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Effect of forest management on the genetic diversity of *Abies hidalgensis*, a threatened species with restricted distribution

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Abstract: *Abies hidalgensis* is an endemic species from the state of Hidalgo, Mexico, that has been registered only in nine fragmented relict populations that have a total of 1 000 individuals among them. Intensive forest management takes place in five of the populations under specific programs focused on *Pinus* spp. Still, it is necessary to know the impact of these activities on the genetic diversity of the threatened species, if restoration and conservation strategies are to be proposed. The aim of this work was to estimate the effect of forest management on the genetic structure of *A. hidalgensis* using seven nuclear molecular markers developed for *A. guatemalensis* (Ab07, Ab08, Ab09, Ab12, Ab15, Ab20, Ab23). The species was sampled growing under two different conditions; (*i*) areas under forest management and (*ii*) conserved areas. Two indexes of genetic diversity were evaluated, observed and expected heterozygosity. The genetic structure was determined by an analysis of molecular variance and a Bayesian assignment model. A bottleneck analysis was also carried out. The populations were found to have a common genetic base (differentiation coefficient F_{ST} = 0.056, number of migrants per generation Nm = 43), which suggests recent fragmentation of the distribution, which in turn increases the bottleneck effect in managed areas (Wilcoxon probability Wp = 0.007 and 0.016). This explains the apparently high heterozygous level (H_e = 0.69) and low inbreeding. Our results are important as they may be used to design strategies for management and conservation of *A. hidalgensis.*

Keywords: bottleneck; fir; genetic structure; genetic variation; population genetics

Diversity and genetic structure are critical population attributes for management and conservation of plant species classified under some level of risk due to their size, fragmentation, sensitivity, or restricted distribution (Gordon et al. 2012; Awad et al. 2014; Wu et al. 2015) because they fluctuate more quickly in response to spatial and temporal factors such as isolation, extent of the original distribution, deforestation, fragmentation, intrinsic demographic factors of the species, and associations such as competition, pollination or predation, life forms, harvesting, stochastic natural events, or anthropogenic dispersal, among others (Hedrick 2005; Conord et al. 2012; Vranckx et al. 2012; Zhang et al. 2012; Wyatt et al. 2021).

In particular, the fragmentation associated with forest management for timber and non-timber purposes has controversial effects on plants, with a varying intensity, depending on silvicultural practices, stand structure, and species characteristics present in the community before and during extraction (Gautam et al. 2021). For example,

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pioneer species benefit from management because it increases their population size while they share a common genetic base (post-colonisation expansion); whereas long-lived trees mostly suffer a reduction in fertility and consequently lose their genetic variability (Poelchau, Hamrick 2013). The negative effects for long-lived species may include a reduction in the number of alleles and genotypes, loss of heterozygosity, and reduction of gene flow and effective population size (Lowe et al. 2015). Nevertheless, for some widely distributed species, there are no differences in genetic diversity between managed and unmanaged stands (Paffetti et al. 2012; Aravanopoulos 2018; Rungis et al. 2019).

However, in principle, relict species with restricted distribution, endemics, and species with low population sizes should not be subjected to forest exploitation. Nevertheless, if their distribution coincides with other important timber species, they will be affected collaterally (Pérez-López et al. 2020). In addition, some species are illegally extracted because they are confused with others of greater distribution or removed due to a change in land use for grazing or clearing. For example, in *Araucaria angustifolia* (Bert.) O. Kuntze, an endangered Brazilian plant species, forest management reduces the number of alleles and therefore genetic diversity (Medri et al. 2003). Phuong Thuy et al. (2020) observed poor genetic diversity (h = 0.2223) in *Pinus kwangtungensis* Chun ex Tsiang compared to other species of the same genus [*Pinus koraiensis* Sieb. et Zucc H_e = 0.61, (Tong et al. 2020); *P. densiflora* Siebold & Zucc H_e = 0.652, (Ahn et al. 2021)] due to reduction of population size and habitat fragmentation. Additionally, it has been documented that relict populations in fragmented landscapes show low genetic diversity and high differentiation due to spatial isolation and high levels of inbreeding (Young et al. 1996; Szczecińska et al. 2016; Li et al. 2018).

Abies hidalgensis Debreczy, Rácz, & Guízar (Debreczy, Rácz 1995) is endemic to the state of Hidalgo, Mexico, and its distribution is extremely limited. To date, nine relict populations with a total of fewer than 1 000 individuals have been identified (Rosales-Islas et al. 2023). It is therefore on the Red List of threatened species in the Vu D2 category but is not covered by Mexican legislation (SEMARNAT 2010).

