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Spatial genetic structure in the very rare and species-rich *Picea chihuahuana* tree community (Mexico)

By C. Z. QUIÑONES-PÉREZ¹⁾, S. L. SIMENTAL-RODRÍGUEZ¹⁾,
C. SÁENZ-ROMERO²⁾, J. P. JARAMILLO-CORREA³⁾ and C. WEHENKEL^{1),*}

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Abstract

In natural plant populations, the spatial genetic structure (SGS) is occasionally associated with evolutionary and ecological features such as the mating system, individual fitness, inbreeding depression and natural selection of the species of interest. The very rare Mexican *P. chihuahuana* tree community covers an area no more than 300 ha and has been the subject of several studies concerning its ecology and population genetics. The

overall aim of most of these studies has been to obtain data to help design preservation and conservation strategies. However, analysis of the fine-scale SGS in this special forest tree community has not yet been conducted, which might help enrich the above mentioned conservation programs. In this study, we examined the SGS of this community, mostly formed by *P. chihuahuana* Martínez, *Pinus strobiformis* Ehrenberg ex Schlechtendahl, *Pseudotsuga menziesii* (Mirb.) Franco, and *Populus tremuloides* Michx, in 14 localities at both the fine and large scales, with the aim of obtaining a better understanding of evolutionary processes. We observed a non-significant autocorrelation in fine-scale SGS, suggesting that the genetic variants of all four tree species are randomly distributed in space within each sampled plot of 50 x 50 m. At the larger scale, the autocorrelation was highly significant for *P. chihuahuana* and *P. menziesii*, probably as a result of insufficient gene flow due to the extreme population isolation and small sizes. For these two species our results provided strong support for the theory of isolation by distance.

Key words: Community genetics, AFLP, gene flow, selection, Sierra Madre Occidental, *Pinus strobiformis* Ehrenberg ex Schlechtendahl, *Pseudotsuga menziesii* (Mirb.) Franco, *Populus tremuloides* Michx.

¹⁾ Instituto de Silvicultura e Industria de la Madera, Universidad Juárez del Estado de Durango, Ciudad Universitaria, Boulevard del Guadiana #501, C.P. 34120, Durango, Dgo. México.

²⁾ Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo Km 9.5 carr. Morelia-Zinapécuaro, Tarímbaro Michoacán 58880, Mexico. Currently at: INRA, UMR 1202 BIOGECO, F-33610 Cestas, France & Univ. Bordeaux, UMR 1202 BIOGECO, F-33615 Pes-sac, France.

³⁾ Instituto de Ecología, Universidad Nacional Autónoma de México, Ciudad Universitaria, Circuito Exterior s/n. Apartado Postal 70-275, 04510 Mexico, D.F.

^{*} Corresponding author: C. WEHENKEL. Ciudad Universitaria, Boulevard del Guadiana #501, C.P. 34120, Durango, Dgo. México. Phone: +52(618) 827-12-15, Fax: (618) 825-18-86. E-Mail: wehenkel@ujed.mx.

Introduction

The geographical distribution of genetic variation, i.e., spatial genetic structure (SGS), is an important theoretical and experimental component of population genetics (EPPERSON, 1993). A decrease in the strength of the genetic relationship between pairs of individuals is expected with increasing distance (WRIGHT, 1943). In natural plant populations, the SGS is occasionally associated with evolutionary and ecological genetics and can be influenced by different processes, like mating system, individual fitness, inbreeding depression and natural selection (EPPERSON, 1993; DEGEN et al., 2001; EPPERSON and GI CHUNG, 2001). Specifically, the SGS is determined by how gene flow through pollen and seed dispersal change as geographical distance increases. Because seed dispersal influences many key aspects of plant biology, including colonization of new sites, metapopulation dynamics and diversity in plant communities (CAIN et al., 2000), analysis of SGS can be helpful for the conservation and management of natural populations (McCUE et al., 1996).

Analysis of spatial genetic structure has been the subject of numerous research studies since the 1940s and remains a relevant issue today (WRIGHT, 1943; TROUPIN et al., 2006; VALBUENA-CARABANA et al., 2007; DE LAFONTAINE et al., 2013). Empirical studies have assessed fine-scale SGS within plant populations by using genetic markers and spatial autocorrelation techniques. These studies have generally provided qualitative descriptions of SGS (VEKEMANS and HARDY, 2004). Spatial genetic analysis complements methods that examine processes occurring at larger geographical scales, such as the landscape scale (CLARK et al., 1999; SÁENZ-ROMERO et al., 2001; SÁENZ-ROMERO and GURIES, 2002; MOCK et al., 2007) or larger scale migration and colonization events (DEGEN et al., 2001). However, detection of SGS is strongly dependent on both the choice of molecular markers and the strategy used to sample the population under study (JUMP and PEÑUELAS, 2007).

