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Abstract

Forests have been globally reduced due to unprecedented rates of deforestation and habitat transformation, which in turn affects the species' genetic structure. Despite harboring a high endemism, temperate forests are the second most degraded terrestrial ecosystem in Mexico. Therefore, understanding the spatial genetic patterns is fundamental to implement management and conservation efforts that contribute with their long-term persistence. By using Single Nucleotide Polymorphisms, this study evaluated the fine-scale spatial genetic structure and the effect of landscape features on gene flow of two conifers, *Abies religiosa* and *Pinus montezumae*, each with different successional affinity, in a temperate forest of central Mexico exhibiting high landscape heterogeneity due to topography and land-use changes. Based on successional affinity of species we expected isolation by resistance in *Abies religiosa* and a panmictic population in *Pinus montezumae*. We analyzed the genetic structure and the effects of altitude, Aspect and land-use changes on the genetic connectivity patterns. A weak isolation by distance pattern was detected in *A. religiosa*, while the spatial component explained only the 3% of its genetic variation. In *P. montezumae*, a very low structure signal was detected between slopes. Overall, the analyses revealed an absence of genetic structure in both species, i.e., no landscape predictor had an effect on gene flow between the populations. We recommend increasing the spatial extent and the assessment of more environmental predictors to further support the study of genetic structure patterns in the species studied. Our study contributes with information for the conservation of Mexican temperate forests.

Keywords: conservation; gene flow; landscape genomics; temperate forest; SNPs.

Introduction

Despite being represented in over 30% of the Earth's surface, forests have been dramatically reduced worldwide mainly due to deforestation and habitat transformation (FAO 2015), which in turn affects the distribution and levels of species' genetic diversity. The isolation and spatial reduction of populations could have profound demographic and negative genetic consequences for species (Li et al. 2021). Landscape heterogeneity caused by land-use changes can prevent gene flow by creating geographic barriers or a matrix of unsuitable habitats across which dispersal cannot occur (Bisbing et al. 2021). Environmental changes

influence the population size, mating, and dispersal of species according to the extent, quality, and configuration of the habitat and the landscape matrix (DiLeo and Wagner 2016; Monteiro et al. 2019).

Landscape and environmental characteristics influence population genetic differentiation and spatial genetic structure (Li et al. 2021). Given the sessile life form of plants, pollen and seed dispersal account for gene flow within and among populations, depending on habitat preferences and dispersal vectors (Cruzan et al. 2020). For this reason, plants are highly sensitive to habitat loss and fragmentation. When a population becomes more isolated, pollen and seed dispersal are restricted within patches, leading to increased relatedness among individuals and a higher genetic structure (Wells and Young 2002; Rico and Wagner 2016).

For decades, the genetic structure has been explained as a result of isolation by distance (IBD); this theory expects genetic differentiation to increase with geographic distance (Wright 1943). Alternatively, it has been proposed that gene flow may be driven by matrix permeability or landscape features (isolation by resistance; IBR), regardless of geographic distance (McRae 2006; McRae and Beier 2007), or by geographic barriers (IBB) that determine gene movement (DiLeo and Wagner 2016). Since eco-evolutionary dynamics are affected by the environment (Allen et al. 2017; Castilla et al. 2019), with implications for the maintenance and conservation of species, it is imperative to assess how habitat loss and fragmentation influence genetic structure in modified landscapes.

Mexican temperate forests are ecosystems undergoing high loss of vegetation cover due to changes in land use (0.8 % per year) (SEMARNAT 2016; Guerra-De la Cruz and Galicia 2017). In central Mexico, La Malinche National Park (LMNP) is a Protected Natural Area considered the country's most isolated mountain (SEMARNAT-CONANP 2013), home to a great diversity of temperate forest species (Fernández and López-Domínguez 2005; Domínguez-Domínguez and Pérez-Ponce de León 2009). Unfortunately, the LMNP has lost 62 % of the original forest cover, and at least 56 % of the current vegetation is fragmented (López-Domínguez and Acosta 2005; Valdez-Pérez et al. 2016). Despite the great ecological and scientific importance of the LMNP, only forest structure (Rojas-García and Villers-Ruiz 2008) and the physiological aspects of some tree species have been studied (Franquiz 2016; Portillo 2017; Franquiz 2018; Montero 2020; George-Miranda et al. 2022).

Since conifers are the main vegetation type of temperate forests, being the habitat of multiple species, supporting ecosystem services (Rzedowski 2006), and having a high dispersal capacity by pollen, they are an interesting target group to investigate genetic differentiation in modified landscapes (Mosca et al. 2014). This study aimed to assess the fine-scale spatial genetic structure of two conifers with contrasting successional affinity (*Abies religiosa* and *Pinus montezumae*) and study the effect of landscape features on gene flow, using LMNP as a case system.