Abies hidalgensis is distributed in the municipalities of Agua Blanca de Iturbide, Tenango de Doria, and Acaxochitlán, Hidalgo, in nine relict populations. Several of them have been subjected to forest extraction (Las Águilas, El Tejocote, Ejido San Pedrito, Los Manantiales, and Zacacuautla), either controlled through the Silvicultural Development Method (SDM) or uncontrolled through elimination directed at corpulent trees, with or without the permission of the owners. In most of the stands under forest management, the cover has been replaced with the genus *Pinus* due to its economic importance. In addition, livestock activities were detected in these areas.

Of the nine populations, only two conserve a partially original cover (Ejido San Cornelio and Ejido San Pedrito). In these, extraction activities are not evident, extraction of specimens of commercial size has not been reported, and there is a scarce presence of stumps or evidence of extraction of trees, either of *A. hidalgensis* or the associated species. In these areas, it is possible to observe different development strata, with high densities of individuals. Two other populations have disappeared because of changes in land use.

The extraction activities are an example of collateral damage, since the scarce knowledge of its morphology, distribution and, in general, about its taxonomic status has resulted in the extraction of specimens in areas under forest management. INEGI (2016, 2017) reports the extraction of 250 $m^3 \cdot r^{-1}$ (m^3 of round wood) and 191 $m^3 \cdot r^{-1}$ of *Abies religiosa* (Kunth) Schltdl. & Cham. in the municipality of Agua Blanca, where this species is not distributed, but *A. hidalgensis* is.

The genetic structure of this type of species is evaluated by means of molecular markers to enable the impact of forest management to be estimated at the genetic level and to identify promising tree populations for the delimitation of conservation units, risk assessment, and design of management strategies (González 2003; Godoy 2009).

The objective of this work was to evaluate the effect of logging on the genetic variation of *A. hidalgensis* using nuclear molecular markers that would enable identification of losses associated with the population reduction caused specifically by the documented logging to assist in the design of restoration and conservation strategies.

MATERIAL AND METHODS

Study sites

The populations at Las Águilas and Ejido San Cornelio (managed and conserved, respectively) located at coordinates 20.369°N, –98.361°E and

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20.361°N, –98.347°E were selected for the study (Figure 1). Forest extraction in Las Águilas is regulated by SDM, and the individuals of *A. hidalgensis* are distributed in regeneration areas of *Pinus patula*. This population has about 30 specimens; in contrast, the population of Ejido San Cornelio has about 396 individuals. Other populations were not considered for contrast since the number of individuals in the managed conditions was small (fewer than ten adults), distributed in small areas and/or geographically close. In addition, the localities under management are changing, some have even disappeared, and the relict individuals are merely ornamental within stands dedicated to timber production. Therefore, free access to the trees is not possible it requires agreements with the owners of the properties.

The populations are located in pine-oak forest and mountain cloud forest (INEGI 2018), between 2 100 m a.s.l and 2 320 m a.s.l. The region is characterised by a humid temperate climate C(m), with a maximum and minimum temperature of 21 °C and 6 °C respectively, precipitation of 1 200 mm to 1 500 mm per year with typical summer rains and 0% to 18% of the total annual precipitation in the winter (García 1973; INEGI 2008).

Field data recording

The group distribution of individuals makes it difficult to take samples with genetic independence, so we worked based on population conditions. However, it is common to obtain samples of individuals 20 m to 30 m apart within the population (Mosca et al. 2012; Stojnić et al. 2019). In the Ejido San Cornelio (conserved), sampled specimens were at least 30 m apart, while in the Las Águilas population (managed), the number of samples collected represents almost the total number of reproductive specimens in the population, so the distance between some individuals is less.

Leaf samples from the middle to the upper part of the crown of 30 reproductive specimens per condition were collected and placed in sealed bags with silica gel, labelled, and kept refrigerated at –20 °C before extracting DNA (Awad et al. 2014).

