GENETIC DIVERSITY AND SPATIAL GENETIC STRUCTURE WITHIN A POPULATION OF AN AROMATIC SHRUB, *LIPPIA ORIGANOIDES* (VERBENACEA), IN THE CHICAMOCHA CANYON, NORTHEASTERN COLOMBIA

ADRIANA SUÁREZ GONZÁLEZ

UNIVERSIDAD INDUSTRIAL DE SANTANDER
FACULTAD DE CIENCIAS
ESCUELA DE BIOLOGIA
2008

GENETIC DIVERSITY AND SPATIAL GENETIC STRUCTURE WITHIN A POPULATION OF AN AROMATIC SHRUB, *LIPPIA ORIGANOIDES* (VERBENACEA), IN THE CHICAMOCHA CANYON, NORTHEASTERN COLOMBIA

ADRIANA SUÁREZ GONZÁLEZ

Trabajo de investigación para optar el titulo de Bióloga

DIRECTOR:
Maria Isabel Chacón Sánchez
PhD Agricultural Botany

CODIRECTOR:
Genis Castillo Villamizar
BsC Biólogo

UNIVERSIDAD INDUSTRIAL DE SANTANDER
FACULTAD DE CIENCIAS
ESCUELA DE BIOLOGIA
2008

CONTENIDO

LISTA DE FIGURAS

Title: Genetic Diversity and Spatial Genetic Structure within a Population of an Aromatic Shrub, *Lippia origanoides* (Verbenacea), in the Chicamocha Canyon, northeastern Colombia*

Author: Adriana Suárez González**

The geographic scale of genetic structure in a population is highly dependent on its breeding system and dispersion capabilities, and this knowledge, in turn, is important for the study of population dynamics and conservation purposes. In this study, the genetic diversity and the scale of geographic structure was studied in a natural population of a prominent aromatic plant, *Lippia origanoides*, native to the Chicamocha Canyon in northeastern Colombia by means of ISSR markers. Genetic diversity, described as *P* (86.21%) and quantified using *I* (0.453) and H_B (0. 484), showed relatively high levels of genetic variation. These levels of genetic diversity are comparable or higher to other plant species with allogamous breeding systems and to other related Verbenaceae species. Cluster (PCO and UPGMA) analyses revealed a low level of differentiation among individuals from two localities of the Chicamocha Canyon. Fine scale autocorrelation analyses, done for the entire region and also separately for each of the two localities, showed results consistent with the classical model of isolation by distance, where there is a decline of genetic similarity with increasing geographical distance.
These results suggest a pattern of moderate but significant levels of local spatial structure in *L. origanoides*. The causes of this pattern are currently unknown and could be influenced by many contemporary factors such as restricted seed dispersal and/or short-distance pollen movement, among others. Our results suggest that sampling individuals at distances greater than 1.4 km may result in the collection of different genotypes, which could help preserve the levels of genetic diversity in a propagation programme.

Key words: Bayesian Analysis, Isolation by distance, ISSR, Neotropics, Spatial autocorrelation,

*Trabajo de Investigación
** Facultad de Ciencias, Escuela de Biología, Maria Isabel Chacón Sánchez - Genis Castillo Villamizar

Titulo: DIVERSIDAD GENETICA Y ESTRUCTURA GENETICA ESPACIAL EN UNA POBLACION DE UN ARBUSTO AROMATICO, *LIPPIA ORIGANOIDES* (VERBENACEA), EN EL CAÑON DEL CHICAMOCHA, NORESTE COLOMBIANO*

Autor: Adriana Suárez González**

Palabras Claves: Análisis Bayesiano, Aislamiento por Distancia, ISSR, Neotrópicos, Autocorrelación Espacial

Contenido: En las poblaciones, la escala de la estructura genética depende directamente del sistema de reproducción y la capacidad de dispersión, y su conocimiento es importante para el estudio de la dinámica de las poblaciones y para propósitos de conservación. En este estudio, la diversidad genética y la escala de la estructura geográfica fueron analizadas en una población natural de una planta aromática promisoria, *Lippia origanoides*, nativa del Cañón del Chicamocha en el noreste Colombiano utilizando cebadores ISSR.
La diversidad genética, descrita como P (86.21%) y cuantificada usando *I* (0.453) y H_B (0. 484), reveló altos niveles relativos de variación genética. Estos niveles de diversidad genética son mayores o comparables con los niveles en otras especies de plantas alogamas y con otras especies relacionadas de la familia Verbenaceae. Análisis de agrupamiento (PCO y UPGMA) revelaron un bajo nivel de diferenciación entre los individuos de dos localidades del Cañón del Chicamocha. Análisis de autocorrelación espacial a escala fina, realizados para toda la región, y por separado para cada localidad, mostraron resultados consistentes con el clásico modelo de aislamiento por distancia, en donde hay una disminución en la símilaridad genética al incrementar la distancia geográfica.
Estos resultados sugieren un patrón moderado pero significativo de estructura genética espacial en *L. origanoides*. Las causas de este patrón son desconocidas y puede estar influenciado por muchos factores contemporáneos como una dispersión limitada de semillas y/o un movimiento de polen a cortas distancias, entre otros. Nuestros resultados sugieren que el muestreo de individuos a distancias mayores de 1.4 km puede resultar en la colecta de diferentes genotipos lo cual ayudaría a conservar lo niveles de diversidad genética en programas de propagación.

*Trabajo de Investigación
** Facultad de Ciencias, Escuela de Biología, Maria Isabel Chacón Sánchez - Genis Castillo Villamizar

1. INTRODUCTION

In natural populations, the spatial genetic structure, i.e. the non-random spatial distribution of genotypes, can result from a number of evolutionary and ecological processes, including the mating system (Kalisz *et al*., 2001), the capacity of the species for gene dispersal (i.e. seed and pollen dispersal mechanisms) (McCauley, 1997; Epperson, 2000; He *et al*., 2000; Miyazaki and Isagi, 2000; Kalisz *et al*., 2001; Hardy *et al*., 2004; Hardesty *et al*., 2005; Chung *et al*., 2006), adult density (Hamrick et al., 1993; Gehring and Delph, 1999; Gitzendanner and Soltis, 2000, Nybom, 2004; Vekemans and Hardy, 2004), thinning among cohorts (Berg and Hamrick, 1995; Parker et al., 2001; Chung *et al*., 2003, Jones et al 2007), spatial and temporal patterns of seedling establishment (Schnabel et al., 1998; Parker et al., 2001), colonization and disturbance history (Linhart and Grant, 1996; Epperson and Chung, 2001; Parker et al., 2001, Wallace, 2002, Llorens *et al*., 2004), and microenvironmental selection (Linhart and Grant, 1996; Gehring and Delph, 1999; Kalisz *et al*., 2001).

The patchy spatial distribution of genotypes can occur at different scales, for example at the level of populations, subpopulations, or even among neighbouring individuals (Escudero *et al.*, 2003). At a fine spatial scale, the most prevalent cause for spatial structure is probably limited gene dispersal, which results in the formation of local pedigree structure (Epperson, 2000; Vekemans and Hardy, 2004). In plants the dispersal function is mediated by

the male gametophyte (pollen) and the young sporophyte (seed) (Petit *et. al*. 2005). Frequently, these two dispersion vehicles show moderate to strong spatial restriction in their dispersal (Vekemans & Hardy 2004), therefore generating processes of fine scale population genetic structure (Gehring and Delph, 1999; Hardy *et al*., 2004; Tero *et al*., 2005). This process is mainly a result of isolation by distance, namely, the significantly positive correlation between genetic relatedness among individuals and their spatial proximity (Hardy *et al*., 2005).

There are different approaches for measuring the structure of genetic diversity in populations. Statistics such as *Fst* (Wright, 1943) or Analysis of Molecular Variance (AMOVA) (Excoffier *et al*., 1992) have been widely used to measure the spatial variance in gene frequencies among populations or other spatial subdivisions. Although these techniques give valuable information about the structure of genetic diversity, they require averaging subpopulations at one or more hierarchical levels (Epperson and Li, 1996), and they do not provide information below the size of the smaller subdivision, so that lower scales cannot be explored (Escudero *et al.*, 2003). Currently, spatial autocorrelation analyses are used to detect and interpret spatial patterns of genetic variation within plant populations (He *et al*., 2000; Chung *et al*., 2004; Zhang *et al*., 2007). Spatial autocorrelation methods make no assumptions about the scale of the structure, which is especially convenient when there is no previous knowledge of the spatial distribution of genotypes in the species under study, and also because

processes that generate spatial structure may operate at different scales and there is thus no single "correct" scale at which to describe populations (Levin, 1992).

