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Fine-Scale Spatial Genetic Structure of Remnant Populations of *Abies religiosa*, in a Temperate Forest in Central Mexico

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Abstract

Genetic structure of a population can be molded by the resistance of the landscape or the distance between populations that function as barriers to gene flow. We analyzed the population genetic structure of *Abies religiosa* on a fine spatial scale and examined isolation models by resistance and distance. We collected vegetative tissue from populations located at the altitudinal extremes of the distribution range of the species on three slopes of La Malinche National Park (LMNP) (South, North, and East) in central Mexico. Genomic DNA was obtained using the CTAB 2X method, and eight microsatellite chloroplast loci were amplified. The genetic structure was identified based on an Analysis of Molecular Variance, a Discriminant Analysis of Principal Components with cross-validation and a spatial Principal Component Analysis using the Gabriel-type connectivity network. The isolation hypotheses were evaluated by constructing partial Mantel tests using Reciprocal Causal Modeling and Maximum Likelihood Population Effects models. A genetic structure of isolation by resistance to elevation was identified, and two genetic groups were recognized: one including populations of the South slope and the other comprising populations of the North and East slopes. We detected in *Abies religiosa* populations of the LMNP an isolation by resistance to elevation that determines the genetic structure, and the greatest genetic exchange between groups of populations located at higher altitudes. It is suggested to promote the connectivity between slopes through assisted migration and immediately halt land-use changes, as part of the actions to preserve genetic diversity in the LMPN. This study contributes to the knowledge of the spatial genetic structure of species at risk in the Mexican temperate forest for their conservation.

Keywords

fir, connectivity, conservation, la malinche national park, chloroplast microsatellites

Introduction

The Mexican temperate forest is the ecosystem ranking second in cover loss due to anthropogenic activities (Guerra-de la Cruz & Galicia, 2017; Semarnat, 2016). Loss of habitat and connectivity seriously affect genetic diversity (Aavik et al., 2019; Monteiro et al., 2019). Changes in the landscape modify the quantity, quality, and configuration of the habitat and the matrix (i.e. the dominant land cover of a landscape, e.g. forest or farming), which influence the effective population size, mating, and migration (DiLeo & Wagner, 2016). All these factors affect evolutionary processes (gene flow and genetic drift) that, as a whole, determine genetic diversity and its spatial structure

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(Goncalves et al., 2019; Herrera-Arroyo et al., 2013; Moran & Clark, 2011). After a disturbance event, often original continuous populations decrease in size and are spatially isolated; the new conditions limit gene flow between populations and increase the effects of genetic drift and inbreeding, with consequences in the levels of genetic differentiation (Evanno et al., 2009; Goncalves et al., 2019; Manel & Holderegger, 2013).

In this scenario, the isolation by resistance (IBR) model proposes that gene flow is driven by the permeability of the matrix, or an environmental or landscape characteristic (McRae, 2006; McRae & Beier, 2007). The response of a species to landscape changes depends on the organism, its life-history traits, and its landscape context (Aavik et al., 2019). For example, in species with specific environmental requirements, alterations in connectivity associated with habitat fragmentation and loss are more severe due to their relative specialization (Aldrich & Hamrick 1998). Alternatively, the distribution of genetic diversity can also be explained by the classic isolation by distance (IBD) model, which assumes that geographic distance limits genetic dispersal and flow (Wright, 1943).

La Malinche National Park (LMNP) is a Protected Natural Area (PNA) that is part of the biological corridor for gene flow between volcanoes of the Trans-Mexican Volcanic Belt (TVB) (e.g., Popocatépetl, Iztaccíhuatl, Nevado de Toluca). This area is home to a temperate forest that covers 206.07 km^2 where a great diversity of species is conserved (Domínguez-Domínguez & Pérez-Ponce de León, 2009; Fernández & López-Domínguez, 2005). The first most important forest in the LMNP is dominated by *Pinus montezumae*, and the second by *Abies religiosa* (Kunth) Schltdl. & Cham. (oyamel fir), covering an area of 13.66 km^2 (6.6%) in the habitat of high mountain species (Anderson & Brower, 1996; Snook, 1993). This forest is characterized by a high carbon uptake capacity and soil formation, making it a forest of great importance for conservation in Mexico. *Abies religiosa* populations show a discontinuous distribution in the LMNP due to severe changes in land use, intentional fires, and illegal logging affecting the habitat of this species (i.e., high humidity, low temperature, and shade; Gallardo-Salazar et al. 2019) and having fragmented at least 56% of the original forest cover in the park (López-Domínguez & Acosta, 2005; Valdéz-Pérez et al., 2016).