Considering that habitat specialization can influence gene flow in plants (Cruzan et al. 2020), we expected to identify a differential fine-scale spatial genetic structure between study species: isolation by resistance in *A. religiosa* (fir) because it is a relative specialist, shade-tolerant species associated with late succession (Gallardo et al. 2019); and a panmictic population in *P. montezumae* (Moctezuma pine) because is a highly dominant pioneer species with the ability to colonize highly disturbed sites (Carrillo-Anzures 1998; Richardson et al. 2007; George-Miranda et al. under review).

Materials and Methods

Study Area

The LMNP is located in central Mexico between coordinates 19°08'–19°20' N and 98°08'–97°55' W (Figure 1). Altitude ranges between 2,200 and 4,461 meters above sea level (m asl). The local climate changes with altitude, but the dominant climate is temperate subhumid, with a mean annual temperature of 14 to 16 °C and a mean annual precipitation of 800 to 1,000 mm, with heavier rains from June to September. Vegetation also varies according to altitude, slope, orography, and anthropogenic activities (Rojas-García and Villers-Ruiz 2008); the dominant species are *Pinus montezumae* Lamb., *P. hartwegii* Lindl., *P. leiophylla* Schiede ex Schltdl. & Cham., *Abies religiosa* (Kunth) Schltdl. & Cham., and *Alnus jorullensis* Kunth (Rojas-García and Villers-Ruiz 2008).

Sampling Design

Sampling was carried out in the four slopes of the LMNP between 2,800 and 3,200 m asl because both target species are distributed within this range (Rojas-García and Villers-Ruiz 2008). Three collection sites were established on each slope of the LMNP (12 in total), which were considered separate populations in this study (Figure 1). In each population, five 1,000 m² circular plots were randomly located (Ramírez-Marcial et

al. 2001), separated by at least 50 m from edge to edge. Within each plot, vegetative tissue was collected from at least five individuals of the population of each species, separated from one another by at least 30 m. For dehydration and further processing, all samples were stored in resealable bags with silica gel and labeled with the species name, population, cohort (i.e., adult or seedling), and geographic coordinates.

Laboratory work

Genomic DNA was extracted with the Plant Pro Kit (Qiagen) kit. DNA was quantified with the PicoGreen fluorometric method using a NanoDrop™ ND 3300 fluorospectrometer (Thermo Scientific). Libraries were prepared in the Institute of Integrative Biology and Systems (IBIS) at the University of Laval by double digestion (ddRADSeq) using the enzymes PstI and MspI. Sequencing was obtained by the *Genome Quebec Centre D'expertise et de Services* through four lines of Illumina NovaSeq 6000 (PE150).

Analysis

Bioinformatic Processing

Genotyping was carried out using the pipeline in Stacks v. 2.62 (Catchen et al. 2013) with de novo alignment and applying the following criteria: exclude sites containing an indel (remove-indels), a minor allele count (mac) of 1, a maximum missing data per site (max-missing) of 0.2, only biallelic sites displaying Hardy Weinberg Equilibrium, and only loci present in at least 90 % of individuals in each population (-r 0.10). We also did additional filtering with VCFtools 0.1.15 (Danecek et al., 2011) considering a minor allele frequency (MAF) of 0.01, a minimum mean depth (min-meanDP) of 10X, and a maximum mean depth (max-meanDP) of 196X for *A. religiosa* and 306X for *P. montezumae* (twice the mean depth obtained with the site-mean-depth command; Taylor et al. 2020, Yamasaki et al. 2020). Subsequently, we identified outliers through SNP scanning with PCAdapt v. 4.3.3 (Duforet-Frebourg et al. 2015; Luu et al. 2017) using R v. 4.2.2 (R Core Team 2022). Loci identified as adaptive were removed to retain only the neutral ones.

Genetic Diversity and Genetic Structure

Basic diversity statistics were obtained by population of each species, i.e., expected heterozygosity (H_e), observed heterozygosity (H_o), and inbreeding coefficient (F_{is}) with vcfR v. 1.14.0 (Knaus and Grünwald 2017), hierfstat v. 0.5.11 (Goudet et al. 2021), and adegenet v. 2.1.10 (Jombart 2021) packages in R.

An Analysis of Molecular Variance (AMOVA) tested for significant differences in genetic structure between populations, cohorts, and slopes. Subsequently, individual ancestry coefficients were estimated based

on sparse non-Negative Matrix Factorization (SNMF) with K values of 1–10 and 1–15 ancestral populations for *A. religiosa* and *P. montezumae*, respectively, using the entropy option and 100 replicates. In addition, a Discriminant Analysis of Principal Components (DAPC) was performed using cross-validation method (Jombart et al. 2010). These analyses were performed using the R packages poppr v. 2.9.3 (Kamvar et al. 2014), LEA v. 3.10.2 (Frichot and François 2015), and adegenet, respectively.

