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Research

Southward Pleistocene migration of Douglas-fir into Mexico: phylogeography, ecological niche modeling, and conservation of 'rear edge' populations

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Summary

• Poleward Pleistocene plant migration has been an important process structuring modern temperate and boreal plant communities, but the contribution of equatorward migration remains poorly understood. Paleobotanical evidence suggests Miocene or Pleistocene origin for temperate 'sky island' plant taxa in Mexico. These 'rear edge' populations situated in a biodiversity hotspot may be an important reserve of genetic diversity in changing climates.

• We used mtDNA sequences, cpDNA sequences and chloroplast microsatellites to test hypotheses of Miocene vs Pleistocene colonization of temperate Douglas-fir in Mexico, explore geographic patterns of molecular variation in relation to Pleistocene climate history using ecological niche models, and assess the taxonomic and conservation implications.

• We found strong evidence for Pleistocene divergence of Douglas-fir in Mexico (958 thousand yr before present (ka) with the 90% highest posterior density interval ranging from 1.6 million yr before present (Ma) to 491 ka), consistent with the southward Pleistocene migration hypothesis. Genetic diversity was high and strongly partitioned among populations. Spatial patterns of molecular variation and ecological niche models suggest a complex late Pleistocene history involving periods of isolation and expansion along mountain corridors.

• These results highlight the importance of southward Pleistocene migration in establishing modern high-diversity plant communities and provide critical insights into proposals to conserve the unique biodiversity of Mexican Douglas-fir and associated taxa.

Introduction

Poleward plant migration as ice sheets retreated during the late Pleistocene and Holocene has been described in many temperate and boreal plant communities throughout the world (Davis, 1981; Huntley & Birks, 1983; Petit *et al.*, 2003). These range shifts have been important in reshuffling species into the observed modern plant communities (Davis, 1981), and within species this reshuffling has often led to secondary contact of previously isolated populations and allopatric divergence of previously contiguous populations (Critchfield, 1984; Gugger *et al.*, 2010). Poleward shifts have also had the effect of creating gradients in genetic diversity, where poleward (leading edge) populations are often less diverse than their equatorward (rear edge) counterparts because of the effect of successive dispersal bottlenecks during migration (Petit *et al.*, 1997; Hewitt, 2000; Gugger *et al.*, 2008). In principle, migration in any direction would have similar effects but, for example, equatorward migration of temperate species is difficult to observe because of the extinction of rear-edge populations during the most recent postglacial period. Thus little is known about the extent to which modern temperate and subtropical forests comprise combinations of species originating in response to the onset of Pleistocene glaciations.

Given that equatorward migration has been identified as an important process in plant biogeography at deeper geological scales (e.g. North American contribution to South American communities during Great American Biotic Interchange; Wallace, 1876), it seems likely that the effects of such migration during the Pleistocene may still be detectable. In particular, southward migration may have been important in the formation of modern plant communities in subtropical Mexico, where temperate and subtropical genera intermix. The southward Pleistocene migration hypothesis was proposed based on early interpretations of the limited fossil record in Mexico (Deevey, 1949; Dressler, 1954; Perry *et al.*, 1998), but subsequent authors have favored an older Tertiary (primarily Miocene) origin for most temperate taxa in Mexico (Graham, 1999).

Disjunct temperate taxa in Mexico form a major part of the Madrean pine-oak (*Pinus–Quercus*) biodiversity hotspot, which covers the middle to high elevations of the Sierra Madre Occidental, Sierra Madre Oriental, Trans-Mexican Volcanic Belt and Sierra Madre del Sur (Fig. 1). This region contains nearly 4000 endemic plant species, of which at least 20 tree species or subspecies in Pinaceae are considered threatened (Conservation International; Norma Oficial Mexicana, 1994, 2001; Farjon & Page, 1999).

Yet, such rear edge populations may be an important reserve of genetic diversity in changing climates because they have often been long-isolated, display strong differentiation, and may exhibit local adaptation in response to strong selection and lack of gene flow (Hampe & Bairlein, 2000; Chang *et al.*, 2004; Martin & McKay, 2004; Hampe & Petit, 2005; Parisod & Joost, 2010). The rear edge populations of temperate taxa in subtropical Mexico have likely expanded and contracted in response to many climate fluctuations, producing complex patterns of intraspecific and interspecific biodiversity (Pennington *et al.*, 2000).

Douglas-fir (*Pseudotsuga menziesii*) is a wide-ranging, ecologically and economically important tree found from central Mexico to central British Columbia. Fossil and molecular evidence point to a northern North American or Asian Tertiary origin of *Pseudotsuga* (Hermann, 1985; Schorn, 1994; Gernandt & Liston, 1999), which suggests that the isolated Mexican 'sky-island' populations are the result of a past southward expansion. The fossil record suggests two alternative hypotheses: early Miocene (*c.* 20 million yr before present (Ma)) colonization when many other temperate taxa are thought to have arrived (Graham, 1999) or Pleistocene colonization in response to glaciation at high latitudes (Deevey, 1949; Dressler, 1954; Perry *et al.*, 1998). A few putative *Pseudotsuga* pollen grains found in Miocene sediment from Chiapas could suggest Miocene colonization (Palacios-Chavez & Rzedowski, 1993); however, *Pseudotsuga* and *Larix* pollen cannot be distinguished (Barnosky, 1985). Alternatively, Pleistocene colonization is supported by the much later first appearance of fossil *Pseudotsuga* pollen in the southern Rocky Mountains in the early Pleistocene (Gray, 1961) or late Pleistocene (Martin, 1963) in southern Arizona. In Mexico, the limited Pleistocene fossil record contains no evidence of Douglas-fir (Brown, 1985; Gugger & Sugita, 2010).

