DIVERSITY AND GENETIC STRUCTURE OF THE MONOTYPIC GENUS *Colombobalanus* (FAGACEAE) IN SOUTHEAST OF COLOMBIAN ANDEANS

Diversidad y estructura genética del género monotípico *Colombobalanus* (Fagaceae) en el sureste de los Andes colombianos

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ABSTRACT
Colombobalanus is a genus with a single species *Colombobalanus excelsa,* currently categorized as vulnerable (VU) and known from only five localities in the Colombian Andes. We analyzed the diversity and genetic structure of four *C. excelsa* forest remnants in one locality in the coffee-producing area of the southeastern corner of Department of Huila, Colombia. Samples from ten trees were collected from each forest remnant for a total of 40 sample leaves, which were analyzed using 7 microsatellite markers. The resulting data matrix was used to perform genetic diversity (H_e), genetic structure (F_{ST}), and genetic distance analyses, as well as the Jaccard similarity index. Fourteen alleles were found for the entire population, with a heterozygosity (H_e) of 0.2797 and a genetic structure (F_{ST}) of 0.049. These indices suggest that the forest remnants are members of a panmictic population with historical patterns of genetic flow. The results also allow us to conclude that the low number of alleles found in these populations compared with other populations in the country show a signature of historical bottleneck in these forest remnants, where few individuals were seedlings of *C. excelsa* due to the disturbances caused by human activity.

Key words. Conservation genetics, microsatellites, endemic species, population genetics, tropical trees.

RESUMEN
Colombobalanus es un género monotípico con una única especie *Colombobalanus excelsa,* que se encuentra presente en cinco localidades de los andes de Colombia

y actualmente está categorizada como vulnerable (VU). Analizamos la diversidad y estructura genética de poblaciones de *C. excelsa* localizadas en cuatro remanentes de bosque que se encuentran ubicados en una región cafetera en el departamento del Huila, Colombia. Fueron colectadas muestras de 10 árboles de cada remanente de bosque para un total de 40 muestras que fueron analizadas utilizando 7 marcadores microsatélites. Los análisis realizados fueron, diversidad genética (H_e), estructura genética (F_{ST}), distancia genética, y el índice de similaridad de Jaccard. Catorce alelos fueron encontrados para la población, con una heterocigocidad de (H_e) of 0.2797 y una estructura genética de (F_{ST}) of 0.049. Los valores de diversidad genética y las diferencias entre las poblaciones estudiadas en términos de distancia y estructura, al igual que el índice de similaridad, sugieren que los cuatro remanentes de bosque pertenecieron a una población panmítica de *C. excelsa* que tuvo un flujo genético continuo en un pasado reciente. Los resultados además nos permiten concluir que la poca cantidad de alelos encontrados en estas poblaciones con respecto a otras poblaciones del país, podría estar indicando un clásico cuello de botella en estos remanentes de bosque, donde quedan pocos individuos semilleros de *C. excelsa* como consecuencia de los disturbios causados por la actividad humana.

Palabras clave. Conservación genética, microsatélites, especie endémica, genética de poblaciones, árboles tropicales.

INTRODUCTION

Colombobalanus excelsa Nexon & Crepet, is a tree species with a restricted distribution; it was found and described for the first time in the Huila department, in the National Natural Park (NPP) Cueva de los Guácharos (Lozano *et al*. 1979). It was then recorded by Heredia & Álvarez (1981) in the Western Cordillera, in the Valle department, in the NNP Farallones de Cali, and later in the Flora and Fauna Sanctuary Guanentá Alto Río Fonce located in the Santander department. Recently a small population was reported in the northern Central Cordillera in Amalfi municipality, Antioquia department (Ariza *et al.* 2009).

This species was originally placed in the genus *Trigonobalanus*, subfamily Trigonobalanideae, which included two more Asian species (*T. verticillata* and *T. doichangensis*). Later it was transferred to the monotypic genus *Colombobalanus*, subfamily Fagoideae, arguing that the other two *Trigonobalanus* species did not seem to share synapomorphies with *Colombobalanus* (Nixon & Crepet 1989). Complemented by fossil evidence, it was determined that each of the three *Trigonobalanus* species has features that are unique within the Fagaceae family. Therefore, it was supported that each one of them belongs to a separate monotypic genus: *Trigonobalanus, Formanodendron* and *Colombobalanus*. So, *Colombobalanus* became a monotypic genus with its unique representative in Colombia *C. excelsa* (Lozano H.C. & Henao J.E.) Nixon & Crepet (Nixon & Crepet 1989).