Several studies of forest tree species have investigated spatial genetic structure by the use of allozyme markers (e.g., in eastern white pine) (EPPERSON and GI CHUNG, 2001) and microsatellites (e.g., in common ash, white pine, and oak) (HEUERTZ et al., 2003; MARQUARDT and EPPERSON, 2004; VALBUENA-CARABANA et al., 2007). JUMP and PEÑUELAS (2007) conducted spatial genetic analyses of pairwise kinship coefficients with similar loci (SSRs) joined by amplified fragment length polymorphisms (AFLP). They found that the SGS estimated with the last type of markers could be detected at distances nearly four times larger than previously estimated with other molecular markers, thus indicating a persistent effect of restricted gene flow at small spatial scales and a higher reliability of AFLPs for estimating SGS. The usefulness of AFLP-markers to analyze large scale genetic structures was further demonstrated by LEINEMANN et al. (2013, 2014) for two shrub species (*Coryllus avellana* and *Prunus spinosa*) with different gene flow systems.

Picea chihuahuana Martínez is an endemic conifer species in Mexico and is considered endangered, according to the official Mexican ruling (NORMA OFICIAL MEXICANA, 2010). To date, 40 populations comprising a mini-

mum total of 42,600 individuals (LEDIG et al., 2000) have been identified in three separate groups in the Sierra Madre Occidental, at altitudes of between 2,100 and 3,000 m a.s.l. These populations are located in humid valleys and form clusters within gallery forests and often include other tree species of the genera *Pinus*, *Quercus*, *Abies*, *Pseudotsuga*, *Populus*, *Juniperus*, *Prunus*, and *Cupressus* (GORDON, 1968; NARVÁEZ, 1984; LEDIG et al., 2000; QUINONES-PÉREZ et al., 2014).

This very rare pine-spruce-cedar community (hereafter referred to as the *P. chihuahuana* tree community) spans on area of no more than 300 ha that is mostly well preserved and remains largely untouched by humans, because of its isolation at high altitudes in very rugged mountains (LEDIG et al., 1997; LEDIG et al., 2000). The *P. chihuahuana* tree community has been the subject of several studies concerning its ecology and population genetics. The overall aim of most of these studies has been to obtain data to help design preservation and conservation strategies (NARVÁEZ, 1984; LEDIG et al., 1997; LEDIG et al., 2000; JARAMILLO-CORREA et al., 2006; WEHENKEL et al., 2012; WEHENKEL and SÁENZ-ROMERO, 2012; QUINONES-PÉREZ et al., 2012; QUINONES-PÉREZ et al., 2014; WEHENKEL et al., 2014; MENDOZA-MAYA et al., 2015). However, analysis of the fine-scale SGS in this special forest tree community has not yet been conducted, which might help enrich the abovementioned conservation programs.

In this study, we examined the SGS of this community, mostly formed by *P. chihuahuana* Martínez, *Pinus strobiformis* Ehrenberg ex Schlechtendahl, *Pseudotsuga menziesii* (Mirb.) Franco, and *Populus tremuloides* Michx, in 14 localities at both the fine (in 0.25 ha plots) and large (minimum 60 km distance class) scales, with the aim of obtaining a better understanding of the complex interaction between the local dispersal, the mating system of the species (particularly *P. chihuahuana*) and other evolutionary processes. Because of the isolated location and strongly mixed nature of this tree community, which often results in low population densities of the each single tree species, we expected a significant autocorrelation at different spatial scales due to restricted gene flow and increasing drift (HAMPE and PETIT, 2005; DICK et al., 2008).

Materials and Methods

Study area

In order to determine the SGS of the *P. chihuahuana* tree community, 14 populations located in the State of Durango and Chihuahua, Northwestern Mexico, were analyzed. The populations studied are listed in Table 1 and their locations are shown in Fig. 1. Each population was represented by one plot of 50 x 50 m (0.25 ha) placed at the population center (Fig. 2). The target tree species *P. chihuahuana*, *P. strobiformis*, *P. menziesii*, and *P. tremuloides* and all other tree taxa species with diameter at breast height (DBH) ≥ 7 cm were fully scored; the DBH, height and x, y coordinates were also recorded. The total number of tree species within each of the populations in the *P. chihuahuana* tree community is shown in Table 2.