Laboratory analysis

DNA was extracted from leaves following the CTAB method of Doyle and Doyle (1987). 2 g of plant tissue were macerated with liquid nitrogen until a fine powder was obtained, and 1 mL of extraction buffer was added (CTAB-PVP 2% -Tris-HCl 100 mM pH 8, NaCl 1.4 M, EDTA 20 mM pH 8, and 1 µL of 2-β-mercaptoethanol) to continue mashing. The mixture was centrifuged at 800 rpm for 8 min. The precipitate was obtained and washed up to three times with the extraction buffer and then incubated at 37 °C for 1 h with 10 µL of RNAse (1 mg·mL^{-1}). Next, 10 µL of proteinase K (10 mg·mL^{-1}) was added and incubated

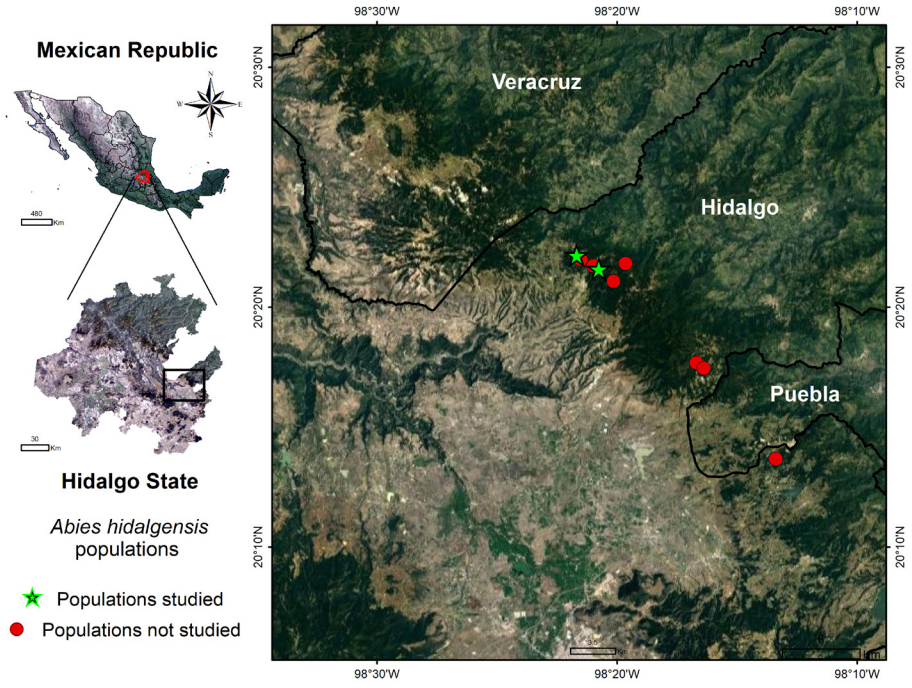

Figure 1. Studied populations of *Abies hidalgensis* in Hidalgo, Mexico

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again at 60 °C for 1 h. Subsequently, 600 µL of chloroform : isoamyl : alcohol (25 : 24 : 1) and 250 µL 2% NaCl were added due to the high resin content (Sánchez-Coello et al. 2012), and shaken at 300 rpm for 1 h at 25 °C. Finally, the mixture was centrifuged at 10 000 rpm for 10 min, and the supernatant was recovered and precipitated with 2/3 parts of the final volume (300–500 µL) of cold isopropanol, allowing it to settle for 12–24 h at –20 °C. After 12 h, it was centrifuged at 12 000 rpm for 10 min, the supernatant was removed without losing the precipitated product, and 1 ml of cold absolute ethanol was added and centrifuged at 14 000 rpm for 10 min. Upon completion, the precipitated product was removed from ethanol and allowed to dry to be resuspended in 50 µL of sterile distilled water or TE buffer (10mM Tris-HCl, 1mM EDTA Na_2). The genetic material was quantified by spectrophotometry in a MAPADA nano spectrophotometer (MAPADA instruments, China).

Seven nuclear microsatellite markers developed for *A. guatemalensis* Rehder by Rasmussen et al. (2008) were used (Table 1). The mix for a tested PCR reaction was 2 µL genomic DNA (> 50 ng·µL^{-1}), 1.6 µL $MgCl_2$ (25 mM), 2.4 µL buffer (5×), 0.5 µL dH_2O, 0.7 µL of forward and reverse primers (5 µM), 0.3 µL of deoxynucleotide triphosphates (dNTPs) (10 mM) and 0.3 µL (1U) of Promega® TaqDNA polymerase for a total of 8.5 µL. The PCR technique was performed in a Thermo Scientific Artik endpoint thermal cycler (Thermo, USA).

The PCR program consisted of an initial denaturation step of 9 min at 94 °C; cycling in three steps repeated 30 times consisting of denaturation at 94 °C for 55 s, annealing for 50 s at 57 °C (primer Ab07 and Ab09), 50 s at 59 °C (primer Ab08 and Ab15), 50 s at 58 °C (primer Ab12 and Ab20), and 50 s at 61 °C (primer Ab23) and annealing at 72 °C for 55 s and the final extension at 72 °C for 7 min. PCR products were visualised on 15% acrylamide gels, run at 90 volts × 90 min and stained with ethidium bromide (10 mg·mL^{-1}) for 10 min. The gels were visualised in a transilluminator and recorded photographically.