In Colombia, it is known by the common names of Black Oak in the Valle and Huila departments, and Purple Oak, Robla, or Encino in Santander department (Calderon 2001). The physiognomy of this species is very similar to the common Andean Oak (*Quercus humboldtii*) but differs by the hardness of the wood and by the presence of a lilaceous exudate in *C. excels*a, that emerges when the bark is wounded (Lozano *et al.* 1979).

This restricted distribution and the endemic nature have caused this species to become a research priority to better understand its population status, diversity, and genetic structure, all of which determine management and conservation policies in the short and long term. Knowing the genetic and ecological patterns and the processes that modify them are crucial to make reasonable decisions about the procedures to preserve the maximum levels of genetic diversity of this species (González 2001), which is vulnerable to extinction in the existing populations in the four departments that have records for Colombia.

Genetic diversity confers an advantage to natural plant populations that can provide enhanced possibilities to survive through environmental changes and selective pressures like human disturbance (Caujapé 2006). This diversity may be affected when population size begins to diminish, either by natural or anthropogenic processes. This is probably the case with the populations of *C. excels*a (Etter 1993). Currently, the forests in the Colombian Andes only represent 27% of the original area (IAvH 1998).

Three genetic studies have been performed in the other populations of this species in Colombia (González 2001, Palacio 2005, Arroyave 2007). In three populations (NNP Farallones de Cali, NNP Cueva de los Guácharos and SFF Alto Guanentá Río Fonce) González (2001) determined the diversity and genetic structure with tree microsattelites markers development for European oaks. In this work she found an intermediate value for genetic structuring within populations (R_{ST}= 0.066) and genetic diversity (H_e= 0.462). On the other hand, Palacio (2005) performed a comparative study of genetic diversity and evolutionary divergence of *C. excels* and *Q. humboldtii* with RAPDs markers in the same populations that González (2001) studied. In this second study, he found slightly higher values of local genetic diversity on populations of C. *excelsa* (I = 0.5188) and a genetic structure between populations of Φ_{ST} = 0.1842. In the final study, Arroyave (2007) determined the genetic diversity of *C. excelsa* using new microsatellite markers developed by Aldrich *et al*. (2002, 2003) to North Americans oaks. She worked with the same population that González (2001) and Palacio (2005), and included one new population (Amalfi, Antioquia). She found a value slightly higher for genetic structuring between populations of F_{ST} = 0.0639, and a genetic diversity of H_e = 0.5640. All these three previous works were made using one population per locality, showing a framework about the population genetic parameters of this species around its natural distribution. Instead, the current research seeks to make a local approximation to the Southeast locality of *C. excelsa*.

The main goal of our study is to solve the following question: What are the patterns in diversity and genetic structure of four forest remnants of *C. excelsa,* located in one of the most southern regions of its distribution in Colombia? This work included a new population reported for Acevedo and Timaná municipalities, in the Huila department, thus completing the molecular studies throughout the known distribution of the species.

MATERIALS AND METHODS

Study area description

Four forest remnants were evaluated and denominated according to their location: Alto Bellavista or San Isidro, La Palma, Alto Santa Barbara, and Marimba (Table 1). These four remnants are located in the south of the country, Southeast of Huila department, in the municipalities of Acevedo and Timaná, more specifically in La Serrania de Peñas Blancas (Fig. 1). The altitudes in this region

range between 1630 and 1900 m.a.s.l, with an average temperature from 16 to 20 °C, a maximum peak of rainfall in July of 235 mm and a minimum in January of 63 mm, for an annual average of 1710 mm (Eslava *et al.* 1986). These forest remnants belong to the sub-Andean forest vegetation type according to the Cleef's classification system (1984), cited by Kappelle (1996).

Table 1. Location of the four forest remnants and their sizes.