Figure 1. – Map of the 40 known *Picea chihuahuana* tree communities and the locations of the 14 populations surveyed herein (black circles) in the State of Durango and Chihuahua, Northwest Mexico. Population codes as in Table 1.

Plant material and AFLP analyses

In order to estimate the SGS, DNA data was obtained by the amplified fragment length polymorphism (AFLP) technique. Needles were sampled from 669 individuals of *P. chihuahuana* (of which 475 were seedlings and saplings < 7 cm DBH from natural regeneration), 129 *Pinus strobiformis* (of which 85 were obtained from natural regeneration), 63 *Pseudotsuga menziesii* (of which 46 were gathered from natural regeneration), and 76 *Populus tremuloides* (of which 58 were natural regeneration) in all 14 locations (Fig. 1).

AFLP fingerprints were generated according to a modified version of the protocol described by VOS et al. (1995). The DNA was extracted with the QIAGEN DNeasy96 plant kit and digested with the restriction enzymes EcoRI and MseI. PCR amplification was carried out with double-stranded EcoRI and MseI adaptors ligated to the end of the restriction fragments to produce template DNA. In pre-AFLP amplification, the PCR products were treated with the primer combination E01/M03 and in the selective AFLP amplification with E35 and M70 for *Picea chihuahuana*, E35 and with M63+C for *Pseudotsuga menziesii*, *Pinus strobiformis*, and *Populus tremuloides*, in a Peltier Thermal Cycler (PTC-200 version 4.0, MJ Research).

Finally, a data matrix was constructed from the presence (coded by 1) or absence (0) at potential band positions. Each band detected (presence) corresponds to a

dominant genetic variant (plus phenotype) with an unknown mode of inheritance of this potential band position (detected fragment length) (KRAUSS, 2000;

Figure 2. – 50 x 50 m plot illustrating the location of each tree with diameter at breast height (DBH) above 7 cm in the very rare and species-rich *Picea chihuahuana* tree community in El Venado location (VN), Chihuahua, Mexico.

BONIN et al., 2004). Absence of a band reflects the recessive genetic variant (minus phenotype) at the given position (locus).

Spatial Structural Analysis of AFLP data

Spatial genetic structure at both the local and broad scales was analyzed using the Spatial Genetic Software (DEGEN et al., 2001). The spatial distance between two data points was calculated as the Euclidean ground distance (DEGEN et al., 2001). Within the 50 x 50 m plots (at fine scale), and provided that there were sufficient trees ≥ 7 cm DBH in each spatial distance-class, all SGS statistics were computed for the 5 and 10 m distance-class distributions, as well as for distributions with minimum 30 tree pairs per distance-class. However, the distance-class distributions did not always comply with Degen's (2000) recommendation of a minimum of 30 tree pairs for each distance class for a more robust permutation test. At the end, ten plots with *P. chihuahuana*, one plot with *P. menziesii*, one plot with *P. strobiformis*, and one plot with *P. tremuloides* were analyzed at this fine scale.

At the large scale (minimum 60 km distance-class distribution), SGS analysis was conducted for a minimum of 100 pairs of trees for each distance class. Because of the lack of sufficient trees ≥ 7 cm DBH of *Pinus strobiformis*, *Pseudotsuga menziesii*, and *Populus tremuloides* in some of the 50 x 50 m plots (Table 2), AFLP data from regeneration of these species, but with unknown x and y coordinates within the plots, were added to complete the analysis of large-scale SGS.

The different AFLP data matrices were used to compute the genetic dissimilarity [D_{ij}] between the binary

vectors of two individuals, i and j , using Tanimoto distance (DEICHSEL and TRAMPISCH, 1985):

$$0 \leq D_{ij} = 1 - \frac{v_{ij}}{v_{ij} + y_i + y_j} \leq 1 \quad [1]$$

where v_{ij} represents the number of shared fragments between individuals i and j , and y_i and y_j denote the fragments that only exist in one individual (i or j , respectively). Genetic distograms were then constructed to infer spatial patterns. The overall mean of genetic distances between all individuals of each species served as a reference for random spatial structure (DEGEN, 2000).

