Isolation by distance or by resistance? Gene flow patterns of *Pinus hartwegii* in a highly human-damaged forest, in central Mexico

Jessica Juan-Espinosa[1], Lorena Ruiz-Montoya[2], Maricela García-Bautista[3], Alejandro Flores-Manzanero[4], José Luis Martínez y-Pérez[5], Bárbara Cruz-Salazar[6]*.

[1]Maestría en Ciencias Biológicas, Centro Tlaxcala de Biología de la Conducta, Universidad Autónoma de Tlaxcala, Tlaxcala, México.
[2]Departamento de Conservación de la Biodiversidad, El Colegio de la Frontera Sur Unidad San Cristóbal de las Casas,Chiapas, México.
[3]Laboratorio de Genética, El Colegio de la Frontera Sur-Unidad San Cristóbal de las Casas, Chiapas, México.
[4]Estación Científica La Malinche-Centro Tlaxcala de Biología de la Conducta, Universidad Autónoma de Tlaxcala, Tlaxcala, México.
[5]Centro de Investigación en Ciencias Biológicas, Universidad Autónoma de Tlaxcala, Tlaxcala México.
[6]Secretaría de Ciencia, Humanidades, Tecnología e Innovación-Centro Tlaxcala de Biología de la Conducta, Universidad Autónoma de Tlaxcala, Tlaxcala, México.

*Corresponding autor: Bárbara Cruz-Salazar. Email: bcruz@secihti.mx; telephone number: 246 462 1167

Abstract

Spatial genetic structure is the result of evolutionary and demographic processes that can be affected by historical and recent environmental changes. *Pinus hartwegii* is distributed in the highest areas of the Mexican mountains (>3400 m a.s.l.); most of its populations in central Mexico have been destroyed by anthropogenic activities. This research assessed the gene flow patterns of *P. hartwegii* in La Malinche National Park (LMNP), a Natural Protected Area severely affected by land use changes and forest extraction. Ten chloroplast microsatellites loci were used to determine the genetic diversity and spatial genetic structure of *P. hartwegii*. Genetic structure was studied using an Analysis of Molecular Variance and a Discriminant Analysis of Principal Components. Spatial genetic structure was evaluated by a Mantel test. Moreover, isolation by distance, and by resistance to land-use changes, and to elevation were analyzed through a partial Mantel test, based on Reciprocal Causal Modeling, and with Maximum Likelihood Population Effects models. Moran's Eigenvector Maps were used to determine genetic neighborhoods. An effect of distance and elevation on spatial genetic structure of *Pinus hartwegi*i in LMNP was detected, likely explained by pollen movement patterns, limited seed dispersal, and microenvironmental selection during germination. This information is of great value for the conservation of the species and the remaining populations in the park and other temperate forests in central Mexico.

Keywords: chloroplast microsatellites, conifer, conservation, La Malinche National Park, spatial genetic structure.

Introduction

The nonrandom spatial distribution of genotypes and alleles is defined as spatial genetic structure; it depends on the population and evolutionary dynamics throughout the history of a species (Troupin et al., 2006). Land-use changes and fragmentation reduce populations´ sizes and modify the habitat of a species, causing changes in dispersal and mating patterns that affect the balance between drift and gene flow (Vekemans and Hardy, 2004); this disturbs genetic structure and may lead to adaptation to new environments, speciation, or extinction after several generations (Wells and Young, 2002).

Limited gene flow promotes genetic structure in populations (Troupin et al., 2006). This impact is greater in plants because they are non-mobile organisms, and gene flow depends on pollen and seed dispersal (Vekemans and Hardy, 2004). In conifers, anemophilous pollination and dispersal increase the ability to reach long distances (~10 km; Molina-Sánchez et al., 2019); however, some landscape features (e.g., types of land use) could limit or favor gene flow. For instance, in *Pinus halepensis*, Troupin et al. (2006) reported that gene flow decreases with high population density because seed dispersal is restricted when wind circulation decreases due to individuals' aggregation; therefore, it could be expected that low tree density would increase the dispersal ability of some pines.

The Mexican temperate forest is the ecosystem with the second highest cover loss due to deforestation and human activities (FAO and PNUMA, 2020). La Malinche National Park (LMNP) is a Protected Natural Area (PNA) that harbors 206.07 km^2 of temperate forest in central Mexico, but undergoes high conversion from forest cover to various types of land use

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(Reyes-González and Rhodes, 2015). Although LMNP was decreed as a conservation area in 1938, human activities such as illegal logging and land use changes, have transformed > 54 % of native forests into farming areas, induced pastures and human settlements; as a result, tree species populations have decreased drastically (Rojas-García and Villers-Ruiz, 2008; George-Miranda et al., 2024).