Lippia origanoides, Kunth, Verbenaceae, is a shrub that can reach up to 3.5 m of height, and whose leaves, flowers and stems present a characteristic Artemisia or marjoram odour (Lopez, 1977). This is a promising species with essential oils of high quality that are used mostly in cosmetics and perfumery, and that have significant antimicrobial activity against *Escherichia coli*, *Staphylococus aureus*, *Staphylococus aureus*, *Candida albicans* and *Candida tropicali*s (Santos *et al*., 2004; Oliveira *et al*., 2007). *L. origanoides* leaves are used like a substitute of common marjoram (Maisch, 1885; Oliveira *et al*., 2007).

L. origanoides occurs in the Neotropical region, specifically in the north and centre of South America, and also in the Caribbean (Giuseppe, 2003). It is generally found in dry environments, where the soils are shallow, stony and poor in organic matter (Albesiano et al., 2003). In Colombia, northern South America, herbarium records of *L. origanoides* suggest the species occurs in the Andean region (Albesiano et al., 2003), the Atlantic Coast (Instituto Alexander Von Humboldt Herbarium) and in the inter-Andean valleys (Álvaro Fernández Pérez Herbarium, Universidad del Cauca). In Santander, in northeastern Colombia, *L. origanoides* exhibits a natural wide distribution in the dry region of the Chicamocha Canyon, especially in small hillsides with smooth or steep slopes. In this locality, the excessive shepherding of

goats has played an important role in the vegetation distribution, although *L. origanoides* is not affected by these herbivores because it contains toxic tannins and defensive structures like spines and thorns (Albesiano *et al*., 2003). Nevertheless, other types of anthropogenic intervention that are taking place on the Chicamocha Canyon, including deforestation and transitory cultivation (Albesiano *et al*., 2003; Malagon *et al*., 1995; Serrato, 2007), could lead to significant concerns regarding the conservation of the species.

Despite being a promising aromatic plant, very little is known about basic biological aspects of *L. origanoides*, for example its breeding system or dispersal capabilities, which have a direct influence on genetic diversity levels and spatial distribution of genotypes inside natural populations (Wright, 1946). It is expected that the odoriferous compounds present in these plants attract pollinators, thus favouring high levels of gene flow within populations (Levin *et al*., 2001; Albre *et al*., 2003).

Highly polymorphic genetic markers are a useful tool to infer genetic structure and dispersal patterns (Hardy *et al*., 2005). ISSR (Intersimple Sequence Repeats) are arbitrary multiloci markers produced by PCR amplification using anchored-microsatellite primers (Zietkiewicz et al. 1994). This method has the advantage that no prior genomic information is required and it generates relatively stable PCR products that show high levels of polymorphisms (Bornet and Branchard, 2004). As a result, ISSR have been widely applied in studies of genetic diversity and population

structure (Camacho and Liston, 2001; Culley and Wolfe, 2001; Sica *et al*., 2005). Additionally, this technique constitutes an alternative for taxa for which little or none research has been done at the molecular level (Liu and Wendel, 2001). Nevertheless, ISSR are dominant markers and their principal limitation for population genetic studies is the biased estimation of allele frequencies, which is usually done by assuming Hardy-Weinberg equilibrium (HWE) inside populations (Zhivotovsky, 1999; Culley and Wolfe, 2001). The estimation bias of this method is particularly serious in presumably outcrossing species where a relatively high proportion of heterozygous individuals are expected. Thus, to counteract this limitation, different types of alternative methods have been proposed, such as a Bayesian approach, which takes full advantage of data provided by dominant markers without assuming HWE (Zhivotovsky, 1999; Holsinger *et al*., 2002).

The aim of the present study was to measure genetic diversity levels of *L. origanoides* from two representative localities of the Chicamocha Canyon, in northeastern Colombia, and to document fine population structure at different spatial scales using spatial autocorrelation analyses.

2. MATERIALS AND METHODS

2.1. Sample Collection

Samples from 111 individuals of *L. origanoides* were collected from one population located on the Chicamocha Canyon, at the western side of the department of Santander in Colombia, during September 2005 and August 2006 (Figure 1). Most of the collections were made at two localities which were chosen for their position along the Chicamocha River, specifically in the municipalities of Pescadero (6º49' N, 73º00' W) and Cepitá (6º45´ N, 72º58´ W) (Figure 1). The sampling strategy reflected the current distribution and abundance of the species in each locality. In Pescadero, *L. origanoides* is more abundant and continuously distributed on both sides of the Chicamocha River, and in this locality sampling was performed at random. On the other hand, in Cepitá, the distribution of *L. origanoides* is restricted to the east side of the Chicamocha River, and at this locality sampling was only possible along two small transects separated by farms and cultivated land. Sixty-two and 39 samples were collected from Pescadero and Cepitá, respectively. Additionally, 10 individuals were sampled between these two localities along the east side of the Chicamocha River, which allowed the examination of larger distance classes. For each individual collected, complete passport data were recorded using a GPS

(Geographical Positional System), GARMIN (USA).

From each individual, 20-30 young leaves were collected and preserved in zip-lock plastic bags containing silica gel and stored at -20ºC until DNA extraction. Herbarium samples were sent to the Colombian National Herbarium (COL) for taxonomic determination under the accession number 522847.

2.2. DNA extraction and PCR amplification

Genomic DNA was isolated from young leaves. Leaf samples were finely ground in liquid nitrogen (-80ºC) and DNA extraction was made using a modification of the CTAB method reported by Pirttilä *et al* (2001). The resulting DNA solution was stored at -20ºC in TE buffer for later use.

A set of 50 ISSR primers (obtained from the University of British Columbia in Vancouver, Canada) was screened for amplification success and polymorphisms in a representative sample of *L. origanoides*. PCR reactions were carried out in a total volume of 15 µl containing 1 unit of *Taq* polymerase (Promega), 1X PCR Buffer (Promega), 0.2mM dNTPs, 0.5µM of the ISSR primer and 10-20 ng of extracted DNA. Amplifications were performed on a PTC-100TM thermocycler (MJ Reserch, Inc) following Wolfe (2005) cycling conditions. PCR products were loaded onto 2% agarose gels in 0.5X TBE buffer, run at constant voltage (50 V) for 3 h and detected by staining with ethidium bromide. Each ISSR band was

considered as an independent character or locus, and polymorphic bands were scored visually as either absent (0) or present (1). Qualitative differences in band intensity were not considered (Assefa *et al*., 2003). Two independent scorings were made on each gel, and only those bands consistently scored were considered for analysis. Also, replicate samples were analyzed for each locus and only reproducible bands were considered for analysis.

2.3. Data analysis

2.3.1. Genetic Diversity

Genetic diversity was described as the percentage of polymorphic loci (*P*), and quantified as Shannon's diversity index (*I*) (Shannon and Weaver**,** 1949) using POPGENE v. 1.31 (Yeh *et al*., 1995), which assumes that populations are in HWE. Also, a Bayesian approach, which does not assume HWE (Zhivotovsky, 1999; Holsinger *et al*., 2002), was used to estimate the average panmictic heterozygosity (H_B) using the HICKORY software (Holsinger and Lewis, 2003). This software uses Markov's chain Monte Carlo simulations to produce posterior distributions based on the data (Holsinger *et al*., 2002).

2.3.2. Genetic Structure

Cluster and autocorrelation analyses were applied for measuring the structure of genetic diversity within the population of *L. origanoides* from the Chicamocha Canyon. In all cases, multi-locus analyses, instead of the classical analysis of one allele at a time, were performed, thus avoiding the stochastic noise (allele to allele and locus to locus) (Smouse and Peakall, 1999). To determine how individuals from the two localities clustered together, a Principal Coordinate Analysis (PCO) was carried out, using a squared Euclidean distance matrix from which principal coordinates were extracted. In addition, the Nei and Li (1979) similarity coefficient was used to measure inter-individual genetic differentiation. This similarity matrix was used to construct a dendrogram with the Unweighted Pairs Group Method using Arithmetic Averages (UPGMA) algorithm. The PCO and Cluster analyses were carried out using MVSP 3.1 (Kovach, 1998).