Municipio	Forest Remnants	Coordinates		Size
Acevedo	Alto Bellavista	01º48`09.2``N	75º59`16.4``W	100 ha
Acevedo	La Palma	01º49`49.6``N	75º58`37.4``W	80 ha
Timaná	Alto Santa Bárbara	01º52`33.6``N	75º56`9.1``W	200 ha
Acevedo	La Marimba	01º46`4.6``N	75º59`33.4``W	400 ha

Field collections

Random samples were taken from 10 adult *C. excelsa* trees in each forest remnant resulting in 40 samples in total. For each tree, two healthy and young leaves (5g approximately) were chosen and stored in sealable plastic bags with 50gr of silica gel to dehydrate the tissue, as per recommended procedures (Adams *et al.* 1999). The samples were brought into the IAvH (Instituto Alexander von Humboldt) Tissue Collection.

Laboratory work

Molecular techniques and data analysis were carried out in the Laboratory of Molecular Biology of IAvH, located at the CIAT facilities (Centro Internacional de Agricultura Tropical), Palmira, Valle department, Colombia. DNA was extracted using a Qiagen® DNA extraction kit for plants (DNeasy® Plant Mini Kit, Cat

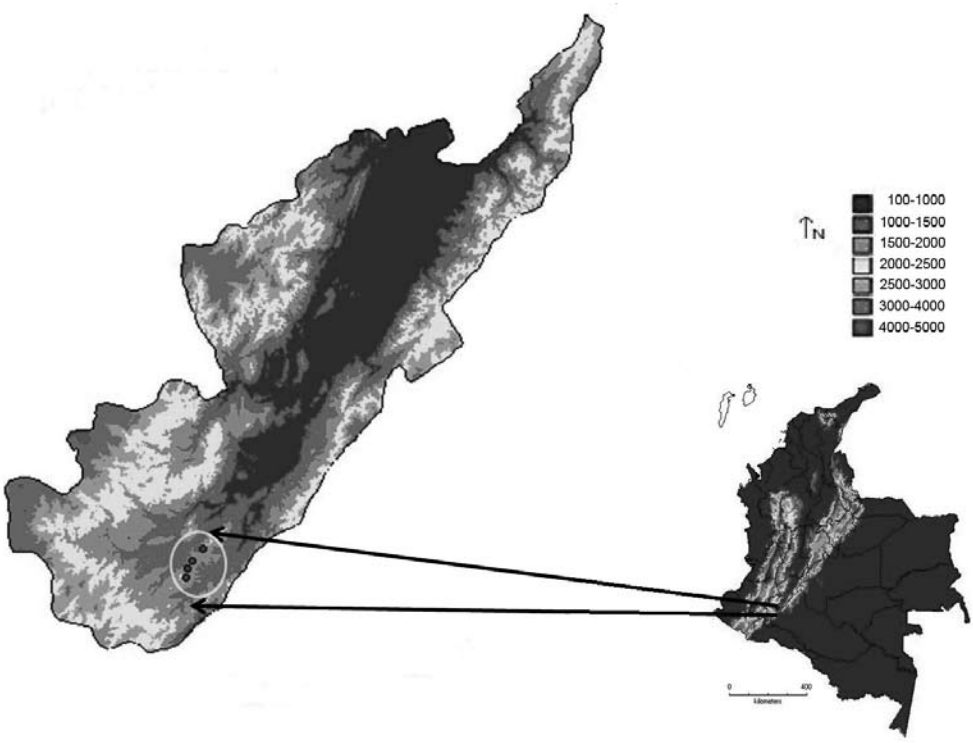

Figure 1. Location of the four forest remnants in Peñas Blancas, Huila Department, Colombia. Program Diva Gis

No. 69104), following the supplied protocol with some modifications. For the DNA evaluation we used agarose gels at 0.8% and UV fluorescence stained with ethidium bromide. Each DNA sample was taken to a final concentration of 5ng/ul for PCR-microsatellites reactions. Polymerase chain reaction (PCR) conditions were set according to the protocol of Aldrich *et al.* (2002, 2003), the concentrations used were: 72 nm of each primer, 0.01U/µl unit of Taq polymerase, 100 µM of each dNTP, 10X PCR buffer, 2.0 mM of $MgCl_2$, 5 ng/µl of DNA and ddH2O (distilled, sterilized and filtered), for a 25 µl total reaction.