A typical conifer of the altitudinal forest-limit in LMNP is *Pinus hartwegii*. This species is restricted to the highest areas of the mountains (3,400–4,000 meters above sea level; m a.s.l.), therefore it is adapted to cold and extreme environments and is considered one of the most vulnerable species to climate change (Viveros-Viveros et al., 2009; Alfaro-Ramírez et al., 2020). Since *P. hartwegii* dominates the high altitudes, it is able to form monospecific forests up to 4200 m. a.s.l, which are the habitat of many high mountain species, making this conifer an important species in ecological and evolutionary terms (Pérez-Suárez et al. 2022). Furthermore, it is essential for the maintenance of different ecosystem services, such as climate and wind regulation, water uptake and carbon sequestration (Alfaro-Ramírez et al., 2020). Unfortunately, *P. hartwegii* populations in LMNP have been reduced by land use changes and illegal logging (George-Miranda et al., 2024), and according to Manzanilla-Quiñones et al. (2019), the populations of this species will lose up to 19.3 % of tree density in the park due to climate change. Despite the potentially high population reduction, the genetic diversity of *P. hartwegii* has only been studied in Pico de Tancíntaro National Park, Michoacan (Viveros-Viveros et al., 2009; Loya-Rebollar et al., 2013), and in Cofre de Perote National Park, Veracruz, Mexico (Iglesias et al., 2006). These studies reported a genetic structure pattern associated with altitude, which underscores the importance of studying other *P. hartwegii* populations.

This research aimed to assess the gene flow patterns of *P. hartwegii* in a zone of high forest cover loss in central Mexico, La Malinche National Park. Considering that *P. hartwegii* has a high dispersal capacity and thrives at the top of the mountain, where wind currents are intense, we expected to detect a panmictic population, i.e., with no spatial genetic structure due to high levels of gene flow.

Materials and Methods

Study Area

La Malinche National Park (LMNP) is located in central Mexico, between coordinates 19°08'–19°20' N and 98°08'–97°55' W (Fig. 1) (CONANP, 2013). The local climate changes with altitude, but the prevailing one is temperate subhumid, with a mean annual temperature of 14 to 16 ºC and summer rains, more intense from June to September (Rojas-García and Villers-Ruíz, 2008). The local vegetation also varies with altitude, slope, and orography; the dominant vegetation types are pine, fir, and pine-oak forests, all of which can be classified as conifer forests (Rzedowski, 2006).

Pinus hartwegii is distributed between 3,400 and 4,000 m a.s.l.; the populations become denser, and the species is progressively dominant as altitude increases until it forms a monospecific forest (Franquiz-Domínguez, 2018).

Sampling

Considering safety aspects and accessibility for fieldwork in the study area, six sampling sites were selected at LMNP: three on the northern and three on the southern slopes. Three altitudinal levels (3,500, 3,750 and 3,900 m a.s.l.) were defined on each slope based on the minimum and maximum distribution limits of the species (CONANP, 2013) and an intermediate level (Fig. 1). A central point was established at each sampling site, from which two transects were laid in opposite orientations. Along each transect, vegetative material was collected from 10 pine individuals separated by at least 30 m, i.e., a total of 20 pines at each altitudinal level (120 total samples), recording their geographic location. Each sample was stored in a 1.5 mL Eppendorf tube with Tris-EDTA buffer at pH 8.0 (Sigma-Aldrich) until DNA extraction.

DNA Extraction and Amplification

Total genomic DNA was extracted from 20 to 30 mg of vegetative tissue (needles) using the CTAB method (Doyle and Doyle, 1990). Ten chloroplast microsatellites designed for *P. thumbergii* Parl. by Vendramin et al. (1996) (Pt26081, Pt30204, Pt45002, Pt63718, Pt71936, Pt87268, Pt107148, Pt107517, Pt109567, Pt110048) were then amplified (Table 1).

PCR reactions were carried out on a BIO-RAD T100TM thermal cycler, in a total volume of 14 µl (i.e., 6 µl of QIAGEN Taq PCR Master Mix, 5 µl of nuclease-free water, 1 µl of each primer (10 µM), and 1 µl of DNA), through an initial denaturation at 94 °C for 5 min, 32 cycles of denaturation at 94 °C for 1 min, an alignment step for 1 minute at a specific temperature (50 and 58.8 °C) (Table 1), and extension step at 72 °C for 1 min; the final extension step was at 72 °C for 8 min (Vendramin et al., 1996).

Definition of Haplotypes

The PCR products were visualized by capillary electrophoresis in a QIAxcel® Advanced system (QIAGEN) using the OM500 method with a 10-base pairs (bp) resolution of 100 to 500 bp for fragments. The size of fragments was determined with the ScreenGel software (QIAGEN 1.6.0.10; © 2010-2016 QIAGEN) provided by the QIAxcel® Advanced system, using the 15 bp/500 bp QX Alignment Marker and the 25–500 bp QX DNA Marker; fragments that differed in more than 10 bp were considered different (QIAGEN, 2008). The binning of the fragments was obtained with the Allelogram v. 2.2 program (Morin et al., 2009). The chloroplast can be considered a locus (Rai and Ginwal, 2018) because there is no recombination in it and inheritance is uniparental (paternal in pines) (Watano et al., 1996); therefore, in this study, haplotypes were defined as the combination of 10 microsatellite fragments (Appendix 1).

