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Genetic variation and demographic contraction of the remnant populations of Mexican Caribbean pine (*Pinus caribaea* var. *hondurensis*: Pinaceae)

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Abstract

• **Introduction** The recognition of endangered species takes into account geographic isolation and small size of populations, as is the case of two populations of *Pinus caribaea* var. *hondurensis* distributed in México.

• **Materials and methods** This study evaluates the genetic variation within and among Mexican populations of *P. caribaea* var. *hondurensis* to make inferences on the historical dynamics of the populations by screening a total of 69 trees with six nuclear microsatellites.

• **Results** The results indicate intermediate levels of genetic variation in both populations ($H_E = 0.465$). The level of inbreeding was significant for both populations (Caobas $F_{IS} = 0.097$; Pioneros $F_{IS} = 0.163$), but the effective population size was higher in the Pioneros population ($N_e = 868$) compared to Caobas ($N_e = 402$). Average genetic differentiation was low ($R_{ST} = 0.033$) and historical migration rate was relatively high ($M = 13.2$).

• **Discussion** These results, together with the analysis of bottlenecks, provide us evidence of historical events of contraction of the population size, which probably occurred between 8,100 and 35,000 years ago. We propose conservation strategies for the emergent recovery of these remaining native forest patches.

Keywords Caribbean pine · Nuclear microsatellites · Population genetics · Conservation · Mexico

1 Introduction

The probability of extinction of a species depends on its intrinsic characteristics and factors that alter the environment where it grows (Lande 1993). Generally, the recognition of species as endangered accounts for both demographic and genetic processes, which are related to geographic isolation and small population sizes (Lande 1993). Geographic isolation can initiate population divergence through mutation and genetic drift (Karhu et al. 2005). Fluctuations in population size can decrease the effective population size and may result in the loss of genetic variation, as well as processes of inbreeding with the resulting local or global population extinction (Shaffer 1981; Boyce 1992). Therefore, small populations are more susceptible to extinction than large populations and extrinsic or environmental factors increase that risk.

Genetic models have been used to infer demographic processes of species, such as colonization, expansion, growth rates, bottlenecks, and founder effects (Maruyama and Fuerst 1985; Cornuet and Luikart 1996). Several studies have used this type of approach in conifers. Karhu et al. (2005) with nuclear microsatellites inferred the presence of expansion events in the populations of *Pinus*

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radiata D Don. on the islands of Guadalupe and Cedros in Mexico. Jaramillo-Correa et al. (2006) used chloroplast microsatellites to infer demographic bottlenecks in some populations of *Picea chihuahuana* Mart. distributed in the Sierra Madre Occidental of Mexico. Al-Rabab'ah and Williams (2004) found, with nuclear microsatellites, that the loss of natural populations of *Pinus taeda* L. in central Texas (USA), is due to the drastic size reductions that occurred during and after the Pleistocene. Ledig et al. (1999) found reduced allozyme variation for the only known population of *Pinus maximartinezii* Rzed., an endemic to Mexico, suggesting a drastic reduction in the population size by recent bottleneck events. These works are representative of species with fragmented and restricted distributions and have demonstrated the utility of the genetic approach for conservation.

Pinus caribaea var. *hondurensis* (Sénécl) Barr. et Golf. (Caribbean pine) is a tropical species with the southernmost and lowest elevation distribution of American pines (from 1 to 1000 m; Farjon and Styles 1997). It is represented by some large and continuous populations (Belize, Nicaragua, Honduras) and several that are restricted and fragmented (Belize, Guatemala, and Mexico; Dvorak et al. 2005). Only a single population was known from Mexico formerly, occurring in the Ejido of Caobas in the locality known as “Jaguactal” in the state of Quintana Roo. According to different authors, this population consists of 150 (Macario et al. 1998; this study) to 1,000 (Dvorak et al. 2005) reproductive trees and is considered endangered (NOM-059-2001). In this study, we report a new population located on the southern boundary of the states of Campeche and Quintana Roo. This population is extremely small, with only 14 reproductive trees. These two populations represent the northernmost distribution reported for the species, and like other populations in Belize, they are found in isolated areas within forest savanna called “swamps”. These habitats are characterized by periods of drought and extreme humidity and very acidic, impermeable soils which floods in the rainy season (June to October).

The current geographic distribution, as well as the ecological conditions in which the Mexican populations occur, suggest the hypothesis that both populations represent remnants of a wider distribution of the species, which diminished due to climate changes, specifically extreme droughts, floods, and the presence of hurricanes.