The presence of statistically significant deviation from a spatial random distribution was tested by randomization permutation methods (MANLY, 1997). The aggregation index (CE) of CLARK and EVANS (1954) was calculated as an indicator of spatial structures of the studied tree positions: values of $[CE] < 1$ indicate a clumped and aggregated structures, $[CE] = 1$ indicates a random distribution and $[CE] > 1$ indicates a regular distribution. The 95% confidence interval (CI) was used. $P(D) < (-CI)$ equals the probability that the observed value is lower than the lower limit of the confidence interval; $P(D) > (+CI)$ equals the probability that the observed value is greater than the upper limit of the confidence interval.

Then, the binary AFLP data matrix was used to perform Principal Coordinate Analysis (PCoA) on GenAlEx 6 (PEAKALL and SMOUSE, 2012) by using the Nei Genetic Distance (NEI, 1972, 1978) for calculating similarities. The first two coordinates were used to represent genetic variation among populations for all four tree species.

Table 1. – 14 Locations of the *Picea chihuahuana* tree community studied: 1) La Tinaja (TN), 2) El Ranchito (RC), 3) El Cuervo (CV), 4) Talayote (TY), 5) Las Trojas (TR), 6) El Venado (VN), 7) La Quebrada (LQ), 8) Paraje Piedra Rayada (PPR), 9) Quebrada de los Durán (Arroyo del Indio Ignacio) (QD), 10) Cebollitas (CB), 11) San José de las Causas (SJ), 12) Santa Bárbara (Arroyo del Infierno) (SB), 13) Arroyo del Chino (ACH), and 14) La Pista (Arroyo de La Pista) (LP).

Code	Location	Property	Municipality	Latitude N	Longitude W	Altitude m
TN	La Tinaja	Ejido El Ranchito	Bocoyna	27°57'27"	107°46'13"	2,380
RC	El Ranchito	Ejido El Ranchito	Bocoyna	27°57'20"	107°45'12"	2,414
CV	El Cuervo	Ejido El Ranchito	Bocoyna	27°57'01"	107°46'18"	2,500
TY	Talayote	Ejido Los Volcanes	Bocoyna	27°55'03"	107°49'01"	2,355
TR	Las Trojas	Ejido El Ranchito	Bocoyna	27°54'27"	107°45'17"	2,395
VN	El Venado	Ejido San Javier	Bocoyna	27°45'41"	107°41'33"	2,311
LQ	La Quebrada	Ejido El Caldillo y su anexo El Vergel	Balleza	26°28'13"	106°21'51"	2,730
PPR	Paraje Piedra Rayada	Ejido Chiqueros	Guanaceví	26°09'15"	106°24'17"	2,600
QD	Quebrada de los Durán	Ejido Chiqueros	Guanaceví	26°08'48"	106°22'53"	2,570
CB	Cebollitas	Private property	Canelas	25°05'55"	106°26'27"	2,450
SJ	San José de las Causas	Ejido San José de las Causas	San Dimas	24°01'07"	105°47'56"	2,480
SB	Santa Bárbara	Ejido El Brillante	Pueblo Nuevo	23°39'44"	105°26'20"	2,725
ACH	Arroyo del Chino	Santa María Magdalena de Taxicaringa	Mezquitil	23°21'05"	104°43'05"	2,600
LP	La Pista	Santa María Magdalena de Taxicaringa	Mezquitil	23°19'52"	104°45'00"	2,685

Because SGS could be also influenced by selection (EPPERSON, 1992), the proportion of candidate AFLP loci under natural selection were calculated using BayeScan v2.1 software (FOLL and GAGGIOTTI, 2008), which is

based on the multinomial Dirichlet model and uses a Reversible Jump Markov Chain Monte Carlo (RJM-CMC) algorithm to obtain posterior distributions. If the results showed a positive value of the locus-specific com-

Table 2. – Number of trees per species in plots of 0.25 ha for each one of 14 locations of *Picea chihuahuana* tree community studied herein.