Data analysis

Genetic diversity. The alleles (bands) were determined with the GelAnalyzer application (Version 19.1). The number of alleles was filtered by Poisson probability; null alleles (frequency less than 5%) were ruled out, which made it possible to eliminate genotyping errors, including non-amplified alleles. Gene frequencies were calculated to determine the average number of amplified samples per locus/condition (N), the average number of alleles (A), the effective number of alleles $N_e = \frac{1}{(\sum p_i)^2}$ by locus and by condition, where the p_i are the allele frequencies, the Shannon informative index for each primer $I = -1 \times \Sigma(p_i \times \ln p_i)$, the observed and expected heterozygosity (H_o and H_e respectively), and the fixation index $f = \frac{H_e - H_o}{H_e}$, which is zero if there are Hardy-Weinberg proportions (Hedrick 2005). The GenAlEx package v. 6.5 (Peakall, Smouse 2006) was used for this.

Genetic structure. The genetic structure was evaluated by Wright's (1951) F statistics (total inbreeding coefficient F_{IT}, inbreeding coefficient F_{IS}, differentiation coefficient F_{ST}) within and between study conditions to determine the distribution of genetic variability within the individual, among individuals in localities, and among the analysed localitiess. The number of migrants per generation (Nm) was also determined pairwise. Molecular analysis of variance (AMOVA) was performed, assuming an infinite allele model with 999 permutations. For these analyses, the GenAlEx v. 6.5 software (Peakall, Smouse 2006) was used. Additionally, to analyse the distribution of genetic variation within conditions, a Bayesian assignment analysis was carried out with the STRUCTURE 2.3.4 program (Pritchard et al. 2000). For each hypothetical genetic group (number or hypothetical genetic groups K = 2–5), 50 000 iterations were performed, with 50 000 Markovian repetitions, assuming the Admixture model and including the correlated allele frequencies (Falush et al. 2003). The process was repeated 10 times to calculate the confidence interval. Structure selector was used to determine the number of genetic groups from the ΔK model (Evanno et al. 2005) and obtain the genetic structure graph (Li, Liu 2018).

Bottleneck. A bottleneck analysis was performed considering a stepwise mutation model (SMM) using the Bottleneck v. 1.2.02 program (Cornuet, Luikart 1996) with resampling of 1 000 steps to the recalculation of the heterozygosity. The mutation-drift equilibrium model assumes that the severe reduction in the effective size of a population is reflected in an expected deficiency or excess of the

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Table 1. Molecular markers used for analysis of the population structure of *Abies hidalgensis* in Hidalgo, Mexico

Locus	Forward primer 5'→ 3'	Reverse primer 3' ← 5'	Size range	T_a (°C)
Ab07	ACTGGCATTTGTCGCATTC	CCTCGGAGGACAAGATTTGC	219–257	57
Ab08	ATCGAGAGGCCCAGGTAGAC	GACATAGCTGATAGTGACGCAAC	128–161	59
Ab09	AGCTTATTTGCACGCTGAAG	TTTTCTTTATGAGAAACCAAGTTCC	129–174	57
Ab12	AGGTTGTGTAAGCCCGTGTAG	CCCTTTGTTGATAGAGGGAAAC	223–244	58
Ab15	AACTAACTCCTATGTGTCAAAATATCC	GCATGGAGGATAAGTAAAGATGG	239–254	59
Ab20	GATCCAGGTTTAGCGTATCTGAG	CAATGAATCTCTGCAACTGACC	134–169	58
Ab23	GGCATTATTTCCCACTTTTCC	TCAGATACATACTTGGGTTGGTG	181–207	61

T_a – annealing temperature

recalculated heterozygosity relative to the observed heterozygosity and whose intensity will depend on the time in which the reduction occurred and the resulted frequency of heterozygous individuals after the population reduction.

RESULTS

Genetic diversity. The microsatellites used were polymorphic. A total of 35 alleles were detected, with a range of 2 to 10 alleles per locus in both study conditions, and with an average of 5 for the managed condition and 4.86 for the conserved condition. The number of alleles for the Ab07, Ab09, Ab12, Ab15, Ab20 and Ab23 loci were 10, 5, 4, 2, 4, 3, respectively, for both study conditions. The Ab08 locus presented seven alleles for the managed condition, one private allele, and six alleles for the conserved condition.

The frequency of some alleles for the Ab07, Ab08, Ab09, Ab20 and Ab23 loci showed differences between the study conditions [Table S1 in Electronic Supplementary Material (ESM)]. At these loci, the most frequent alleles are different between conditions.