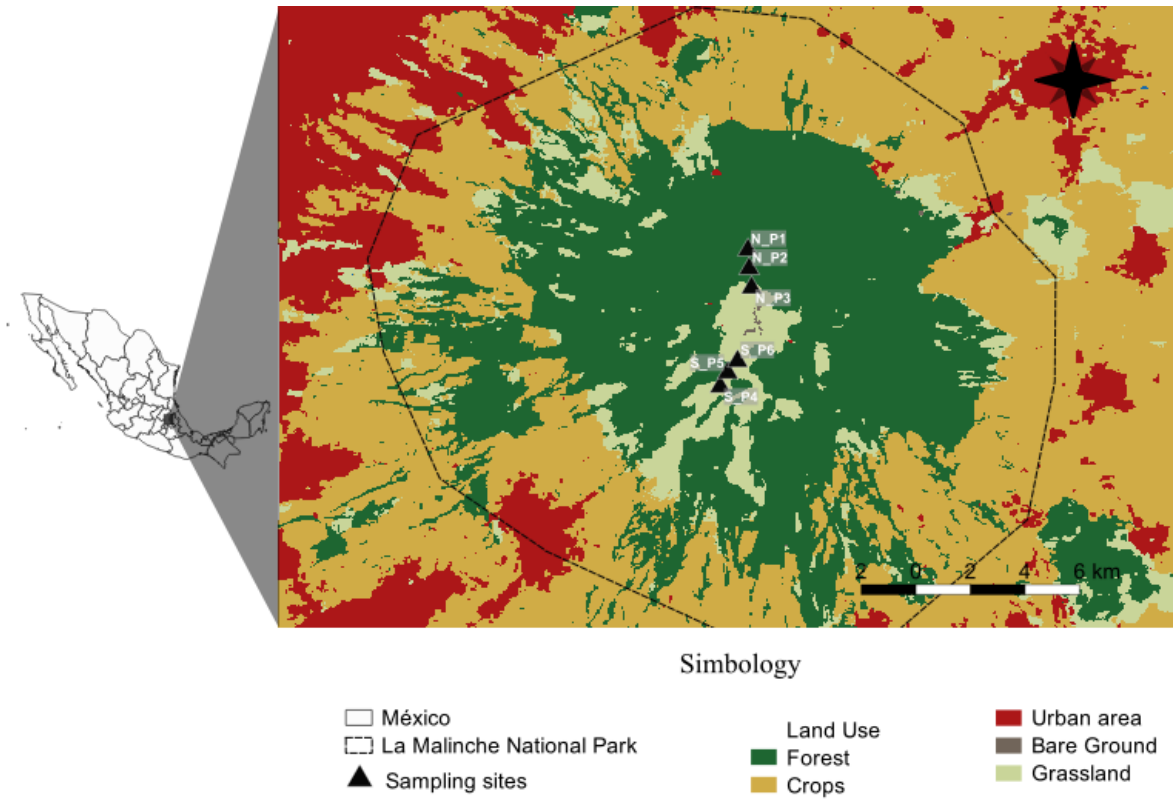

Figure 1
Sampling sites for the collection of vegetative tissue of *Pinus hartwegii* in six sampling sites on the northern (N) and southern (S) slopes in La Malinche National Park. P1 and P4, 3,500 meters above sea level (m a.s.l.); P2 and P5, 3,750 m a.s.l.; P3 and P6, 3,900 m a.s.l.

Table 1
Chloroplast DNA microsatellites loci (cpSSR) designed for *Pinus thunbergii* (Vendramin et al., 1996) and used to determine the diversity and genetic structure of *Pinus hartwegii* in six sampling sites in La Malinche National Park. bp, base pairs; Tm, alignment temperature.

Locus	cpSSR size (bp)	Tm (°C)
Pt45002	145 – 175	58.8
Pt30204	125 – 155	58.8
Pt107517	85 – 115	50
Pt63718	95 – 105	50
Pt71936	125 – 155	50
Pt110048	85 – 135	50
Pt109567	105 – 125	50
Pt87268	125 – 175	50
Pt26081	105 – 125	50
Pt107148	115 – 135	50

Data Analysis

Genetic diversity was described by the haplotype number (h), the effective number of haplotypes (eh), and haplotype diversity (Hd) using the GenAlEx v. 6.5 program (Peakall and Smouse, 2006). Differences in genetic structure between populations and slopes were studied with an Analysis of Molecular Variance (AMOVA) with 1,000 permutations based on Weir and Cockerham (1984) (poppr package); statistical significance was evaluated using 100,000 replicates with the ade4 package (Dray and Dufour, 2007). Genetic groups were identified with a Discriminant Analysis of Principal Components (DAPC) applying cross-validation with the adegenet v. 4.2.3 package in R (R Core Team, 2023) (Jombart et al., 2008; Jombart et al., 2010); this analysis is a multivariate method that weights genetic differentiation among groups for better clustering (Jombart et al., 2010).