Some genetic studies have been carried out in *A. religiosa*. For example, Aguirre-Planter et al. (2000) identified a relationship between gene flow and geographic distances in TVB populations based on isoenzymes. Heredia-Bobadilla et al. (2012) reported a high genetic variability between populations and low levels of gene flow in populations inhabiting the Nevado de Toluca National Park, using chloroplast DNA (cpDNA; cp-trnK, cp-TRNG-trnQ) and mitochondrial DNA (mtDNA; nad-5, coz3in). Also, Jaramillo-Correa et al. (2008), through mtDNA and cpDNA, revealed that the Mesoamerican *Abies* species share a recent common ancestor and that their divergence and speciation have been determined mainly by genetic drift and isolation during warm interglacial periods. Méndez-González et al. (2017) identified a high genetic diversity and a variable population genetic structure according to the genetic marker, i.e., four genetic groups with amplified fragment length polymorphism (AFLP) and two with chloroplast microsatellites (cpSSRs). Genetic diversity, genetic differentiation between populations, and the associated factors are unknown in LMNP. For this reason, the objective of the present study was to determine whether the remaining populations of *A. religiosa* are genetically structured and, if so, what factors best explain its gene flow.

Since chloroplast microsatellites (cpSSR) (a) are highly polymorphic, (b) are of a non-recombinant genome, and (c) reveal paternal inheritance (pollen) in conifers, they are considered suitable markers for studying the genetic diversity and structure associated with demographic changes and landscape factors such as habitat fragmentation (Mittal & Dubey, 2009; Navascues & Emerson, 2005; Wheeler et al., 2014). Therefore, we consider that cpSSRs are adequate markers to explore the consequences of landscape changes on gene flow in *A. religiosa*. Since LMNP maintains a high environmental heterogeneity given the altitudinal range and changes in land use (Semarnat-Conanp, 2013), we expected to find high genetic differentiation levels between populations, explained by isolation by resistance (IBR).

This study contributes to the knowledge of the impacts of anthropogenic activities on populations of native tree species distributed in areas with heavy forest cover loss from changes in land use. This information is essential for defining conservation strategies for vulnerable temperate forest species.

Methods

The LMNP is located in central Mexico, to the east of the TVB, in the states of Tlaxcala and Puebla, between coordinates 19°08'–19°20' N and 98°08'– 97°55'. The park covers an area of 460.93 km^2 with an altitudinal range from 2,200 to 4,461 m a.s.l. (Figure 1). Depending on the altitude, the mean annual temperature varies between 2 °C and 17 °C, with rains in summer (Fernández & López-Domínguez, 2005). The local vegetation also varies according to altitude, slope, orography, and anthropogenic activities; the dominant species are *Pinus montezumae*, *A. religiosa*, *Pinus hartwegii*, *Pinus leiophylla*, and *Alnus jorullensis* (Rojas-García & Villers-Ruíz, 2008). The predominant land use is farming, followed by forest and secondary vegetation (Figure 1c).

Vegetative Tissue Collection

We toured three slopes of the LMNP that are relatively easy to access (North, East, and South) because of the orography and the type of ownership (social) of agricultural plots (López-Téllez et al., 2019), aiming to identify the relative abundance of the species within its altitudinal range (2400–3600 m a.s.l.; Sáenz-Romero et al., 2012). Two collection sites were selected on each slope, which were assumed to be distinct populations located at the upper and lower limits of its altitudinal range, with a total of six populations throughout the LMNP (2 populations × 3 slopes) (Figure 1). We established a central point in each population, from which two transects were drawn in opposite orientations. Along each transect, vegetative material was collected from 20 individuals separated by at least 30 m. The geographic location of each individual was recorded; then, young undamaged needles were collected, transported, and stored in 1.5 mL Eppendorf tubes with Tris-EDTA pH 8.0 (Sigma-Aldrich) buffer.

Laboratory

Tissue samples were first ground with liquid nitrogen (Doyle, 1990); afterward, genomic DNA was extracted using the CTAB 2X method. The extractions were visualized in 1% agarose electrophoresis to determine their viability. Unsuccessful samples