The number of effective alleles is generally low for the loci studied. In particular, the Ab12, Ab15, Ab23 loci present less than three effective alleles. Consistent with this, the Shannon index is low for the Ab15 locus in both conditions, while the Ab23 locus is low only for the managed condition (Table 2).

Table 2. Number of alleles and effective alleles, Shannon index, observed and expected heterozygosity and fixation index by locus

Study condition	Locus	N_a	N_e	I	H_o	H_e	f
Forest management	Ab07	10	7.595	2.139	0.933	0.868^{ns}	−0.075
	Ab08	7	5.732	1.838	0.567	0.826^{***}	0.314
	Ab09	5	3.734	1.456	0.433	0.732^{***}	0.408
	Ab12	4	2.456	1.013	0.600	0.593^{*}	−0.012
	Ab15	2	1.946	0.679	0.833	0.486^{***}	−0.714
	Ab20	4	3.509	1.313	0.500	0.715^{*}	0.301
	Ab23	3	1.972	0.742	0.400	0.493^{ns}	0.188
Conserved	Ab07	10	7.229	2.098	0.800	0.862^{ns}	0.072
	Ab08	6	5.187	1.706	0.433	0.807^{***}	0.463
	Ab09	5	3.854	1.461	0.433	0.741^{***}	0.415
	Ab12	4	3.789	1.358	0.967	0.736^{ns}	−0.313
	Ab15	2	1.897	0.666	0.633	0.473^{ns}	−0.340
	Ab20	4	3.742	1.352	0.333	0.733^{***}	0.545
	Ab23	3	2.605	1.012	0.667	0.616^{ns}	−0.082

$^{ns}P > 0.05$; $^{*},^{**},^{***}$ $P < 0.05$, $P < 0.01$, $P < 0.001$ respectively; N_a – number of alleles; N_e – effective number of alleles; I – Shannon index; H_o – observed heterozygosity; H_e – expected heterozygosity; f – fixation index, significant difference between H_o and H_e

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The Ab07 and Ab23 loci for the managed condition are in the Hardy-Weinberg (HW) equilibrium; in contrast, the Ab08, Ab09 and Ab20 loci are heterozygous deficient, and Ab15 locus has an excess of heterozygotes. In the conserved condition, the Ab07, Ab12, Ab15, Ab23 loci are in the HW equilibrium, while the Ab08, Ab09 and Ab20 locus shows a high excess of homozygotes. (Table 2). The fixation index *f* showed that the Ab08, Ab09 and Ab20 loci have greater inbreeding in both study conditions (Table 2).

The H_o mean for both conditions was significantly different from the H_e mean, so they are not under the HW equilibrium. The *f* fixation index generally showed that the species has little inbreeding. In particular, the conserved condition presented more inbreeding (Table 3).

Genetic structure. F_{ST} indicates small but significant genetic differentiation between study conditions ($P < 0.001$), while F_{IS} and F_{IT} indicate significantly different inbreeding between conditions (Table 4). In particular, the Ab08, Ab09 and Ab20 loci for the F_{IS} and F_{IT} statistics indicate an excess of homozygotes. The Ab15 locus indicates an excess of heterozygotes for both statistics; however, it is not statistically different. The F_{ST} statistic for five of the seven evaluated loci indicates small but significant differentiation between the study conditions.

The estimated number of migrants (*Nm*) per generation is 4.22 between conditions, which indicates a high gene flow, but when we compare by locus, these values are diverse (Table 4). This high gene flow indicates a common origin; therefore, the ob-