The process of DNA amplification by PCR was performed using a MJ Research PTC-100 Programmable Thermal Controller at the following conditions: an initial denaturation at 94°C for 1 minute, followed by 30 cycles of denaturation at 94°C for 30 seconds, a mating to 45-56ºC for 45 seconds, a DNA synthesis at 72°C for 30 seconds, and a final extension at 72°C for 10 minutes and cooled to 4°C for five minutes. The primer used in this study were developed to analyze microsatellite in *Quercus rubra* (Aldrich *et al.* 2002, 2003), standardized and selected by Arroyave (2007) for *C. excelsa*. To evaluate the PCR product we used 1.5% agarose gel and UV fluorescence stained with ethidium bromide. Finally, genotypes for each locus were scored in 6% polyacrylamide gel using a vertical electrophoresis chamber, stained with silver nitrate and a standard weight marker 10-330 bp from Gibco® (Bassam *et al*. 1991).

Data analysis

Using seven microsatellite loci (Table 2), two types of matrices were generated according to the type of analysis to develop: in the first matrix, each allele was assigned a different letter in order to define the genotype and then, from this matrix, a second binary matrix of presence (1) or absence (0) of each allele was prepared. For the analysis of genetic diversity the number and frequency of alleles were evaluated and genetic diversity was calculated in terms of expected heterozygosity H_e (Nei 1987), using the program GenAlEx 6 (Peakall & Smouse 2006).

Population differentiation was determined by calculating the genetic structure (F_{ST}), using GenAlEx 6 (Peakall & Smouse 2006) and by calculating the distance between populations with Nei's unbiased distance (1978), using

Table 2. General description of the microsatellites tested

Locus[1]	$(GA)_n$[2]	Primers (5´-3')[3]	$MgCl_2$[4]	Ta[5] °C	Pb[6]
0M05	$(GA)_{20}$	F CTACAAGTTACATGCCCAATCA R CTTTGCGCAGGTCCATTAC	2.0	53	184-215 *(195)
0M07	$(GA)_{19}$	F TTTAGCATCACATTTCCGTT R TTTTGTGTCATCCGGTATTA	2.0	45	185-209 *(308-315)
0C11	$(GA)_{15}$	F ATACCCAGCTCCATGACCA R TCCCCAAATTCAGGTAGTGT	1.5	53	204-222 *(204-208)
0I01	$(GA)_{16}$	F GGGCTATCAAGTAAGTGCTTAAC R ACGCCATCCCTATAACACA	1.3	56	196-218 *(205-210)
1i15	$(GA)_{23}$	F CAGCCTCATCGATTACCCCAAAC R GGTCGCTGAGGGGGAAAG	1.3	51	186-226 *(212)
1F02	$(GA)_{15}$	F CCAATCCACCCTTCCAAGTTCC R TGGTTGTTTTGCTTTATTCAGCC	1.3	56	164-184 *(140)
1J11	$(GA)_{20}$	F AGTTTGGGTCAAATACCTCC R AGATAATCCTATGATTGGTCGAG	1.5	51	194-240 *(230-238)

Nomination of the Locus[1], pattern type and number of repetitions[2], primer sequences[3], $MgCl_2$ concentration used in PCR[4], Mating temperature used in PCR [5]and pairs of alleles recorder register by Aldrich (2002, 2003) [6]- *pairs of alleles recorder register in this study.

POPGENE version 3.2 (Yeh *et al.* 1997). A similarity analysis was performed using the statistical program NTSYS-PC version 2.0 (Rohlf 1993), using the Jaccard similarity index and UPGMA clustering method, generating a similarity tree. To complement this analysis, we conducted a Mantel test to assess the correlation between genetic distance and geographic distance.

RESULTS

Genetic diversity

In total, we found 14 alleles for all loci, three alleles at loci 0M07 and 0I01, (these being the most polymorphic loci), and one allele at loci 1F02 and 0M05 (Table 3). The heterozygosity values of the most polymorphic loci were, H_e= 0.6130 for 0I01, H_e= 0.4216 for the 0M07 and the average heterozygosity for the four fragments studied was H_e= 0.2797 (Table 4). The effective number of alleles N_e was 1.5396, indicating that among the analyzed loci there was an average of 1.53 alleles per locus.

Genetic distance

According to the genetic distance of Nei (1978), the closest forest remnants were La Palma and La Marimba (0.0127) and the farthest remnants were Alto Bellavista and La Marimba (0.0272) (Table 5). The dendrogram (output for program POPGENE through UPGMA method), based on the unbiased distance of Nei (1978) showed that the high forest fragment Bellavista, which was the farthest, was genetically closer and clustered between the remnants of forest La Palma and La Marimba (Fig. 2). The Mantel test indicated that there was no correlation between genetic and geographical distance (r=0.678, p=0.325).

