Genetic diversity and spatial genetic structure within a population of an aromatic shrub, *Lippia origanoides* (Verbenaceae), in the Chicamocha Canyon, northeastern Colombia

**ADRIANA SUÁREZ G.**, **GENIS CASTILLO** and **MARIA I. CHACÓN S.**

*1 Laboratorio de Biología Molecular, Escuela de Biología, Universidad Industrial de Santander, Km 2 vía Refugio, Piedecuesta, Colombia
2 Centro Nacional de Investigações para la Agroindustrializacióe de Espécies Vegetales Aromáticas y Medicinales Tropicais (CENIVAM), Universidad Industrial de Santander, Carrera 27, Calle 9, Bucaramanga, Colombia
3 Facultad de Agronomía, Universidad Nacional de Colombia, Bogotá, Colombia*

(Received 30 May 2008 and in revised form 17 October 2008)

**Summary**

The geographical scale of genetic structure in a continuous population is highly dependent on its breeding system and dispersion capabilities, and this knowledge is important for the study of population dynamics as well as for conservation purposes. In the present study, spatial autocorrelation statistics and intersimple sequence repeat (ISSR) markers were used to describe the genetic structure of a natural population of a prominent aromatic plant, *Lippia origanoides*, native to the Chicamocha Canyon in northeastern Colombia. For this purpose, individuals were sampled from two localities within the Chicamocha Canyon, where the species is abundant and continuously distributed. Cluster (principal coordinate analysis (PCO) and unweighted pair group method using arithmetic averages (UPGMA)), analysis of molecular variance (AMOVA) and Bayesian analyses revealed a low level of genetic differentiation among the two localities, suggesting that they belong to a single population. Genetic diversity levels in this population, described as the percentage of polymorphic loci ($P = 86.21\%$) and quantified using Shannon’s diversity index ($I = 0.453$) and the average panmictic heterozygosity ($H_B = 0.484$), were shown to be comparable to or higher than that in other plant species with allogamous breeding systems and to other related Verbenaceae species. Fine-scale autocorrelation analyses showed a pattern consistent with the classical model of isolation by distance with moderate but significant levels of local spatial structure. Our results suggest that sampling individuals at distances greater than ~1.2 km may result in the collection of different genotypes, which could help preserve the levels of genetic diversity in a propagation programme. The causes of this spatial 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.

**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) and the capacity of the species for gene dispersal (i.e. seed and pollen dispersal mechanisms) (Epperson, 2000; Kalisz *et al.*, 2001; Hardy *et al.*, 2004). The patchy spatial distribution of genotypes can occur at different scales, e.g. 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 & Hardy, 2004). In sexually reproducing plants, the dispersal function is mediated by the male gametophyte (pollen) and the young sporophyte (seed), and other propagules may also be involved in vegetatively reproducing plants (Ward, 2006). Frequently, pollen and seeds show moderate to strong spatial restriction in their dispersal (Vekemans

https://doi.org/10.1017/S0016672308009841 Published online by Cambridge University Press
& Hardy, 2004), therefore generating processes of fine-scale population genetic structure (Gehring & Delph, 1999; Hardy et al., 2004; Tero et al., 2005). These processes are mainly a result of isolation by distance, namely, the significantly positive correlation between genetic relatedness among individuals and their spatial proximity (Wright, 1946; Hardy et al., 2005).

Currently, spatial autocorrelation analyses are used to detect and interpret spatial patterns of genetic variation within plant populations (He et al., 2000; 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).

The species under study, Lippia origanoides, Kunth, Verbenaceae, is a shrub that can reach up to 3-5 m in 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 antioxidant activity and antimicrobial activity against Escherichia coli, Staphylococcus aureus, Candida albicans and Candida tropicalis (Santos et al., 2004; Oliveira et al., 2007; Stashenko et al., 2008). The leaves of L. origanoides are used as a substitute for common marjoram (Maisch, 1885; Oliveira et al., 2007).