Species	TN	RC	CV	TY	TR	VN	LQ	PPR	QD	CB	SJ	SB	ACH	LP	Total
<i>Abies durangensis</i> Martínez	-	-	-	-	-	-	-	-	-	18	9	14	-	-	41
<i>Cupressus lindleyi</i> Klotzsch ex Endl.	-	-	-	-	-	-	-	-	-	10	24	99	10	4	47
<i>Juniperus deppeana</i> Steud.	-	-	-	-	-	-	-	-	1	-	-	3	-	1	5
<i>Picea chihuahuana</i> Martínez	33	11	22	28	12	27	33	23	23	11	12	6	5	24	270
<i>Pinus arizonica</i> Engelm.	2	27	8	5	4	8	5	6	89	-	-	-	-	-	154
<i>Pinus strobiformis</i> Ehrenberg ex Schlechtendahl	33	30	32	4	23	11	40	-	-	34	4	-	4	-	215
<i>Pinus cooperi</i> Martínez	-	-	-	-	-	-	-	-	-	-	30	4	-	61	95
<i>Pinus durangensis</i> Martínez	4	-	19	1	5	8	-	-	-	4	-	1	-	-	42
<i>Pinus leiophylla</i> Schl. & Cham.	-	-	-	-	-	1	-	-	-	-	1	-	-	-	2
<i>Pinus teocote</i> Schiede ex Schltdl. & Cham.	-	-	-	-	-	-	-	-	-	-	-	2	-	-	2
<i>Populus tremuloides</i> Michx.	-	12	-	-	33	-	16	1	11	-	-	-	-	-	73
<i>Prunus serotina</i> Ehrh.	2	3	1	4	1	1	-	-	-	-	-	-	-	-	12
<i>Pseudotsuga menziesii</i> (Mirb.) Franco	-	-	-	15	-	-	-	8	9	10	-	35	11	-	88
<i>Quercus spp.</i>	2	4	-	1	11	9	14	-	-	-	10	20	-	1	72
Total	76	87	82	58	89	65	108	38	133	87	90	184	30	91	1218

Table 3. – Analysis of fine-scale spatial genetic structure in *P. chihuahuana* tree community by using a distance class of size 5 meters, 5,000 permutations and a 95% confidence interval.

Loc	SP	N	MD (m)	CA	P(D)<(- CI)	P(D)>(+ CI)	MT
TY	Pch	23	5.84	0.58	ns	ns	8/(0-5)
TN	Pch	31	3.40	0.55	ns	ns	24/(10-15)
CV	Pch	16	4.56	0.53	ns	ns	4/(40-45,45-50)
VN	Pch	23	3.44	0.50	ns	ns	4/(30-35)
TR	Pch	12	7.46	0.49	ns	ns	3/(20-25)
RC	Pch	40	3.61	0.49	ns	ns	25/(45-50)
LQ	Pch	25	5.83	0.48	ns	ns	10/(0-5)
QD	Pch	20	5.13	0.47	ns	ns	5/(45-50)
PPR	Pch	20	5.45	0.42	ns	ns	8/(45-50)
LP	Pch	17	6.30	0.27	0.998**/(15-20)	ns	6/(0-5,15-20,45-50)
TN	Pst	10	7.71	0.60	ns	ns	1/(30-35)

Note: Loc = location of the *P. chihuahuana* tree population; SP = species; N = tree number; MD = mean distance to the next tree; CA = calculated average; MT = minimum tree pairs per class (distance class); P(D)<(-CI), P(D)>(+CI) = autocorrelation P value; Pch = *Picea chihuahuana*; Pst = *Pinus strobiformis*; asterisks indicate significance: (**) at 1%/99% and (***) at 0.1%/99.9%; ns = not significant.

Table 4. – Analysis of fine-scale spatial genetic structure in *P. chihuahuana* tree community by using a distance class of size 10 meters, 5,000 permutations and a 95% confidence interval.

Loc	SP	N	MD (m)	CA	P(D)<(- CI)	P(D)>(+ CI)	MT
TY	Pch	23	5.84	0.58	ns	ns	28/(0-10)
TN	Pch	31	3.40	0.55	ns	ns	59/(10-20)
CV	Pch	16	4.56	0.53	ns	ns	8/(40-50)
VN	Pch	23	3.44	0.50	ns	ns	14/(30-40)
TR	Pch	12	6.43	0.49	ns	ns	6/(20-30)
RC	Pch	40	3.61	0.49	ns	ns	85/(40-50)
LQ	Pch	25	5.83	0.48	ns	ns	24/(0-10)
QD	Pch	20	5.13	0.47	ns	ns	13/(40-50)
PPR	Pch	20	5.45	0.42	ns	0.997**/(0-10)	20/(0-10)
LP	Pch	17	6.30	0.27	ns	ns	14/(40-50)
TN	Pst	10	7.71	0.60	ns	ns	3/(30-40)