Recently, Dvorak et al. (2005) used a predictive climatic model to propose that *P. caribaea* var. *hondurensis* had a larger distribution in the past (7,000–8,000 years BC), and today, it should occur along the east coast of Yucatan, as far north as Puerto Juarez, Quintana Roo. In addition, Furman (1997) using RAPDs from several populations, found that two localities of Belize and the Caobas locality in Mexico are very similar, but very different genetically from populations

in Honduras and Nicaragua, suggesting that they might be satellite populations derived from a core population.

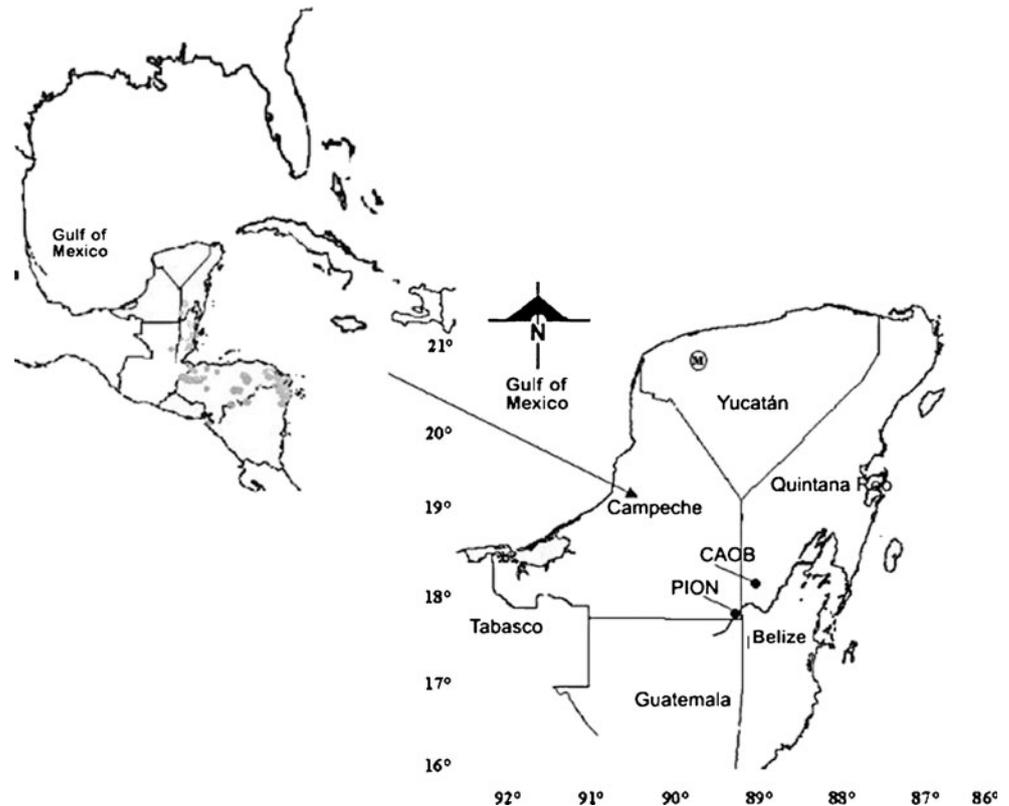
In this sense, the study of the Mexican population of *P. caribaea* var. *hondurensis* is of great relevance for both evolutionary ecology and conservation biology because (1) as the northernmost populations of the species, it could represent evidence that this region was an important past route of dispersal among America (from the northeast of the Yucatan Peninsula) and the Caribbean islands, (2) these populations could contain unique genetic variants which could be of great adaptive value, and (3) their extremely small population size requires the generation of basic information that allows specific strategies for restoration and conservation. Currently, no work exists that includes genetic material from these two populations (Furman (1997) only used material from one individual of the locality of Caobas), nor any demographic study that could help us understand their population dynamics. In the present study, we analyze molecular data (nuclear microsatellites: nSSR) to determine the dynamics of local extinction of these populations as well as to infer demographic events that have influenced their evolutionary history. We selected nuclear microsatellites as genetic markers because they are highly polymorphic, codominant, and compatible with population genetic theory (Petit et al. 2005). The goals of this study were to: (1) estimate levels of variation and genetic structure, inbreeding and the effective size of the Mexican population of *P. caribaea* var. *hondurensis*, (2) test the hypothesis that a bottleneck diminished the levels of genetic variation and population differentiation, and (3) generate specific strategies for conservation of these remnant native forest patches.