Fine-Scale Spatial Genetic Structure

First, we investigated isolation by distance with a Mantel test using ecodist v. 2.0.9 R package (Goslee & Urban 2022) with 10,000 replicates; to this end, pairwise geographic (Euclidean) and genetic distances were calculated, the latter through the proportion of shared alleles (D_{ps}) between individuals of each species (Bowcock et al. 1994). Afterward, the spatial genetic structure was studied using a spatial Principal Components Analysis (sPCA) in adegenet (Jombart et al. 2010). Considering the potential high dispersal capacity of the two species even in this modified landscape, cryptic spatial genetic structure was explored with a genetic neighborhood analysis in MEMGENE v1.0.2 R package (Galpern et al. 2014). This method produces Moran's eigenvector maps (MEMs) that describe spatial autocorrelation patterns among the analyzed samples. Then, a regression analysis (adjusted R^2) is conducted between MEMs and genetic distances to identify spatial patterns in the genetic data (Galpern et al. 2014). For this analysis we used D_{ps} , $\alpha = 0.05$ for significant vectors and 10,000 permutations.

The effect of three landscape surfaces on gene flow was evaluated: (1) Mexican Continuous Elevation (MCE), (2) orientation (Aspect), and (3) land use (land cover). The MCE raster was downloaded from INEGI (<https://inegi.org.mx>, 15 m $\times$ 15 m resolution), from which the Aspect raster was created using raster package v. 3.6.20 (Hijmans 2022) in R. The land use raster was obtained from the supervised visual classification of Landsat 5 images (30 m $\times$ 30 m resolution) downloaded from GloVis (<https://glovis.usgs.gov>) and analyzed with ArcGIS v. 10.4 (ESRI 2018). All rasters were resampled to a 200 m $\times$ 200 m resolution with the raster R package, based on a smaller area than the dispersal capacity of conifers (~314 km²; Molina-Sánchez et al. 2019).

We optimized the resistance surfaces by following ResistanceGA v. 4.2.8 (Peterman 2018) method in R. This procedure applies functional transformations to the original surfaces to maximize the relationships between the pairwise genetic and effective (landscape resistance) distances. After the transformation of the

surfaces, ResistanceGA calculates the effective distances using least-cost path or circuit theory algorithms (among individuals or populations), and fits the genetic data by performing linear mixed-effects models using the maximum likelihood population effects (MLPE) method to identify the dependence of the values within pairwise distance matrices (Clarke et al. 2002; van Strien et al. 2012; Burkhart et al. 2017). In addition, the procedure evaluates a geographic distance model, i.e., Euclidean instead of effective-resistance distances.

In our analysis we used individuals pairwise D_{ps} as the genetic data and least-cost path effective distances in all the analyses. Two independent optimization runs were performed for each single surface to verify convergence in their transformation functions and parameter estimates. Finally, the support of the optimization was evaluated using the corrected AIC (AICc; Hurvich and Tsai 1989).

Results

We recovered 7,326 neutral SNPs of *A. religiosa* and 34,423 of *P. montezumae*. In *A. religiosa*, the mean expected heterozygosity (H_e) was 0.14, the observed heterozygosity (H_o) was 0.10, and the mean inbreeding coefficient (F_{is}) was 0.23 (Table 1). For *P. montezumae*, these values were mean $H_e = 0.16$, mean $H_o = 0.12$, and mean $F_{is} = 0.17$ (Table 1).

The AMOVA showed non-significant genetic structure in any hierarchy of interest (i.e., populations, cohorts, and slopes) in *A. religiosa*; the greatest variation in this conifer was detected within samples (~69 %; Table 2). In *P. montezumae*, the highest variation was also found within samples (~76 %), but a low and significant genetic structure between slopes was detected (0.11 % variation, $P = 0.03$; Table 2). The SNMF identified an optimal $K = 1$ in both species (Figure 2a, b), whereas the DAPC indicated an absence of structure (Figure 2a, b).

The Mantel test revealed a low but significant positive correlation between genetic and geographic distances ($r = 0.12$, $P = 0.0004$) for *A. religiosa*, indicating a weak isolation by distance (IBD) pattern; for *P. montezumae*, a lower and non-significant correlation ($r = 0.04$, $P = 0.16$) was detected. The sPCA identified neither a local [$\max(t) = 0.13$, $P = 0.98$ in *A. religiosa*; $\max(t) = 0.02$, $P = 0.93$ in *P. montezumae*] nor a global structure [$\max(t) = 0.03$, $P = 0.59$ in *A. religiosa*; $\max(t) = 0.02$, $P = 0.49$ in *P. montezumae*]. The MEMGENE analysis detected eight negative Moran's eigenvectors in *A. religiosa*, with the first two explaining 35.9 % of the variation (20.4 % and 15.5 %, respectively) (Figure 4a-b). However, only 3 % of the

genetic variation was spatially structured ($R^2 = 0.03$); for *P. montezumae*, two positive Moran's eigenvectors explained the total variation (50.9 % and 49.1 %), but the model showed a poor adjustment ($R^2 = -0.01$).

Finally, no landscape resistance model had a greater effect than distance model (Table 3).