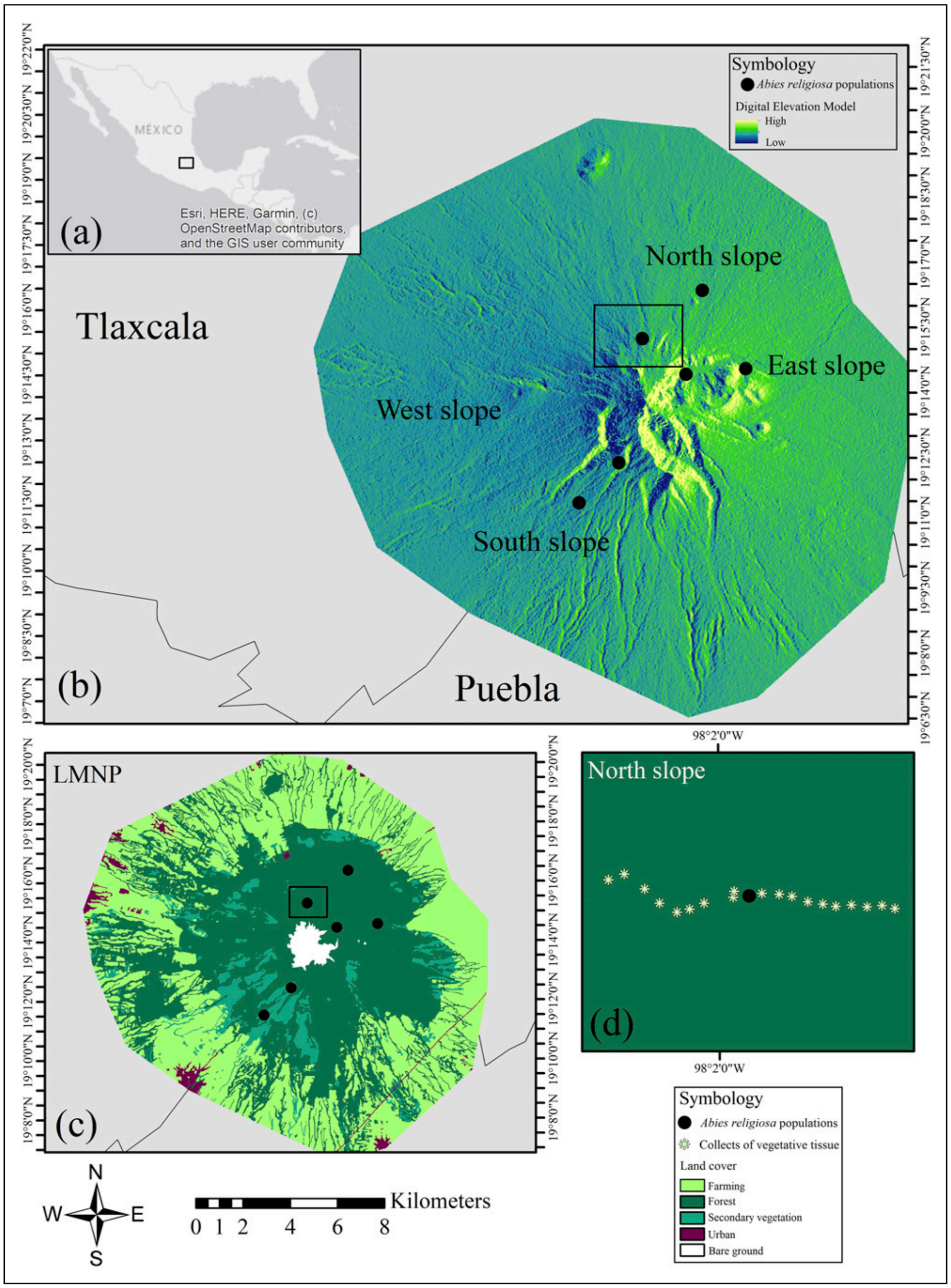

Figure 1. Location of the populations of *Abies religiosa* in La Malinche National Park used for collecting vegetative tissue and describing the spatial genetic structure.

were purified using Wizard ® SV Minicolums (Promega) columns and Wash Buffer 2 (Qiagen) following the vendor-standardized procedure, although modifying the concentrations to improve the result.

Ten chloroplast microsatellites (cpSSR) designed for *Pinus thunbergii* and *P. leucodermis* were amplified (Vendramin et al., 1996). Amplifications were run with a final volume of 14 µl using the Master Mix (*Taq* DNA Polymerase; Qiagen) solution in a T100 Touch^{TM} Thermal Cycler (Bio-Rad). The PCR conditions proposed by Vendramin et al. (1996) were modified: initial denaturation at 95 °C for 5 min, then 32 cycles with denaturation at 94 °C for 1 min, alignment at 50 °C–58 °C for 1 min (see Table 1), followed by extension at 72 °C for 1 min. The final extension step was at 72 °C for 8 min. The PCR product was read by capillary electrophoresis (QIAxcel, Qiagen) using the method OM500, with a 10-bp resolution for fragments of 100–500 bp. Amplicons were considered different when they were >10 bp (Qiagen, 2008); amplicon size was determined with the ScreenFel software (Qiagen v 1.0.2.0; Ambion Inc., Austin TX) provided by the QIAxcel system, using the 15 bp/500 bp QX Alignment Marker and the 25–500 bp QX DNA Marker. The binning of the fragments obtained was carried out with the program Allelogram v. 2.2 (Morin et al., 2009).