Mexican Douglas-fir is presently geographically isolated from USA populations by large deserts. To varying degrees Mexican populations are ecologically (Vargas-Hernández *et al.*, 2004; Acevedo-Rodríguez *et al.*, 2006), morphologically (Reyes-Hernández *et al.*, 2005, 2006) and genetically (Li & Adams, 1989) distinct from those in the USA and Canada. Consequently, Mexican Douglas-fir populations

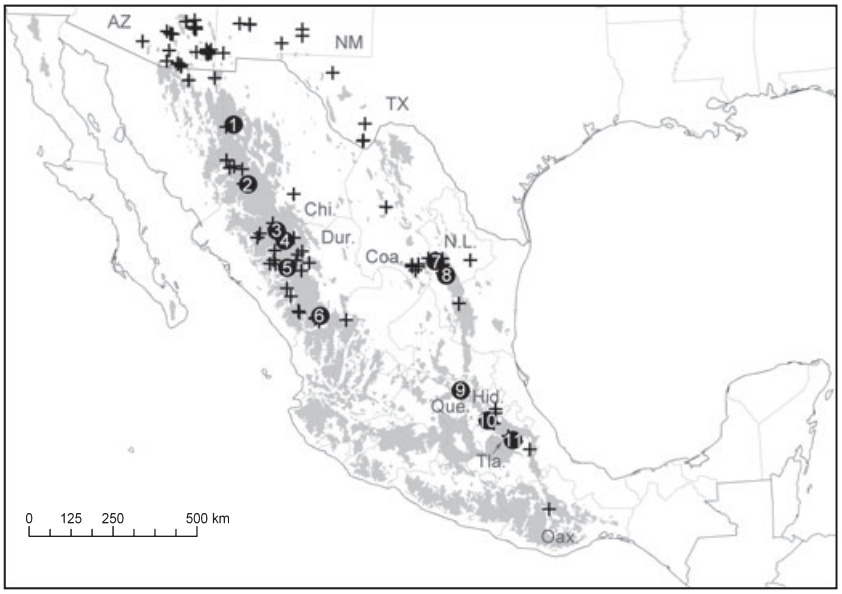

Fig. 1 Map of Mexico with Madrean pine–oak region shown in light gray (Conservation International Foundation); *Pseudotsuga* range as approximated by location of herbarium samples (from GBIF) and shown as crosses; sample sites shown as black points numbered according to Table 1. States mentioned in the text are labeled with abbreviations: AZ, Arizona; Chi., Chihuahua; Coa., Coahuila; Dur., Durango; Hid., Hidalgo; N.L., Nuevo León; NM, New Mexico; Oax., Oaxaca; Que., Querétaro; Tla., Tlaxcala; TX, Texas.

have been classified as multiple separate species (Flous, 1934a,b; Martínez, 1949), a separate variety (Reyes-Hernández *et al.*, 2006; Earle, 2009) or part of the Rocky Mountain variety of Douglas-fir (*P. menziesii* var. *glauca*), whose northern limit extends into central British Columbia (Little, 1952; Hermann & Lavender, 1990). Compared with northern Mexican populations, central Mexican populations are morphologically and phenologically more distinct from USA populations (Reyes-Hernández *et al.*, 2005, 2006; Acevedo-Rodríguez *et al.*, 2006).

Mexican Douglas-fir is listed as 'subject to special protection' (Norma Oficial Mexicana 1994, 2001) because all the Mexican Douglas-fir populations are small and fragmented, ranging from a few dozen to a few thousand individuals (Mápula-Larreta *et al.*, 2007; Velasco-García *et al.*, 2007), and a number of studies suggest low fertility and seedling recruitment rates because of inbreeding depression (Vargas-Hernández *et al.*, 2004; Mápula-Larreta *et al.*, 2007; Velasco-García *et al.*, 2007). Within Mexico, the lowest fertility and seedling recruitment rates and highest inbreeding rates were found in central populations, which are among the smallest and most isolated in Mexico (Juárez-Agis *et al.*, 2006; Mápula-Larreta *et al.*, 2007; Velasco-García *et al.*, 2007; Cruz-Nicolás *et al.*, 2008). Leaf and cone morphology (Reyes-Hernández *et al.*, 2005, 2006) and bud phenology (Acevedo-Rodríguez *et al.*, 2006) are also least variable in central Mexican populations compared with northern Mexican populations. Therefore, we expect genetic diversity to be positively correlated with latitude, consistent with the signature of dispersal bottlenecks during southward migration and because latitude is correlated with morphological and phenological diversity, fertility rates, and population size.

Here, we investigate mitochondrial (mtDNA) and chloroplast DNA (cpDNA) sequence and cpDNA microsatellite (cpSSR) variation in 11 populations throughout Mexico to test Miocene vs Pleistocene southward migration hypotheses to explain the origins of temperate Douglas-fir in Mexico and test the association of geographic patterns of molecular variation and population size changes with Pleistocene climate history using ecological niche models. We assess the implications of these results for the taxonomic status of Mexican populations and for conservation strategies in an understudied biodiversity hotspot.