Discussion

Evolutionary processes such as drift and dispersal commonly occur on a geographic scale (MacManus et al. 2020). Habitat modification and fragmentation may cause the loss of connectivity between populations (Balkenhol et al. 2015) by modifying ecological and evolutionary processes, being the primary threat to the conservation of some species (Arroyo-Herrera et al. 2013), especially those inhabiting ecosystems under high anthropogenic impact, like temperate forests in LMNP. The results of this research work partially met our predictions since no isolation by resistance was detected in *A. religiosa*, but high gene flow was observed between populations in both species despite the altitudinal variation and the intense changes in land use in the LMNP. However, it is necessary to expand the study area to evaluate other populations and environmental variables that may contribute to explaining gene flow.

In general, genetic diversity was lower in *A. religiosa* than in *P. montezumae*, and the inbreeding coefficient was higher in the former (fir). This finding may be related to smaller population sizes in *A. religiosa*, consistent with the distribution of this species in the LMNP in shaded areas with high humidity and low temperature (Gallardo et al. 2019), *P. montezumae* occurs in open areas, even with high disturbance (Carrillo-Anzures 1998). Ern (1976) reported associations between *A. religiosa* and other conifers in areas where the former is currently absent (Cruz-Salazar 2021, personal observation), while it appears that *P. montezumae* is expanding its distribution as a result of its affinity to areas with changes in land use (George-Miranda et al. under review).

In addition to inbreeding, an important consequence of reduced population size is increased genetic drift that can promote genetic differentiation (Eckert et al. 2010; Leroy et al. 2018). Although this phenomenon was not detected in *A. religiosa*, it has been observed with other markers that are more sensitive to demographic changes, such as chloroplast microsatellites (Wheeler et al. 2014; Cruz-Salazar et al. under review). Also, in long-lived species such as conifers, the effects of anthropogenic disturbance are delayed (Ehrlén and Lehtila 2002; Krauss et al. 2010; Aavik 2019); hence, it is recommended to study recent generations (e.g., seedlings). In this regard, Cruz-Salazar et al. (2023) reported an association between

environmental distances and genetic differentiation in *A. religiosa* seedlings from LMNP based on SNPs. This information suggests that genetic structure by cohort may be identified in future generations, although the data reported herein did not detect it, probably due to the small spatial scale of this study.

In conifers, dispersal through pollen (10 km) is greater than through seeds (31 m) (Briggs et al. 2009; Molina-Sánchez et al. 2019; Ortiz-Bibian et al. 2019), and although anemochoric dispersal promotes gene flow, small population sizes prevent effective cross-pollination and lead to increased inbreeding levels (Mantilla 2006). According to Rojas-García and Villers-Ruíz (2008), *A. religiosa* accounts for only 6.6 % (1,366 ha) of the LMNP forest cover, while *P. montezumae* is the dominant conifer with 59 % of the cover (12,170 ha); this difference may contribute to explain the variation in heterozygosity levels between species.

Environmental conditions, such as environmental gradients, variation in area, and connectivity between habitat patches can affect the spatial genetic structure because allele frequencies depend on the rate of migration (gene flow) and the increase in genetic drift (Wright 1931; Hein et al. 2021). Fine-scale spatial genetic structure in wind-pollinated and dispersed tree species is low because they have a high gene flow by pollen dispersal over large geographic distances (Steinitz et al. 2011; Kremer et al. 2012; Uchiyama et al. 2014). According to Elleouet and Aitken (2018), the long life and high probability of long-distance pollen dispersal contribute to panmixia and maintain high genetic diversity because the founder effect is reduced when founders of different origins accumulate before local reproduction occurs.

We identified a low contribution of space in genetic variation and weak isolation by distance (IBD) in *A. religiosa*, indicating that genetic differentiation is barely explained by space and geographic distances (Manel et al. 2003; Hein et al. 2021). Furthermore, the fine-scale spatial genetic structure was not explained by any resistance surface studied, either in *A. religiosa* or in *P. montezumae*, suggesting that the strong environmental changes evaluated (i.e., altitude, orientation, and land use) cause no effect on gene flow among the populations of these species at this spatial scale.

The absence of structure identified in this study is consistent with other investigations in conifers in which spatial genetic structure was evaluated with SNPs. For example, Chhatre et al. (2013) found a weak genetic structure pattern in *Pinus taeda*, but a high genetic differentiation in second-generation breeding populations was detected. Dillon et al. (2013) investigated adaptation signatures and genetic structure among mainland populations of *Pinus radiata*, observing low genetic differentiation among them ($F_{st} = 0.043$). For

their part, Mosca et al. (2014) studied the effect of isolation by distance (IBD) and isolation by adaptation (IBA) on population genetic structure in *Abies alba* and *Larix decidua* based on 231 and 233 SNPs, respectively; these authors reported that IBD had a stronger effect than IBA, with a weak but significant genetic structure in both species and higher values in *L. decidua*. Lastly, Tian et al. (2022) reported local patterns of IBD and genetic differentiation in *Pinus bungeana* in China, with populations in the central and eastern regions clustered together while those from the western region were separated.