According to herbarium records (Missouri Botanical Garden Herbarium, St. Louis, MO, USA), L. origanoides occurs mainly in the Neotropical region, specifically in northern South America (Colombia, Venezuela and Guyana), with one record occurring in central Chile. 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 that the species occurs in the Andean region of Santander in northeastern Colombia (specifically in the Chicamocha Canyon) (Albesiano et al., 2003), the Atlantic Coast in northern Colombia (Herbarium Federico Medem Bogotá, Instituto Alexander von Humboldt (FMB)) and in the inter-Andean valleys in southern Colombia (Universidad del Cauca (CAUP) Herbario, Popayán, Cauca, Colombia). According to Albesiano et al. (2003), in Santander, L. origanoides is associated with the vegetation type known as thorny scrub, which is distributed in the semi-arid area of the central basin of the Chicamocha River in the localities of Pescadero and Ceptá, especially in small hillsides with smooth or steep slopes, soils with poor content of organic matter where lixiviation processes are frequent and with relatively high erosion rate. In these localities, the excessive shepherding of goats has played an important role in the vegetation distribution and has favoured the proliferation of L. origanoides and other species that are not directly grazed by these herbivores due to toxic tannins and defensive structures like spines and thorns (Albesiano et al., 2003). Although there is no evidence that L. origanoides is an endangered species in Colombia (Betancur et al., 2006), it has been suggested that human disturbance, including deforestation, wildfires and transitory cultivation, has played an important role in reducing the distribution of this species in the Chicamocha Canyon (Malagon et al., 1995; Albesiano et al., 2003; Serrato, 2007). Also, in the Atlantic Coast in northern Colombia, recent urbanization activities in areas around the city of Santa Marta in the department of Magdalena have reduced and fragmented the distribution extent of L. origanoides (A. Suárez, unpublished observations).

Despite being a promising aromatic plant, very little is known about basic biological aspects of L. origanoides, e.g. its breeding system and dispersal capabilities, which have a direct influence on genetic diversity levels and the 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).

In this sense, highly polymorphic genetic markers are a useful tool to infer genetic structure and dispersal patterns (Hardy et al., 2005). Intersimple sequence repeats (ISSRs) are arbitrary multilocus markers produced by PCR amplification using anchored-microsatellite primers (Zietkiewicz et al., 1994). This method has the advantage of generating relatively stable PCR products that show high levels of polymorphisms without requiring prior genomic information (Borne & Branchard, 2004). As a result, ISSRs have been widely applied in studies of genetic diversity and population structure (Camaecho & Liston, 2001; Culley & Wolfe, 2001; Sica et al., 2005). Additionally, this technique constitutes an alternative for taxa for which little or no research has been done at the molecular level (Liu & Wendel, 2001). Nevertheless, ISSRs 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 (Zhitovotvsky, 1999; Culley & Wolfe, 2001). The estimation bias of this method is particularly serious in presumably outcrossing species where the frequency of the null allele may not be confidently estimated from the proportion of individuals that lack the marker since a relatively high proportion of the null allele will be present in
the non-identifiable heterozygotes (Lynch & Milligan, 1994). 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).

In this context, the aim of the present study was to measure genetic diversity levels of *L. origanoides* from the central basin of the Chicamocha River, in northeastern Colombia, and to document fine population structure at different spatial scales using spatial autocorrelation analyses.

2. Materials and methods

(i) Sample collection

Samples from 111 individuals of *L. origanoides* were collected in the central basin of the Chicamocha River in the localities of Pescadero (6°49'N, 73°00'W) and Cepitá (6°45'N, 72°58'W) (Fig. 1). Sampling was performed in these two localities because they provide relatively homogeneous areas dominated by the species thus minimizing the possible effects of selection on genetic structure due to microhabitat heterogeneity. The sampling strategy reflected the current distribution and abundance of the species within each locality. In Pescadero, *L. origanoides* is abundant and continuously distributed on both sides of the Chicamocha River, whereas in Cepitá, *L. origanoides* is found on the east side of the Chicamocha River, and at this locality, sampling was performed along two small transects separated by farms and cultivated land. Samples from 62 and 39 individuals were collected in Pescadero and Cepitá, respectively, at elevations ranging from 550 to 830 m.a.s.l. For the purpose of examining larger distance classes, samples were also taken from ten individuals along a transect connecting these two localities on the east side of the Chicamocha River where *L. origanoides* is relatively less abundant (Fig. 1). For each sample collected, latitudinal, longitudinal and elevation data were recorded using a GPS (geographical positional system) from Garmin (USA).