The correlation between a genetic Euclidean distance matrix and a geographic distance matrix was tested using Mantel tests implemented in GENALEX version 6.1 (Peakall and Smouse, 2006). Since Mantel tests are apparently insufficiently powerful to detect fine-scale structure because they are only sensitive to linear relationships of spatial autocorrelation (Heywood, 1991), additional spatial autocorrelation analyses were done under the Moran's *I* and the Normalized Mantel *rm* approaches. The Moran's *I*

coefficient is comparable to the Pearson correlation coefficient and its purpose is to quantify the correlation of the values of a single variable (in this case the first extracted axis of a PCO) between pairs of individuals located at a given physical distance (Fortin *et al.*, 2002). On the other hand, the Normalized Mantel *rm*, which is based on a Mantel statistic, estimates the linear relationship between two sets of variables taken at the same sampling locations (Escudero *et al.*, 2003). Normalized Mantel *rm* coefficients were calculated using the pairwise Euclidean genetic distances extracted from GENALEX version 6.1 (Peakall and Smouse, 2006).

It has been suggested that the sampling design can affect the estimation of the spatial genetic structure (Vekemans and Hardy, 2004). Vekemans and Hardy (2004) proposed that sampling nearby individuals at different locations, and then organizing them into subgroups could be an alternative for characterizing fine genetic structure when there is not a homogeneous sampling. For this reason, the autocorrelation analyses were performed not only for the entire region (all 111 individuals) but also separately for each of the two localities (Pescadero and Cepitá). Moran's *I* and Normalized Mantel *rm* statistics usually range from -1 to 1, where significant negative values indicate negative spatial autocorrelation (pairs of individuals in the distance class have dissimilar allele frequencies), whereas significant positive values indicate positive spatial autocorrelation (pairs of individuals in the distance class have similar allele frequencies). Every possible pair of individuals was considered as a join and assigned to one of 10 distance

classes (according to the Euclidean distance separating the pair) on Pescadero and Cepitá; and 20 distance classes on the entire region. The distance classes were based on arbitrarily chosen intervals of equal size to which the samples were assigned. Several intervals were tested, ranging from 20 to 200 m and maximum distance classes of 2.5 km and 1.4 km were used for the entire region and Pescadero, respectively. For Cepitá, tested intervals ranged from 40 m to 80 m, with a maximum distance class of 590 m. These distance classes accounted for the smallest and largest spatial resolution possible for our dataset. All distance class intervals analyzed were chosen to allow for at least 30 pairs of data in each class. The analyses were done following the classical rule of only considering pairs of points separated by less than half the maximum distance observed among all pairs (Wagner *et al*., 2005). Moran's *I* and Normalized Mantel *rm* coefficients were calculated using the PASSAGE software (Rosenberg, 2001). For these two approaches, the significance of individual coefficients was determined from their moments (Sokal and Oden, 1978), and since autocorrelation coefficients among the distance classes are not independent from each other, significance for the entire correlograms was calculated using a Bonferroni criterion (Oden, 1984).

3. RESULTS

3.1. Genetic Diversity

A total of 58 scorable bands, ranging from 300 bp to more than 1000 bp, were generated using five ISSR primers containing CT or TC repeat units in 111 individuals. The five primers used are the following: #813 (CTC TCT CTC TCT CTC TT), #814 (CTC TCT CTC TCT CTC TA), #824 (TCT CTC TCT CTC TCT CG), #843 (CTC TCT CTC TCT CTC TRA) and #845 (CTC TCT CTC TCT CTC TRG). Of these bands, 50 were polymorphic, accounting for 86.21% of ISSR polymorphism. Genetic diversity indices calculated either assuming HWE (*I*) or with the Bayesian approach (H_B) did not differ significantly (0.453± 0.009 and 0.484± 0.227, respectively).

3.2. Genetic Structure

Results of the PCO analysis are shown in Figure 2. The high overlap of individuals shows a pattern of very low differentiation between the two localities. In addition, the dendrogram constructed with UPGMA using the similarity matrix based on the Nei and Li coefficient did not suggest the presence of discrete geographic groups among individuals (data not shown). The average genetic similarity as measured by the Nei and Li coefficient (1979) was 0.84 and ranged from 0.987 to 0.672. Pairs of genetically

distant individuals (Nei and Li coefficient close to 0.672) were found within the two localities (inside Pescadero and inside Cepitá) and also between the two localities. Supporting this finding of relatively low level of differentiation among individuals from the two localities, the Mantel test showed a weak but significant correlation among genetic and geographic distance ($r=0.074$, $P<0.05$).

Fine scale autocorrelation analyses were carried out for all 111 individuals from the entire region as well as separately for Pescadero and Cepitá. For the entire region, significant autocorrelations were observed with Moran's *I* and Normalized Mantel *rm* autocorrelograms in all distance classes analyzed. The autocorrelograms for distance class intervals smaller than 150 m showed positive spatial autocorrelations (data not shown) and negative correlations were only observed when larger intervals were considered (i.e. 150 m and 200 m). The correlograms for 150 m intervals are shown in Figure 3. The Moran's *I* autocorrelogram (Figure 3a) shows significantly positive values at the first interval (0-150 m) and at the intervals 750–900 m, 900–1050 m and 1050–1200 m, indicating high genetic similarity shared among individuals within these distances. At intervals from 150 m to 750 m values were not significant. Figure 3a also shows significantly negative values at distances between 1200 and 1350 m, indicating that individuals separated for more than 1.2 km are likely to be genetically more differentiated than expected from a spatial random distribution of genotypes. Similar results were obtained for the correlogram

based on the Normalized Mantel *rm* coefficient (Figure 3b).

Autocorrelation analyses were also performed separately for the localities of Pescadero and Cepitá using distance class intervals of 150 m and 60 m, respectively. For Pescadero, Moran's *I* autocorrelogram showed significant positive autocorrelation at the first interval (0-150 m) and significant negative autocorrelation at the last interval of 1200-1350 m (Figure 4a). This result was similar for the Normalized Mantel *rm* autocorrelogram (Figure 4b). In Cepitá, a similar correlation pattern to that observed for the entire region and for Pescadero was found, but at a smaller scale. The Moran's *I* autocorrelogram exhibited positive significant correlations at distance intervals ranging from 0 to 120 m, while at the last distance interval of 420–490 m significantly negative correlations were observed (Figure 5a). Similar results were obtained for the Normalized Mantel *rm* autocorrelogram (Figure 5b).

4. DISCUSSION

4.1. Genetic diversity

L. origanoides from the Chicamocha Canyon exhibited high levels of genetic variation indicated by a high *P* (86.21%) and relatively high *I* and H_B (0.453 and 0.484, respectively). This level of genetic variation for *L. origanoides* is higher in comparison to previous reports from widely distributed species (Hamrick, 1979; Loveless and Hamrick, 1984; Gitzendanner and Soltis, 2000; Nybom, 2004; Wallace 2004). However, for a more meaningful comparison of genetic diversity, data from close relatives (e.g. congeners) should be analyzed (Gitzendanner and Soltis, 2000). As far as we know, there have been no reports of genetic diversity in *Lippia* species, except for an unpublished study of *L. alba* and *Aloysia triphylla* (a species related to the genus *Lippia*) using ISSR markers (Martinez *et al.* personal communication). In that study, *L. alba* showed high diversity values as measured by *P* (85.23%), *I* (0.418) and H_B (0.436), and *A. triphylla* also showed a similar pattern for the same diversity indexes: *P* (95.45%), *I* (0.436) and H_B (0.461). Genetic diversity levels of these two widely distributed Verbenaceae species are similar or slightly higher than those obtained for *L. origanoides*. Other reports from widely distributed Verbenaceae species, which are not so closely related to *L. origanoides*, show comparatively similar or lower levels of genetic diversity. For

example, reports from *Vitex negundo* L. in China, have shown *P* ranging from 81% to 85% in some populations analyzed (Su et al 2003, Zhang *et al* 2007). *Vitex rotundifolia* L., an aromatic and medicinal plant from China, has shown moderate *P* (22.6%) and *I* (0.275) (Hu *et al* in press).

ISSR polymorphisms used in this study are presumably mostly from nuclear DNA. In contrast, a previous analysis of DNA polymorphisms in four non-coding chloroplast DNA regions showed no genetic diversity among a set of *L. origanoides* individuals from the Chicamocha Canyon (Suarez *et al*., 2007). These apparently contrasting results may be explained in part by a higher mutation rate of microsatellite regions from the nuclear genome compared to the chloroplast genome (Morgante and Olivieri, 1993), and by the different modes of transmission of the cpDNA (usually maternally) and ISSR (usually biparentally) (Birky 1995). The levels of genetic variation within populations may not only be influenced by the mating system of the species but also by its effective population size (Culley and Wolfe, 2001; Muir *et al*., 2004; Mable and Adam, 2007; Wright, 1946), which is expected to be smaller for uniparentally than for biparentally inherited markers. The dominant nature of the ISSR markers used in this study does not allow us to draw conclusions about the mating system in this species, therefore, this is an aspect that deserves further investigation.