Materials and Methods

Sampling

We restricted our sampling and most analyses of Douglas-fir *Pseudotsuga menziesii* (Mirb.) Franco to Mexico because the USA/Mexico border coincides with a natural gap in the distribution caused by large deserts and because previous morphological studies have shown that USA and Mexican populations differ. Leaf tissue was collected from 9 to 16 individuals (mean = 11.6) from 11 natural populations throughout most of the range of Douglas-fir in Mexico (Fig. 1; see the Supporting Information, Table S1). Samples were kept on ice and then stored at −80°C. In addition, pressed herbarium vouchers were made for each sample. Representative vouchers from each population were deposited in Herbario Nacional de México (MEXU; Universidad Nacional Autónoma de México, México, DF) and Herbario del Centro Regional del Bajío (IEB; Pátzcuaro, Michoacán), and the remaining vouchers are stored in the A. González-Rodríguez laboratory.

DNA preparation

Total genomic DNA was extracted from leaf tissue using the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA, USA) according to the manufacturer's instructions.

Two mtDNA and two cpDNA segments shown to neutrally vary in USA and Canadian populations of Douglas-fir were amplified, sequenced, and aligned according to previously established procedures (Gugger *et al.*, 2010). The cpDNA segments were *rps7-trnL* and *rps15-psaC*, containing *ndh* pseudogenes, intergenic spacers, and an intron (Wakasugi *et al.*, 1994; Braukmann *et al.*, 2009). The mtDNA segments were variable region seven of the small-subunit ribosomal RNA gene (*V7*; Duff & Nickrent, 1997, 1999) and the first intron of the *nad7* gene (*nad7i1*; Jaramillo-Correa *et al.*, 2004). Douglas-fir mtDNA is maternally inherited (Marshall & Neale, 1992) and cpDNA is paternally inherited (Neale *et al.*, 1986), thus neither generally undergoes heterologous recombination (Birky, 2001) and each acts as a single locus. Therefore, we concatenated the two cpDNA sequences to produce a single cpDNA sequence and the two mtDNA sequences to produce a single mtDNA sequence.

Three chloroplast DNA simple sequence repeats (cpSSR) shown to be variable in Canadian populations of Douglas-fir were assayed (*Pt*26081, *Pt*63718, *Pt*71936; Vendramin *et al.*, 1996; Viard *et al.*, 2001). The three loci were simultaneously amplified using the Qiagen Multiplex PCR kit in 5 μl reactions as follows: 1× multiplex PCR master mix, 2 μM each primer, deionized water and 20 ng DNA (Cortés-Palomec *et al.*, 2008). The thermal cycling program consisted of one cycle at 94°C for 2 min and then 35 cycles, each at 94°C for 1 min, 55°C for 1 min and 72°C for 2 min. A final extension step at 72°C for 15 min was included. The PCR products were diluted 1 : 1 in deionized water, combined with the GenScan-500 LIZ size standard (Applied Biosystems, Foster City, CA, USA) and analysed in an ABI-PRISM 3100-avant sequencer. Peak Scanner 1.0 (Applied Biosystems) was used for fragment analysis and final sizing.

Parsimony networks for all Mexican sequences plus representative sequences of haplotypes found only north of Mexico and outgroups (Gugger *et al.*, 2010) were produced separately for mtDNA and cpDNA using TCS 1.21 (Clement *et al.*, 2000) with insertion–deletions coded as a fifth state. A network for cpSSR haplotypes was constructed manually in TCS assuming a stepwise mutation model (SMM; Ohta & Kimura, 1973).

Genetic diversity

Patterns of genetic diversity were quantified for each population and for three geographic regions commonly defined for Mexican Douglas-fir: Sierra Madre Occidental (I), Sierra Madre Oriental (II) and central Mexico (III). For each population and region, we estimated haplotype richness (h), haplotype richness after correcting for unequal sample sizes with rarefaction (h_r; Hurlbert, 1971) and haplotype diversity (H; Nei, 1987) using CONTRIB 1.01 (Petit *et al.*, 1998). For sequence data, we calculated nucleotide diversity (π; Tajima, 1983; Nei, 1987) using ARLEQUIN 3.11 (Excoffier *et al.*, 2005) and for cpSSR data, we calculated $\bar{D}^2_{SH}$, which is the mean pairwise genetic distance among individuals within a population under a SMM (Goldstein *et al.*, 1995b; Vendramin *et al.*, 1998). Latitudinal trends in the diversity (h_r and H for combined cpDNA and cpSSR; π for cpDNA sequences; $\bar{D}^2_{SH}$ for cpSSR) of populations were investigated with linear regression.

Changes in population size

To test for population expansion with DNA sequence data, we calculated F_S (Fu, 1997) and assessed its significance with coalescent simulations (Hudson, 1990) in DNASP 5.0 (Librado & Rozas, 2009). For cpSSR, we calculated F_S in ARLEQUIN by binary coding haplotypes following Navascués *et al.* (2006). Significant negative values of F_S suggest population expansion or selection.