In volcanic areas such as LMNP, anemochoric dispersal depends on regional wind patterns resulting from the global variation of wind regimes, either facilitating or restraining the displacement of pollen and seeds (Kling and Ackerly 2021), which could be related to the dispersal rate among slopes. In this sense, *P. montezumae* showed a low and significant genetic structure between slopes with the AMOVA; however, since the greatest genetic variation was conserved within samples (~76 %), we can conclude that all the individuals are part of a panmictic population (Excoffier et al. 1992)

Single Nucleotide Polymorphisms (SNPs) are markers with high coverage and depth that can reveal high variation across the genome (Morin et al. 2004). The high mutation rate and variation of SNPs in plant genomes (Oraguzie et al. 2007) make them an excellent marker for assessing gene flow (Sousa and Hey 2013). However, Tsykun et al. (2017) indicate that the observed patterns in SNP markers reflect the ancient divergence of distant populations (~1000 km). This may be one reason for the lack of genetic structure observed in our results; therefore, we suggest increasing the spatial scale of the study to further support the conclusions about genetic diversity, population genetic structure and gene flow patterns in the species studied.

Likewise, given the importance of the LMNP as a key element for the connectivity of the populations of high-mountain species inhabiting temperate forests in central Mexico, which are currently isolated in the middle of a matrix of non-forest land uses (Reyes-González and Rhodes 2015), it is essential to obtain information from other temperate forests in the area (e.g., Popocatepetl, Iztaccíhuatl, Mount Tlaloc), which will allow for determining gene flow on a regional scale and inform strategies to conserve or restore the connectivity of tree species and their associated fauna.

Since landscape effects have been scarcely assessed in trees (Bisbing et al. 2021), the present study contributes to the genomic knowledge, especially for temperate forest tree species. In addition, it advances the analyses of the effect of changes in the landscape on neutral evolutionary processes that can determine the

permanence of populations and species. The information reported herein is useful for conserving the remaining temperate forest fragments in a landscape with high anthropogenic impact in Mexico.

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Statements and Declarations

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Competing Interest

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

Bárbara Cruz-Salazar carried out the study conception and design, as well as the laboratory work. Bárbara Cruz-Salazar and Alejandro Flores-Manzanero performed the analyses. The first draft of the manuscript was written by Bárbara Cruz-Salazar. All authors commented, read, edited, and approved the final manuscript.

Data Availability

The datasets generated during this study are available from the corresponding author upon reasonable request.

Table 1. Genetic diversity of populations of *Abies religiosa* and *Pinus montezumae* from La Malinche

National Park, based on neutral SNPs. S, Southeast slope; E, East slope; N, North slope; W, West slope; H_e , expected heterozygosity; H_o , observed heterozygosity; F_{is} = inbreeding coefficient.

Population	H_e	H_o	F_{is}
<i>Abies religiosa</i>			
S1	0.14	0.09	0.27
S2	0.15	0.11	0.15
S3	0.13	0.07	0.34
E1	0.15	0.10	0.23
E2	0.13	0.10	0.21
E3	0.15	0.11	0.21
W1	0.13	0.10	0.18
W2	0.13	0.08	0.27
W3	0.14	0.10	0.21
Average	0.14	0.10	0.23
<i>Pinus montezumae</i>			
S1	0.15	0.11	0.20
S2	0.16	0.12	0.16
S3	0.16	0.12	0.16
E1	0.15	0.12	0.15
E2	0.16	0.12	0.18
E3	0.16	0.12	0.17
N1	0.16	0.12	0.18
N2	0.16	0.12	0.19
N3	0.15	0.11	0.20
W1	0.16	0.12	0.16
W2	0.16	0.12	0.18
W3	0.16	0.12	0.16
Average	0.16	0.12	0.17

Table 2. Covariance components of the Analysis of Molecular Variance of *Abies religiosa* and *Pinus**montezumae* based on neutral SNPs. %, variation percentage; ^{ns}, non-significant; *, $P \leq 0.05$; **, $P \leq 0.01$.

Component	Sigma	%
<i>Abies religiosa</i>		
Between slopes	-0.1218726	-0.02 ^{ns}
Between populations	-2.3417697	-0.47 ^{ns}
Between cohorts	4.7915604	0.96 ^{ns}
Between samples	154.480522	30.95*
Within samples	342.26752	68.58*
Total	499.075961	100
<i>Pinus montezumae</i>		
Between slopes	3.05	0.11*
Between populations	-4.06	-0.15 ^{ns}
Between cohorts	6.18	0.23 ^{ns}
Between samples	626.52	23.45**
Within samples	2040.22	76.36**
Total	2671.91	100

Table 3. Independent runs results for the resistance surfaces optimization with ResistanceGA in populations of *Abies religiosa* and *Pinus montezumae* from La Malinche National Park based on the proportion of shared alleles obtained with neutral SNPs. CME, Continuous Mexican Elevations raster; Aspect, slope raster; Land use, land-use changes raster. AICc, average of the corrected Akaike Information Criteria (Hurvich and Tsai 1989) obtained for each model in 10,000 bootstrap iterations; k , number of parameters; mR^2 , proportion of the overall variation explained by the model fixed effects; R^2c , conditional R^2 value.