Table 3. Alleles found at each locus for the four *C. exelsa* forest fragments.

Locus	Allele	Fr1	Fr2	Fr3	Fr4
OMO5	**1**	1.000	1.000	1.000	1.000
OC11	**1**	0.200	0.000	0.000	0.050
	2	0.800	1.000	1.000	0.950
OMO7	**1**	0.850	0.750	0.800	0.550
	2	0.150	0.150	0.100	0.150
	3	0.000	0.100	0.100	0.300
1FO2	**1**	1.000	1.000	1.000	1.000
1J11	**1**	0.700	0.600	0.750	0.650
	2	0.300	0.400	0.250	0.350
1I15	**1**	0.800	0.800	0.700	0.700
	2	0.200	0.200	0.300	0.300
OIO1	**1**	0.550	0.450	0.500	0.550
	2	0.250	0.250	0.000	0.150
	3	0.200	0.300	0.500	0.300

Fr1: Forest remnant Alto Bellavista, **Fr2:** Forest remnant La Palma, **Fr3:** Forest remnant Alto Santa Bárbara and **Fr4:** Forest remnant La Marimba.

Table 4. General genetic diversity for the four forest remnants.

Locus	Size Sample	Hom Obs	Het Obs	Hom Exp*	Het Exp*	Nei**	Average Het
OMO5	40	1.000	0.000	1.000	0.000	0.000	0.000
OMO7	40	0.725	0.275	0.573	0.427	0.422	0.396
OC11	40	0.975	0.025	0.881	0.118	0.117	0.103
OIO1	40	0.675	0.325	0.387	0.613	0.605	0.581
1I15	40	1.000	0.000	0.620	0.379	0.375	0.370
1FO2	40	1.000	0.000	1.000	0.000	0.000	0.000
1J11	40	0.400	0.600	0.555	0.444	0.438	0.432
Media	40	0.825	0.175	0.717	0.283	0.279	0.269
Est Desv		0.233	0.233	0.242	0.242	0.239	0.231

***** Expectation of homozygous and heterozygous.
** Expectation of heterozygous by Nei (1973)

Table 5. Nei (1978) unbiased genetic distance for the *C. excelsa* forest remnants – bottom of the diagonal – and Nei (1978) unbiased genetic identity – upper the diagonal.

Forest Remanents	Alto Bellavista	La Palma	Alto Santa Bárbara	La Marimba
Alto Bellavista	****	0.9863	0.974	0.9731
La Palma	0.0137	****	0.9836	0.9874
Alto Santa Bárbara	0.0263	0.0166	****	0.9821
La Marimba	0.0272	0.0127	0.018	****

The genetic structure F_{ST} statistics (calculated using GenAlEx program) gave a value of 0.049, indicating that the four remnants differ little. Likewise, we found the Jaccard similarity index (program NTSYS–PC) did not show any particular among each group forest remnant analyzed (Fig. 3), suggesting that differences exist within but not between the remnants.

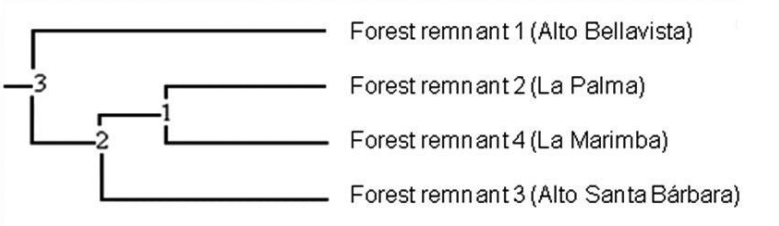

Figure 2. Nei distance tree (1978), based on UPGMA method for the four *C. excelsa* populations