A Bayesian skyline plot (Drummond *et al.*, 2005) of changes in effective population size (N_e) through time based on all Mexican cpDNA sequence data was estimated using BEAST 1.5.3 (Drummond & Rambaut, 2007). We chose the Hasegawa–Kishino–Yano substitution model (Hasegawa *et al.*, 1985) with empirical base frequencies, a strict molecular clock, and a piecewise-constant coalescent Bayesian skyline tree prior with 10 starting groups (results same for five groups; not shown). Two runs of 20 million steps and effective sample size (ESS) > 200 were compared to ensure convergence. Outputs were combined in LOGCOMBINER 1.5.3 and visualized in TRACER 1.5 (Drummond & Rambaut, 2009). To convert the x-axis (subs per site) to demographic units (years), we used a previously published mutation rate for this locus (4.41×10^{-10} subs per site yr^{-1}; Gugger *et al.*, 2010).

Population structure

We computed genetic differentiation among populations for all loci as G_{ST} (Nei, 1973; Pons & Petit, 1995). We also calculated differentiation among populations considering genetic distance as N_{ST} for sequence data (Lynch & Crease 1990; Pons & Petit, 1996) and R_{ST} for cpSSR data (Slatkin, 1995) using PERMUT-CPSSR 2.0 (Pons & Petit, 1996). N_{ST} or R_{ST} significantly greater than G_{ST} indicates phylogeographic structure.

For mtDNA and cpDNA sequence data, we performed a spatial analysis of molecular variance (SAMOVA) in SAMOVA 1.0 (Dupanloup *et al.*, 2002) to identify groups of genetically similar populations. SAMOVA uses a simulated annealing approach to group geographically close populations to maximize the variance (F_{CT}) among a user-defined number of groups (K). We performed this analysis for K = 2–6, and chose the number of groups that gave the highest F_{CT}.

To identify clusters of populations sharing similar cpSSR compositions, we computed pairwise genetic distance among populations as $(\delta\mu)^2$ (Goldstein *et al.*, 1995a), and used this distance matrix to create a UPGMA dendrogram (Sneath & Sokal, 1973) in PAUP* 4.0b10 (Swofford, 2003). Given that all cpSSRs are linked, we modified $(\delta\mu)^2$ from Goldstein *et al.* (1995a) for the case of multiple microsatellite markers (m) for a single nonrecombining locus:

$$(\delta\mu)^2 = \left(\sum_{k=1}^{m} |\mu_{Ak} - \mu_{Bk}| \right)^2, \qquad \text{Eqn 1}$$

where μ_{Ak} and μ_{Bk} are the mean allele size in populations A and B at the kth microsatellite marker. Bootstrap support is always 100% based on one microsatellite locus, so we approximated support values for major branches in the dendrogram assuming that each of the three cpSSR markers was a separate locus in POPTREE2 (Takezaki *et al.*, 2009).

Divergence from Rocky Mountain variety

Divergence time between Mexican and Rocky Mountain populations (Gugger *et al.*, 2010) was estimated using an isolation-with-migration model (Nielson & Wakeley, 2001) in IMa (Hey & Nielson, 2007). The full IMa model simultaneously estimates six parameters scaled by substitution rate: divergence time (t), migration from population one to two (m_1), migration from population two to one (m_2), effective population size of each population (N_1 and N_2), and effective population size of the ancestor (N_A). The IMa model assumes constant population size, neutral molecular markers, no recombination within loci, free recombination among loci and a particular mutation model. We chose mutation models and priors as described in Gugger *et al.* (2010), and at least three runs with

ESS > 50 were compared to ensure convergence. We only trusted estimates whose posterior distribution dropped to zero within the prior intervals investigated. To scale the outputs to demographic units, we used a generation time of 100 yr and per locus per yr substitution rates of 4.81×10^{-7} for mtDNA and 6.66×10^{-7} for cpDNA (Gugger *et al.*, 2010). We also tested whether or not migration among Rocky Mountain and Mexican populations was important during divergence by conducting a two-step likelihood ratio test described in Gugger *et al.* (2010). If neither test was significant, the model with $m_1 = m_2 = 0$ was not rejected, so we ran the IMa analysis again with migration set to zero.

Ecological niche modeling

The potential distribution of *P. menziesii* was modeled for present climate conditions and two Last Glacial Maximum (LGM; *c.* 21 thousand yr before present (ka)) general circulation models provided by the Paleoclimate Modelling Intercomparison Project Phase II (Braconnot *et al.*, 2007): the Community Climate System Model (CCSM; Collins *et al.*, 2006) and the Model for Interdisciplinary Research on Climate (MIROC; Hasumi & Emori, 2004). The present-day distribution was projected into the A2a story-line scenario (Nakicenovic & Swart, 2000) using three global climate change models: the Canadian Centre for Climate Modelling and Analysis model (CGCM2), the Australian Commonwealth Scientific and Research Organization model (MK2) and the Hadley Centre for Climate Prediction and Research model (HadCM3). All potential distributions were modeled using the Maximum Entropy algorithm implemented in MAXENT 3.3.1 (Phillips *et al.*, 2006).

We used a set of 74 presence points for *P. menziesii* in Mexico obtained from the Global Biodiversity Information Facility (GBIF; http://data.gbif.org/species/browse/taxon/), the Herbario Nacional de México collections, and personal observations. Climatic data were obtained from the WorldClim dataset (Hijmans & Graham, 2006) for LGM and current climate scenarios with 30 arcsec and 2.5 arcmin resolutions, respectively (available at http://www.worldclim.org/download). Climatic data used for the future scenarios were provided by the CIAT downscaled GCM Data Portal (http://gisweb.ciat.cgiar.org/GCMPage/) with a 30 arcsec resolution. As a threshold-independent method for model validation, we used the area under the receiver operating characteristic curve (AUC). The LGM reconstructions were developed independently with respect to the present-day distribution, but models for the future were projected using the present climate layers with the same resolution (30 arcsec) and using the 'Projection' option in MAXENT. Finally, a jackknife test was performed to measure the relative importance of climatic variables on the occurrence prediction for every distribution model.