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

(ii) DNA extraction and PCR amplification

Genomic DNA was isolated from young leaves. Leaf samples were finely ground in liquid nitrogen (−80 °C) 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 −20 °C in TE buffer for later use.

A set of 50 ISSR primers (obtained from the University of British Columbia, Vancouver, ON, 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), 1× PCR buffer (Promega), 0.2 mM dNTPs, 0.5 μM of the ISSR primer and 10–20 ng of extracted DNA. Amplifications were performed on a PTC-100TM thermocycler (MJ Research) following Wolfe (2005) cycling conditions. PCR products were loaded onto 2% agarose gels in 0.5× TBE buffer, run at constant voltage (50 V) for 3 h and detected by staining with ethidium bromide. Each ISSR band was considered 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 analysed for each locus and only reproducible bands were considered for analysis.

(iii) Data analysis

Genetic diversity was described as the percentage of polymorphic loci (P), and quantified as Shannon’s diversity index (I) (Shannon & 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 (Hb) using the HICKORY software (Holsinger & Lewis, 2003). This software uses Markov chain Monte Carlo simulations to produce posterior distributions based on the data (Holsinger et al., 2002).

The genetic structure of L. origanoides from the two localities of the central basin of the Chicamocha River was first explored by means of a principal coordinate analysis (PCO) using a squared Euclidean distance matrix from which principal coordinates were extracted. Also, a matrix of pairwise similarities among individuals was obtained using the Nei & Li (1979) coefficient and this matrix was employed to build a dendrogram by using the unweighted pair group method using arithmetic averages (UPGMA) algorithm. The PCO and UPGMA analyses were carried out using MVSP 3.1 (Kovach, 1998). Population structure was further examined by means of analysis of molecular variance (AMOVA) with the program GENALEX version 6.1 (Peakall & Smouse, 2006), and by applying the Bayesian hierarchical model implemented in HICKORY (Holsinger & Lewis, 2003).

The correlation between a genetic Euclidean distance matrix and a geographical distance matrix was assessed by means of Mantel tests using GENALEX version 6.1 (Peakall & Smouse, 2006). Since Mantel tests are not so 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 purpose of the Moran’s I coefficient 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 & Smouse, 2006).

It has been suggested that the sampling design can affect the estimation of the spatial genetic structure (Vekemans & Hardy, 2004). Vekemans & 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 Cepita). Every possible pair of individuals was considered as a join and assigned to 1 of 10 distance classes (according to the Euclidean distance separating the pair) in Pescadero and Cepita, and 20 distance classes in the entire region. Distance classes were based on either equally sized or arbitrarily chosen intervals, each one containing at least 30 pairs of data. For the entire region and Pescadero, several intervals were tested ranging from 20 to 200 m, at regular increments of 10 m, and maximum distance classes of 4-8 and 1-4 km, respectively. For Cepita, tested intervals ranged from 40 to 80 m, at regular increments of 10 m, with a maximum distance class of 590 m. These distance classes accounted for the smallest and largest spatial resolution possible for our dataset. 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 & Oden, 1978), and significance for the entire correlograms was calculated using a Bonferroni criterion (Oden, 1984).

3. Results

(i) 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: no. 813 (CTC TCT CTC TCT TT), no. 814 (CTC TCT CTC TCT CT TA), no. 824 (CTC TCT CTC TCT CG), no. 843 (CTC TCT CTC TCT TRG), and no. 845 (CTC TCT CTC TCT TRG). Of these bands, 50 were polymorphic (P = 86.21%). Genetic diversity indexes, calculated either assuming HWE (I) or with the Bayesian approach (Hb), did not differ significantly (0.453 ± 0.009 and 0.484 ± 0.227, respectively). Additionally, there was
not a significant difference between the genetic diversity values between the localities of Pescadero and Cepitá ($I = 0.435 \pm 0.006$ and $H_B = 0.495 \pm 0.229$ for Pescadero; $I = 0.441 \pm 0.005$ and $H_B = 0.493 \pm 0.231$ for Cepitá).