2 Materials and methods

2.1 Study area

The two Mexican populations of *P. caribaea* var. *hondurensis* are distributed in the southern states of Quintana Roo and Campeche. The population of Caobas (CAOB) is located in the area known as the “Jaguactal” in the Ejido of Caobas, Municipality of Othon P. Blanco, Quintana Roo (between 18° 15' 12.5" N and 88° 57' 45.6" W; Fig. 1). The population of Pioneers (PION) is in the Ejido Pioneros del Río Nuevo Centro Poblacional within the natural reserve of Calakmul, on the southern limit of the states of Campeche and Quintana Roo (between 17° 53' 05" N and 89° 10' 44" W). The CAOB population is represented by 150 to 200 trees with most individuals (>60%) between 50 and 80 years and fewer seedlings and saplings (<15%), whereas the PION population is represented by only 14 mature trees older than 80 years, and no regeneration (Rebolledo et al. unpublished

Fig. 1 Geographic location of the Mexican populations of *P. caribaea* var. *hondurensis*. Gray color represents the natural distribution of the variety



data). This species grows in Gleysols characterized by possessing a great quantity of clay with a strong plasticity and adhesiveness when humid, while when dry, it shrinks and develops cracks and fissures (Macario et al. 1998). The topography is relatively flat and uniform; the altitudes of the CAOB and of the PION population are 130 and 68 msnm, respectively. The vegetation is characterized by representative species of the forest savanna (*Curatella americana* L, *Crescentia cujente* L, and *Scleria* sp.) and temporally flooded lowland forest (*Haematoxylum campechianum* L, *Clusia chanekiana* Lundell, *Mimosa bahamensis* Benth, and *Erythroxylum confusum* Britton). The climate is tropical in both populations with an average annual rainfall of 1,300 mm and an average temperature of 26°C (Macario et al. 1998).

2.2 Collection of material

In February 2006, ecological data and biological material were obtained. For the CAOB population, a census of its entire distribution area was conducted (~4 ha), and three transects were placed in the area with the greatest density of individuals. Along these transects, one small branch per tree was collected from a total of 60 individuals. For the PION population, samples were collected from all 14 trees found in an area of about 2 ha. The samples were labeled and stored at -70°C until DNA extraction.

2.3 Microsatellites

Genomic DNA was extracted using a Miniprep CTAB method (Vázquez-Lobo 2006). Eighteen primers were tested; 14 derived from *P. taeda* (Elsik et al. 2000) and four from *Pinus resinosa* (Boys et al. 2005), of which six polymorphic microsatellites with good resolution were selected (Table 1). The amplification reactions and PCR conditions were performed using the methodology developed for *P. taeda* (Elsik et al. 2000). The fragments were separated on polyacrylamide gels 6% (7 M of urea) and run at 55 W for 1.5 to 3.5 h, depending on the size of the fragments. The polymorphisms were displayed with the silver nitrate staining method (Echt et al. 1996), and fragment size was determined visually using a 10-bp reference marker.

According to the molecular analyses of Shepherd et al. (2002), five of the six microsatellites used in this study did not show null alleles. However, we estimated null allele frequencies for each locus with Micro-Checker 2.2.3 (Van Oosterhout et al. 2004). The results indicate a relatively low proportion of null alleles for CAOB population in two loci (PtTX2146, proportion 0.166; PRE13, proportion 0.132). To remove these null alleles, we performed this analysis again excluding five individuals that the Micro-Checker program suggested. Based on this result, the analysis of genetic variation of CAOB population was realized on a total of 55 individuals that did not present null alleles.

Table 1 Allele frequencies obtained for Mexican populations of *P. caribaea* var. *hondurensis*

Locus	Population		
	Alleles	Caobas (CAOB)	Pioneros (PION)
PtTX2123	201	0.916	0.964
	191	0.027	0.036
	198	0.063	0
PtTX3025	267	0.009	0
	268	0.718	0.785
	252	0.236	0.178
	272	0.036	0.036
PtTX3013	142	0.109	0
	134	0.872	0.607
	125	0.009	0.714 _s
	145	0.009	0.321
PtTX2146	181	0.566	0.679
	177	0.400	0.321
	165	0.033	0
PtTX3020	187	0.259	0.214
	181	0.241	0.214
	167	0.037	0.071
	195	0.463	0.500
PRE13	240	0.239	0.500
	244	0.185	0.231
	252	0.271	0.269
	230	0.087	0
	270	0.065	0
	255	0.152	0
No. of alleles/locus		4.000	2.833