Results

Genetic diversity

Mexican populations were genetically distinct from USA and Canadian populations, but more closely related to the Rocky Mountain variety than the coastal variety (Fig. 2; Gugger *et al.*, 2010).

We observed four *rps7-trnL* and nine *rps15-psaC* haplotypes that combined for 12 cpDNA haplotypes based only on cpDNA sequences (GenBank accessions in Table S2). These formed two major clades: one found primarily in northern Mexico (C21–C24) and the other found throughout the rest of Mexico (C25–C32; Fig. 2). None of these haplotypes were observed in the USA or Canada but one (C20) from southern Arizona and New Mexico fell into the clade from northwestern Mexico. In central Mexico, two geographically structured subclades were also observed (C26/C28 and C30/C31).

The mtDNA data also support the distinction of northwestern populations (Fig. 2). We observed three *V7* and two *nad7i1* haplotypes that combined for four mtDNA haplotypes (Table S2). The most common (M4) was also common in the southwestern USA and predominates in Durango and northeast Mexico. The three other combined mtDNA haplotypes were private to populations in Chihuahua (M8, M9) and the northernmost population in Durango (M10). No individuals from central Mexico could be PCR-amplified for either mtDNA marker, and neither could many individuals from the rest of Mexico. Chloroplast DNA was easily amplified from those same individuals, suggesting the problem was not caused by poor quality DNA extract. We varied PCR conditions and used alternative primer pairs without improved success. Therefore, we believe that the mitochondrial genome in those individuals may have undergone major rearrangements or insertion–deletion events, rather than point mutations at the primer site. Such rearrangements are thought to be common in the plant mitochondrial genome (Palmer & Herbon, 1988; Birky, 2001).

Fragment lengths for each cpSSR marker were 99–100 and 102–105 bp for *Pt*26081, 90–93 bp for *Pt*63718, and 148–152 bp for *Pt*71936. These combined for 21 cpSSR haplotypes (Table S3). Some cpSSR haplotypes were common to all three geographic regions (S5, S11, S12, S15), some distinguished regions (S10, S16) and many were private to populations (especially in Durango; Fig. 2). The cpSSR variation in Mexico was highly divergent from that in the coastal variety of Douglas-fir (*P. menziesii* var. *menziesii*) in British Columbia, with each marker containing *c.* 20 fewer repeats in Mexico than British Columbia (Table S3; Viard *et al.*, 2001).

Overall, genetic diversity was high for cpDNA and cpSSR (Table 1) and moderately high for mtDNA (Table 2).