(ii) Population structure

Results of the PCO analysis are shown in Fig. 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 geographical groups among individuals (data not shown). The average genetic similarity as measured by the Nei and Li coefficient (Nei & Li, 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. The AMOVA results indicate that most of the molecular variation in *L. origanoides* from the Chicamocha Canyon is found within localities (97%), with much less amount of variation among localities (3%) ($\Phi_{PT} = 0.034$, $P < 0.001$). Similarly, the Bayesian analysis shows a low level of genetic differentiation among localities ($\theta_{II} = 0.032 \pm 0.021$). All these results suggest the existence of a single population of *L. origanoides* in the central basin of the Chicamocha River.

(iii) Spatial genetic structure

The presence of spatial genetic structure in the population of *L. origanoides* from the Chicamocha Canyon was evaluated by means of a Mantel test and fine-scale autocorrelation analyses. The Mantel test was carried out for the entire region (all 111 individuals) and showed a weak but significant correlation among genetic and geographical distance ($r = 0.074$, $P < 0.05$), suggesting that genetic variation within this population may be spatially structured. To further explore this, three separate fine-scale autocorrelation analyses (for the entire region, for Pescadero and for Cepitá) were conducted using both Moran’s *I* and normalized Mantel $r_m$. For the entire region and for Pescadero, autocorrelograms were conducted at distance class intervals ranging from 20 to 200 m. In both cases, significant autocorrelations were observed in all distance class intervals analysed and for intervals smaller than 150 m only positive spatial autocorrelations were observed (data not shown). Negative correlations were only observed when larger intervals were considered (i.e. 150–200 m). The correlograms for 150 m intervals are shown in Figs 3 and 4. For the entire region, the Moran’s *I* autocorrelogram (Fig. 3a) shows significant 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 by individuals within these distances. Figure 3a also shows significant negative values at the intervals 1200–1350 m and 3500–4000 m, indicating that individuals

---

Fig. 2. The first two coordinates from the PCO, using squared Euclidean genetic distance, explained 28.8% of the total variability. The high overlap of individuals shows a pattern of very low differentiation between the two localities.
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 $r_m$ coefficient (Fig. 3b) with significant positive values for the intervals 0–150 m and 300–450 m, and significant negative values for the intervals 1200–1350 m and 3500–4000 m. For Pescadero, the 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 (Fig. 4a). This result was similar for the normalized Mantel $r_m$ autocorrelogram with an additional significant positive value at the interval 300–450 m (Fig. 4b). In Cepitá, autocorrelograms for distance class intervals ranging from 40 to 80 m were obtained and a similar correlation pattern to that observed for the entire region and for Pescadero was found, but at a smaller scale (Fig. 5). The Moran's $I$ autocorrelogram exhibited significant positive correlations at the first two distance intervals ranging from 0 to 120 m, whereas at the distance interval of 420–490 m, significant negative correlations were observed (Fig. 5a). Similar results were obtained for the normalized Mantel $r_m$ autocorrelogram with an additional significant negative value for the last interval of 490–590 m (Fig. 5b).
Genetic structure of Lippia origanoides

4. Discussion

(i) Genetic diversity

L. origanoides from the Chicamocha Canyon exhibited relatively 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 is higher than those reported previously for widely distributed species (Loveless & Hamrick, 1984; Gitzendanner & Soltis, 2000; Nybom, 2004). However, for a more meaningful comparison of genetic diversity, data from close relatives (e.g. congeners) should be analysed (Gitzendanner & Soltis, 2000). As far as we know, there have been no reports of genetic diversity in Lippia species, except for an unpublished study of Lippa alba and Aloysia triphylla (a species related to the genus Lippia) using ISSR markers (F. Martinez, G. Castillo & M. I. Chacón, 2008, personal communication). In that study, L. alba showed relatively 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 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 (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., 2008).