Note: Loc = location of the *P. chihuahuana* tree population; SP = species; N = tree number; MD = mean distance to the next tree; CA = calculated average; MT = minimum tree pairs per class (distance class); P(D)<(- CI), P(D)>(+ CI) = autocorrelation P value; Pch = *Picea chihuahuana*; Pst = *Pinus strobiformis*; asterisks indicate significance: (**) at 1%/99% and (***) at 0.1%/99.9%; ns = not significant.

ponent (α) and a posterior probability > 0.95, we expected diversifying selection, whereas negative α values with posterior probabilities > 0.95 indicated possible balancing or purifying selection (http://cmpg.unibe.ch/software/bayescan/files/BayeScan2.1_manual.pdf; FOLL and GAGGIOTTI, 2008; FOLL et al., 2010).

The parameters of the chain and of the model were as follows: output number of iterations (5,000), thinning interval size (10), pilot runs (20), length of pilot runs (5,000), additional burn in (50,000), prior odds for the neutral model (10), lower boundary for uniform prior on the inbreeding coefficients F_{is} (0) and the higher boundary for uniform prior on F_{is} (1).

Results

The AFLP primer combination resulted in 243 polymorphic bands of 75–450 base pairs across all individuals of *Picea chihuahuana*, while the respective numbers of bands for *Pinus strobiformis*, *Pseudotsuga menziesii*, and *Populus tremuloides* were 250, 207 and 237. At the fine scale (5 m, 10 m and maximal 28 m distance-class distributions) and for the 10 plots analyzed, autocorrelations were not significant ($P \geq 0.01$ or $P \leq 0.99$), except for the 3th distance class in the LP plot (Table 3) and for the 1st distance class in PPR (Tables 4 and 5). The mean genetic distance between *P. chihuahuana* trees in LP (the southern most population) was the lowest in this study. The CE index was not significant, except in the population TN (Table 5), indicating a generally spatial random distribution of tree positions.

At the large scale, the SGS analysis included a set of 14 locations of the tree community under study. This analysis revealed highly significant autocorrelations ($P \geq 0.99$) for all four tree species (Table 6). However, only *Picea* and *Pseudotsuga* followed a clear trend and a significant decline in the strength of the genetic relationship with increasing distance between pairs of individuals (Fig. 3a, 3b, 3c, and 3d). In this case, the CE was also highly significant, indicating a clumped and aggregated spatial distribution of tree positions.

Principal Coordinate Analysis revealed an informative separation of populations. The first three coordinates explained 86.4% of the *P. chihuahuana*, 82.5% of the *P. strobiformis*, 88.0% of the *P. tremuloides*, and 94.1% of the *P. menziesii* of AFLP variation. By plotting the first two coordinates (which together explained 78.2% of the *Picea* AFLP variation, 71.1% of the *Pinus* AFLP variation, 79.9% of the *Populus* AFLP variation and 86.2% of the *Pseudotsuga* AFLP variation) the northern populations of these four trees species almost always clustered separately from those of the central and southern regions (Fig. 4).

Table 5. – Analysis of fine-scale spatial genetic structure in *P. chihuahuana* tree community by using a minimum the 30 pairs of data points for *Picea chihuahuana* and maximum possible number of tree pairs for *Pinus strobiformis*, *Populus tremuloides* and *Pseudotsuga menziesii*, 5,000 permutations and a 95% confidence interval.

Loc	SP	N	MD (m)	CE	CA	P(D)<(- CI)	P(D)>(+ CI)	MT
TY	Pch	23	5.84	1.22 ns	0.58	ns	ns	33/(42-63)
TN	Pch	31	3.40	0.62 ***	0.55	ns	ns	45/(64-80)
CV	Pch	16	4.56	0.68 ns	0.53	ns	ns	29/(27-54)
VN	Pch	23	4.74	0.94 ns	0.50	ns	ns	30/(0-11)
TR	Pch	12	6.43	0.99 ns	0.49	ns	ns	34/(0-28,28-56)
RC	Pch	40	3.61	0.91 ns	0.49	ns	ns	50/(45-60)
QD	Pch	20	5.13	1.14 ns	0.47	ns	ns	33/(0-11,33-44)
LQ	Pch	25	4.59	1.03 ns	0.47	ns	ns	40/(11-22)
PPR	Pch	20	5.45	1.19 ns	0.42	ns	0.998**/(0-14)	36/(0-14)
LP	Pch	17	6.30	1.28 ns	0.27	ns	ns	30/(0-14)
TN	Pst	10	7.71	1.24 ns	0.60	ns	ns	9/(28-56)
TR	Pop	9	2.10	0.82 ns	0.53	ns	ns	14/(25-50)
QD	Pme	7	3.17	1.19 ns	0.48	ns	ns	7/(10-20)