Genetic Diversity

Since the chloroplast has a haploid genome, it has paternal inheritance in conifers and is not subject to recombination (Neale & Sederoff, 1989; Watano et al., 1996); hence, it can be considered as a locus (Rai & Ginwal, 2018). It can also be perceived as a circular haploid chromosome in which sequence variation generates different alleles within individual non-recombinant loci (Echt et al., 1998). Therefore, this study analyzed genetic diversity based on haplotypes formed by combining fragments from the eight successfully amplified microsatellite loci (cpSSR) (Rai & Ginwal, 2018). Genetic diversity was described through the number of observed haplotypes (H_a), number of effective haplotypes (H_e), Shannon-Weiner index (I), haplotype diversity (h), and genetic distances (Nei, 1987), using the program GenAlEx v. 6.4 (Peakall & Smouse, 2006).

Genetic Structure

Genetic structure was evaluated between populations with F_{st} index, based on Weir and Cockerham (1984), by Analysis of Variance Molecular (AMOVA) routine with 1000 permutations and using ARLEQUIN v. 3.1 (Excoffier and Lischer, 2010). The pattern of spatial genetic variation was evaluated using a Discriminant Analysis of Principal Components (DAPC) with cross-validation (Jombart et al., 2010). Connectivity was determined by constructing a spatial Principal Components Analysis (sPCA) (Jombart et al., 2008) through a Gabriel-type network. This network was selected because it has moderate saturation, which makes networks more informative than saturated ones (Dyer & Nason, 2004; Naujokaitis-Lewis et al., 2013). All analyses were carried out

Table 1. Loci of Chloroplast Microsatellites (cpSSR) Designed for *Pinus Thunbergii* and *P. Leucodermis* (Vendramin et al., 1996) Used to Estimate the Genetic Diversity and Structure of *Abies Religiosa* in La Malinche National Park, Mexico. AT, Alignment Temperature; ✓, Successfully Amplified; ×, Not Amplified.

Locus	Sequence (5'–3')	AT (°C) in *Abies religiosa*	Amplified
Pt26081-F	CCCGTATCCAGATATACTTCCA	58	✓
Pt26081-R	TGGTTTGATTCATTCGTTCAT		
Pt30204-R	TCATAGCGGAAGATCCTCTTT	58	✓
Pt30204-F	CGGATTGATCCTAACCATACC		
Pt45002-F	AAGTTGGATTTTACCCAGGTG	50	×
Pt45002-R	GAACAAGAGGATTTTTTCTCATACA		
Pt63718-F	CACAAAAGGATTTTTTTTCAGTG	50	✓
Pt63718-R	CGACGTGAGTAAGAATGGTTG		
Pt71936-F	TTCATTGGAAATACACTAGCCC	58	✓
Pt71936-R	AAAACCGTACATGAGATTCCC		
Pt87268-F	GCCAGGGAAAATCGTAGG	50	✓
Pt87268-R	AGACGATTAGACATCCAACCC		
Pt107148-F	GTTCATTCGGGATCCTTAAAA	50	✓
Pt107148-R	GTACTTTCCTTCAGCCAATCTG		
Pt107517-F	AAAGCTTTTATTGCGGCC	58	×
Pt107517-R	ATGGCAGTTCCAAAAAAGC		
Pt109567-F	TATTATCGAACAACGAGAATAATCC	58	✓
Pt109567-R	TCACTGTCACTCTACAAAACCG		
Pt110048-F	TAAGGGGACTAGAGCAGGCTA	58	✓
Pt110048-R	TTCGATATTGAACCTTGGACA		

with the software R v. 4.0.3 (packages adegenet, akima, poppr, ResistanceGA, and tess3r; R Core Team, 2020).

To evaluate the hypotheses of isolation, we constructed matrices of resistance to land-use changes ($IBR_{l\text{-}uc}$) and elevation (IBR_e) between pairs of populations, with the program Circuitscape v. 4 (McRae & Shah, 2009). To this end, we elaborated a raster from the visual categorization of land use (farming, forest, secondary vegetation, urban, bare ground) with the program ArcGIS v. 10.4 (ESRI, 2018), from Landsat 5 images (30 m × 30 m resolution) downloaded from GloVis (https://glovis.usgs.gov). And for altitude, we obtained an elevation raster of the study area using the Digital Elevation Model downloaded from ASF Data Search Vertex (https://search.asf.alaska.edu/#/; Alos Pasar images, 12.5 m resolution). In the case of the IBD, we used linear geographic distances between population pairs calculated with the program GenAlEx v. 6.4 (Peakall & Smouse, 2006).

Once the matrices of each isolation hypothesis were constructed, partial Mantel tests were performed with the genetic distances (Nei, 1987), using Reciprocal Causal Modeling (RCM) to avoid erroneous conclusions due to the high correlation of alternative models (Cushman et al., 2006; Cushman & Landguth, 2010). In addition, to identify the factors that contribute to gene flow, Maximum Likelihood Population Effects (MLPE) models were performed, these linear random effects models account for the lack of independence between pairwise comparisons (Row et al. 2017; Burgess & Garrick 2020). The analyses were carried out using the software R v. 4.0.3 (packages MuMIn, vegan, and ComMLPE; R Core Team, 2020).