In contrast with these results, a parallel 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 (Suárez et al., 2007). ISSR polymorphisms used in the present study are presumably mostly from nuclear DNA. In this way, these apparently contrasting results may be explained by a higher mutation rate of microsatellite regions from the nuclear genome compared with the chloroplast genome (Morgante & Olivieri, 1993), and by the different modes of transmission of the complete chloroplast DNA (cpDNA) (usually maternally) and ISSRs (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 (Wright, 1946; Culley & Wolfe, 2001; Muir et al., 2004; Mable & Adam, 2007), which is expected to be smaller for uniparentally than for biparentally inherited markers. However, it is important to note that the sampling scheme analysed in Suárez et al. (2007) was rather small; thus more samples are needed to assess intraspecific variation in the chloroplast genome.

There are several reports where outcrossing may yield high levels of genetic variation in a population (Culley & Wolfe, 2001; Muir et al., 2004; Mable & Adam, 2007). Given the relatively high levels of genetic diversity found in L. origanoides and the lack of a significant difference between the genetic diversity indexes calculated either assuming HWE or with the Bayesian approach, an important influence of outcrossing on the reproduction of this species could be inferred. However, this is a subject that deserves further investigation.

(ii) Population genetic structure

In this study, different approaches were used to estimate the level of genetic differentiation between individuals within and among localities in the central basin of the Chicamocha Canyon (Pescadero and Cepitá). Results from the PCO, UPGMA, AMOVA and the Bayesian approach (based on partitioned heterozygosity) showed a very low genetic differentiation among localities, which may suggest that in this region there is a single population where neither
the river nor human disturbance is acting as a barrier to gene flow.

However, the spatial autocorrelation analyses using the Moran’s I and normalized Mantel $r_m$ coefficients indicate that the distribution of genetic diversity within this population 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 (Fig. 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 greater distances, only significant negative values or no significant values were displayed.

In general, there was a strong positive spatial association between individuals distributed within 0–1200 m from each other (0–120 m for Cepitá), whereas individuals distributed at a distance greater than ~1200 m from each other (490 m for Cepitá) showed significant negative autocorrelation (Figs 3–5). 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 (Epperson, 2000; Vekemans & Hardy, 2004). A variety of empirical data indicate that in several cases seeds show a localized dispersal rate resulting in patches of plants, whereas 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; Ouborg et al., 1999; Kalisz et al., 2001). 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. 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. Kalisz et al., 2001; Hardy et al., 2004; Hardesty et al., 2005). Also, it could be the result of short-distance pollen movement, as it has been reported for some shrubs and herbs (Marr et al., 2000; Barthelmess et al., 2006; Byrne et al., 2007). 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 (McCauley, 1994; Trapnell & Hamrick, 2004; Petit et al., 2005) and their influence on fine-scale population structure in this species.

The results from the present study are comparable with those obtained for tree species where distance classes of hundreds of metres were analysed (Doligez & Joly, 1997; Degen et al., 2001) and where fine genetic structure at distance classes of hundreds of metres was found (Latouche-Hallé et al., 2003; Hardesty et al., 2005). Numerous analyses in shrubs have found spatial genetic structure at finer scales, from 1 to 10 m (Loiselle et al., 1995; England et al., 2003; Tang et al., 2007). Future analyses may include a sampling strategy where shorter distances are considered.

Autocorrelation analyses are greatly affected by the sampling strategies used (Vekemans & Hardy, 2004). However, in the three autocorrelation analyses performed (Figs 3–5), a relatively large number of pairs of data were assigned to each distance class analysed; therefore, it is unlikely that the significant genetic structures detected in our analyses within proximate distances are artefacts of the sampling scheme.

(iii) Implications for conservation and breeding

The wide distribution and the relatively high genetic diversity estimated in the present study lead 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 because of the overharvesting of natural stands (Muchugi et al., 2006; Tang et al., 2007). Currently, human disturbance is taking place in regions where L. origanoides exhibits a natural wide distribution (Malagon et al., 1995; Albesiano et al., 2003; 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 & Hardy, 2004). For these reasons, the present study does not allow us to predict the patch size appropriate for an accurate collection, but it does allow us to suggest that sampling individuals at distances greater than ~1.2 km along the transects where the samplings were conducted could result in the collection of different genotypes, which could help preserve the levels of genetic diversity in a propagation programme. Since different chemotypes have been
identified within *L. origanoides* in the Chicamocha Canyon (Stashenko *et al.*, 2008), additional research is needed to establish the relationship between essential oil composition and genetic variation, which will undoubtedly contribute to future breeding initiatives.