4.2. Genetic structure

In this study, different approaches were used to estimate the level of genetic differentiation among individuals from two localities in the Chicamocha Canyon (Pescadero and Cepitá) and also from individuals within localities. Results from the PCO and Cluster analyses showed very low genetic differentiation among localities, which could lead to suggest that neither the river nor anthropogenic intervention are acting like barriers to gene flow.

However, the spatial autocorrelation analyses using the Moran's *I* and Normalized Mantel *rm* coefficients indicate that the distribution of genetic diversity is not random among and within localities. The shape of the correlograms revealed significant fine-scale genetic structure within the Chicamocha Canyon. The Moran's *I* correlogram corresponding to the entire region (Figure 3) showed what seems to be a sinusoidal variation in the first nine distance classes. This pattern may indicate a patchy distribution of the variation (Radeloff et al., 2000). However, at larger distances only significantly negative or no significant values were displayed. In general, there was a strong positive spatial association between individuals distributed within 450-1200 m from each other (120 m for Cepitá), while individuals distributed at a distance larger than 1200 m from each other (490 m for Cepitá) showed significant negative autocorrelation (Figures 3 to 5).

These spatial patterns of genetic relatedness are consistent with the classical model of isolation by distance, where there is a decline of genetic similarity with increasing geographical distance (Wright, 1946). Among the different evolutionary and ecological processes that may lead to the establishment of this spatial genetic structuring, the most widely studied is limited gene dispersal. Additionally, it has been suggested that limited gene dispersal is probably the most prevalent cause for spatial structure at a fine spatial scale (Epperson, 2000; Vekemans and Hardy, 2004). In plants, dispersal function is mediated by pollen and seeds (Petit *et al*., 2005). A variety of empirical data indicate that in several cases seeds show a localized dispersal rate resulting in patches of plants, while pollen may show a long-distance dispersal occurring at a higher frequency, which is enough to counteract the effects of genetic drift in small populations (Wright 1946; McCauley, 1997; Ouborg *et al*., 1999; Kalisz *et al*., 2001; Chung *et al*., 2004; Chung *et al*., 2006). ISSR polymorphisms are mostly located in the nuclear genome, which in most angiosperms shows a biparental inheritance, thus reflecting the combined effect of the relative movement of seeds and pollen. In *L. origanoides*, like in other plants, the presence of odoriferous compounds are likely the cause of attraction of pollinators (Levin *et al*., 2001; Albre *et al*., 2003), which could serve as vehicles for pollen dispersal in relatively long distances. When seed dispersal is random within a population, spatial genetic structure will not develop (Kalisz *et al*., 2001; Chung *et al*., 2003). The moderate but significant levels of local spatial structure in *L.*

origanoides, revealed by the spatial autocorrelation analyses, could be due in part to relatively restricted seed dispersal capabilities as it has been reported for different plant populations (e.g. He *et al*., 2000; Miyazaki and Isagi, 2000; Kalisz *et al*., 2001; Hardy *et al*., 2004; Hardesty *et al*., 2005; Chung *et al*., 2006). Also it could be the result of relatively short-distance pollen movement, as it has been reported for some shrubs and herbs (Richards *et al*., 1999; Marr *et al*., 2000; Barthelmess *et al*., 2006; Byrne *et al*., 2007). As far as we know, there are no reports on the seed and pollen dispersal mechanisms in this species. For this reason, it is not possible to make a precise estimation of the cause (or causes) for the non-random distribution of genotypes in *L. origanoides* from the Chicamocha canyon. Additional studies, including those that make use of different types of genetic markers (e.g. markers from cpDNA), will permit to evaluate the relative movement of seeds and pollen (Trapnell & Hamrick 2004, Petit *et. al*. 2005) and their influence on fine scale population structure in this species.

The results obtained in this study are comparable to those obtained for tree species where distance classes of hundreds of meters were analyzed (Doligez and Joly, 1997; Degen *et al*., 2001) and where fine genetic structure at distance classes of hundreds of meters was found (Latouche-Halle *et al*., 2003; Hardesty *et al*., 2005). Numerous analyses in shrubs have found spatial genetic structure at finer scales, from 1 to 10 meters (Loiselle *et al*., 1995; England *et al*., 2003; Chung *et al*., 2006; Tang *et al*., 2007).

Future analyses may include a sampling strategy where shorter distances are considered.

It must be taken into account that autocorrelation analyses are greatly affected by the sampling strategies used (Vekemans and Hardy, 2004). For example, the precision of the autocorrelation estimation increases with the increasing number of samples belonging to the distance class analyzed. In the three autocorrelation analyses performed (Figures 3-5), a relatively large number of pairs of data separated by short distances were assigned to each distance class analyzed, therefore, it is unlikely that the significant genetic structures detected in our analyses within proximate distances are artifacts of the sampling scheme.

4.3. Implications for conservation and breeding

The present results have important implications for conservation and breeding purposes. *L. origanoides* is a species with high quality essential oils that are used mostly in cosmetics and perfumery and also have significant antimicrobial activity (Santos et al., 2004, Oliveira *et al*., 2007). Additionally, Oliveira *et al*. (2007) have suggested that *L. origanoides* can be considered as promising for future utilization as a food ingredient since it shares the chemical composition of essential oils with other cultivated species. The wide distribution and the relative high genetic diversity estimated in this study leads us to conclude that *L. origanoides* is not yet an

endangered species. However, appropriate conservation and breeding strategies should be implemented if a massive oil production is planned. Previous studies on promising species from Africa and China have reported how species with wide distributions have become vulnerable by the over-harvesting of natural stands (Muchugi *et al*., 2006; Tang *et al*., 2007). Currently, anthropogenic interventions are taking place in regions were *L. origanoides* exhibits a natural wide distribution (Albesiano *et al*., 2003; Malagon *et al*., 1995; Serrato, 2007). Habitat fragmentation caused by deforestation and transitory cultivation could lead to significant concerns regarding the conservation of this species. The estimation of the average size of genetic patches can be helpful in designing collection strategies for proper propagation and breeding purposes. Usually, the first x-intercept in the correlogram has been used as an estimate of the diameter of the patch (Peakall et al., 2003). However, this interpretation might be problematic since this distance is not a characteristic of the populations studied and depends strongly on the sampling scheme (Vekemans and Hardy, 2004). For these reasons, this study does not allow us to precise the patch size appropriate for an accurate collection, but it does allow us to suggest that sampling individuals at distances larger than 1.4 km could result in the collection of different genotypes, which could help preserve the levels of genetic diversity in a propagation program. Additional research on the relationship between the essential oil composition and the genotypic variation will contribute to future breeding initiatives, since different

chemotypes have been identified within *L. origanoides* (Oliveira *et al*., 2007).

ACKNOWLEDGEMENTS

We are grateful to COLCIENCIAS, Colombia, and to the Centro Nacional de Investigaciones para la Agroindustrialización de Especies Vegetales Aromáticas y Medicinales Tropicales- CENIVAM, Colombia, for providing funding to carry out this research. We are also grateful to the Dirección de Investigaciones, Universidad Nacional de Colombia, Bogotá, Colombia, for their financial support for this research. We thank the Fundación Alejandro Angel Escobar, Colombia, for providing a scholarship to Adriana Suárez to perform this study. We are especially grateful to Professor Jorge Hernández Torres for his technical and logistic assistance and to Lucio Navarro E. for his technical support. We are also especially grateful to Félix Martínez for his assistance in the development of this research and to Stefanie Hartmann for revising the manuscript and her valuable comments.