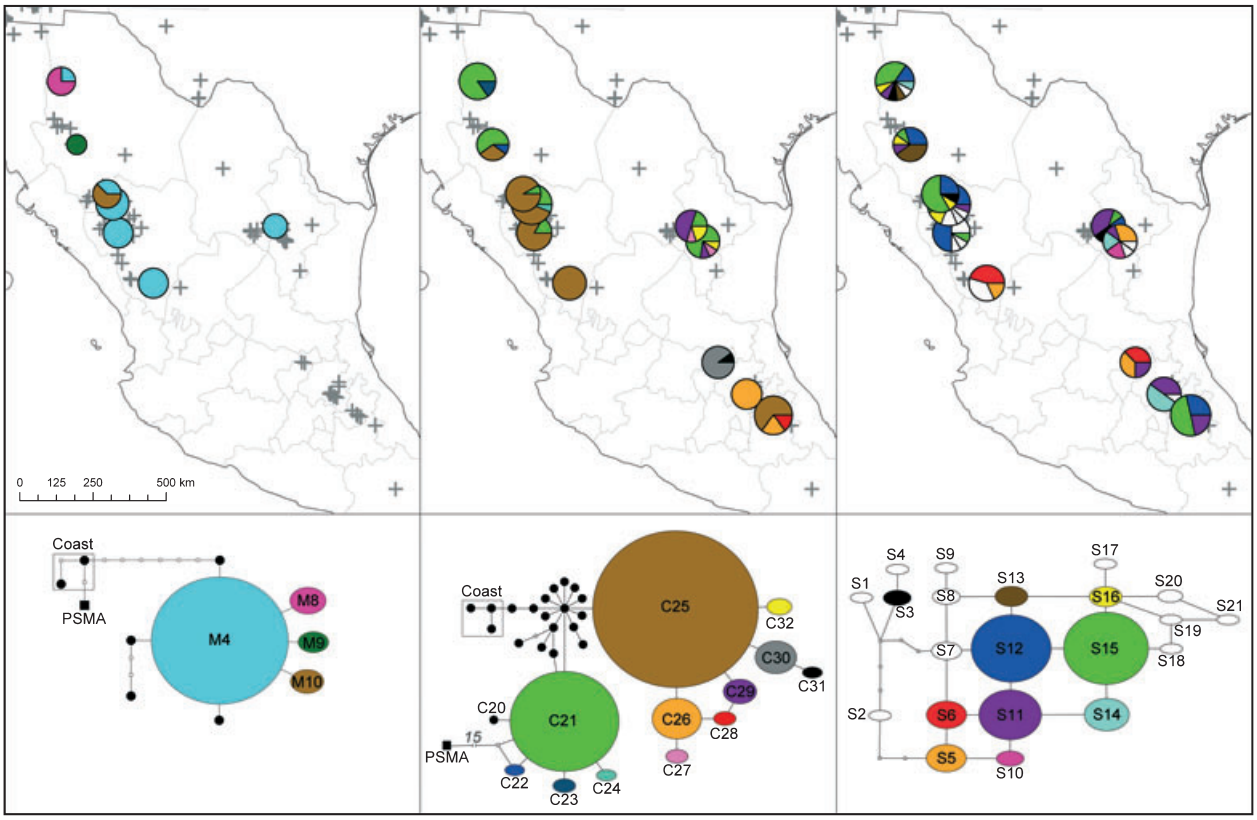

Fig. 2 Maps with sample sites colored according to mitochondrial DNA (mtDNA, left), chloroplast DNA (cpDNA, center) sequence and cpDNA microsatellite (cpSSR, right) haplotype composition and proportioned according to sampling intensity. Below each, parsimony networks show the relationship among haplotypes observed in Mexico (colored elliptical nodes, proportional to the frequency in the dataset) and haplotypes observed in the USA and Canada (small circular black nodes; Gugger *et al.*, 2010). Haplotypes from the coastal variety (*Pseudotsuga menziesii* var. *menziesii*) are marked; all other USA or Canadian haplotypes are the Rocky Mountain variety (*P. menziesii* var. *glauca*). Black square nodes indicate the outgroup, *Pseudotsuga macrocarpa* (PSMA), and small, open points indicate inferred, unobserved haplotypes. Internodes are one mutational step unless otherwise noted. In the cpSSR panel, white indicates haplotypes private to one population. Colors do not relate from one map/network to the next or to those in Gugger *et al.* (2010), except M4. Haplotype numbering is consistent with Gugger *et al.* (2010).

Genetic diversity was positively correlated with latitude for all cpDNA and cpSSR diversity measures investigated, but those correlations were not statistically significant ($0.41 < r < 0.43$, $0.18 < P < 0.22$). Trends in diversity were not measured for mtDNA because of the small sample size.

Changes in population size

For sequence data, there was no evidence of population expansion based on F_S (Tables 1 and 2). For cpSSR data, no populations showed significant evidence of expansion based on F_S. However, both populations in Chihuahua had marginally significant F_S and Region I and the overall F_S had a significant F_S, suggesting expansion (not significant after Bonferroni correction). Finally, a Bayesian skyline plot of N_e through time constructed using all cpDNA sequence data for Mexico shows an increase in population size starting in the late Pleistocene (*c.* 100 ka; Fig. 3).

Population structure

N_{ST} was not significantly greater than G_{ST} for mtDNA (0.80 ± 0.10; 0.77 ± 0.12, respectively) or cpDNA (0.55 ± 0.12; 0.56 ± 0.10) sequence data, but R_{ST} (0.32 ± 0.09) was greater than G_{ST} (0.16 ± 0.03) for cpSSR data ($P = 0.038$). G_{ST} for mtDNA and cpSSR were similar to average values in other conifers (0.76 and 0.15, respectively), but the value for cpDNA was much higher than average (0.16; Petit *et al.*, 2005). The fact that Douglas-fir's 'heavy' pollen does not disperse as far as pine pollen may account for this discrepancy (Sugita, 1993).

A SAMOVA of mtDNA defined four groups ($F_{CT} = 0.84$): El Largo, Chureachi, Guanaceví and the rest of Mexico

Table 1 Diversity measures for chloroplast DNA (cpDNA) sequence, cpDNA microsatellite (cpSSR), and combined cpDNA and cpSSR for each population, region, and the overall dataset

			cpDNA				cpSSR				Combined		
	Population	n[1]	h	H (SD)	π (SD)[2]	F_S[3]	h	H (SD)	$\bar{D}^2_{SH}$[2]	F_S[3]	h	h_r[2,4]	H (SD)[2]
1	El Largo, Chihuahua	13	2	0.28 (0.14)	0.000186 (0.000239)	0.24	8	0.86 (0.09)	4.27	−2.68	8	4.6	0.86 (0.09)
2	Chureachi, Chihuahua	10	3	0.60 (0.13)	0.000749 (0.000599)	0.72	5	0.80 (0.10)	0.59	−1.99	6	5.2	0.84 (0.10)
3	Guaneceví, Durango	12	2	0.17 (0.13)	0.000220 (0.000265)	0.43	4	0.64 (0.13)	2.10	0.24	4	3.3	0.64 (0.13)
4	Guaneceví 2, Durango	16	3	0.49 (0.12)	0.000688 (0.000541)	1.09	7	0.85 (0.06)	1.40	−2.26	8	5.2	0.86 (0.06)
5	Altares, Durango	12	2	0.30 (0.15)	0.000401 (0.000384)	1.38	5	0.73 (0.11)	1.35	−0.72	6	4.6	0.80 (0.10)
6	Las Flores, Durango	11	1	0	0	0	3	0.69 (0.09)	0.38	0.24	3	2.9	0.69 (0.09)
	Region I (Sierra Madre Occidental)	74	5	0.51 (0.04)	0.000701 (0.000521)	0.41	17	0.86 (0.03)	2.33	**−7.57**	23	11.3	0.91 (0.02)
7	Jamé, Coahuila	10	4	0.73 (0.12)	0.000734 (0.000590)	0.68	6	0.84 (0.10)	3.08	−1.66	8	6.8	0.96 (0.06)
8	Cerro Potosí, Nuevo León	11	4	0.49 (0.18)	0.000817 (0.000631)	1.02	6	0.89 (0.08)	3.23	−1.23	7	6.0	0.91 (0.08)
	Region II (Sierra Madre Oriental)	21	4	0.70 (0.07)	0.000932 (0.000663)	2.17	9	0.88 (0.04)	3.25	−2.54	14	14	0.96 (0.03)
9	Cerro Pingüica, Querétaro	10	2	0.20 (0.15)	0.000132 (0.000201)	−0.34	3	0.75 (0.10)	0.46	0.14	3	3.0	0.75 (0.10)
10	Estanzuela, Hidalgo	10	1	0	0	0	3	0.64 (0.10)	3.03	1.67	3	2.9	0.64 (0.13)
11	Villa Real, Tlaxcala	14	3	0.56 (0.13)	0.000327 (0.000333)	1.14	3	0.67 (0.08)	0.45	0.64	6	4.5	0.79 (0.09)
	Region III (central Mexico)	34	5	0.74 (0.04)	0.000661 (0.000508)	0.5	7	0.84 (0.03)	1.75	−0.77	11	9.6	0.91 (0.02)
Overall		129	12	0.79 (0.07)	0.000897 (0.000618)	−1.28	21	0.91 (0.02)	2.63	**−9.16**	44	–	0.97 (0.02)