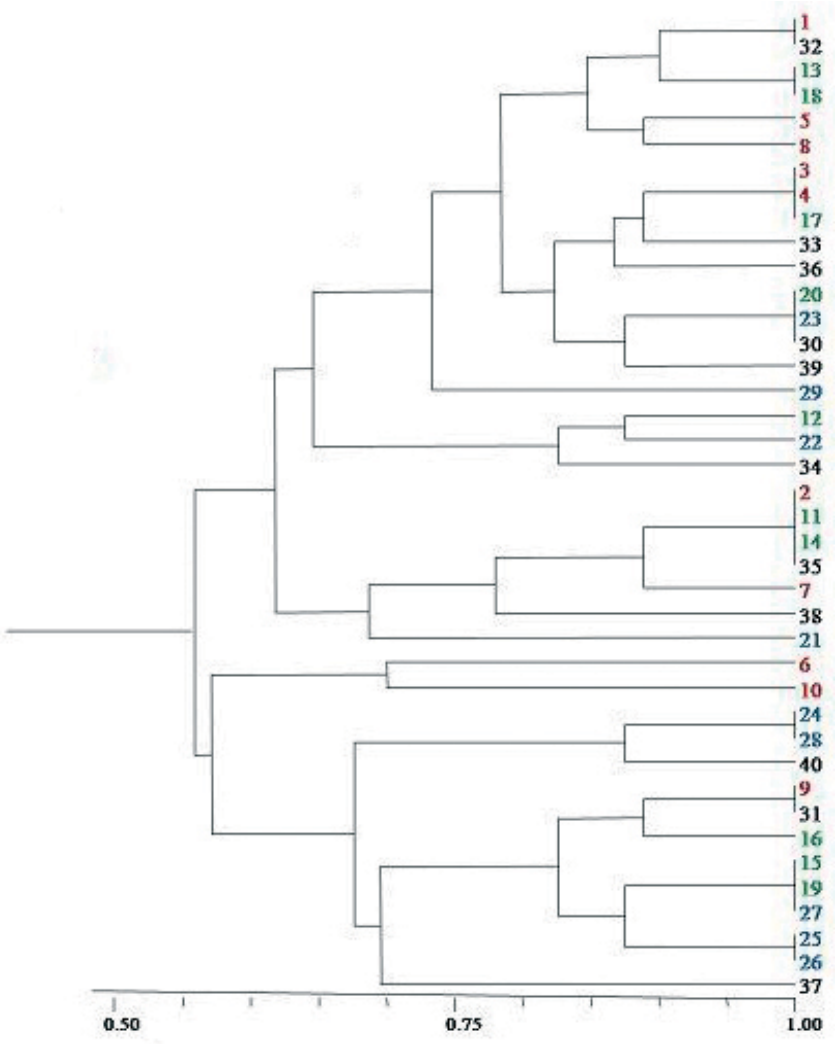

Figure 3. Dendrogram of Jaccard genetic distance as in the four forest remnants from seven microsatellite loci developed with the program NTSYS-PC. Similarity index of Nei and Li (1979). Red: Individuals of Alto Bellavista, Green: Individuals of La Palma, Blue: Individuals of Alto Santa Bárbara and Black: Individuals of La Marimba

DISCUSSION

The 14 alleles found in 40 individuals of this study contrast with the 39 alleles found in 30 individuals of NNP Cueva de los Guacharos (Arroyave 2007), located about 60 kilometers south on the same side of Cordillera Oriental. Despite this lower number of alleles, half of the alleles found in this study are unique and are not present in the population of NNP Cueva de los Guacharos. This means that, although the diversity values are low, some of this low diversity may be unique and therefore likely to be preserved.

Our results suggest that the genetic diversity of *C. excelsa* in these forest remnants from southeastern (H_e= 0.2797) was lower compared with previous works, where to the closest population of NNP Cueva de los Guacharos show highest values: H_e= 0.462 (González 2001), I= 0.4712 (Palacio 2005), and H_e= 0.5623 (Arroyave 2007). In contrast, genetic diversity f *C. excelsa* was similar to natural populations of close lineages of *Trigonobalanus* from Asia. The genetic diversity in seven populations of *Trigonobalanus verticillata* studied by AFLP in Malaysia showed a value of H_e= 0.198 (Kamiya *et al.* 2002), while *T. doichangensis*, in a study conducted in 5 distant towns in China with RAPD, found a diversity value of H_e= 0.160 (Sun *et al.* 2007). Both genetic diversity values are lower than those found in this study for *C. excelsa*.