We are grateful to COLCIENCIAS, Colombia, for financial support through grant number RC 432-2004 and to the CENIVAM, Bucaramanga, Colombia, for providing support to carry out this research. We are also grateful to the Dirección de Investigaciones, Universidad Nacional de Colombia, Bogotá, Colombia, for their financial support of 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 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 Stefanie Hartmann for revising the manuscript and providing valuable comments.

**References**

Albesiano, S., Rangel, J. O. & Cadena, A. (2003). La vegetación del cañón del río Chicamocha (Santander, Colombia). *Caldasia* 25, 73–99.

Albre, J., Quilichini, A. & Gibernau, M. (2003). Pollination ecology of *Arunum 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 *Eragrostis tef* (Zucc.) Trotter. *Hereditas* 139, 174–183.

Barthelmess, E. L., Richards, C. M. & McCauley, D. E. (2006). Relative effects of nocturnal vs diurnal pollinators and distance on gene flow in small *Silene alba* populations. *New Phytologist* 169, 689–698.

Betancur, J., García, N., Fernández-Alonso, J. L., Rivera-Díaz, O. & Hernández, A. (2006). *Libro Rojo de Plantas de Colombia*, vol. 3. Ministerio del Medio Ambiente, Vivienda y Desarrollo Territorial de Colombia; Instituto de Investigación de Recursos Biológicos Alexander von Humboldt; Instituto de Ciencias Naturales, Universidad Nacional de Colombia; Ministerio de Ambiente, Vivienda y Desarrollo Territorial.

Birky, C. W. (1995). Uniparental inheritance of mitochondrial and chloroplast genes: mechanisms and evolution. *Proceedings of the National Academy of Sciences of the USA* 92, 11331–11338.

Borner, 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, C. F., Yates, C. & Coates, D. J. (2007). Extensive pollen dispersal in a bird-pollinated shrub, *Cabochnamus quadrifidus*, in a fragmented landscape. *Molecular Ecology* 16, 1303–1314.

Camacho, F. J. & 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.

Culley, T. M. & Wolfe, A. D. (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, M. H., Leveau, A. & Kremer, A. (2001). Fine-scale spatial genetic structure of eight tropical tree species as analysed by RAPDs. *Heredity* 87, 497–507.

Dolgez, A. & Joly, H. I. (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, P. R., Whelan, R. J. & Ayre, D. J. (2003). Effects of seed bank disturbance on the fine-scale genetic structure of populations of the rare shrub *Grevillea maclayana*. *Heredity* 91, 475–480.

Emperson, B. K. (2000). Spatial genetic structure and non-equilibrium demographics within plant populations. *Plant Species Biology* 15, 269–279.

Escudero, A., Iriondo, J. M. & Torres, M. E. (2003). Spatial analysis of genetic diversity as a tool for plant conservation. *Biological Conservation* 113, 351–365.

Fortin, M. J., Dale, M. R. T. & Ver Hoef, J. (2002). Spatial analysis in ecology. In *Encyclopedia of Environmetrics* (ed. A. H. El-Shaarawi & W. W. Piegmors), pp. 2051–2058. Chichester, UK: John Wiley and Sons.

Gehring, J. L. & Delph, L. F. (1999). Fine-scale genetic structure and clinal variation in *Silene acaulis* despite high gene flow. *Heredity* 82, 628–637.

Gitzendanner, M. A. & Soltis, P. S. (2000). Patterns of genetic variation in rare and widespread plant congeners. *American Journal of Botany* 87, 783–792.