		Run 1				Run 2			
Surface	Type	AIC _c	<i>k</i>	m <i>R</i> ²	<i>R</i> ² c	AIC _c	<i>k</i>	m <i>R</i> ²	<i>R</i> ² c
<i>Abies religiosa</i>									
CME	Continuous	-8199.35	4	0.0004	0.55	-8199.35	4	0.0004	0.55
Aspect	Continuous	-8199.47	4	0.0002	0.55	-8199.47	4	0.0002	0.55
Land use	Categorical	-8194.08	6	0.00008	0.55	-8194.08	4	0.00008	0.55
Distance	Uniform	-8204.47	2	0.0003	0.55	-8204.47	2	0.0003	0.55
<i>Pinus montezumae</i>									
CME	Continuous	-22769.97	4	0.002	0.78	-22769.07	4	0.001	0.78
Aspect	Continuous	-22770.22	4	0.0007	0.79	-22770.22	4	0.0007	0.79
Land use	Categorical	-22765.06	6	0.0007	0.79	-22763.72	6	0.0007	0.79
Distance	Uniform	-22772.67	2	0.0002	0.78	-22772.67	2	0.0002	0.78

Figure Captions

Fig. 1 Study area for collecting vegetative tissue of *Abies religiosa* and *Pinus montezumae* in La Malinche National Park (polygon marked in black). Green dots depict the populations from where samples of both species were collected. Gray scale shows the absence (lighter) and presence of vegetation (darker).

Fig. 2 Sparse non-Negative Matrix Factorization analysis (SNMF) of populations of *Abies religiosa* (a) and *Pinus montezumae* (b) from La Malinche National Park based on neutral SNPs. The arrow indicates the most probable number of ancestral populations (K), $K = 1$.

Fig. 3 Discriminant Analysis of Principal Components (DAPC) of populations of *Abies religiosa* (a) and *Pinus montezumae* (b) from La Malinche National Park based on neutral SNPs and cross-validation (Jombart et al. 2010). S, Southeast slope; E, East slope; N, North slope; W, West slope.

Fig. 4 Spatial genetic patterns of the proportion of shared alleles (D_{ps}) in *Abies religiosa* based on neutral SNPs using MEMGENE (Galpern et al. 2014) and projected into a land-use raster. Circles are individuals. The size and color of circles depict the genetic similarity among individuals. x -axis and y -axis represent longitude and latitude in UTM, respectively. (a) first MEMGENE that explains 20.4 % of the variation. (b) second MEMGENE that explains 15.5 % of the variation.

Figure 1.