5. FIGURES

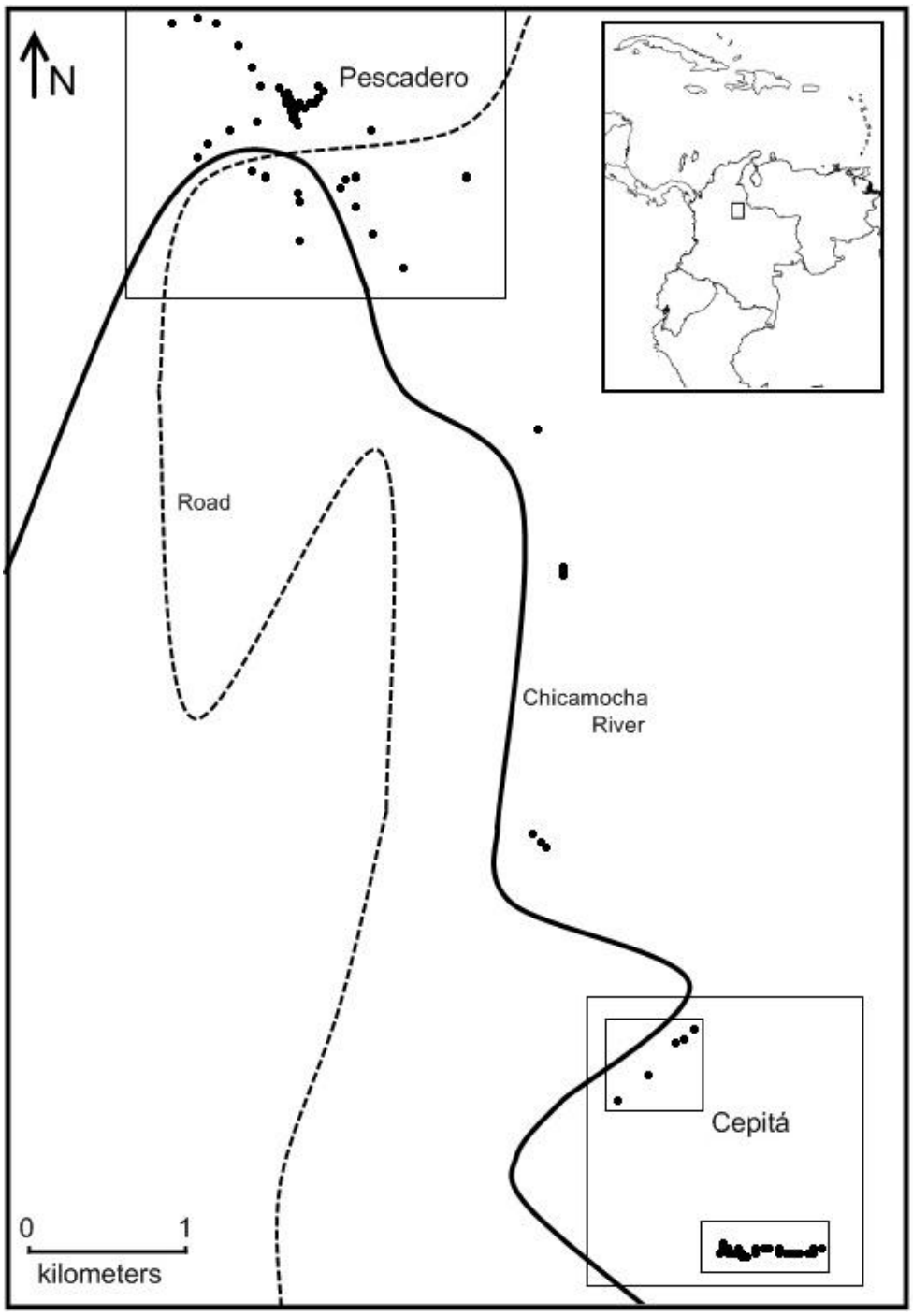

Fig. 1. Map of the distribution of the *L. origanoides* individuals sampled from the Chicamocha Canyon.

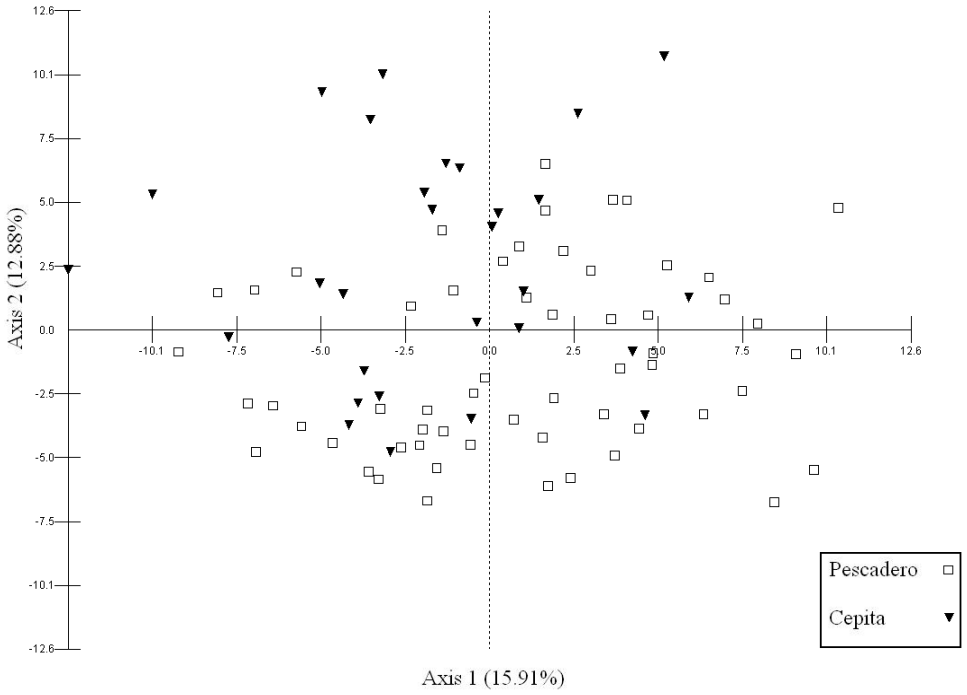

Fig. 2. Principal Coordinate analysis (PCO) using squared euclidean genetic distance. The first two coordinates explain 28.8% of the total variability.

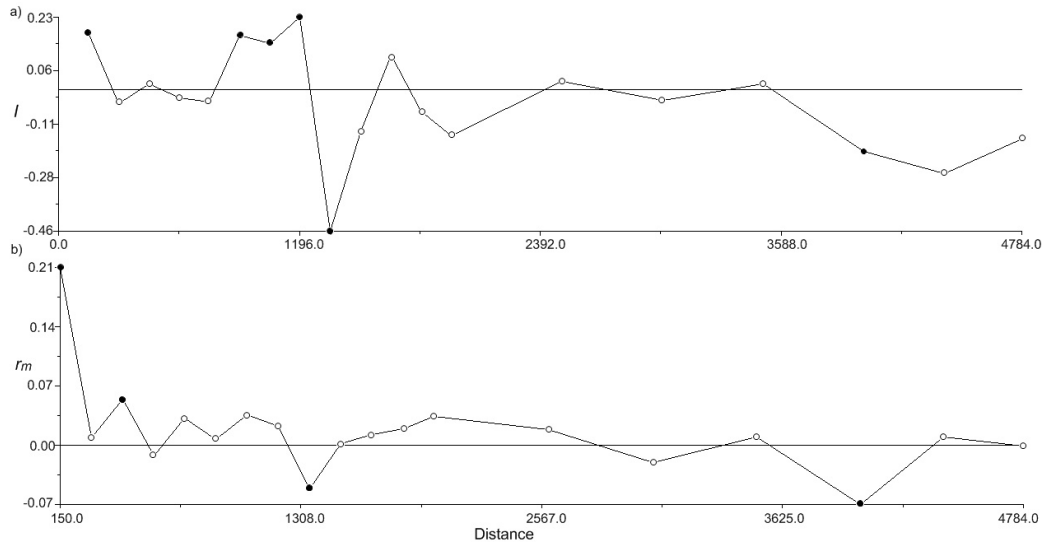

Fig. 3. Spatial genetic structure in the entire region. a) Moran's *I* correlogram. b) Mantel correlogram. Distance classes were defined at 150 m intervals. Black dots represent significant ($p<0.05$) Moran's *I* and Normalized Mantel *rm* values.