Genetic diversity was determined by observed (H_O) and expected (H_E) heterozygosity, the repeat size range of the alleles, the number of alleles per locus per population, and the mean number of alleles per locus. The genetic structure (R_{ST}) was obtained according to the stepwise mutation model (SMM; Kimura and Ohta 1978) and inbreeding coefficient (F_{IS}) according to Wright (1965). These estimates were made with the program Arlequin ver. 3.0 (Excoffier et al. 2005). The effective population size (N_e) and the effective migration rates ($M_R = N_e m$) were estimated with a simulated annealing procedure of maximum likelihood assuming a mutation rate (μ) of 10^{-3} per generation (Boys et al. 2005), using the program Migrate ver. 3.0 (Beerli 2008). The N_e was calculated as $\Theta/4 \times 10^{-3}$ (Boys et al. 2005), and M_R as m/μ (Beerli 2006). To infer the consequences of demographic processes on the levels of genetic diversity of populations, we tested the “bottleneck” hypothesis with two methods: the first was “heterozygosity excess”, which assumes that when a population experiences a recent reduction of their effective population size, it tends to exhibit

a reduction in the number of alleles and in heterozygosity (Cornuet and Luikart 1996). The heterozygosity (H_E), theoretically is higher than expected heterozygosity (H_{Eq}) at mutation-drift equilibrium calculated from allele number (Cornuet and Luikart 1996; Luikart et al. 1998). The second method was the graphic representation of the “mode shift indicator” (Luikart et al. 1998). The reduction or loss of classes of allele frequencies “shifts” can be detected by comparing the distribution of alleles in a population to the distribution that would be expected at mutation-drift equilibrium (Maruyama and Fuerst 1985; Luikart et al. 1998). Both analyses assume a stepwise mutation model and were performed using Bottleneck 1.2.02 software (Piry et al. 1999).

3 Results

3.1 Genetic variation

For the six selected loci, the repeat size of the alleles ranged from 2 to 20 bp showing higher frequency alleles with repeat sizes from 2 to 6 bp. A total of 24 alleles were found in the CAOB population, and 17 alleles in the PION population, with an average of 4.00 and 2.83 alleles per locus, respectively. The most frequent alleles for both populations were 201 (PtTX2123), 168 (PtTX3025), 134 (PtTX3013), 181 (PtTX2146), 195 (PtTX3020), and 240 (PRE13) (Table 1). The average expected and observed heterozygosity of CAOB population ($H_E=0.471$, $H_O=0.459$) were slightly higher than PION ($H_E=0.459$, $H_O=0.386$). Both populations had a deficit of heterozygotes compared to random mating expectation, and thus a significant coefficient value (CAOB $F_{IS}=0.097$, $P=0.045$; PION $F_{IS}=0.163$, $P=0.003$; Table 2). The estimated effective size (N_e) was higher in the population of PION ($N_e=868$) than in CAOB ($N_e=402$).

The genetic structure among populations was low but significant ($R_{ST}=0.033$, $P=0.037$), and the average gene flow was relatively high ($M=13.2$). Gene flow from PION to CAOB ($M=16.1$) was slightly higher than from CAOB to PION ($M=10.3$), suggesting historical asymmetric gene flow.

3.2 Demographic history

For three loci (PtTX2123, PtTX 3025, and PtTX3013), the bottleneck analysis based on the “heterozygosity excess” model had heterozygosity values (H_E) that were lower than expected under mutation-drift equilibrium (H_{Eq}) for each population and for the total gene pool (Table 3). This suggests a significant loss of genetic diversity due to fragmentation and decrease in population size.

The mode shift analysis revealed more than one allele class, with medium and high frequencies (0.3 to 0.7), as well

Table 2 Parameters of genetic diversity estimated for Mexican populations of *P. caribaea* var. *hondurensis*

Population	N	A	H_O	H_E	F_{IS}	Θ	N_e
CAOB	55	24	0.459 (0.226)	0.471 (0.247)	0.097 ^a	1.60	402
PION	14	17	0.386 (0.207)	0.459 (0.222)	0.163 ^a	3.27	868
Average	37	20.5	0.422 (0.216)	0.465 (0.235)	0.053 ^a		

Values in parentheses are standard deviation

N size, A allele number, H_O and H_E observed and expected heterozygosity, F_{IS} fixation index, Θ genetic diversity parameter ($4N_e u$), N_e population effective size

^aSignificant deviations from $F_{IS}=0$ at 0.05 probability

as a the highest frequency (0.8) of two and three class of alleles for PION and CAOB, respectively. The CAOB population contains the largest number of allele classes with low frequencies, lower than 0.1. Also, both populations have two gaps with loss of classes of allelic frequencies (gaps 4 and 7 for CAOB and 8 and 9 for PION; Fig. 2). These results corroborate the existence of events of population size contraction with the consequent loss of allelic variants.