Results

Of the ten microsatellites tested, successful amplifications were obtained for only eight (Table 1). Of these, we identified 27 fragments or alleles that formed a total of 116 haplotypes; of these, only four haplotypes were shared among individuals.

The number of observed haplotypes (H_a) and the number of effective haplotypes (H_e) were higher in the populations inhabiting the East and North slopes ($H_a = 20$, $H_e = 20$) and lower in the populations of the South slope ($H_a = 19$, $H_e = 18$). The Shannon-Weiner index (I) ranged from 2.93 in the two populations of the South slope to 2.99 in the populations of the East and North slopes. Haplotype diversity (h) was high in all populations (h = 0.94–0.95) (Table 2).

The AMOVA identified the highest percentage of variation within populations (97.11%), and a low but significant genetic differentiation (F_{st} = 0.03, P = <0.05). The DAPC grouped together the two populations of the South slope and the high-altitude population of the North slope (NP4); another group included the populations of the East slope (EP1, EP2) and the low-altitude population of the North slope (NP3) (Figure 2). Separately, the connectivity analysis identified a global spatial structure (max(t)= 0.007, P = 0.05), and the connectivity network identified two genetic groups: one including the populations of the South slope (SP5 and SP6) and the other the populations of the East and North slopes (Figure 3).

Table 2. Genetic Diversity of Six Populations (Pop) of *Abies religiosa* in La Malinche National Park, Based on Eight Chloroplast Microsatellites (Vendramin et al., 1996). H_a, Number of Observed Haplotypes; H_e, Number of Effective Haplotypes; I, Shannon-Weiner Index; h, Haplotype Diversity.

Slope	Altitude	Pop	H_a	H_e	I	h
East	3126	EP1	20	20	2.99	0.95
	3440	EP2	20	20	2.99	0.95
North	3131	NP3	20	20	2.99	0.95
	3554	NP4	20	20	2.99	0.95
South	3128	SP5	19	18	2.93	0.94
	3551	SP6	19	18	2.93	0.94
		Mean	20	19	2.97	0.95

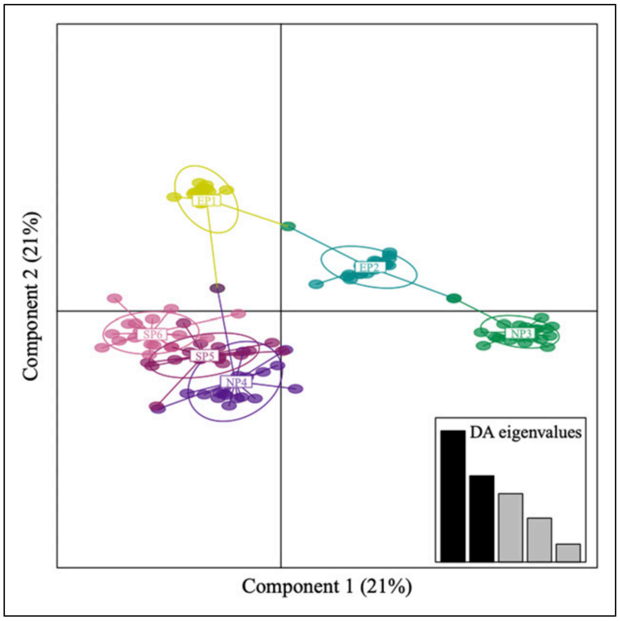

Figure 2. Discriminant Analysis of Principal Components of six populations of *Abies religiosa* in La Malinche National, based on eight chloroplast microsatellites (Vendramin et al., 1996). DA, discriminant analysis; EP1, low-altitude population of the East slope; EP2, high-altitude population of the East slope; NP3, low-altitude population of the North slope; NP4, high-altitude population of the North slope; SP4, low-altitude population of the South slope; SP5, high-altitude population of the South slope.

The partial Mantel tests showed a statistically significant association between genetic distances (GD) and elevation (IBR_e) (Table 3). The Reciprocal Causal Model detected altitude as the best explanatory variable, followed by distance (Table 4). Likewise, the MPLE determined that the model where genetic distances are explained by IBR_e is the best model (AICc = -60,088, logLik = 36.04, P = < 0.001; Table 5). The contribution of variables to explain GD was