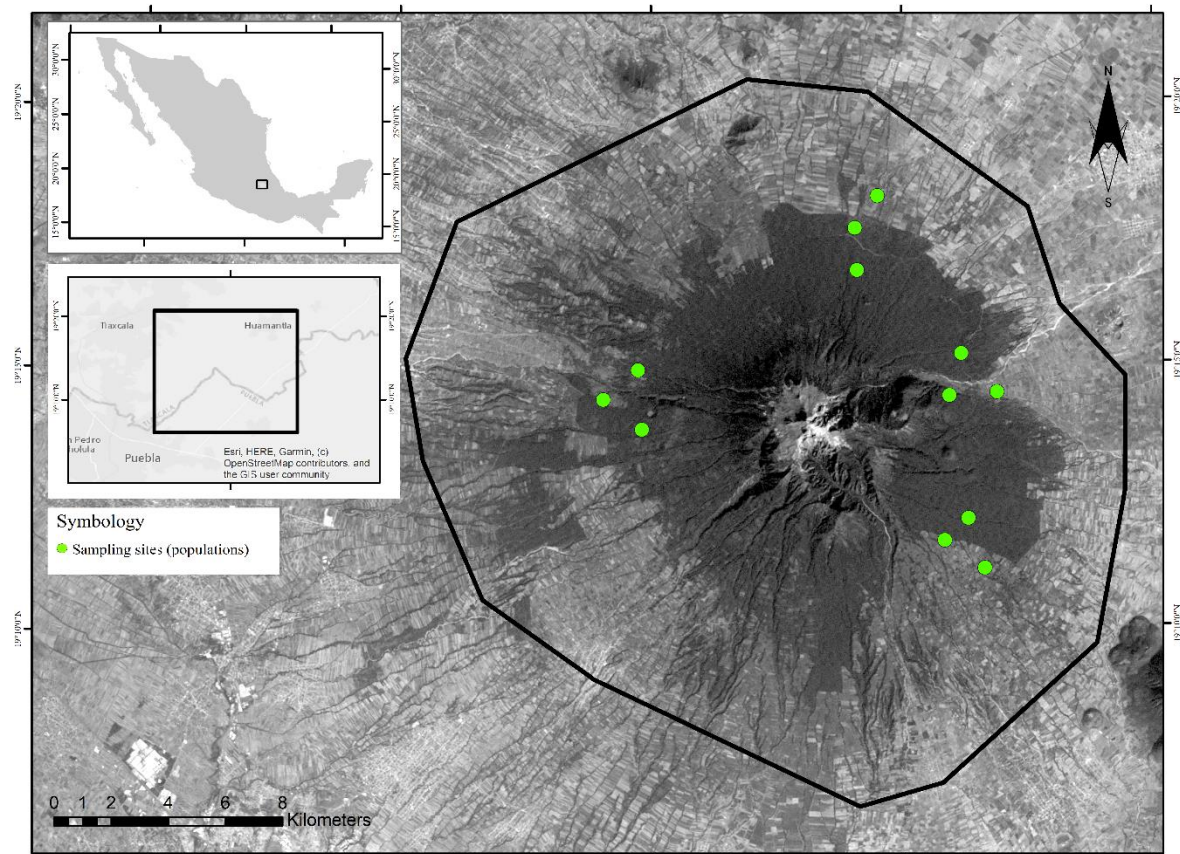


Figure 2.

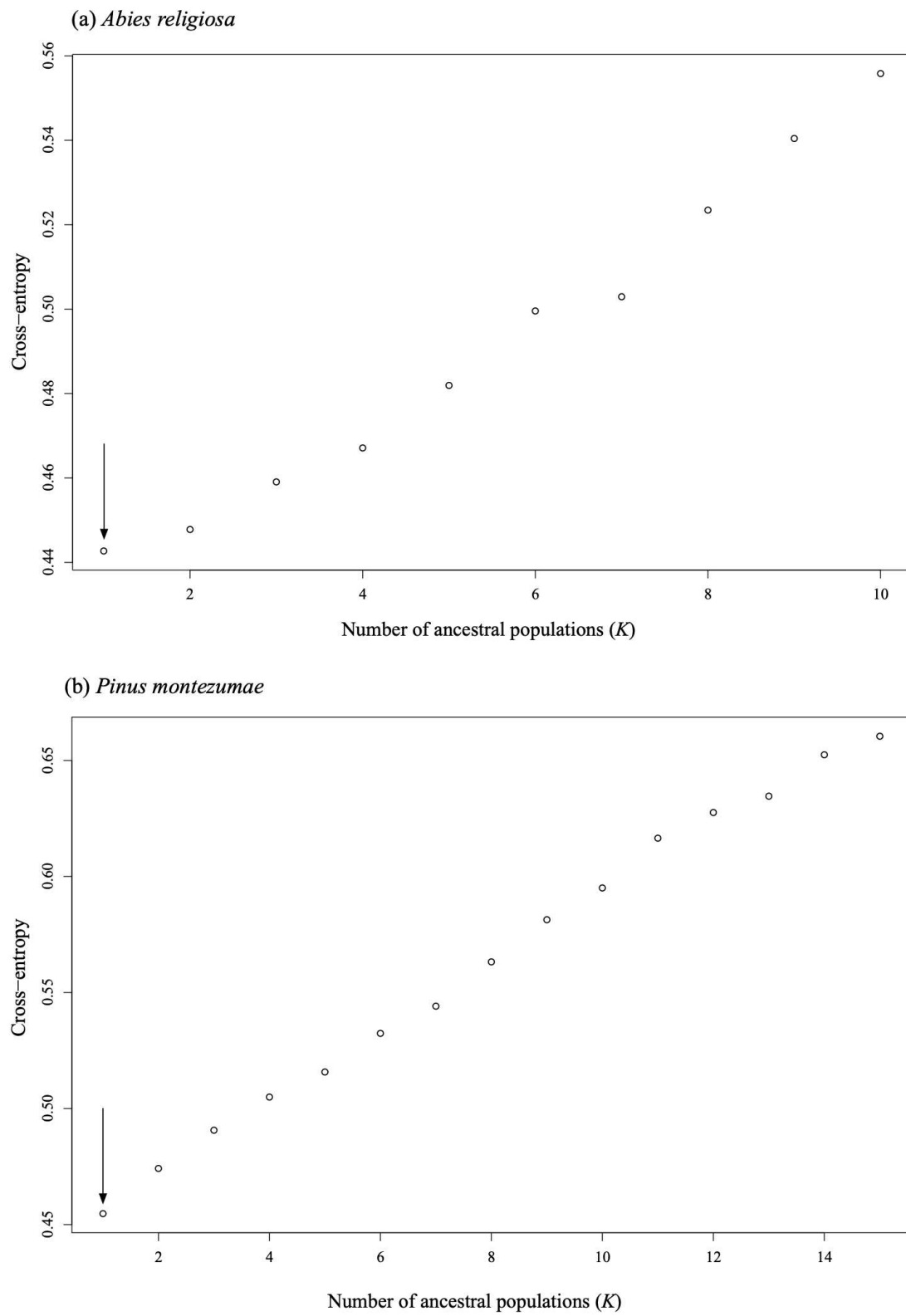
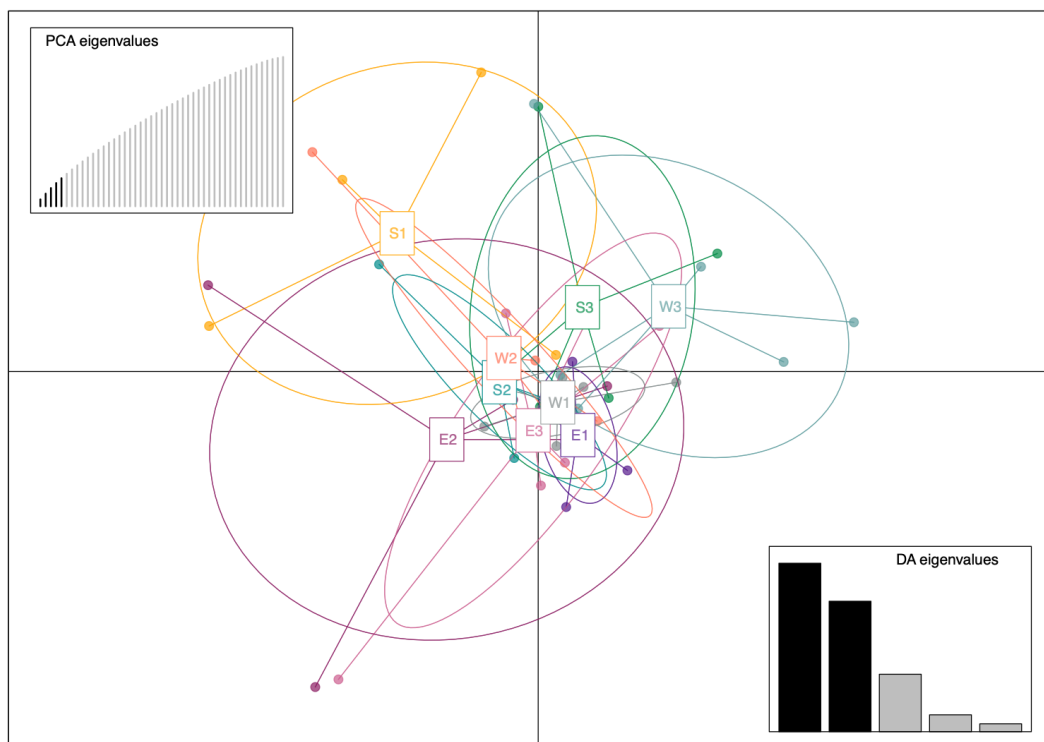