4 Discussion

4.1 Genetic variation and demographic history

Our results show significant levels of genetic variation in CAOB and PION, although they are lower than those obtained with nSSR for other pine species (e.g., *P. radiata* $H_O=0.625$, (Smith and Devey 1994); $H_O=0.670$, (Karhu et al. 2005); *Pinus strobus* $H_O=0.515$, (Echt et al. 1996), *P. taeda* $H_O=0.559$, (Elsik et al. 2000); *P. caribaea* var.

hondurensis $H_O=0.490$, (Shepherd et al. 2002); $H_O=0.527$, (Rebolledo et al., unpublished data), and *Pinus elliottii* var. *elliottii* $H_O=0.500$, (Shepherd et al. 2002)). For the study of Shepherd et al. (2002), the estimation was based on a sample size of five individuals and should be taken with care. However, the study by Rebolledo et al. (unpublished data) was based on an average of 20 individuals from seven populations distributed in Belize and Honduras, and with four of the nSSR used in this study. Therefore, the genetic diversity of the two Mexican populations is still lower than that obtained in other populations of *P. caribaea* var. *hondurensis* and most pine species analyzed with nSSR.

An exceptional case is *P. resinosa*, a species of wide but fragmented distribution, for which Boys et al. (2005) reported an average of $H_O=0.185$, below the one obtained in this study. They consider that this low value may be due to recent colonization event. At the population level, H_E from CAOB and PION were higher than H_O , and F_{IS} was significant (CAOB $F_{IS}=0.097$; PION $F_{IS}=0.163$), which suggest a higher average rate of inbreeding. In contrast, the

Table 3 Results of the bottleneck analyses performed with the heterozygosity excess model in the Mexican populations of *P. caribaea* var. *hondurensis*

Population	Locus	K_n	H_E	H_{Eq}	SD	DH/sd	P
CAOB	ptTX2123	3	0.172	0.471	0.139	-2.151	0.054*
	ptTX3025	4	0.435	0.603	0.107	-1.578	0.050*
	ptTX3013	4	0.230	0.608	0.101	-3.738	0.004*
	ptTX2146	3	0.530	0.494	0.130	0.277	0.471
	ptTX3020	4	0.672	0.608	0.103	0.621	0.301
	PRE13	6	0.818	0.749	0.065	1.059	0.076
PION	ptTX2123	2	0.072	0.342	0.146	-1.901	0.0000*
	ptTX3025	3	0.362	0.565	0.108	-1.809	0.0430*
	ptTX3013	3	0.542	0.570	0.109	-0.081	0.3930
	ptTX2146	2	0.452	0.345	0.147	0.796	0.3190
	ptTX3020	4	0.677	0.691	0.077	0.161	0.4860
	PRE13	3	0.649	0.573	0.108	1.143	0.0750

SMM Stepwise mutation model, K_n number of alleles per locus, H_E expected heterozygosity, H_{Eq} expected heterozygosity at mutation-drift equilibrium, SD standard deviation of the mutation-drift equilibrium distribution of the heterozygosity, $H_O - H_{Eq}/sd$ standardized difference for each locus

* $P=0.05$, probability of deficit for the expected heterozygosity (H_E)

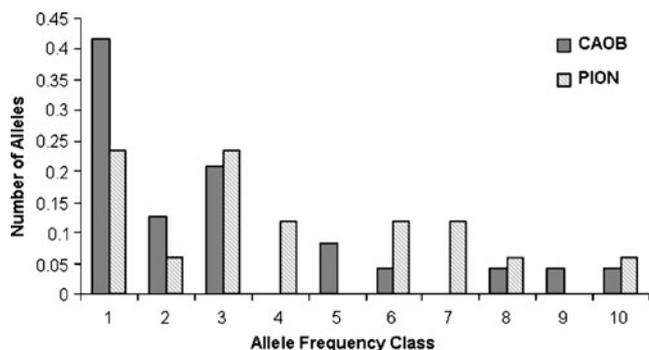


Fig. 2 Frequency distribution of allele classes for the Mexican populations of *P. caribaea* var. *hondurensis* obtained with the mode shift indicator

preliminary estimation of F_{IS} obtained by Rebolledo et al. (unpublished data) was not significant ($F_{IS}=0.035$). This estimate is similar to that reported by Dvorak et al. (2005), with isozymes for populations of this variety from Belize and Guatemala ($F_{IS}=0.011$).