Spatial genetic structure was explored by a Mantel test with 10,000 replicates by using the geographic distances obtained with GenAlEx v. 6.5 (Peakall and Smouse, 2006), and proportion of shared alleles (Dps; Bowcock et al., 1994) between pairs of individuals. Additionally, we tested isolation hypotheses (isolation by distance, IBD; isolation by resistance to land-use changes, IBR_{LU}; and isolation by resistance to elevation, IBR_{E}) through a partial Mantel test and Reciprocal Causal Modeling (RCM) (Cushman and Landguth, 2010), using the genetic and geographic distances previously calculated. To this end, we also constructed matrices of isolation by resistance to land-use changes (IBR_{LU}) and isolation by resistance to elevation (IBR_{E}) between pairs of individuals using the Circuitscape v. 4 program (McRae and Shah, 2009). The layers used were (1) a land-use change raster elaborated from visual categorization of land use (farming, forest, secondary vegetation, urban, and bare ground) through ArcGIS v. 10.4 (ESRI, 2018), with Landsat 5 images (30 m x 30 m resolution) downloaded from Glo Vis (https://glovis.usgs.gov); and (2) an elevation raster obtained from Digital Elevation Model (12.5 m x 12.5 m resolution) downloaded from ASF Data Search Vertex (https://search.asf.alaska.edy/#/; Alos Pasar images).

Likewise, to remove the dependence between pairwise comparisons, the factors contributing to gene flow were evaluated by performing Maximum-Likelihood Population-Effects (MLPE) models. Finally, we evaluated the presence of genetic neighborhoods with MEMGENE v. 4.2.3 in R (Galpern et al., 2014; R Core Team, 2023), which merges Moran's eigenvector maps (MEM) and regression analysis to explain the spatial genetic structure (Galpern et al., 2014). To this end, we used the Dps as the genetic dissimilarity metric, considering 10,000 permutations, an alpha = 0.05, and 90 % truncation.

Results

Genetic Diversity

A total of 103 different haplotypes were identified from a total sample of 120 trees. The effective number of haplotypes (eh) ranged from 15 to 18. The mean haplotype diversity (Hd) was 0.99 (Table 2).

Table 2

Genetic diversity of *Pinus hartwegii* in six sample sites in La Malinche National Park based on 10 chloroplast microsatellites loci (Vendramin et al., 1996). N, sample size; h, number of haplotypes; eh, effective number of haplotypes; Hd, haplotype diversity; N_P1, North slope, 3,500 meters above sea level (m a.s.l.); N_P2, North slope, 3,750 meters above sea level (m a.s.l.); N_P3, North slope, 3,900 m a.s.l.; S_P4, South slope, 3,500 m a.s.l.; S_P5 South slope, 3750 m a.s.l.; S_P6, South slope, 3,900 m a.s.l

Sampling site	N	h	eh	Hd
N_P1	20	18	17	0.99
N_P2	20	18	15	0.98
N_P3	20	18	17	0.99
S_P4	20	19	18	0.99
S_P5	20	18	17	0.99
S_P6	20	19	18	0.99
Mean		18	17	0.99

Spatial Genetic Structure

The AMOVA showed a higher genetic variation within sampling sites (99.23 %) than between sampling sites (0.76 %) (P = 0.0004), and within slopes (99.40 %) than between slopes (0.59 %) (P = 0.000). The DAPC showed only one gene cluster, whose first and second discriminant components explained 10.38 % and 7.34 % of the genetic variation, respectively (Fig. 2).