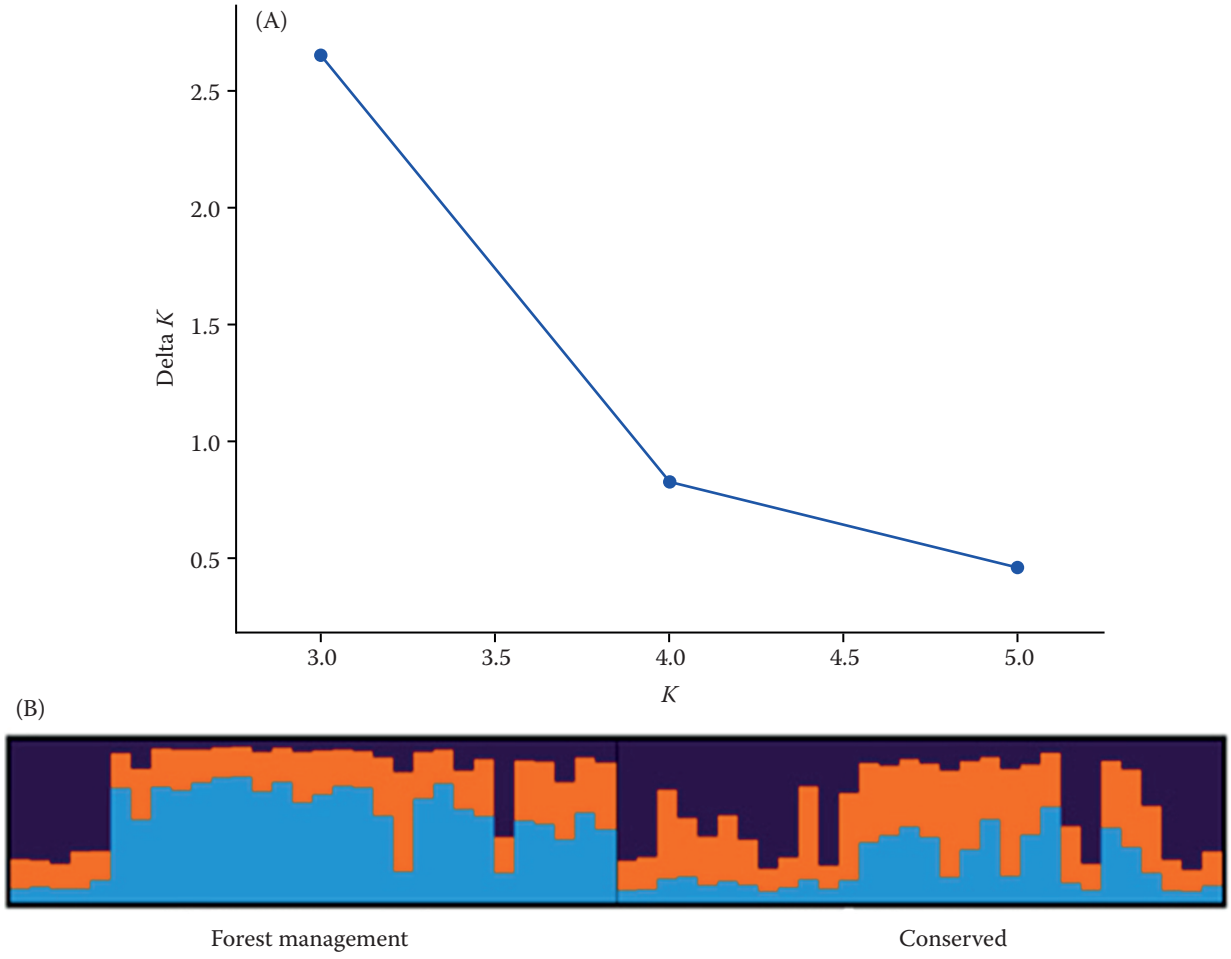

Figure 2. Results of Structure analysis using Structure selector; each vertical bar represents the inferred ancestry in each genetic group (A) estimation of the most probable *K* using the delta *K* method by Evanno et al. (2005); (B) bar plot representing the genetic structure at $K = 3$.

K – number or hypothetical genetic groups; delta *K* (the method) – mean ($|L''(K)|$)/sd($L(K)$); $L(K)$ – likelihood distribution difference; $L''(K)$ – values of the second-order rate of change of the likelihood distribution; sd – standard deviation

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Table 3. Shannon index, observed and expected heterozygosity, and fixation index by study condition and population mean

Study condition	I	H_o	H_e	f
Forest management	1.31 ± 0.21	0.61 ± 0.08	0.67 ± 0.06*	0.06 ± 0.14
Conserved	1.38 ± 0.17	0.61 ± 0.09	0.71 ± 0.05*	0.11 ± 0.14
Mean	1.35 ± 0.13	0.61 ± 0.05	0.69 ± 0.04	0.08 ± 0.10

*$P < 0.05$ significant differences of the Hardy-Weinberg equilibrium; I – Shannon index; H_o – observed heterozygosity; H_e – expected heterozygosity; f – fixation index

Table 4. F coefficients and number of migrants by locus and total population

Locus	F_{ST}	F_{IS}	F_{IT}	Nm
Ab07	0.026**	0.015	0.041	11.28
Ab08	0.029**	0.402***	0.419***	9.13
Ab09	0.059**	0.426***	0.459***	5.66
Ab12	0.044*	–0.162	–0.111	8.19
Ab15	–0.006	–0.517	–0.526	215.75
Ab20	–0.015	0.438***	0.430***	52.12
Ab23	0.240***	0.055	0.282***	1.47
Mean	0.056***	0.135***	0.183***	43.37 ± 29.44

*,**,**$P < 0.05$, $P < 0.01$, $P < 0.001$ respectively; F_{ST} – differentiation coefficient; F_{IS} – inbreeding coefficient; F_{IT} – total inbreeding coefficient; Nm – number of migrants per generation

served differences are caused by the different management between the study conditions.