These results suggest that the four *C. excelsa* forest remnants of mature trees maintain diversity values are lower than those that have historically been maintained and that the challenge now is to promote the necessary actions for the conservation of existing *C. excelsa* forest remnants. These heterozygosity differences are indicative of a possible bottleneck effect, since the diversity of alleles found is drastically smaller in relation to the number of alleles found in other populations of the country (14 alleles in this study in relation to 39 alleles found in NNP Cueva de los Guacharos for Arroyave (2007)) evaluated by means of the same microsatellite molecular markers. A possible cause for this scenario is the disturbance caused by anthropogenic processes in the area, like wood extraction.

In addition, genetic structure (F_{ST} 0.049) could be considered as a moderate differentiation between forest remnants evaluated in this study. Since this study was conducted on a local scale (no more than 13 km between the most distant populations) and Arroyave (2007) results were obtained from a larger scale (she covered the other three populations found in Colombia where the most distant populations are around 600 km of distance), she found an F_{ST} of 0.0639.

Also, in the closely related species of Trigonobalanus F_{ST} values are much larger than those found in the present study, *T. verticillata* F_{ST} = 0.153 (Kamiya *et al.* 2002) and *T. doichangensis* F_{ST} = 0.530 (Sun *et al.* 2007). These values are associated with broad ranges of the distribution of the species, confirming that the genetic structure of populations studied in this work can be important considered the small geographic scale.

The genetic distances found among the four forest fragments do not correspond to their geographical distances (Table 5), since the order between forest remnants from south to north in the Sierra de Peñas Blancas is: Marimba, Alto Bellavista, Alto La Palma, and Santa Barbara respectively. Genetic distance values found indicated that the remnants genetically closer to each other are La Palma and La Marimba and the farthest are Alto Bellavista and La Marimba. This genetic proximity between forest remnants La Palma and La Marimba could be explained by nonlinearity of the terrain topography and the processes of pollination of black oak, primarily by wind, which allows a

greater flow between nonlinear remnants but geographically more accessible. This result is associated with the lack of correlation between genetic distance and geographical distance from the Mantel test.

The dendrogram generated from similarity analysis (Fig. 3) clearly showed that individuals do not have a grouping pattern. However, they are mixed throughout the tree. Individuals from different populations show identical genetic composition of 100% similarities (eg. individuals 2, 12, 14 and 35 are similar and belong to different forest remnants) and confirm the results found in previous analysis of genetic structure, genetic distance, and the Mantel test on low differentiation of the remnants.

The four forests were strongly disturbed in the seventies by the looting of precious woods. Currently, they are in various successional stages dominated mainly by black oak and associated with *Q. humboldtii*. In addition, they are immersed in a landscape matrix dominated by large tracts of land used for agriculture activities. The remnant Alto Bellavista and La Palma mainly are in a coffee matrix. Whereas, Alto Santa Barbara and La Marimba are in a grass matrix for cattle, alternated with coffee. According to the results, we can conclude that the four studied forest remnants were part of a panmictic population, which, in the recent past, was part of a continuous forest with free flow of pollen and seeds. This is because the genetic distance and the Mantel test did not show an effect of geographical isolation on genetic distances. Finally, the similarity analysis shows no pattern of grouping individuals and populations. This idea is supported by the closeness of remnant as they are one after the other in the Serrania de Peñas Blancas, with distances to High Bellavista to La Marimba of 4 km, High Bellavista to La Palma of 3.69 km, and La Palma to High Santa Barbara of 6.19 km, respectively.

We concluded that the remnants of these forests are the result of a bottleneck generated by anthropogenic disturbances, which may have led to a drastic fall in the number of alleles observed. These forest remnants should be included in management plans where populations can recover viable population sizes.

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LITERATURE CITED

Adams, R., M. Zhong & Y. Fei. 1999. Preservation of DNA in plant specimens: inactivation and re-activation of DNases in field specimens. Molecular Ecology 8:681-684.

Aldrich, P.R., C.H. Michler, W. Sun & J.R. Severson. 2002. Microsatellite markers for northern red oak (Fagaceae: *Quercus rubra*). Molecular ecology Notes 2:472-474.

Aldrich, P.R., M. Jagtap, H. Michler & J.R. Severson. 2003. Amplification of North American Red Oak Microsatellites

Markers in European White Oaks and Chinese Chestnut. Silvae Genetica 52:3-4.