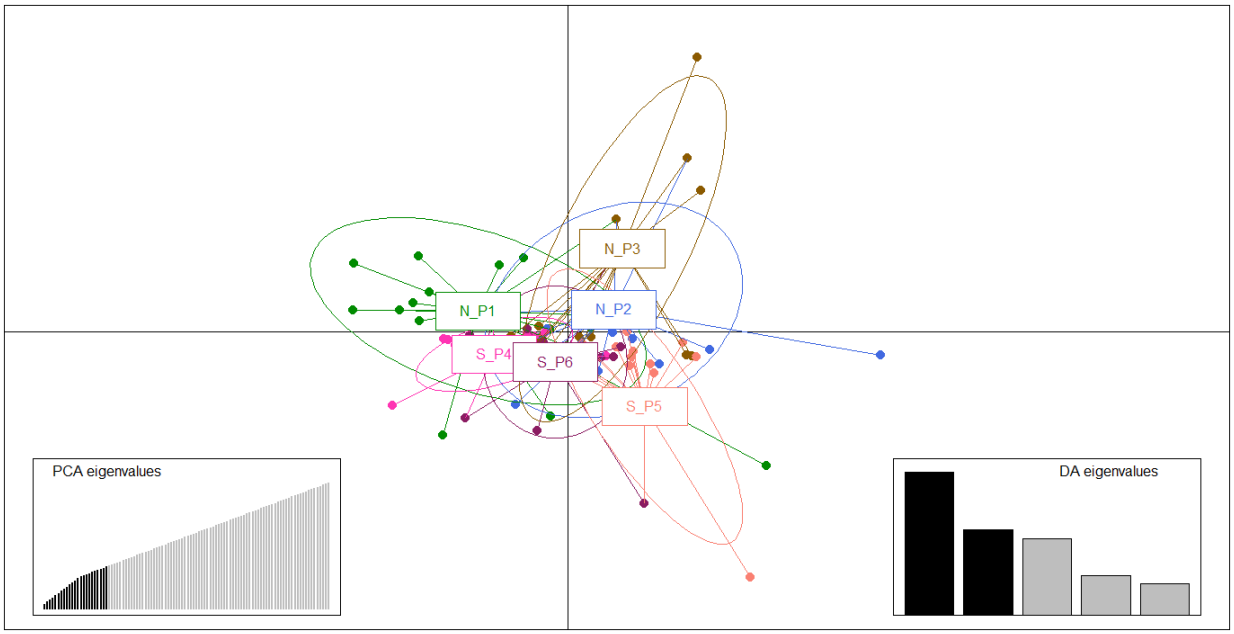

Figure 2
Discriminant Analysis of Principal Components of *Pinus hartwegii* in six sampling sites in La Malinche National Park based on 10 chloroplast microsatellites loci (Vendramin et al., 1996) using 20 PCA Eigenvalues (black bars) and two Discriminant Functions (DA Eigenvalues). N_P1, North slope, 3,500 meters above sea level (m a.s.l.); N_P2, North slope, 3,750 m a.s.l.; N_P3, North slope, 3,900 m a.s.l.; S_P4, South slope, 3,500 m a.s.l.; S_P5 South slope, 3,750 m a.s.l.; S_P6, South slope, 3,900 m a.s.l.

The Mantel test identified an isolation by distance pattern (r^2 = 0.13, P <0.001) with two-point clouds grouping the individuals, and the first flow interruption around 1.5 km distance (Fig. 3). Likewise, Partial Mantel tests and the Reciprocal Causal Model identified distance as the best explanatory variable (Table 3). Meanwhile, the MPLE model detected that genetic distances were best explained by both IBD and IBR_E (AIC = -37197.50, log-Lik = 18603.76, P < 0.01) (Table 4).

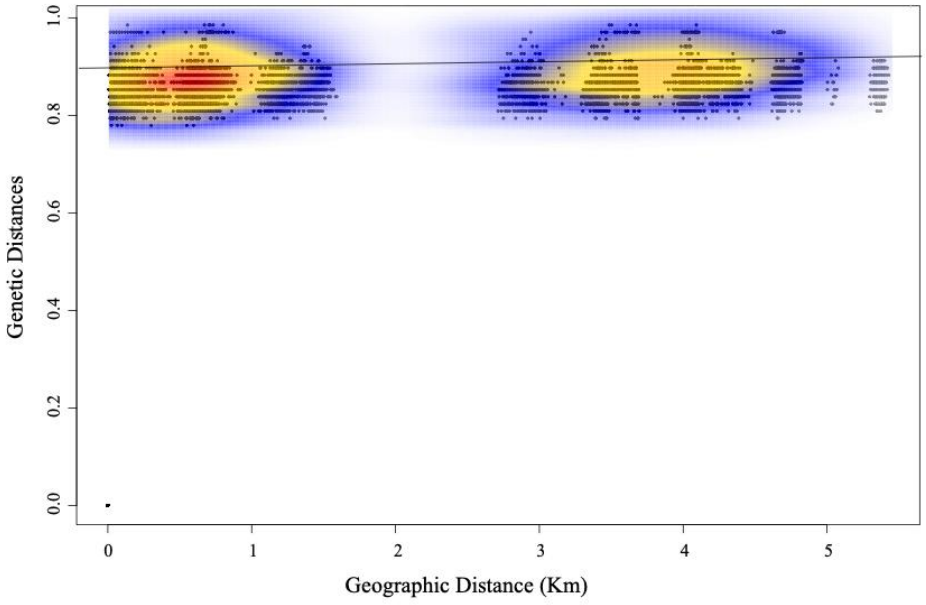

Figure 3
Mantel test to evaluate isolation by distance in six sampling sites in La Malinche National Park, based on proportion of shared alleles (Bowcock et al., 1994), estimated from 10 chloroplast microsatellites loci (Vendramin et al., 1996).

Table 3
Partial Mantel tests using Reciprocal Causal Modeling (RCM) between proportion of shared alleles (Bowcock et al., 1994) (GD), geographic distances, and ecological distances, based on land-use changes (LU) and elevation (E), between pairs of individuals of *Pinus hartwegii* in La Malinche National Park, to assess Isolation by Distance (IBD) and Isolation by Resistance (IBR). The most supported relationships for each pair of tests according to the relative RCM value are marked in bold.