A low Nei's genetic distance (0.195) and high identity (0.823) were observed, which indicates that it is the same species.The AMOVA results indicate that the genetic divergence between populations is 5.6% and among individuals 12.8%, while the divergence within individuals is 81.36% (Table S2 in the ESM). The greater observed divergence within individuals is influenced by a private allele, the 146 allele (frequency = 0.2) for the Ab08 locus, in the managed condition.

The number of genetic groups estimated from the Bayesian analysis in STRUCTURE is three ($K = 3$) (Figure 2A). The managed condition shows a larger predominance of one genetic group, while the rest seem to be more evenly distributed, although for the first evaluated specimens, one of the last two genetic groups is predominant. In the conserved condition, there is apparently a greater predominance of two genetic groups that present less dominance compared to the managed condition (Figure 2B).

Bottleneck. Six loci in the managed condition (Wilcoxon test, $P = 0.007$) and all loci in the conserved condition have excess heterozygosity under the mutation-drift equilibrium model. These results indicate that both conditions show evidence of a recent genetic bottleneck, which is stronger in the conserved condition.

DISCUSSION

Both conditions are in disequilibrium and maintain an apparently high connectivity, high levels of heterozygosity (slightly higher in the conserved condition), and greater genetic homogeneity among individuals under management conditions. This is because forest management activities are aggressive and are fragmenting the landscape, so genetic connectivity is a reflection of the past; a relatively recent history of extraction has eradicated all the individuals in some areas, and the few isolated trees are related adults grouped within a radius of less than 30 m. Similarly, low genetic diversity has been observed for some relict species in fragmented landscapes, but unlike our results, they show high differentiation between populations due to spatial isolation and high levels of inbreeding (Young et al. 1996; Szczecińska et al. 2016; Li et al. 2018) which in these specific cases is due to the time elapsed since fragmentation, since a few generations (one to four) are enough for population genetic divergence to manifest (Lavigne et al. 2001).

However, this can take decades in long-lived trees, like the ones sampled in this work (Eguiarte et al. 2007; Wiberg et al. 2016). Thus, the loss of genetic diversity depends on the degree of fragmentation related to gene flow and dispersal mechanisms, the demographic structure and the time elapsed since the reduction in population size (Bialozyt et al. 2006; Aldrich et al. 1998).

However, even when heterozygosity is high for *A. hidalgensis*, Rasmussen et al. (2008) observed 114 alleles using the same microsatellites as in this study on *A. guatemalensis*, a related species (Cruz-Nicolás et al. 2021), but unlike this study, 18 populations were evaluated in a wide distribution range. In our work, such population representativeness is impossible due to the conservation conditions

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described. We only obtained between 4 and 5 effective alleles average depending on the condition, so there is a real loss of allelic diversity but an artificial, short-lived extreme increase in the heterozygosity, which is explained by recent bottlenecks, since allelic diversity is much more sensitive to genetic changes (Spencer et al. 2000).

High levels of genetic diversity have also been observed in other conifers with relict populations such as *Picea omorika* (Panč) Purk (Aleksić et al. 2009) and *Picea chihuahuana* Martinez (Jaramillo-Correa et al. 2006), and in other species of the genus *Abies* (Table 5). Therefore, the high levels of H_o and H_e are the product of frequent past events of mixing of genetically differentiated populations and the recent drastic population reduction (Luikart et al. 1998), which decreases the number of alleles in a microsatellite concerning its allelic range (Garza, Williamson 2001), although such effects are temporary.

For example, in the particular case of *A. ziyuanensis*; another highly threatened fir species endemic to China, which like *A. hidalgensis* has low population numbers and declining population size mainly due to human activities, the species has a reduced total number of nuclear alleles (29) (Tang et al. 2008), similar to *A. hidalgensis* (35). The number of polymorphic loci found in *A. ziyuanensis* was five; at three of the eight evaluated loci ,only one allele was identified. Despite being polymorphic in *A. hidalgensis*, the Ab15 locus only had two alleles identified, so it is evident that the reduction in genetic diversity is related to the loss of alleles.