Another interesting result is the average number of alleles per locus, which was lower for both populations (CAOB, four alleles and PION, three alleles) than the average reported for other pine species, such as *P. resinosa* (nine alleles, (Boys et al. 2005)), *P. radiata* (six alleles, (Smith and Devey 1994); seven alleles, (Karhu et al. 2005)), *Pinus oocarpa*, *Pinus patula*, and *Pinus tecunumanii* (five alleles, (Dvorak et al. 2009)), and similar to other populations of *P. caribaea* var. *hondurensis* distributed in Belize and Honduras (four alleles, Rebolledo et al. unpublished data). In general, these results suggest that the Mexican populations of *P. caribaea* var. *hondurensis* are losing genetic diversity due possibly to genetic drift, and this is also reflected by an increase of inbreeding.

The decrease in effective population size (N_e) has been found to be the main cause for the loss of genetic diversity (Shaffer 1981; Karhu et al. 2005). For the CAOB population, which is represented by 150 to 200 individuals, the estimated N_e was 402, less than the value obtained for PION (868), although the latter includes only 14 trees. Few studies have estimated the effective size with nSSR, but if we compare the N_e of the Mexican populations of this pine with the N_e obtained from *P. resinosa* (N_e between 62 and 222; (Boys et al. 2005)), these values are relatively higher, which shows that the two populations have suffered a drastic reduction in census size and suggest that inbreeding has played an important role in the decline of genetic variation. In particular, for the PION population, the lower genetic diversity could be the result of a recent reduction in population size, without having a direct impact on the estimated N_e . The latter, if we compare the N_e of PION with CAOB population, was higher than in CAOB. An alternative explanation for these N_e values is that the population size of PION is larger than we observed.

These results combined with those obtained with the bottleneck analysis indicate that both populations have declined in size during their evolutionary history. H_E values for three of six loci were lower than expected under mutation-drift equilibrium for both populations, whereas the remaining three loci had opposite estimates, high values of H_E compared to H_{Eq} (e.g., PRE13 locus; Table 3). Such results are comparable to those obtained with other species of conifers (*P. taeda* (Al-Rabab'ah and Williams 2004); *P. chihuahuana* (Jaramillo-Correa et al. 2005)). In particular, in the study of *P. taeda* (Al-Rabab'ah and Williams 2004), which tested the hypothesis of bottlenecks in a population (Lost Pines) represented by 14 individuals, the H_E values obtained were lower than expected at drift-mutation equilibrium (H_{Eq}) for several loci, suggesting a reduction in allele diversity. The authors conclude that the Lost Pines population underwent events of ancestral bottlenecks (between 3,000 and 30,000 years ago). Our results are similar since H_E was lower than H_{Eq} for three loci, indicating a relatively ancient reduction of population size. The results obtained from the analysis of the classes of allelic frequencies corroborate this hypothesis, indicating a decline and loss of alleles in both populations, with a greater loss of alleles in the PION population. Small populations tend to lose rare alleles more rapidly than large populations (Maruyama and Fuerst 1985). Therefore, the PION population has suffered a great loss of alleles due to the effect of genetic drift, while the CAOB population has experienced a reduction in both census and effective sizes.

Dvorak et al. (2005) offered the hypothesis that *P. caribaea* var. *hondurensis* populations were distributed more continuously in the past. The results obtained in this work support two central aspects of this hypothesis: the first relates to the patterns of genetic structure found for pine species with geographically isolated or fragmented distributions, which are much higher than those obtained for species with wide distributions (Ledig 1998). Nevertheless, the value obtained in this study was low ($R_{ST}=0.033$), and relatively similar to the value obtained by Dvorak et al. (2005); ($F_{ST}=0.023$), suggesting that *P. caribaea* had a wider distribution in the past. This is also reflected in the high rates of ancestral gene flow obtained between Mexican populations ($M=13.1$) regardless of the actual geographical fragmentation. The second aspect relates to the results obtained by Dvorak et al. (2005) with the predictive analysis of the distribution of the species. They conclude that *P. caribaea* var. *hondurensis* had a wider distribution in the Yucatan Peninsula in the past, with a demographic dynamics of contraction and expansion events generated by climate changes of drought and extreme floods that occurred during the last 10,000 years. We conclude that events of contraction that were detected in the Mexican populations could not have occurred in the recent past, or within $4N_e$ generations (less than 80