Model	Partial Mantel Test	RCM
1	GD, IBR_E, IBD	-0.04
	GD, IBD, IBR_E	
2	GD, IBR_{LU}, IBD	-0.17
	GD, IBD, IBR_{LU}	
3	GD, IBR_{LU}, IBR_E	-0.21
	GD, IBR_E, IBR_{LU}	

Table 4.
Maximum-Likelihood Population-Effects models to assess Isolation by Resistance for land-use changes (IBR_{LU}) and elevation (IBR_E) and Isolation by Distance (IBD) of *Pinus hartwegii* in six sampling sites in La Malinche National Park, based on proportion of shared alleles (Bowcock et al., 1994), obtained from 10 chloroplast microsatellites loci (Vendramin et al., 1996). df, degree freedom; logLik, Model Fitted by Maximum Likelihood; AICc, Corrected Akaike Information Criteria (Hurvich and Tsai, 1991); Delta, Variance. **, P < 0.01.

Model	df	logLik	AICc	Delta
GD ~ IBR_E + IBD**	5	18603.76	-37197.50	0.00
GD ~ IBR_E + IBD + IBR_{LU}**	6	18604.22	-37196.40	1.07
GD ~ IBD + IBR_{LU}**	5	18594.53	-37179.10	18.45
GD ~ IBR_E**	4	18592.38	-37176.80	20.75
GD ~ IBR_E + IBR_{LU}**	5	18592.41	-37174.80	22.70
GD ~ IBR_{LU}**	4	18580.58	-37153.10	44.36
GD ~ IBD**	4	18567.22	-37126.40	71.06

The Mantel test identified an isolation by distance pattern (r^2 = 0.13, P <0.001) with two-point clouds grouping the individuals, and the first flow interruption around 1.5 km distance (Fig. 3). Likewise, Partial Mantel tests and the Reciprocal Causal Model identified distance as the best explanatory variable (Table 3). Meanwhile, the MPLE model detected that genetic distances were best explained by both IBD and IBR_E (AIC = -37197.50, logLik = 18603.76, P < 0.01) (Table 4).

Regarding genetic neighborhoods, only 1 % of the genetic variation was explained by the landscape, detecting seven negative Moran's eigenvectors, of which the first four MEMGENE variables explained 21.70 %, 17.20 %, 14 % and 13.20 % of the variance, respectively (Fig. 4).

Discussion

The diversity and spatial genetic structure in plants is the result of interacting ecological and evolutionary processes, particularly limited dispersal of pollen and seeds (Vekemans and Hardy, 2004). Our results showed a low but significant spatial genetic structure with a pattern of isolation by distance, and isolation by resistance to elevation in *P. hartwegii* at La Malinche National Park. In part, this could be due to the limited seed dispersal, perhaps influenced by population density (lower gene flow as higher density) and microenvironmental selection during germination and seedling establishment (Iglesias et al., 2006), since paternal inheritance of chloroplast DNA in conifers mainly reflects patterns of pollen dispersal. Intense deforestation and forest degradation in recent decades (George-Miranda et al. 2024) has not interrupted gene flow, which is consistent with our hypothesis.

The levels of genetic diversity observed in *P. hartwegii* forests of La Malinche National Park were similar to those reported in other *pine* species in which chloroplast microsatellites were also used (e.g., *P. uncinata*, Hd = 0.99; Dzialuk et al., 2009; *P. sylvestris*, Hd = 0.98; Robledo-Arnuncio and Gil, 2005). Despite its highland-restricted distribution, *P. hartwegii* showed higher genetic diversity than other species with wider distributions, such as *P. kesiya* (Hd = 0.65; Rai and Ginwal, 2018) and *P. leiophylla* (Hd = 0.76; Rodríguez-Banderas et al., 2009). This high genetic diversity recorded in *pine* species could be

explained by the characteristics of the marker used, since chloroplast microsatellites (cpSSR) show a high polymorphism due to length variability, which is the result of the expansion or contraction of the repeated region (Provan et al., 2001). Another explanation is the evidence of homoplasy in cpSSR. This process may cause the evolution of two different microsatellites lineages through independents mutational events from a similar number of repetitions (Navascués and Emerson, 2005). However, the estimation of haplotype-based indices appears to be unaffected by homoplasy, suggesting that the levels of diversity and structure obtained are trustworthy (Navascués and Emerson, 2005).