Note: Loc = location of the *P. chihuahuana* tree population; SP = species; N = tree number; MD = mean distance to the next tree; CE = aggregation index; CA = calculated average; MT = minimum tree pairs per class (distance class); P(D)<(- CI), P(D)>(+ CI) = autocorrelation P value; Pch = *Picea chihuahuana*; Pst = *Pinus strobiformis*; Pop = *Populus tremuloides*; Pme = *Pseudotsuga menziesii*; asterisks indicate significance: (**) at 1%/99% and (***) at 0.1%/99.9%; ns = not significant.

Table 6. – Analysis of large-scale spatial genetic structure in *P. chihuahuana* tree community, by using 5,000 permutations and a 95% confidence interval.

SP	N	MD (km)	CE	CA	P(D)<(- CI)	P(D)>(+ CI)	MT
Pch	669	1.00	0.000 ***	0.55	0.9999***/(0-60)	0.9999***/(60-120) 0.9999***/(240-300) 0.9999***/(360-420)	5,000/(360-420)
Pst	130	1.39	0.000 ***	0.66	0.996**/(90-180)	ns	400/(90-180)
Pop	76	1.41	0.000 ***	0.63	0.9999***/(0-150) 0.9999***/(300-450)	0.9999***/(150-300)	280/(300-450)
Pme	63	1.41	0.000 ***	0.60	0.9999***/(0-150) 0.9999***/(300-450)	0.9999***/(150-300) 0.9999***/(450-600)	250/(450-600)

Note: SP = species; N = tree number; MD = mean distance to the next tree; CE = aggregation index; CA = calculated average; MT = minimum tree pairs per class (distance class); P(D)<(-CI), P(D)>(+CI) = autocorrelation P value; Pch = *Picea chihuahuana*; Pst = *Pinus strobiformis*; Pop = *Populus tremuloides*; Pme = *Pseudotsuga menziesii*; asterisks indicate significance: (**) at 1%/99.9% and (***) at 0.1%/99.9%; ns = not significant. The distance class size is given in km.

Figure 3. – Large-scale spatial genetic structure analysis, based on AFLP data. Distogram calculated using mean Tanimoto distance for *Picea chihuahuana* (a), *Pinus strobiformis* (b), *Populus tremuloides* (c), and *Pseudotsuga menziesii* (d), with a minimum of 100 tree pairs per distance class. CI = confidence interval.

Figure 4. – Separation of *Picea chihuahuana*, *Pinus strobiformis*, *Populus tremuloides*, and *Pseudotsuga menziesii* populations by the first two coordinates in Principal Coordinates analysis. The populations codes are: TN = La Tinaja, RC = El Ranchito, CV = El Cuervo, TY = Talayote, TR = Las Trojas, VN = El Venado, LQ = La Quebrada, PPR = Paraje Piedra Rayada, QD = Quebrada de los Durán (Arroyo del Indio Ignacio), CB = Cebollitas, SJ = San José de las Causas, SB = Santa Bárbara (Arroyo del Infierno), ACH = Arroyo del Chino, and LP = La Pista (Arroyo de La Pista). N = northern, C = central, and S = southern populations.

According to the outlier analysis (BayeScan), diversifying selection should be significantly influencing 6.3% of the AFLP loci in *P. chihuahuana*, 1.4% in *P. strobiformis*, 6.5% in *P. tremuloides*, and 0.5% in *P. menziesii*. However, balancing or purifying selection should be affecting 8.3% of the AFLP loci in *P. chihuahuana*, and no AFLP loci in the other three species.