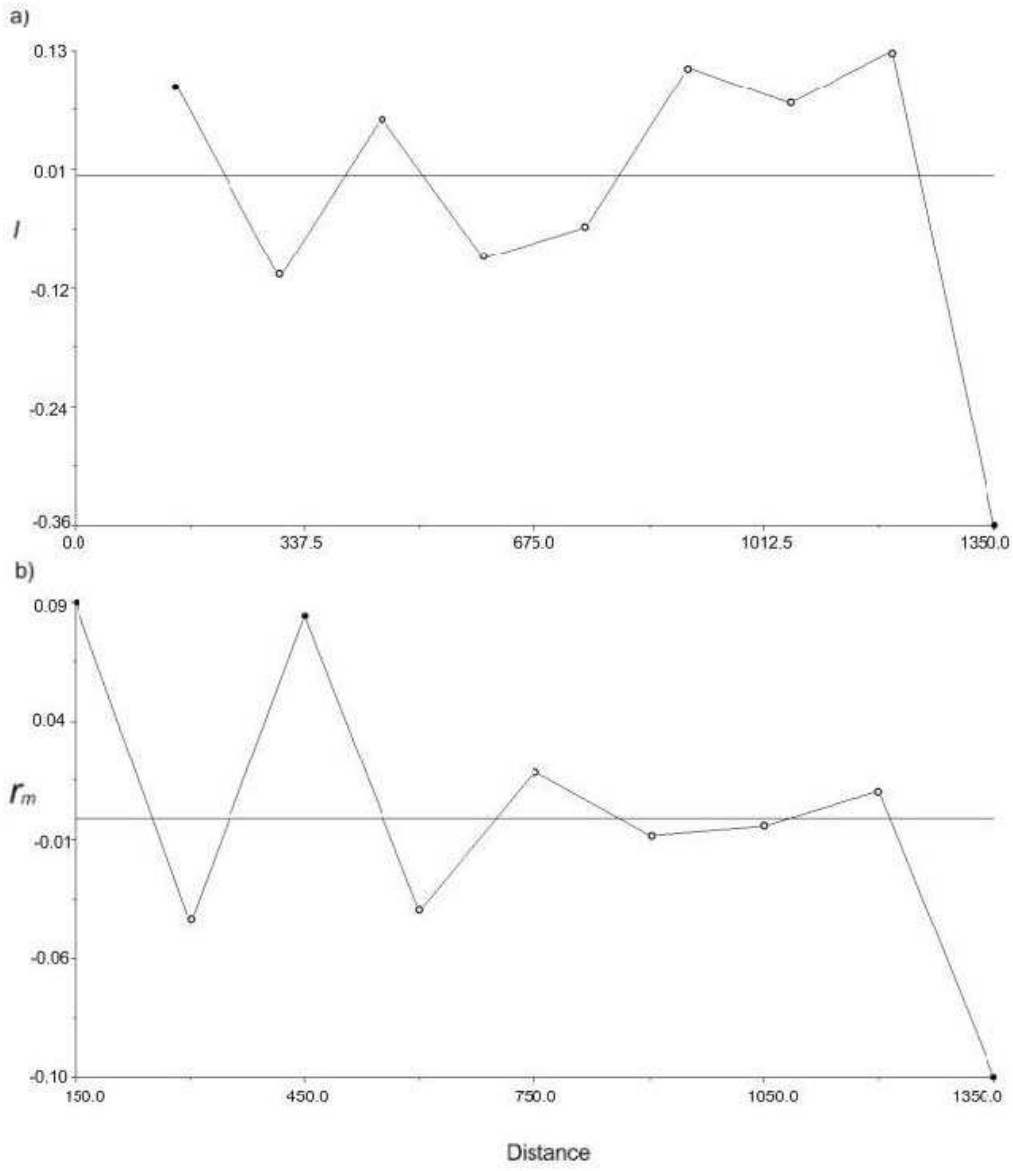

Fig. 4. Spatial genetic structure in Pescadero. a) Moran's *I* correlogram. b) Mantel correlogram. Distance classes were defined at 150 m intervals. Black dots represent significant ($p<0.05$) Moran's *I* and Normalized Mantel *rm* values.

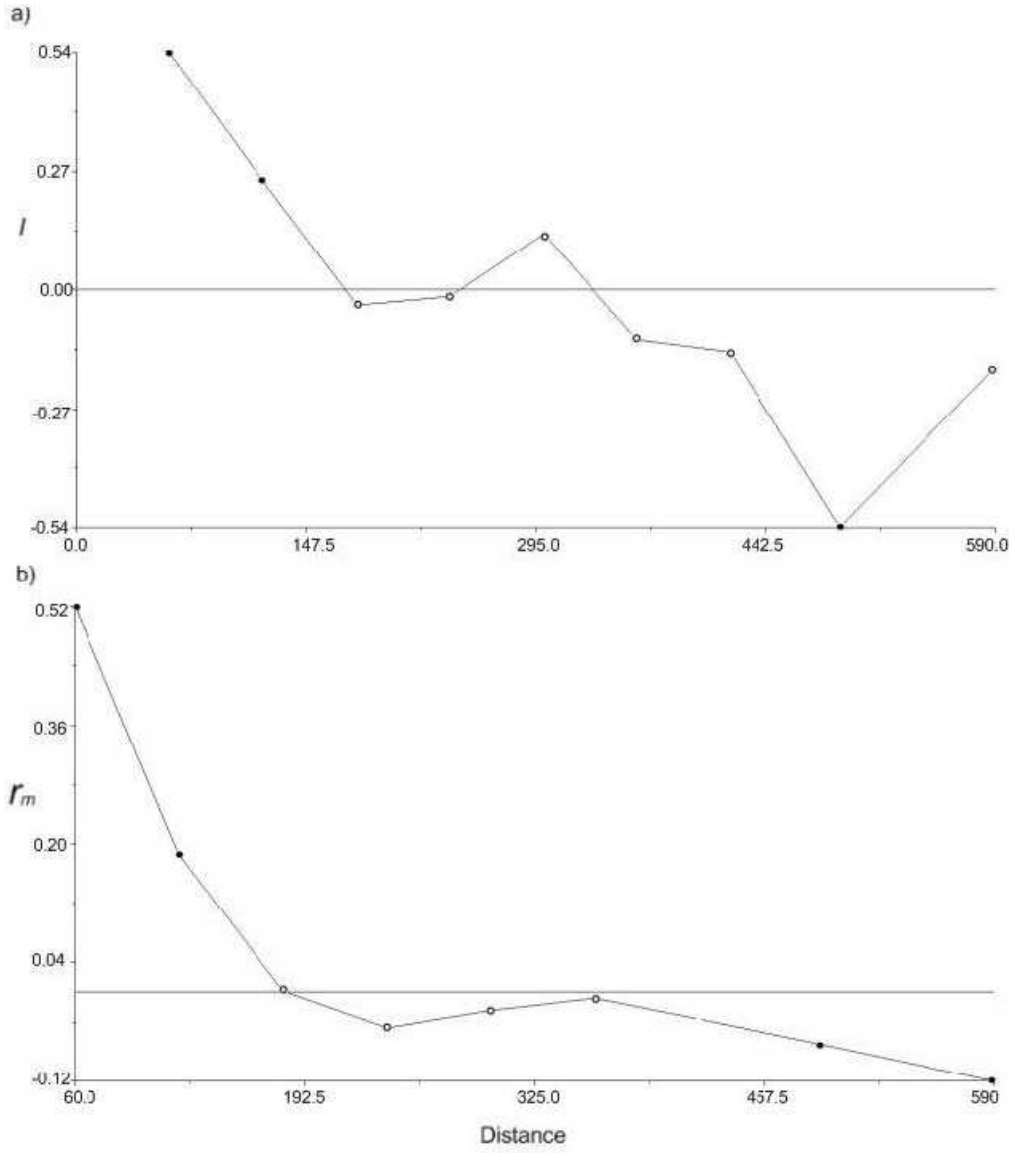

Fig. 5. Spatial genetic structure in Cepitá. a) Moran's *I* correlogram. b) Mantel correlogram. Distance classes were defined at 60 m intervals. Black dots represent significant ($p<0.05$) Moran's *I* and Normalized Mantel *rm* values.

6. LITERATURE CITED

Albesiano S, Rangel JO, Cadena A. 2003. La Vegetación del Cañon del Rio Chicamocha (Santander, Colombia). *Caldasia* **25:** 73-99.

Albre J, Quilichini A, Gibernau M. 2003. Pollination ecology of *Arum italicum* (Araceae). *Botanical Journal of the Linnean Society* **141:** 205-214.

Assefa K, Merker A, Tefera H. 2003. Inter simple sequence repeat (ISSR) analysis of genetic diversity in tef [*Eragrostis tef* (Zucc.) Trotter]. *Hereditas* **139:** 174–183.

Barthelmess EL, Richards CM, McCauley DE. 2006. Relative effects of nocturnal vs diurnal pollinators and distance on gene flow in small *Silene alba* populations. *New Phytologis* **169:** 689–698.

Berg EE, Hamrick JL. 1995. Fine-scale genetic structure of a Turkey oak forest. *Evolution* **49:** 110-120.