Evidence of this is the high variation detected within populations of *P. laricio* (96.6 %) and *P. sylvestris* (92.79 %) (Dzialuk et al., 2009; Bonavita et al., 2016), such as was identified by AMOVA in *P. hartwegii* (99.23 % within-sampling site, and 99.40 % within-slope). This result agrees with the distribution of genetic variation in a single group observed with the DAPC, indicating that the sampled sites correspond to a panmictic population (Excoffier and Lischer, 2010), possibly favored by the biological and life history characteristics of the species, i.e., reproductive system, high pollen dispersal capacity (~69 km reported for other pine species; Pérez-Alva et al., 2024) and longevity (up to 600 years; Villanueva-Díaz et al., 2010). Longevity can delay changes in genetic diversity and structure because the gene flow that gave rise to mature populations reflects environmental conditions spanning decades or hundreds of years (Villanueva-Díaz et al., 2015; Aavik et al., 2019). Whereas, the small study area (461.12 km2; CONANP, 2013) in relation to high dispersal of the focal species could cause the observation of only a part of the population, and avoid prohibit the detection of gene clusters, therefore it is very important to extend the study to nearby mountains where the species is distributed.

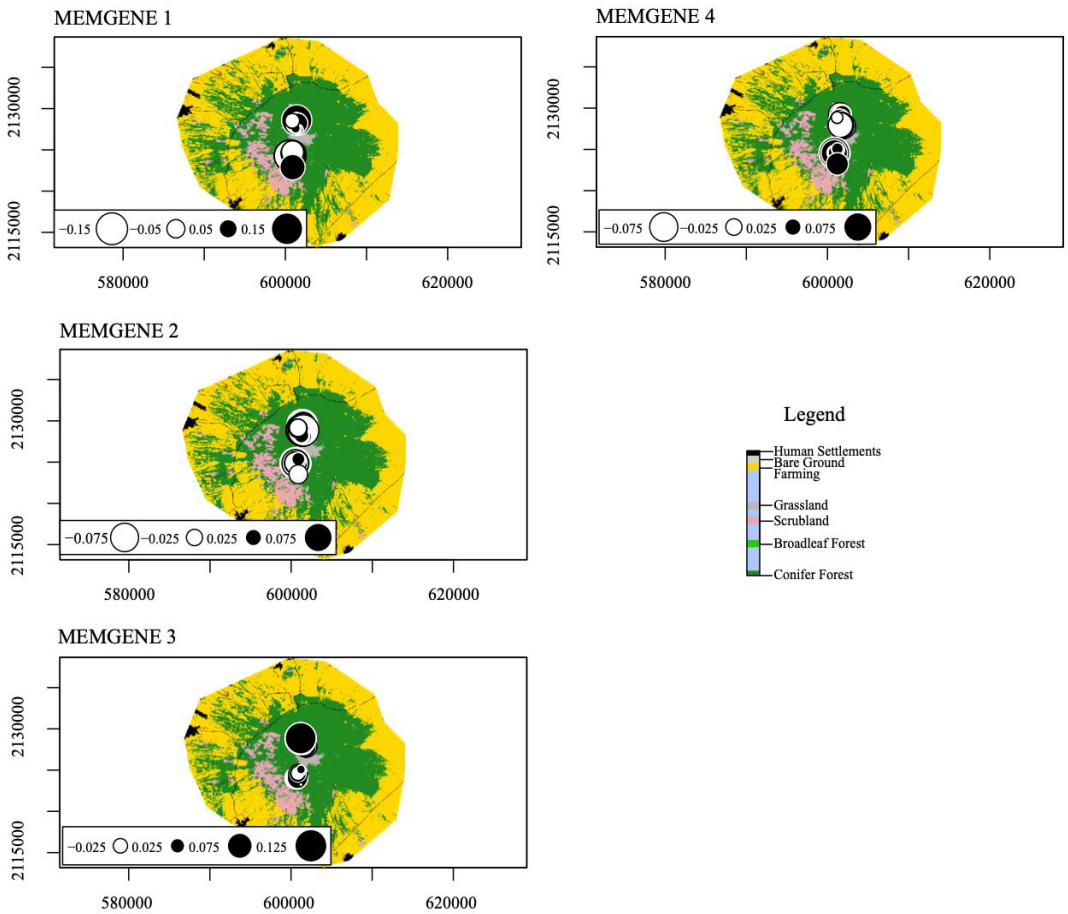

Figure 4
Spatial genetic structure based on 10 chloroplast microsatellites loci (cpSSR; Vendramin et al., 1996) of *Pinus hartwegii* in six sampling sites in La Malinche National Park using MEMGENE (Galpern et al., 2014) and projected on the land use raster. Circles depict the scores of individuals in each Moran's Eigenvectors Maps (MEM). The size and color of each circle are proportional to its positive (black) or negative (white) autocorrelation value. (a) MEMGENE 1 explained 21.70 % of the variation; (b) MEMGENE 2, 17.2 %; (c) MEMGENE 3, 14 %; and (d) MEMGENE 4, 13.2 %.

Limited gene dispersal may result in excess mating by proximity and an isolation by distance pattern (Wright, 1946). In *P. hartwegii*, an isolation by distance was detected with various methods, probably due to limited seed dispersal (Iglesias et al., 2006). In anemophilous species such as pines, limited seed dispersal generally determines spatial genetic structure (De-Lucas et al., 2009), as pollen reaches greater dispersal distances (e.g., 12.19 m for seeds vs. 112 m for pollen in *P. pinaster*; González-Martínez et al., 2006; De-Lucas et al., 2008).