Figure 3.

(a) *Abies religiosa*



(b) *Pinus montezumae*

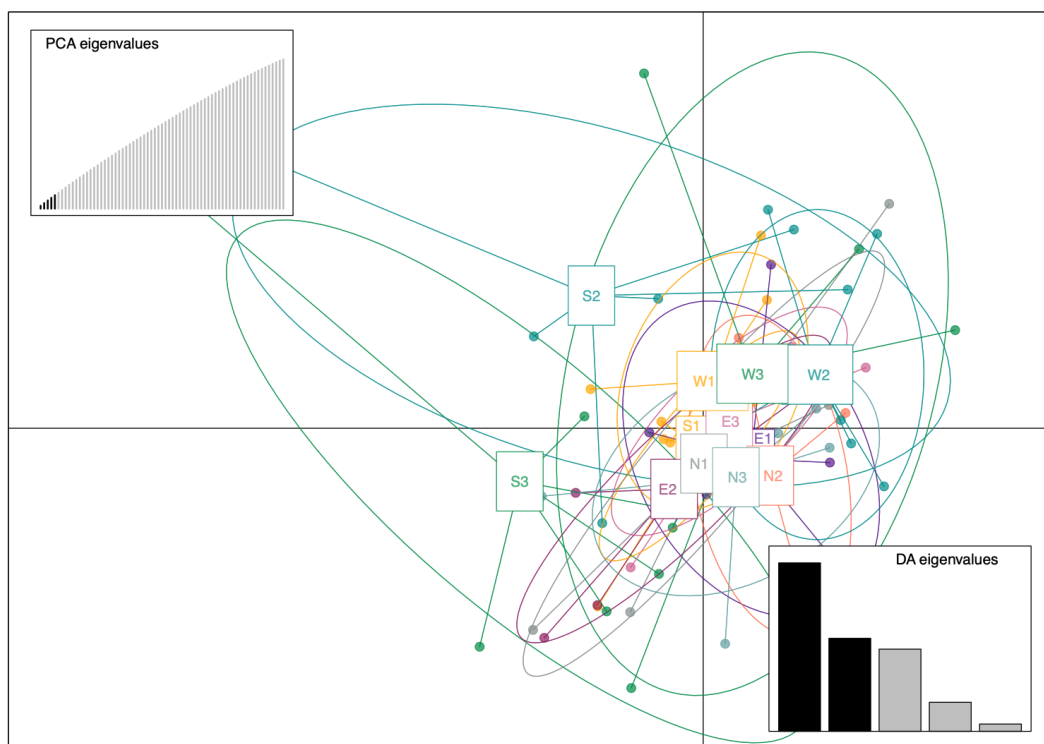


Figure 4.