In *A. nebrodensis*, a highly threatened species with a single population, Vendramin et al. (1995), observed a higher H_o than H_e, and indicated that it could be due to selection in favour of heterozygotes. In contrast, in this study, four loci were observed with an excess of homozygotes, which, based on the population structure, could be due to the Wahlund effect, which homogenises diversity within populations and ends up generating high inbreeding in later stages of succession (Litrico et al. 2005). All tested loci would be expected to exhibit the same trend in the balancing selection hypothesis, still both excess and deficiency can be observed in both conditions. Considering that selection is unlikely to act on more than one locus, the observed deviations are consistent with the drastic reduction in population size that occurs stochastically and randomly.

On the other hand, based on the *f* fixation index, *A. hidalgensis* shows little inbreeding. However, if the reduction in effective population size is recent and since only adult trees were assessed,

Table 5. Comparison of genetic diversity of *A. hidalgensis* with other species of the genus *Abies*

Species	Molecular marker	Mean N_e	Mean *Ar*	H_o/H_e	H_e	F_{ST}	F_{IS}	Reference
Abies guatemalensis	SSR	28.600	–	0.822	0.862	0.051	0.045	Rasmussen et al. (2010)
Abies guatemalensis	isoenzyme	–	–	–	0.069	–	–	Aguirre-Planter et al. (2000)
Abies religiosa		–	–	–	0.108	–	–	
Abies flinckii		–	–	–	0.113	–	–	
Abies hickelii		–	–	–	0.100	–		
Abies pinsapo	nSSR	2.825	–	0.528	0.596	–	0.120	Cobo-Simón et al. (2020)
	ISSR	1.058	–	–	0.035	–	–	
Abies ciclica	SSR	–	2.77	0.578	0.595	–	0.029	Awad et al. (2014)
Abies marocana		–	2.86	0.405	0.572	–	–	
Abies bornmuelleriana		–	4.22	0.668	0.799	–	–	
Abies cephalonica		–	4.17	0.713	0.821	–	–	
Abies alba		–	3.93	0.663	0.808	–	–	
Abies ziyuanensis	SSR	3.600	–	–	0.437	0.250	–	Tang et al. (2008)

N_e – effective number of alleles; *Ar* – rarefied allelic richness; H_o – observed heterozygosity; H_e – expected heterozygosity; F_{ST} – differentiation coefficient; F_{IS} – inbreeding coefficient; nSSR –nuclear microsatellites; ISSR – inter-microsatellites; SSR – Simple Sequence Repeats

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it is unlikely to be observed. In this regard, Leding et al. (2006), found significantly different levels in the inbreeding coefficient in *A. bracteata* D. Don ex Poiteau between samples of adult trees and seeds, so that in the next generations of *A. hidalgensis* more significant inbreeding could be expected, which would be a critical factor for the design of management and conservation strategies. Consequently, the level of inbreeding concerning Wright's *F* statistics is low (Wright 1951).

Implications for conservation. The patterns of genetic diversity detected in this study can be used in designing management and conservation strategies for *A. hidalgensis*. The total of the populations known up to now was not analysed. However, two populations are evaluated, where the most significant distance between specimens occurs and where one has a good degree of conservation.

Conserving alleles in the context of populations is a fundamental strategy because, as already mentioned, they are more susceptible to loss due to bottlenecks. Since they are unique, it is important to obtain representative seeds of all populations for breeding purposes, especially of those specimens with the 146 allele. Given the climate fluctuations that imply new adverse scenarios, any allele is a potential benefit. Increasing the population size with the greatest possible diversity is urgently necessary since its ability to adapt to changes depends on it (Gargiulo et al. 2019).

In the case of *A. hidalgensis*, conservation efforts should focus on preventing habitat loss due to human impacts and future climate change impacts, as well as assessing the feasibility of carrying out *ex situ* conservation of genetic resources or assisted migration, given its limited ability to migrate. In addition, the seeds are difficult to obtain because they are found in isolated and tall individuals; the species does not form a seed bank, the seedlings emerge infrequently (Rosales-Islas, personal observation), and most of them die before reaching the juvenile stage. So, seeds and seedlings are a reservoir of genetic variability (Mosseler et al. 2003) where all recovery, propagation, and management efforts should be directed.

CONCLUSION

Abies hidalgensis has a high heterozygosity but suffers a negative effect of logging associated with a reduction of the population size and show a limited allelic richness distributed differently in response to forest management. However, its populations maintain a common genetic base that has not yet been differentiated because fragmentation and reduction in population size are very recent. We are observing a corpse that still does not know it is dead, since the total eradication of individuals in some areas generates a total loss of connectivity, which increases the level of threat to the species and accelerates its extinction rate; therefore, conservation actions are paramount.

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