Likewise, the strong effect of elevation on *P. hartwegii* gene flow coincides with Viveros-Viveros et al. (2009) and Loya-Rebollar et al. (2013), who found a high genetic differentiation associated with altitude and climate in populations from Michoacan, Mexico; climate, given by altitude, could also determine the seed establishment, and in turn the isolation by resistance to elevation we detected in *P. hartwegii*, as has also been previously identified in other conifers (e.g., Abies religiosa; Cruz-Salazar et al. 2023). In this regard, recent studies have reported that *P. hartwegii* germination rates in the study area decrease as elevation increases, due to resource limitation and habitat conditions (Franquiz-Domínguez, 2018; George-Miranda et al., 2022), therefore suggesting that microenvironmental selection may intervene in seed establishment (Vekemans and Hardy, 2004; Troupin et al., 2006).

In addition, pollen dispersal rates could be influenced by environmental factors (e.g., wind or updrafts) and biological factors (e.g., reproductive phenology) (Robledo-Arnuncio and Gil 2005; Kavaliauskas et al., 2022). Accordingly, Franquiz-Domínguez (2018) found that reproductive synchrony in *P. hartwegii* decreases at lower altitudes in LMNP. Another aspect is the proportion of female and male individuals between slopes and altitudes; for instance, on the southern slope, more individuals were reported in the female stage (>58 %) at all altitudes (Franquiz-Domínguez, 2018), whereas on the northern slope, the male stage only predominated in the high-elevation area of its distribution (71 %) (Franquiz-Domínguez, 2018); these conditions probably affect gene flow throughout the study area, which in turn promotes a panmictic population and explains the importance of orography for genetic connectivity.

Population density influences the spatial genetic structure in plants (Vekemans and Hardy, 2004). In populations with high densities, spatial genetic structure may be greater because seed dispersal is limited by a reduction in wind intensity and the interruption of seed self-rotation by collision with trees (Troupin et al., 2006). In LMNP, forest conversion to agricultural and livestock activities has resulted in an annual loss of 0.08 % of forest coverage (CONANP, 2013), and 30 % of forest remnants is fragmented (Padilla et al., 2014), which has significantly decreased tree density; the greatest *P. hartwegii* density is found on the north slope, mainly in higher-altitude populations (George-Miranda et al., 2022). Nevertheless, we did not identify an isolation by resistance to land use, and only 1 % of the genetic variation of *P. hartwegii* was structured by the landscape. The low contribution of landscape characteristics to gene flow is possibly due to the sampling design, hence we propose to increase the number of sampling sites to evaluate other resistance of surfaces (e.g., temperature, humidity, precipitation) that can influence microevolutionary processes, such as gene flow, genetic drift, or natural selection, which altogether determine genetic diversity and structure between populations or groups of individuals (Garrido-Garduño and Vázquez-Domínguez, 2013).

Since the chloroplast genome is not subjected to recombination, is inherited paternally in the Pinaceae family, and is dispersed first by pollen and then by seeds, this information does not disclose maternal gene flow or fertilization (pollination), limiting conclusions in this regard (Mogensen, 1996). However, propagation by both vectors in each generation (i.e. pollen and seeds) favors migration-drift balance more rapidly than in other genomes, making chloroplast molecular markers highly efficient to reveal recent stochastic processes (Heuertz et al 2003; Méndez-González et al., 2017; Petit et al., 2005). Despite the high genetic diversity and continuous gene flow we detected using cpSSR markers, it is suggested to include biparental molecular markers (e.g., Single Nucleotide Polymorphisms and nuclear microsatellites) and recent populations (seedlings) in future studies, which will allow assessing inbreeding levels, estimating effective population sizes, and identifying changes in genetic structuring and gene flow patterns, both paternal and maternal, over time.

This study reports high levels of haplotype diversity, a panmictic population, isolation by distance and isolation by resistance to elevation in *P. hartwegii* forest in LMNP. Distance and altitude associated gene flow are related to limited seed dispersal in some areas of the park, possibly influenced by population density and microenvironmental selection in germination and seedling establishment. This information contributes to understand the gene flow patterns and to recognize altitude and distance as factors that constrain pollen dispersal and, probably, seed establishment; however, in further research, it is a priority to evaluate the landscape effects on genetic structure, for which random sampling and a larger spatial scale are required.

Considering the interruption of gene flow associated with distance, we suggest that for restoration and/or germplasm conservation activities, the collection of seeds from sites separated by > 1.50 km should be considered in order to recover greater genetic diversity. We also recommend further studies addressing the effect of different landscape elements (e.g., land use changes, environmental traits) on gene flow at a larger scale, including different cohorts and biparental markers, and continuing population monitoring. To conserve the remaining *P. hartwegii* forest in the LMNP, it is urgent to immediately prevent further land use changes and illegal logging, as these activities are detrimental to the conservation of genetic diversity and, ultimately, the long-term survival of this species in the park.

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