Discussion and Conclusions

SGS within plant populations has been investigated at both fine (FRELICH et al., 2003; TROUPIN et al., 2006; VALBUENA-CARABANA et al., 2007; KETTLE et al., 2011) and large scales (FRECKLETON and WATKINSON, 2002) often using spatial autocorrelation methods. In the tree populations under study here, most of the times we observed a non-significant autocorrelation for fine-scale SGS (Tables 3, 4 and 5), suggesting that the genetic variants of *P. chihuahuana*, *P. strobiformis*, *P. menziesii*, and *P. tremuloides* are randomly distributed in space within each sampled plot. Such a pattern can be explained by sufficient gene flow (both by pollen and seed) within each particular stand. Furthermore, the low population density of each tree species imposed by mixed stand structure did not form a significant family-related structure in the plots and, therefore, did not support an isolation-by-distance process at the fine scale (in 50 x 50 m plots), as observed for other wind dispersed tree species

in Europe and North America (GEHRING and DELPH, 1999; MARQUARDT and EPPERSON, 2004; VEKEMANS and HARDY, 2004; TROUPON et al., 2006). Moreover, in the study plots used here (of only 0.25 ha), the impact of uniformly-acting selection countering significant autocorrelation would probably be weak, although micro-environmental selection cannot be excluded (EPPERSON, 1992; QUINONES-PÉREZ et al., 2014). In this study, AFLP loci affected by possible balancing or purifying selection were only detected in *P. chihuahuana*. Moreover, their proportion was low with 8.3%. Previous studies in *Picea abies*, *Picea glauca* and *Pinus monticola* have reported low proportions of putative adaptive AFLP loci (2–12%) (ACHERÉ et al., 2005; NAMROUD et al., 2008; RICHARDSON et al., 2009), thus suggesting that the observed patterns are likely produced by stochastic forces. Such patterns can actually be explained by the strong inbreeding depression at early seedling stages as observed for most tree taxa (PETIT and HAMPE, 2006).

At the larger scale, the autocorrelation was highly significant for *P. chihuahuana* and *P. menziesii* (Table 6), probably as a result of limited gene flow due to the extreme population isolation and small sizes of (Fig. 3 and 4, Table 1). Such features should prompt genetic drift resulting in high genetic divergence (HAMPE and PETIT, 2005), as previously shown for *P. chihuahuana* with both allozymes and chloroplast markers (LEDIG et

al., 1997; JARAMILLO-CORREA et al., 2006). For *P. chihuahuana* and *P. menziesii* at the large scale, our results provided strong support for the theory of isolation by distance that predicts the expected pattern of SGS at drift-dispersal equilibrium (VEKEMANS and HARDY, 2004). The SGS analyze of *P. strobiformis* showed a very weak spatial distance – genetic distance relationship (Fig. 3 and 4), probably caused by a strong seed interchange, likely prompted by birds, such as the Mexican Jay (*Aphelocoma wollweberi*), and due to the continuous and broad geographic distribution of this tree species (LOONEY and WARING, 2013). However, we did not find an explanation for why *P. tremuloides* individuals in the distance class of 300 to 450 km were genetically more similar than more closely located individuals (Fig. 3). Further research might be needed to clarify such unexpected finding.

Differential selection could play a role generating the SGS found in response to environmental clines, because of the important hitchhiking effects expected at a high selfing rate in small and isolated tree populations/communities (VEKEMANS and HARDY, 2004). However, the low proportion of putatively adaptive AFLPs found in the four analyzed tree species (0.5 to 6.5%) suggests that the impact of differential selection is not important.

In view of the vital relevance of gene flow in generating, maintaining and conserving genetic diversity, understanding spatial genetic structure would be valuable in reproductive, morphological and demographic studies carried out with the aim of designing strategies for the conservation and sustainable management of the very small, isolated and rare *P. chihuahuana* tree community (WEHENKEL and SÁENZ-ROMERO, 2012; WEHENKEL et al., 2014). For example, other studies have been suggested to decrease the accumulated inbreeding in small *Picea chihuahuana* populations by introducing new genotypes (seedlings originated from seeds collected from different populations than the targeted population) (LEDIG, 2012; MENDOZA-MAYA et al., 2015). This raises the question as to whether we should mimic natural spatial structures when planting new genotypes.

The following management actions could be also helpful for the preservation of the isolated and rare *P. chihuahuana* tree community: a) *in situ* conservation by regulating livestock and controlling forest fires, b) elimination of competing vegetation (other tree species) in the vicinity of priority populations, c) establishment of artificial regeneration using reproductive local material gathered from well-selected sites outside the priority population's boundaries (but not from inside the priority population), d) increasing the size of the smallest populations by planting individuals at the endangered populations edges, and e) *ex situ* conservation along with assisted migration in response to climate change, and f) long-term seed conservation by seed storage in gene banks (WEHENKEL and SÁENZ-ROMERO, 2012; QUINONES-PÉREZ et al., 2014).

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