Hardesty, B. D., Dick, C. W., 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, O. J., González-Martínez, S. C., Fréville, H., Boquien, G., Mignot, A., Colas, B. & Olivier, 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, O. J., Magia, L., Bandou, E., Breyne, P., Caron, H., Chevallier, M. H., Dolgez, A., Dutech, C., Kremer, A., Latouche-Hallé, C., Troispoux, V., Veron, V. & Degen, B. (2005). Fine-scale genetic structure and gene dispersal differences in 10 neotropical tree species. *Molecular Ecology* 15, 559–571.

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

Heywood, J. S. (1991). Spatial analysis of genetic variation in plant populations. *Annual Review of Ecology and Systematics* 22, 335–355.

Holsinger, K. E. & Lewis, P. O. (2003). *HICKORY*: A Package for Analysis of Population Genetic Data. v. 1.0. Available at http://www.eeb.uconn.edu

Holsinger, K. E., Lewis, P. O. & Dey, D. K. (2002). A Bayesian approach to inferring population structure. *Heredity* 87, 1560–1568.

Hu, Y., Zhu, Y., Zhang, Q. Y., Xin, H. L., Qin, L. P., Lu, B. R., Rahman, K. & Zheng, H. C. (2008). Population genetic structure of the medicinal plant *Vitex rotundifolia* in China: implication for its use and conservation. *Journal of Integrative Plant Biology* 50, 1118–1129.

Kalisz, S., Nason, J. D., Hanzawa, F. M. & Tonsor, S. J. (2001). Spatial population genetic structure in *Trillium grandiflorum*: the roles of dispersal, mating, history, and selection. *Evolution* 55, 1560–1568.
Kovach, W. (1998). MVA * A Multivariate Statistical Package for Windows, Version 3.0. Pentraeth, Wales, UK: Kovach Computing Services.

Latouche-Hallé, 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, R. A., Raguobob, R. A. & McDade, L. A. (2001). Fragment chemistry and pollinator affinities in *Nyctaginaceae*. *Phytochemistry* 58, 429–440.

Levin, S. (1992). The problem of pattern and scale in ecology. *Ecology* 73, 1943–1967.

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

Loiselle, B. A., Sork, V. L., Nason, J. & Graham, C. (1995). *Flora de Venezuela. Verbenaceae*. *Botany* 82, 1420–1425.

Lopez, S. (1977). *Flora de Venezuela. Verbenaceae*, pp. 338–441. Mérida, Venezuela: Consejo de Publicaciones, Facultad de Farmacia, Universidad de los Andes.

Loveless, M. D. & Hamrick, J. L. (1984). Ecological determinants of genetic structure in plant populations. *Annual Review of Ecology and Systematics* 15, 65–95.

Lynch, M. & Milligan, B. G. (1994). Analysis of population genetic structure with RAPD markers. *Molecular Ecology* 3, 91–99.

Mable, B. K. & Adam, A. (2007). Patterns of genetic diversity in outcrossing and selfing populations of *Arabidopsis lyrata*. *Molecular Ecology* 16, 3565–3580.

Maisch, J. M. (1885). On some useful plants of the natural order of Verbenaceae. *American Journal of Pharmacy* 57, 189–199.

Malagon, D., Llinas, R. D. & Chamorro, C. (1995). *Suelos de Colombia: Origen, Evolución, Clasificación, Distribución y Uso*. Bogotá, Colombia: IGAC.

Marr, D. L., Leebens-Mack, J., Elms, L. & Pellmyr, O. (2000). Pollen dispersal in *Yucca filamentosa* (Prodoxidae). *American Journal of Botany* 87, 670–677.

McCauley, D. E. (1994). Contrasting the distribution of chloroplast DNA and allozyme polymorphism among local populations of *Silene alba*: implications for studies of gene flow in plants. *Proceedings of the National Academy of Sciences of the USA* 91, 8127–8131.

Morgante, M. & Olivieri, A. M. (1993). PCR-amplified microsatellites as markers in plant genetics. *The Plant Journal* 3, 175–182.

Muchugi, A., Lengkeek, A. G., Kadu, C. A., Muluvu, G. M., Njagi, E. N. & Dawson, I. K. (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, A. J., Fleming, C. C. & Vogt, 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, W. H. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proceedings of the National Academy of Sciences of the USA* 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, N. L. (1984). Assessing the significance of a spatial correlogram. *Geographical Analysis* 16, 1–16.