generations; (Luikart et al. 1998)). To support this hypothesis, we can estimate the time during which these populations have decreased in size, based on a timeframe of $2N_e$ to $4N_e$ generations (Luikart et al. 1998) and assuming a generation time based on the age of first reproduction of approximately 10 years (Okoro 1983). The generation times were of 812 to 1,624 generations for CAOB and 1,730 to 3,472 for PION, and the likely decline in the size of populations could have occurred between 8,120 and 34,720 years ago. The time interval of population decline estimated coincides with periods of abundant rainfall and prolonged floods in the region (Leyden et al. 1998). The occurrence of *P. caribaea* var. *hondurensis* on raised platforms is consistent with the hypothesis that the water level and the duration of flooding in the marshes are directly correlated to Caribbean pine abundance (Dvorak et al. 2005).

4.2 Conservation recommendations

The information obtained in this study is of great use for delineating specific conservation strategies: the CAOB population needs to be formally delimited, and all anthropogenic activity (such as cutting trees, introduction of livestock, and burning) should be avoided. The implementation of these strategies ought to promote an increase in the currently low regeneration rate and provide an environment where seedlings can survive, grow, and increase the size of the natural reproduction. For the PION population, more explorations of the area are needed to confirm the census size that was reported in this work. We also recommend that this small population be listed in the Mexican red list (NOM-059-SEMARNAT-2001) and recognized as vulnerable. Since no regeneration was observed for this population, we recommend in situ conservation measures such as performing a general cleaning of the zone to generate gaps that allow for seed germination and establishment. For ex situ conservation of both populations, seeds should be collected from maturing cones (from June to August), with seed germination carried out offsite followed by reintroduction of seedlings to the area. These measures offer a highly desirable solution for the repopulation of these sites.

In summary, the use of molecular microsatellites markers enabled us to recognize intermediate levels of genetic variation, high levels of historical gene flow, and relatively high values of effective population sizes in Mexican populations of *P. caribaea* var. *hondurensis*. These results, together with the bottleneck tests, suggest that the Mexican populations come from the fragmentation of a large population that had a more continuous distribution in the past. Currently, populations are isolated, have extremely small census sizes, and exhibit evidence of loss of allelic variants and signs of inbreeding. Therefore, we recommend that a conservation strategy must be incorpo-

rated into the management programs developed by the institutions responsible for safeguarding forest resources of Mexico.

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References

- Al-Rabab'ah MA, Williams CG (2004) An ancient bottleneck in the Lost Pines of central Texas. *Mol Ecol* 13:1075–1084
- Beerli P (2006) Comparison of Bayesian and maximum-likelihood inference of population genetic parameters. *Bioinformatics* 22:341–345
- Beerli P (2008) Migrate version 3.0, a maximum likelihood and Bayesian estimator of gene flow using the coalescent. Available at <http://popgen.scs.edu/migrate.html>
- Boyce MS (1992) Pollination viability analysis. *Ann Rev Ecol Syst* 23:481–506
- Boys J, Cherry M, Dayanandan S (2005) Microsatellite analysis reveals genetically distinct populations of red pine (*Pinus resinosa*, Pinaceae). *Am J Bot* 92:833–841
- Cornuet JM, Luikart G (1996) Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. *Genetics* 144:2001–2014
- Dvorak WS, Hamrick JL, Gutiérrez EA (2005) The origin of Caribbean pine in the seasonal swamps of the Yucatán. *Int J Plant Sci* 166:985–994
- Dvorak WS, Potter KM, Hipkins VD, Hodge GR (2009) Genetic diversity and gene exchange in *Pinus oocarpa*, a Mesoamerican pine with resistance to the pitch canker fungus (*Fusarium circinatum*). *Int J Plant Sci* 170:609–626
- Echt CS, Marquardt PM, Hsieh M, Zahorchak RR (1996) Characterization of microsatellite markers in eastern white pine. *Genome* 39:1102–1108
- Elsik CG, Minihan VT, Hall SE, Scarpa AM, Williams CG (2000) Low-copy microsatellite markers for *Pinus taeda* L. *Genome* 43:550–555
- Excoffier L, Laval G, Schneider S (2005) Arlequin, ver 3.0: an integrated software package for population genetics data analysis. *Evol Bioinf Online* 1:47–50
- Farjon A, Styles BT (1997) *Pinus* (Pinaceae). *Flora Neotropical*, monograph 75. New York Botanical Garden, New York, 246 pp
- Furman BJ (1997) Phylogenetic informative markers as a diagnostic tool to identify species, hybrids and introgression in Central American and Mexican pines. Dissertation, Department of Genetics, North Carolina State University, Raleigh, 233 pp
- Jaramillo-Correa JP, Beaulieu J, Ledig T, Bousquet J (2006) Decoupled mitochondrial and chloroplast DNA population structure reveals Holocene collapse and population isolation in a threatened Mexican-endemic conifer. *Mol Ecol* 15:2787–2800
- Karhu A, Vogl C, Moran GF, Bell JC, Savolainen O (2005) Analysis of microsatellite variation in *Pinus radiata* reveals effects of genetic drift but no recent bottlenecks. *J Evol Biol* 19:167–175
- Kimura M, Ohta T (1978) Stepwise mutation model and distribution of allelic frequencies in finite populations. *Proc Natl Acad Sci USA* 75:2868–2987