[1]Total sample size. No data could be obtained for one cpSSR sample in Population 8, two cpSSR samples in Population 9 and one cpDNA sample in Population 10.
[2]Used in regressions of latitude on diversity.
[3]Bold values were significant under coalescent simulations.
[4]Rarefaction to eight for populations and 20 for regions.

Table 2 Mitochondrial DNA (mtDNA) diversity measures for populations, regions and overall dataset

		mtDNA				
	Population	n	h	H (SD)	π (SD)	F_S[1]
1	El Largo, Chihuahua	8	2	0.43 (0.17)	0.000459 (0.000523)	0.54
2	Chureachi, Chihuahua	4	1	0	0	0
3	Guaneceví, Durango	8	2	0.54 (0.12)	0.000574 (0.000601)	0.87
4	Guaneceví 2, Durango	11	1	0	0	0
5	Altares, Durango	9	1	0	0	0
6	Las Flores, Durango	9	1	0	0	0
	Region I (Sierra Madre Occidental)	49	4	0.50 (0.08)	0.000599 (0.000553)	−0.58
7	Jamé, Coahuila	6	1	0	0	0
Overall		55	4	0.59 (0.18)	0.000539 (0.000516)	−0.75

[1]No values were statistically significant under coalescent simulations.

(Fig. 4). The remaining variation resided within populations (17.6%) rather than among populations within groups (−2.0%; Table S4). The SAMOVA of cpDNA also defined four groups (F_{CT} = 0.56): El Largo, Chureachi and Cerro Potosí; Estanzuela; Cerro Pingüica; and the rest. Most of the remaining variation was within populations (37.4%) with some among populations within groups (6.5%). All variance components were significant ($P < 0.025$).

An UPGMA of cpSSR data based on $(\delta\mu)^2$ defined two major groups: northwestern Mexico plus Villa Real, Tlaxcala and eastern Mexico plus Las Flores, Durango (Fig. 4).

Divergence from Rocky Mountain variety

Divergence time estimates among Mexican and Rocky Mountain populations did not converge under the full model, although a clear peak in the posterior distribution of t corresponding to 1.01 Ma was observed (Fig. S1, Table 3). The zero migration model could not be rejected, so the analysis was repeated without migration. With $m_1 = m_2 = 0$, divergence time converged at 958 ka with the 90% highest posterior density interval (HPD) ranging from 1.63 Ma to 464 ka, which falls entirely within the

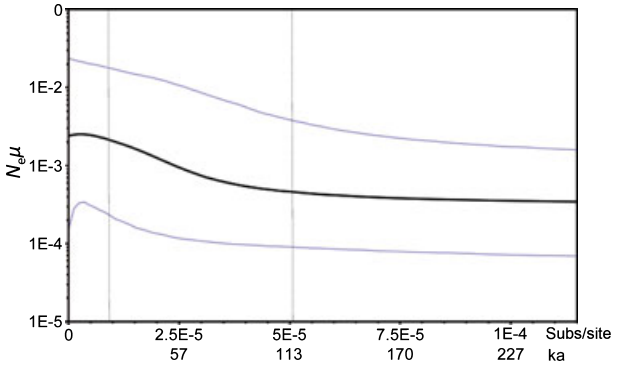

Fig. 3 Bayesian skyline semilog plot of median $N_e\mu$ through time based on chloroplast DNA (cpDNA) sequence data, where μ is the mutation rate per generation. Confidence intervals are shown as pale lines. The *x*-axis is shown in two scales: substitutions per site and calendar time in thousands of years before present (ka). A mutation rate of 4.41×10^{-10} substitutions per site yr^{-1} was used to convert substitutions per site to years (Gugger *et al.*, 2010). Vertical lines mark the start of the Wisconsinan glaciation (*c.* 115 ka) and Last Glacial Maximum (*c.* 21 ka).

Pleistocene. When run separately, mtDNA and cpDNA gave similar divergences of 878 ka and 866 ka, respectively.