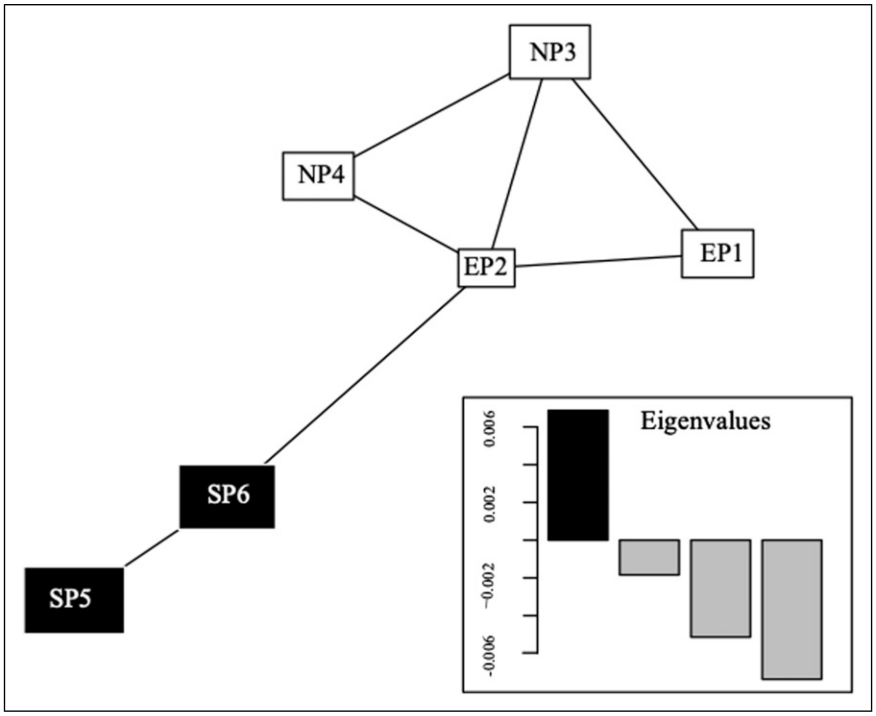

Figure 3. Spatial Principal Component Analysis (sPCA) using the Gabriel-type connectivity network of six populations of *Abies religiosa* in La Malinche National Park, based on eight chloroplast microsatellites (Vendramin et al., 1996). EP1, low-altitude population of the East slope; EP2, high-altitude population of the East slope; NP3, low-altitude population of the North slope; NP4, high-altitude population of the North slope; SP4, low-altitude population of the South slope; SP5, high-altitude population of the South slope. The color of boxes indicates genetic groups.

Table 3. Partial Mantel Tests Between Genetic Distances (GD) of Pairs of Populations of *Abies religiosa* in La Malinche National Park and Isolation by Landscape Resistance for Land-Use Changes ($IBR_{l\text{-}uc}$) and Elevation (IBR_e), and Isolation by Geographic Distance (IBD). *y*, Dependent Variable, Genetic Distances; *x*, Explanatory Variables for Genetic Distances; z, Controlled Variables for Partial Mantel Tests.

	y, GD		
x	z, IBD	z, IBR_e	z, $IBR_{l\text{-}uc}$
$IBR_{l\text{-}uc}$	—	0.26	-0.42
IBD	0.28	—	0.28
IBR_e	0.69*	0.63*	—

Table 4. Partial Mantel Tests, with Reciprocal Causal Modeling (RCM) of Genetic Distances (GD) Between Populations of *Abies religiosa* in La Malinche National Park, Based on Eight Chloroplast Microsatellites, and Isolation by Resistance for Land-Use Changes ($IBR_{l\text{-}uc}$) and Elevation (IBR_e), and Isolation by Distance (IBD). The Most Supported Relationships for Each Pair of Tests According to the Relative Value of the Reciprocal Causal Modeling are Marked in Bold.

Model	Parcial mantel test	RCM
1	**A ← (GD, IBR_e, IBD)** B ← (GD, IBD, IBR_e)	0.344
2	C ← (GD, $IBR_{l\text{-}uc}$, IBD) **D ← (GD, IBD, $IBR_{l\text{-}uc}$)**	-0.024
3	E ← (GD, $IBR_{l\text{-}uc}$, IBR_e) **F ← (GD, IBR_e, $IBR_{l\text{-}uc}$)**	-1.108

75% for IBR_e and 20% for IBD in the MPLE. $IBR_{l\text{-}uc}$ was not detected with either analysis (Tables 4 and 5).

Discussion

We identified a spatial genetic structure in the populations of *A. religiosa* inhabiting LMNP, probably resulting from isolation by resistance to elevation. Gene flow is seemingly not prevented by changes in land use since high functional connectivity (gene flow performed) was observed between the populations of the East and North slopes; however, the evident isolation of the populations of the South slope is explained by the increase in genetic drift as a consequence of the reduction in population size.