Birky W. 1995. Uniparental inheritance of mitochondrial and chloroplast genes: Mechanisms and evolution. *Proccedings of the National Academy of Sciences of the Unites States of America* **92:** 11331-11338

Bornet B, Branchard M. 2004. Brief report: Use of ISSR fingerprints to detect microsatellites and genetic diversity in several related *Brassica* taxa and *Arabidopsis thaliana*. *Hereditas* **140:** 245-248.

Byrne M, Elliott CP, Yates C, Coates DJ. 2007. Extensive pollen dispersal in a bird-pollinated shrub, *Calothamnus quadrifidus*, in a fragmented landscape. *Molecular Ecology* **16:** 1303–1314.

Camacho FJ, Liston A. 2001. Population structure and genetic diversity of *Botrychium Pumicola* (Ophioglossaceae) based on Inter-Simple Sequence Repeats (ISSR). American *Journal of Botany* **88:** 1065–1070.

Chung MY, Nason JD, Epperson BK, Chung MG. 2003. Temporal aspects of the fine-scale genetic structure in a population of *Cinnamomum insularimontanum* (Lauraceae). *Heredity* **90:** 98–106.

Chung MY, Nason JD, Chung MG. 2004. Spatial genetic structure in populations of the terrestrial orchid *Cephalanthera longibracteata* (Orchidaceae). *American Journal of Botany* **9:** 52-57.

Chung JM, Lee BC, Kim JS, Park C, Chung MY, Chung MG. 2006.

Fine-scale genetic structure among genetic individuals of the clone-forming monotypic genus *Echinosophora koreensis* (Fabaceae). *Annals of Botany* **98:** 165–173.

Culley TM, Wolfe AD. 2001. Population genetic structure of the cleistogamous plant species *Viola pubescens* Aiton (Violaceae), as indicated by allozyme and ISSR molecular markers. *Heredity* **86:** 545-556.

Degen B, Caron H, Bandou E , Maggia L, Chevallier MH, Leveau A, Kremer A. 2001. Fine-scale spatial genetic structure of eight tropical tree species as analysed by RAPDs. *Heredity* **87***:* 497-507.

Doligez A, Joly H. 1997. Genetic diversity and spatial structure within a natural stand of a tropical forest tree species, *Carapa procera* (Meliaceae), in French Guiana. *Heredity* **79:** 72-82.

England PR, Whelan RJ, Ayre DJ. 2003. Effects of seed bank disturbance on the fine-scale genetic structure of populations of the rare shrub *Grevillea macleayana*. *Heredity* **91:** 475– 480.

Epperson BK, Li T. 1996. Measurement of genetic structure within populations using Moran's spatial autocorrelation statistics. *Proceedings of the National Academy of Sciences* **93:** 10528–10532.

Epperson BK. 2000. Spatial genetic structure and non-equilibrium demographics within plant populations. *Plant Species Biology* **15**: 269-279.

Epperson BK, Chung MG. 2001. Spatial genetic structure of allozyme polymorphisms within populations of *Pinus strobus* (Pinaceae). *American Journal of Botany* **88:** 1006–1010.

Escudero A, Iriondo JM, Torres ME. **2003.** Spatial analysis of genetic diversity as a tool for plant conservation. *Biological Conservation* **113**: 351-365.

Excoffier L, Smouse PE, Quattro JM. 1992. Analysis of molecular variance inferred from metric distances among dna haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* **131**: 479-491

Fortin MJ, Dale MRT, ver Hoef J. 2002. Spatial analysis in ecology. In: El-Shaarawi A.H., Piegorsch W.W. (Eds.), Encyclopedia of Environmetrics, John Wiley and Sons Ltd, Chichester. 2051–2058

Gehring JL, Delph LF. 1999. Fine-scale genetic structure and clinal variation in *Silene acaulis* despite high gene flow. *Heredity* **82:** 628-637.

Gitzendanner MA, Soltis PS. 2000. Patterns of genetic variation in rare and widespread plant congeners. *American Journal of Botany* **87**: 783–792.

Giuseppe A. 2003. *Ficha de reconocimiento de Lippia origanoides*. Centro Jardín Botánico de Mérida. Facultad de Ciencias. Universidad de los Andes.

Hamrick JL. 1979. Relationships between life history characteristics and electrophoretically detectable genetic variation in plants. *Annual Review of Ecology and Systematics* **10:** 173-200.

Hamrick JL, Murawski DA, Nason JD. 1993. The influence of seed dispersal mechanisms on the genetic structure of tropical tree populations. *Vegetatio* **107/108**: 281-297

Hardesty BD, Dick CW, Kremer A , Hubbell S, Bermingham E. 2005. Spatial genetic structure of *Simarouba amara* Aubl (Simaroubaceae), a dioecious, animal-dispersed Neotropical tree, on Barro Colorado Island, Panama. *Heredity* **95:** 290-297.

Hardy OJ, Gonzalez SC, Freville H, Boqueen G, Mignot A, Colas B, Olivieri I. 2004. Fine-scale genetic structure and gene dispersal in *Centaurea corymbosa* (Asteraceae) I. Pattern of pollen dispersal. *Journal of Evolutionary Biology* **17:** 795–806.

Hardy OJ, Magia L, Bandou E, Breyne P, Caron P, Chevallier MH, Doligez A, Dutech C, Kremer A, Latouche-Hallé C, Troispoux V, Veron V, Degen B. 2005. Fine-scale genetic structure and gene dispersal inferences in 10 Neotropical tree species. *Molecular Ecology* **15:** 559-71.

He T, Rao G, You R, Ge S, Hong D. 2000. Spatial autocorrelation of genetic variation in three stands of *Ophiopogon xylorrhizus* (Liliaceae s.l.). *Annals of Botany* **86:** 113–121.

Heywood JS. 1991. Spatial Analysis of Genetic Variation in Plant Populations. *Annual Review of Ecology and Systematics* **22:** 335-55.

Holsinger KE, Lewis PO, Dey DK. 2002. A Bayesian approach to inferring population structure from dominant markers. *Molecular Ecology* **11:** 1157–1164.

Holsinger KE, Lewis PO. 2003. HICKORY: a package for analysis of population genetic data V1.0. http://www.eeb.uconn.edu.

Hu Y, Zhu Y, Zhang QY, Xin HL, Qin LP, Lu B, Rahman K, Zheng HC. Population Genetic Structure of the Medicinal Plant *Vitex rotundifolia* in China, the Implication for Utilization and Conservation. *Journal of*

Integrative plant Biology. In press**.**

Jones TH, Vaillancourt RE, Potts BM. 2007. Detection and visualization of spatial genetic structure in continuous *Eucalyptus globules* forest. *Molecular Ecology* **16:** 697–707.

Kalisz S, Nason JD, Hanzawa FM, Tonsor SJ. 2001. Spatial population genetic structure in *Trillium grandiflorum*: The roles of dispersal, mating, history, and selection. *Evolution* **55:** 1560–1568.

Kovach W. 1998. *MVSP* - A Multivariate Statistical Package for Windows, ver. 3.0. Kovach Computing Services, Pentraeth, Wales, U.K.

Latouche-Halle C, Ramboer A, Bandou E, Caron H, Kremer A. 2003. Nuclear and chloroplast genetic structure indicate fine-scale spatial dynamics in a neotropical Tree population. *Heredity* **91:** 181–190.

Levin S. 1992. The Problem of Pattern and Scale in Ecology. *Ecology* **73:** 1943-1967.

Levin R, Ragusob R, McDadec L. 2001. Fragrance chemistry and pollinator affinities in *Nyctaginaceae Phytochemistry* **58:** 429–440.

Linhart YB, Grant MC. 1996. Evolutionary significance of local genetic differentiation in plants. *Annual Review of Ecology and Systematics* **27:** 237–77.

Liu B, Wendel JF. 2001. Intersimple sequence repeat (ISSR) polymorphisms as a genetic marker system in cotton. *Molecular Ecology Notes* **1:** 205–208.

Llorens TM, Ayre DJ, Whelan RJ. 2004. Evidence for ancient genetic subdivision among
recently fragmented populations of the endangered shrub *Grevillea caleyi* (Proteaceae)
Heredity **92**: 519–526.

Loiselle BA, Sork VL, Nason J, Graham C. 1995. Spatial genetic structure of a tropical understory shrub, *Psychotroia officinalis* (Rubiaceae). *American Journal of Botany* **82:** 1420-142.