Oliveira, D. R., Leitão, G. G., Bizzo, H. R., Lopes, D., Alviano, D. S., Alviano, C. S. & Leitão, S. G. (2007). Chemical and antimicrobial analyses of essential oil of *Lippia origanoides* H.B.K. *Food Chemistry* 101, 236–240.

Ouborg, N. J., Pipquet, Y. & van Groenendael, J. M. (1999). Population genetics, molecular markers and the study of dispersal in plants. *Journal of Ecology* 87, 551–568.

Peakall, R. & Smouse, P. E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes* 6, 288–295.

Peakall, R., Ruibal, M. & Lindenmayer, D. B. (2003). Spatial autocorrelation analysis offers new insights into gene flow in the Australian bush rat, *Rattus fasciatus*. *Evolution* 57, 1182–1195.

Petit, R. J., Duminil, J., Fineschi, S., Hampe, A., Salvini, D. & Vendramin, G. G. (2005). Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. *Molecular Ecology* 14, 689–701.

Pirttiläi, A. M., Hirskiropi, M., Kämäriäinen, T., Jaakola, L. & Hohtola, A. (2001). DNA isolation methods for medicinal and aromatic plants. *Plant Molecular Biology Reporter* 19, 273a–273f.

Radeloff, V. C., Miller, T. F., He, H. S. & Mladenoff, D. J. (2000). Periodicity in spatial data and geostatistical models: autocorrelation between patches. *Ecography* 23, 81–91.

Rosenberg, M. S. (2001). *PASSAGE: Pattern Analysis, Spatial Statistics, and Geographic Exegesis, Version 1*. Tempe, AZ: Department of Biology, Arizona State University.

Santos, F. J., Lopes, J. A., Cito, A. M., Oliveira, E. H., Lima, S. G. & Reis, F. (2004). Composition and biological activity of essential oils from *Lippia origanoides* H.B.K. *Journal of Essential Oil Research* 16, 504–506.

Serrato, P. K. (2007). *Los Cactos Colombianos: Una Síntesis Geográfica*, pp. 119–135. Bogotá, Colombia: IGAC.

Shannon, C. E. & Weaver, W. (1949). *The Mathematical Theory of Communication*. Urbana, IL: University of Illinois Press.

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.

Sokal, R. R. & Oden, N. L. (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.

Stashenkon, E., Ruiz, C., Muñoz, A., Castañeda, M. & Martínez, J. (2008). Composition and antioxidant activity of essential oils of *Lippia origanoides* H.B.K. grown in Colombia. *Natural Product Communications* 3, 563–566.

Su, H., Qu, L. J., 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.

Suárez, A. R., Martínez, F. O., Castillo, G. A. & Chacón, M. I. (2007). Molecular characterization of aromatic species of the genus *Lippia* from the Colombian tropics. *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* Y.) at the margin of its range in Ireland. *Heredity* 90, 563–566.

Tero, N., Aspi, J., Siikamäki, P. & Jäkäläniemi, A. (2005). Local genetic population structure in an endangered...
plant species, *Silene tatarica* (Caryophyllaceae). *Heredity* **94**, 478–487.

Trapnell, D. W. & Hamrick, J. L. (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, O. J. (2004). New insights from fine-scale spatial genetic structure analyses in plant populations. *Molecular Ecology* **13**, 921–935.

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

Ward, S. (2006). Genetic analysis of invasive plant populations at different spatial scales. *Biological Invasions* **8**, 541–552.

Wolfe, A. D. (2005). ISSR techniques for evolutionary biology. *Methods in Enzymology* **395**, 134–144.

Wright, S. (1946). Isolation by distance under diverse systems of mating. *Genetics* **31**, 39–59.

Yeh, F., Yang, R. C. & Boyle, T. (1995). *POPGENE*, the User-friendly Shareware for Population Genetic Analysis. Edmonton, AB, Canada: Molecular Biology and Biotechnology Center, University of Alberta.

Zhang, Z. Y., Zheng, X. M. & 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, L. A. (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 sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics* **20**, 176–183.