The present study did not detect isolation by resistance associated with land-use changes ($IBR_{l\text{-}uc}$), despite the fact that a continuous and drastic change in land use occurs in the study area, likely because the effects of anthropogenic disturbance are delayed in conifer species due to their longevity (Aavik et al., 2019). Land-use changes disrupt the spatial

Table 5. Maximum Likelihood Populations Effects Models to Assess Isolation by Resistance for Land-Use Changes ($IBR_{l\text{-}uc}$) and Elevation (IBR_e), and Isolation by Distance (IBD) of Six Populations of *Abies religiosa* in La Malinche National Park, Based on Eight Chloroplast Microsatellites. logLik, Model Fitted by Maximum Likelihood; AICc, Corrected Akaike Information Criteria (Hurvich & Tsai, 1989); Δ, Variance.

Model	Formula	logLik	AICc	Δ
1	$GD \sim IBR_e$***	36.04	-60.09	0
2	$GD \sim IBR_e + IBR_{l\text{-}uc}$**	37.51	-58.36	1.73
3	$GD \sim IBD$***	34.72	-57.44	2.65
4	$GD \sim IBR_e + IBD$***	36.04	-55.42	4.67
5	$GD \sim IBD + IBR_{l\text{-}uc}$**	35.52	-54.38	5.71
6	$GD \sim IBR_{l\text{-}uc}$*	32.74	-53.48	6.61
7	$GD \sim IBR_e + IBD + IBR_{l\text{-}uc}$***	37.98	-53.46	6.63

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

continuity of the habitat, which inevitably decreases population size and isolation, both of which reduce gene flow and increase genetic drift, thus impacting genetic variation and differentiation (Eckert et al., 2010; Leroy et al., 2018). Conifers usually show high genetic diversity and low genetic differentiation with nuclear markers due to anemochoric dispersal. However, this is not the case with single-parent genomes such as chloroplast DNA, in which the effective population size is approximately half that of nuclear genes, reducing the time to fix an allele because of the greater influence of genetic drift (Clark et al., 2000). In the family Pinaceae, the chloroplast genome is subject to paternal inheritance and dispersed first by pollen and later by seeds. As a result, propagation by both vectors in each reproductive cycle means that the migration-drift balance is reached more rapidly in chloroplast than in other genomes (e.g., nuclear, mitochondrial) (Mogensen, 1996); consequently, markers efficiently reveal recent stochastic processes (Heuertz et al., 2003; Méndez-González et al., 2017; Petit et al., 1993, 2005), which could suppose the observation of the demographic dynamics associated to the resistance of the landscape.

In *A. religiosa*, each generation comprises about 27 years since sexual maturity is reached at 17 to 25 years, and the cycle from pollen release to seed takes another two years (Mantilla, 2006; Ortiz-Bibian et al., 2019). The detected genetic structure may not reveal the actual effect of land-use changes, so it would be important to include recent generations (i.e., seedlings) to determine potential changes in connectivity.

Despite we do not rule out the possible underestimation of genetic diversity due to genotyping technique used (capillary electrophoresis) which does not have a detection of variation < 10 bp (Qiagen, 2008), this study detected a high haplotype diversity ($h = 0.94–0.95$), at levels consistent with the results of previous studies using chloroplast microsatellites in *Abies* species. For example, Clark et al. (2000) reported an *h* value of 0.95 in *A. balsamea*, whereas Parducci et al. (2001) reported *h* values of 0.93–0.99 in *A. alba*, 0.97 in *A. numidica*, and 0.98–1.0 in *A. cephalinica*. For their part, Jaramillo-Correa et al. (2008) found haplotype diversity values of 0.93 in *A. gualmensis*, 0.94 in *A. hickeli*, and 0.91 in populations of *A. religiosa* in the TVB; the last data is similar to the one reported by Méndez-González et al. (2017) in populations of this same species at El Ajusco, in central Mexico ($h = 0.94$).

An important result of our research is the lower haplotype diversity identified in the populations of the South slope compared to *h* values in populations of the East and North slopes, which may be due to a more intense genetic drift associated with the significant reduction in population size (Leroy et al., 2018; Song et al., 2006); it is possible that the climatic conditions of the North and East slopes that favor the best development of *A. religiosa* populations (Allende et al., 2016) help to maintain genetic diversity in these populations. An intense change in land use has been observed in the LMNP since the decade of 1980 (Ríos, 2014). For example, Ern (1976) reported associations between *A. religiosa* and other conifers in areas where the former is currently absent (Cruz-Salazar, 2021, personal observation). The loss of tree cover, including *A. religiosa* forests, is even more serious on the South slope, where there has been uncontrolled illegal logging (López-Téllez et al., 2019; Rojas-García and Villers-Ruiz, 2008; Valdez-Pérez et al., 2016). This activity has dramatically reduced populations of tree species of economic interest, affecting the microhabitat conditions necessary for germination and recruitment of these species, particularly those that need shade and moisture, such as *A. religiosa* (e.g. shade, humidity) (Gallardo-Salazar et al., 2019; Rzedowski, 2006).