- Lande R (1993) Risk of population extinction from demographic and environmental stochasticity and random catastrophes. *Am Nat* 142:911–927
- Ledig FT (1998) Genetic variation in *Pinus*. In: Richardson DM (ed) *Ecology and biogeography of Pinus*. Cambridge University Press, Cambridge, pp 251–280
- Ledig FT, Conkle MT, Bermejo-Velazquez B, Eguiluz-Piedra T, Hodgskiss PD, Johnson DR, Dvorak WS (1999) Evidence for an extreme bottleneck in a rare Mexican pinyon: genetic diversity, disequilibrium, and the mating system in *Pinus maximartinezii*. *Evolution* 53:91–99
- Leyden BW, Brenner M, Dahlin BH (1998) Cultural and climatic history of Cobá, a lowland Maya City in Quintana Roo. *México Quat Res* 49:11–122
- Luikart G, Allendorf FW, Cornuet JM, Sherwin WB (1998) Distortion of alleles frequency distributions provides a test for recent population bottlenecks. *J Hered* 89:238–247
- Macario MPA, Torres PS, Cabrera CE (1998) Estructura y composición de una comunidad de *Pinus caribaea* var. *hondurensis* (Sénecl) Barr. Et Golf., en el estado de Quintana Roo. *México Caribb J Sci* 34:50–57
- Maruyama T, Fuerst PA (1985) Population bottlenecks and nonequilibrium models in population genetics II. Number of alleles in a small population that was formed by a recent bottleneck. *Genetics* 111:675–689
- Okoro OO (1983) Influence of flowering habit of *Pinus caribaea* var. *hondurensis* Barr. et Golf on seed yield. *Niger J For* 13:36–42
- Petit JR, Duminil J, Fineschi S, Hampe A, Salvini D, Vendramin GG (2005) Comparative organization of chloroplast, mitochondrial and nuclear diversity in plant populations. *Mol Ecol* 14:689–701
- Piry S, Luikart G, Cornuet JM (1999) Bottleneck: a computer program for detecting recent reductions in the effective population size using allele frequency data. *J Heredity* 90:502–503
- Shaffer ML (1981) Minimum population sizes for species conservation. *Bioscience* 31:131–134
- Shepherd M, Cross M, Maguire TL, Dieters MJ, Williams CG, Henry RJ (2002) Transpecific microsatellites for hard pines. *Theor Appl Genet* 104:819–827
- Smith DN, Devey ME (1994) Occurrence and inheritance of microsatellites in *Pinus radiata*. *Genome* 37:977–983
- Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) MicroChecker: software for identifying and correcting genotyping errors in microsatellite data. *Mol Ecol Notes* 4:535–538
- Vázquez-Lobo A (1996) Evolución de hongos endófitos del género *Pinus* L: implementación de técnicas moleculares y resultados preliminares. Dissertation, Universidad Nacional Autónoma de México, 66 pp
- Wright S (1965) The interpretation of population structure by *F*-statistics with special regard to systems of mating. *Evolution* 19:395–420