Lopez S. 1977. *Flora de Venezuela. Verbenaceae.* Universidad de los Andes. Consejo de Publicaciones. Facultad de Farmacia. Venezuela, 338-441.

Loveless MD, Hamrick JL. 1984. Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* **15**: 65-95.

Mable BK, Adam A. 2007. Patterns of genetic diversity in outcrossing and selfing populations of *Arabidopsis lyrata*. *Molecular Ecology* **16:** 3565–3580.

Maisch J. 1885. On some useful plants of the natural order of Verbenacea. *American Journal of Pharmacy* **57:** 189-199

Malagon D, Llinas RD, Chamorro C. 1995. Suelos de Colombia: origen, evolución, clasificación, distribución y uso. IGAC, Bogotá, D.C.

Marr DL, Leebens-Mack J, Elms L, Pellmyr O. 2000. Pollen dispersal in *Yucca filamentosa* (Agavaceae): The paradox of self-pollination behavior by *Tegeticula yuccasella* (Prodoxidae). *American Journal of Botany* **87:** 670–677.

McCauley DE. 1997. The Relative Contributions of Seed and Pollen Movement to the Local Genetic Structure of *Silene alba. Journal of Heredity* **88**: 257-263

Miyazaki Y, Isagi Y. 2000. Pollen flow and the intrapopulation genetic

structure of *Heloniopsis orientalis* on the forest floor as determined using microsatellite markers. *Theoretical and Applied Genetics* **101:** 718–723.

Morgante M, Olivieri AM. 1993. PCR-amplified microsatellites as markers in plant. *Genetics* **3:** 175–182.

Muchugi A, Lengkeek AG, Kadu CA, Muluvi GM, Njagi EN, Dawson IK. 2006. Genetic variation in the threatened medicinal tree Prunus africana in Cameroon and Kenya: Implications for current management and evolutionary history. *South African Journal of Botany* **72:** 498-506.

Muir G, Lowe AJ, Fleming CC, Vogl C. 2004. High nuclear genetic diversity, high levels of outcrossing and low differentiation among remnant populations of *Quercus petraea* at the margin of its range in Ireland. *Annals of Botany* **93:** 691-697.

Nei M, Li WH. 1979. Mathematical model for studing genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences* **76:** 5269-5273.

Nybom H. 2004. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. *Molecular Ecology* **13**: 1143–1155.

Oden NL. 1984. Assessing the significance of a spatial correlogram. *Geographical Analysis*
16: 1–16.

Oliveira DR, Leitao GG, Bizzo HR, Lopes D, Alviano DS, Alviano CS, Leitao SG. 2007. Chemical and antimicrobial analyses of essential oil of *Lippia origanoides* H.B.K. *Food Chemistry* **101**: 236–240.

Ouborg NJ, Piquot Y, Van Groenendael JM. 1999. Population genetics, molecular markers and the study of dispersal in plants. *Journal of Ecology* **87:** 551-568.

Parker KC, Hamrick JL, Parker AI, Nason JD. 2001. Fine-scale genetic structure in *Pinus clausa* (Pinaceae) populations: Effects of disturbance history. *Heredity* **87:** 99-113.

Peakall R, Ruibal M, Lindenmayer DB. 2003. Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, Rattus fuscipes. *Evolution* **57:** 1182-1195.

Peakall R, Smouse PE. 2006. *GENALEX 6*: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology*

Notes **6:** 288–295.

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

Pirttilä AM, Hirsikorpi M, Kämäräinen T, Jaakola L, Hohtola A. 2001. DNA Isolation Methods for Medicinal and Aromatic Plants. *Plant Molecular Biology Reporter* **19:** 273a–f.

Radeloff VC, Miller TF, He HS, Mladenoff DJ. 2000. Periodicity in spatial data and geostatistical models: autocorrelation between patches. *Ecography* **23:** 81–92.

Richards CM, Church S, McCauley DE. 1999. The influence of population size and isolation on gene flow by pollen in Silene alba. *Evolution* **53:** 63-73.

Rosenberg MS. 2001. *PASSAGE*. Pattern Analysis, Spatial Statistics, and Geographic Exegesis. Version 1. Department of Biology, Arizona State University, Tempe, AZ.

Santos FJB, Lopes AD, Cito GL, Oliveira EH, Lima SG. 2004.

Composition and Biological Activity of Essential Oils from *Lippia origanoides* H.B.K. *Journal of Essential Oil Research* **16:** 504–506.

Schnabel A, Nason JD, Hamrick JL. 1998. Understanding the population genetic structure of *Gleditsia triacanthos* L.: seed dispersal and variation female reproductive success. *Molecular Ecology* **7**: 819-832.

Serrato PK. 2007. Los cañones Colombianos: Una síntesis geográfica. IGAC. Bogota, D.C. 119-135.

Shannon CE, Weaver W. 1949. *The Mathematical Theory of Communication.* University of Illinois Press, Urbana.

Sica M, Gamba G, Montieri S, Gaudio L, Aceto S. 2005. ISSR markers show differentiation among Italian populations of *Asparagus acutifolius* L. BMC *Genetics* **6:** 17. Published online 2005 March 18.

Smouse PE, Peakall R. 1999. Spatial autocorrelation analysis of individual multiallele and multilocus genetic structure. *Heredity* **8:** 561-573.

Sokal RR, Oden NL. 1978. Spatial autocorrelation in biology. 2. Some biological implications and four applications of evolutionary and ecological interest. *Biological Journal of the Linnean Society* **10:** 229–249.

Sokal RR, Jacquez GM, Wooten MC. 1989. Spatial Autocorrelation Analysis of Migration and Selection. *Genetic* **121:** 845-855.

Su H, Qu LJ, He K, Zhang Z, Wang J, Chen Z, Gu H. 2003. The Great Wall of China: a physical barrier to gene flow?. *Heredity* **90:** 212–219

Suarez AR, Martinez F, Castillo GA, Chacon MI. 2007. Molecular Characterization of Aromatic Species of the Genus *Lippia* from the Colombian Neotropics. *Acta Horticulturae*
756: 129-138.

Tang S, Li Y, Geng Y, Zhang G, Wang L, Zhong Y. 2007. Clonal and spatial genetic structure in natural populations of Luohanguo (*Siraitia grosvenorii*), an economic species endemic to South China, as revealed by RAPD markers. Biochemical *Systematics and Ecology* **35:** 557-565.

Tero N, Aspi J, Siikamaki P, Jakalaniemi A. 2005. Local genetic population structure in an endangered plant species, *Silene tatarica* (Caryophyllaceae). *Heredity* **94:** 478-487.

Trapnell DW, Hamrick JL. 2004. Partitioning nuclear and chloroplast variation at multiple spatial scales in the neotropical epiphytic orchid, *Laelia rubescens. Molecular Ecology* **13:** 2655–2666.

Vekemans X, Hardy OJ. 2004. New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology* **13:** 921–935.

Wagner H, Holderegger R, Werth S, Gugerli F, Hoebee S, Scheidegger C. 2005.
Variogram analysis of the spatial genetic structure of continuous populations using multilocus microsatellite data. *Genetics* **169:** 1739–1752.

Wallace LE. 2002. Examining the effects of fragmentation on genetic variation in
Platanthera leucophaea (Orchidaceae): Inferences from allozyme and random amplified polymorphic DNA markers. *Plant Species Biology* **17**: 37–49.

Wallace LE. 2004. A comparison of genetic variation and structure in the allopolyploid *Platanthera huronensis* and its diploid progenitors, *Platanthera aquilonis* and *Platanthera dilatata* (Orchidaceae). *Canadian Journal of Botany* **82**: 244-252

Wolfe AD. 2005. ISSR Techniques for Evolutionary Biology. *Methods in Enzymology* **395:** 134–144.

Wright S. 1943. Isolation by distance. *Genetics* **38**: 114-138

Wright S. 1946. Isolation by distance under diverse systems of mating. *Genetic* **31:** 39-59

Yeh F, Yang RC, Boyle T. 1995. *POPGEN*, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Center, Univesity of Alberta, Edmonton.

Zhang ZY, Zheng XM, Ge S. 2007. Population genetic structure of *Vitex negundo* (Verbenaceae) in Three-Gorge Area of the Yangtze River: The riverine barrier to seed dispersal in plants. *Biochemical Systematics and Ecology* **35:** 506-516.

Zhivotovsky LA. 1999. Estimating population structure in diploids with multilocus dominant DNA markers. *Molecular Ecology* **8:** 907-913.

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