The structure genetic observed in this study is possibly due to the exchange of genes through pollen. In conifers, pollen dispersal can reach up to 10 km (Molina-Sánchez et al., 2019), while seeds reach only 31 m in *A. alba*, transported mainly by wind and some animals (Briggs et al., 2009; Ortiz-Bibian et al., 2019). At elevated sites such as volcanoes, anemochoric dispersal depends on regional wind patterns resulting from the global variation of wind regimes, which either facilitates or restrains the displacement of pollen and seeds (Kling & Ackerly, 2020). In the LMNP, the dominant winds flow from the southeast during autumn and winter and from the northeast in spring and summer (Fernández & López-Domínguez, 2005); this may result in constant gene flow and low genetic structure despite the changes in land use and the obvious geographic barriers (e.g. Barranca Grande).

In *A. religiosa*, sexual structures are produced in winter (December), while pollination takes place in spring (April-June), followed by seed dispersal (October-December). However, the limited period of pollen dispersal (from six days up to one month) and the lack of formation of female cone production due to limited resources may prevent effective cross-pollination and increase inbreeding levels (Mantilla, 2006). Likewise, it has been reported that low population density decreases seed viability and germination percentage with influence on inbreeding (Ortiz-Bibian et al., 2019); in this study low population density may be due to deforestation or habitat loss but we do not rule out the effect of sampling conducted at the extremes of the altitudinal distribution of the species, where population density might decrease.

Our results indicate a spatial genetic structure determined mainly by IBR to elevation and two genetic groups; these findings are consistent with those reported by Méndez-González et al. (2017) in populations of El Ajusco, Mexico, based on chloroplast microsatellites. Interestingly, the populations inhabiting the South slope of the LMNP were genetically connected with the higher-altitude populations of the other slopes, suggesting that dispersal seemingly occurs through the volcano peak. This assumption is supported by the presence of an important geographic barrier (Barranca Grande) between the East and South slopes (Semarnat-Conanp, 2013), which possibly limits gene flow.

Implications for Conservation

Studying landscape resistance to gene flow is fundamental for the management and conservation of viable populations in modified landscapes (Oyler-McCance et al., 2013). A management unit is a group of populations that maintain a constant gene flow (Barbosa et al., 2009; Coates et al., 2018). In the present study, we detected two management units, one formed by the populations of the South slope and the other by the populations inhabiting the East and North slopes. Although land-use changes do not seem to determine isolation, these are a threat that will eventually affect the population genetic structure. Therefore, starting assisted migration planning is advisable (Sáenz-Romero et al., 2012) to decrease inbreeding and promote the conservation of the genetic diversity of *A. religiosa* in the LMPN, as it has occurred in other conifers species (e.g. Mendoza-Maya et al 2015). Assisted migration may consist of two steps: (1) migration between populations with the least genetic differentiation, i.e., between the high-altitude populations of the East and North slopes and the populations of the South slope; and (2) migration between populations with the greatest genetic differentiation, that is, between low-altitude populations inhabiting the East and North slopes and populations of the South slope. In addition, it is essential to obtain information from other temperate forests in the region (e.g., Popocatépetl, Iztaccíhuatl, Mount Tlaloc) to set out conservation strategies for the oyamel fir at a regional level.

It is also essential to immediately halt land-use changes throughout the LMNP, especially on the South slope, because pollen scarcity in small, isolated populations increase self-pollination and inbreeding (Morales-Velázquez et al., 2010; Ortiz-Bibian et al., 2019). Otherwise, habitat loss and population decline of tree species in LMNP (Ern, 1976; López-Domínguez and Acosta, 2005; Valdez-Pérez et al., 2016) may adversely affect recruitment and genetic structure of recent populations because these conditions enhance genetic drift (Waples, 2016).

This study reports a spatial genetic structure derived from isolation by resistance to elevation and two genetic groups that show a clear separation of the populations inhabiting the South slope, which may indicate a greater effect of genetic drift on these. The insignificant effect of IBR to land-use changes on the genetic diversity of *A. religiosa* is possibly due to the late effect of fragmentation on the genetic diversity of long-live species or anemochoric dispersal that can preserve pollen movement despite high fragmentation. However, it is imperative to limit changes in land use to conserve the habitat required by this species and thus ensure the establishment of future populations. In addition, in order to define conservation strategies focused on the conservation and promotion of connectivity, it is necessary to include populations data from other temperate forests of the TVB. The information reported herein is highly relevant for the conservation of *A. religiosa*, the associated fauna, and the ecosystem services provided by the temperate forest of central Mexico.

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Data Availability Statement

Haplotypes of chloroplast microsatellite loci: Dryad doi:10.5061/dryad.18931zd22 (https://datadryad.org/stash/share/Xsy9_dWK4_ic1XmDP8xpupQ1yMeFpKMJN7m8aS_AjDU).

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