Ariza-Cortés, W., J. Toro-Murillo & A. Lores-Medina. 2009. Análisis florístico y estructural de los bosques pre-montanos en el municipio de Amalfi (Antioquia, Colombia). Colombia Forestal 12:81-102.

Arroyave, M. 2007. Análisis de la diversidad genética de la especie vulnerable *Colombobalanus excelsa* (Lozano *et al.*) Nixon & Crepet (Fagaceae) por medio de marcadores moleculares microsatélites. Tesis de pregrado. Universidad del Valle.

Bassam, B., G. Caetano-Anolles & P. Gresshoff. 1991. Fast y sensitive silver staining of DNA in polyacrilamide gels. Analytical Biochemistry. 19:680-683.

Calderón, E. 2001. Plantas colombianas en peligro, extintas o en duda. Bogotá: Instituto Alexander von Humboldt.

Caujapé, J. 2006. *Brújula para botánicos desorientados en la genética de poblaciones*. Ediciones Exegen. Departamento de Biodiversidad Molecular y Banco de ADN Jardín Botánico Canario ¨Viera y Calvijo¨. 133pp.

Eslava, J., V. López & G. Olaya. 1986. Contribución al conocimiento del régimen térmico y pluviométrico de Colombia. Colombia Geográfica 12(2).

Etter, A. 1993. Diversidad ecosistémica en Colombia hoy. *En:* S. Cárdenas y H. D. Correa (eds.), 43-61 pp. *Nuestra Diversidad Biológica*. Bogotá: Fundación Alejandro Escobar-CEREC.

González, E. 2001. Diversidad Genética de tres poblaciones de *Colombobalanus excelsa* (Forman) Nixon & Crepet (Fagaceae), especie endémica de los Andes Colombianos. Tesis de pregrado. Universidad del Valle.

Heredia, M. & H. Álvarez. 1981. Presencia de *Trigonobalanus excelsa* en la cordillera occidental. Cespedesia 10 (37-38):181-186.

Instituto de Investigación en Recursos Biológicos Alexander von Humboldt. 1998. Informe Nacional sobre el estado de la biodiversidad en Colombia. Bogotá, Colombia.

Kapelle, M. 1996. *Los Bosques de Roble (Quercus) de la Cordillera de Salamanca, Costa Rica: biodiversidad, ecología, conservación y desarrollo*. 1ed. Heredia, C.R.: Instituto Nacional de Biodiversidad: Universidad de Amsterdanm. 336pp.

Kamiya, K., K. Harada, M. Clyde & A. Mohamed A. 2002. Genetic variation of *Trigonobalanus verticillata*, a primitive species of Fagaceae, in Malaysia revealed by chloroplast sequences and AFLP markers. Genes & Genetic Systems 77 (3):177-186.

Lozano, G., J. Hernández J & J. Henao. 1979. Hallazgo del género *Trigonobalanus* Forman, 1962 (Fagaceae) en el Neotrópico-I. Caldasia 12 (60): 517-537.

Nei, M. 1978. Estimation of average heterozigosity and genetic distance from a small number of individuals. Genetics 89:583-590.

Nei, M. 1987. *Molecular Evolutionary Genetics.* Columbia University Press, New York. 512 pp.

Nixon, K. & W. Crepet. 1989. *Colombobalanus* Fagaceae taxonomic status y phylogenetic relationships. American Journal of Botany 76(6):828-841.

Palacio, J. 2005. Estudio comparativo de la diversidad genética y divergencia evolutiva entre la especie vulnerable *Colombobalanus Excelsa* (Lozano *et al*.) Nixon & Crepet y el roble común *Quercus humboldtii* Bonpl.: Implicaciones para la ecología de la conservación. Tesis de Maestría. Universidad Nacional de Colombia.

Peakall, R. & P. Smouse. 2006. GenAlex 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288-295.

Rohlf, J. 1993. NTSYS-PC, *Numerical taxonomy and multivariate analysis system*. Setauket, NY., Exeter Publ.

Sun, W., C. Han & C. Wilson. 2007. Genetic diversity of the rare Asian plant, *Trigonobalanus doichangensis* (Fagaceae). Australian Journal of Botany 55 (1):10-17.

Yeh, C., C. Yang & T. Boyle. 1997. *PopGene*. Alberta, Canada, University of Alberta and Center for International Forestry research.

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