc Lond 359:515–537

Pennington RT, Lavin M, Oliveira-Filho A (2009) Woody plant diversity. Evolution, and ecology in the tropics: perspectives from seasonally dry tropical forests. Annu Rev Ecol Evol Syst 40:437–457

Perrier X, Jacquemoud-Collet JP (2006) DARwin software. http://darwin.cirad.fr/darwin

Petit RJ, El Mousadik A, Pons O (1998) Identifying populations for conservation on the basis of genetic markers. Conserv Biol 12(4):844–855

Petit RJ, Duminil J, Fineschi S, Hampe A, Salvini D, Vendramin GG (2005) Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. Mol Ecol 14:689–701

Prado DE, Gibbs PE (1993) Patterns of species distributions in the dry seasonal forests of South America. Ann Mo Bot Gard 80:902–927

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959

Provan J, Soranzo N, Wilson NJ, Golstein D, Powel W (1999) A low rate for chloroplast microsatellites. Genetics 153:943–947

Quiroga MP, Pacheco S, Malizia L, Premoli AC (2012) Shrinking Forests under Warming: evidence of *Podocarpus parlatorei* (pino del cerro) from the Subtropical Andes. J Hered 103(5):682–691

Ravi V, Khurana JP, Tyagi AK, Khurana P (2008) An update on chloroplast genomes. Plant Systematic Evol 271:101–122

Raymond M, Rousset F (1995) GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Heredity 86:248–249

Shama LS, Kubow KB, Jokela J, Robinson CT (2011) Blottlenecks drive temporal and spatial genetic changes in alpine caddisfly metapopulations. Evol Biol 11:278

Slatkin M (1987) Gene flow and the geographic structure of natural populations. Science 236:787–792

Szpiech ZA, Jakobsson M, Rosenberg NA (2008) *ADZE:* a rarefaction approach for counting alleles private to combinations of populations. Bioinformatics 24(21):2498–2504

Templeton AR (2006) Population genetics and microevolutionary theory. Wiley-Liss Publication, New Jersey

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MICROCHECKER: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

von Altschul SR (1964) A taxonomic study of the genus *Anadenanthera*. Contr Gray Herb 193:3–65

Weising K, Gardner R (1999) A set of conserved PCR primers for the analysis of simple sequence repeat polymorphism in chloroplast genomes of dicotyledonous. Genome 42:9–19

Werneck FP, Costa GC, Colli GR, Prado DE, Sites JW (2011) Revisiting the historical distribution of Seasonally Dry Tropical Forests: new insights based on palaeodistribution modelling and palynological evidence. Global Ecol Biogeogr 20:272–288

White T, Adams W, Neale DB (2007) Forest Genetics. CAB International Publishing, Cambridge

Wright S (1951) The genetical structure of populations. Ann Eugen 15:323–354

Young A, Boyle T, Brown T (1996) The population genetic consequences of habitat fragmentation for plants. Trends Ecol Evol 11:413–418