Ecological niche modeling

The AUC values for the training and test data in the present-day, LGM1 (MIROC), and LGM2 (CCSM) models were 0.973/0.986; 0.972/0.986 and 0.981/0.985, respectively. These values indicate a good performance for the three models. The future projections under the three global change models showed the same AUC values for the training and test data (0.957/0.979) and resulted in similar predicted distribution areas, and thus only the HadCM3 model is shown (Fig. 5).

The distribution models suggest a reduction in suitable distribution area in the present-day distribution model of Mexican Douglas-fir in comparison with the models under both LGM climate scenarios (Fig. 5). Some areas in the western portion of the Trans-Mexican Volcanic Belt, the Central Plateau, and the Sierra Madre Oriental appear as suitable for the species during the LGM, particularly under the CCSM model. It is also probable that larger areas in the Sierra Madre Occidental were occupied by Douglas-fir during the LGM. However, the MIROC model suggests that the northernmost portion of the Sierra Madre Occidental was less suitable for the species during the LGM conditions than under present-day conditions. The future projections under the HadCM3 climate change model suggest a severe reduction in the area suitable for Mexican Douglas-fir by 2050 and the almost complete loss of the species by 2080.

The jackknife analysis indicated that the variables with the highest relative contributions to the present day and to LGM models were mean annual temperature, minimum temperature of the coldest month, mean temperatures of the warmest, coldest and driest quarters, annual precipitation and precipitation of the driest quarter (Table S5). By contrast, for the future projections the annual precipitation had the highest effect in explaining the distribution. This suggests that the predicted reduction in suitable area for the species will be caused mainly by a reduction in precipitation rather than by an increase in temperature.

Discussion

Colonization of Mexico and divergence from Rocky Mountain variety

We find strong support for the Pleistocene southward migration hypothesis as an explanation for the origins of

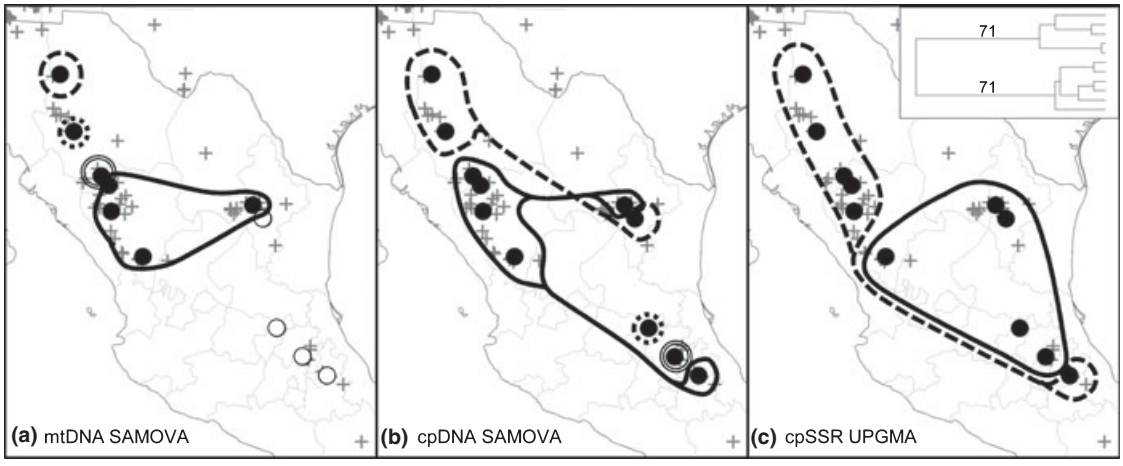

Fig. 4 Population structure identified in (a) mitochondrial DNA (mtDNA) SAMOVA (b) chloroplast DNA (cpDNA) SAMOVA and (c) UPGMA clustering analysis of cpDNA microsatellite (cpSSR) $(\delta\mu)^2$ values (dendrogram with approximate bootstrap support for two major groups shown as inset). Each group is delineated with different line styles. For (a), open circles indicate populations in which no samples could be PCR-amplified.

Table 3 IMa results showing divergence time (t), migration from population one to two (m_1) and migration from population two to one (m_2) for a run with the full model, followed by results of the likelihood ratio tests and then t with its 90% highest posterior density interval (HPD) for a run with $m_1 = m_2 = 0$

	Full model				$m_1 = m_2$		$m_1 = m_2 = 0$		Model without migration ($m_1 = m_2 = 0$)		
	Priors (t, m_1, m_2, q_1, q_2, q_A)[1]	t (ka)	m_1	m_2	-2Λ	P	-2Λ	P	t (ka)	90% HPD low	90% HPD high
Both loci	5, 2, 3, 10, 10, 10	1011[2]	0.0000001	0.0000001	0.3506	0.55	$-0.188 \approx 0$	≈ 0.5	958	464	1628
mtDNA	5, 10, 6, 10, 10, 10	878[2]	0.0000002	0.0000001	0.702	0.40	$-0.0032 \approx 0$	≈ 0.5	878	265	2789
cpDNA	5, 1, 3, 20, 7, 7	806[2]	0	0.0000001	0.3729	0.54	0.405	0.26	866	378	1765

In each case, the values for the run with the highest effective sample size (ESS; Hey & Nielson, 2007) are shown. ka, thousand years before present .

[1]Priors given as the maximum values of a truncated uniform distribution.

[2]Posterior distribution for this parameter never dropped to zero, suggesting unreliable estimates.

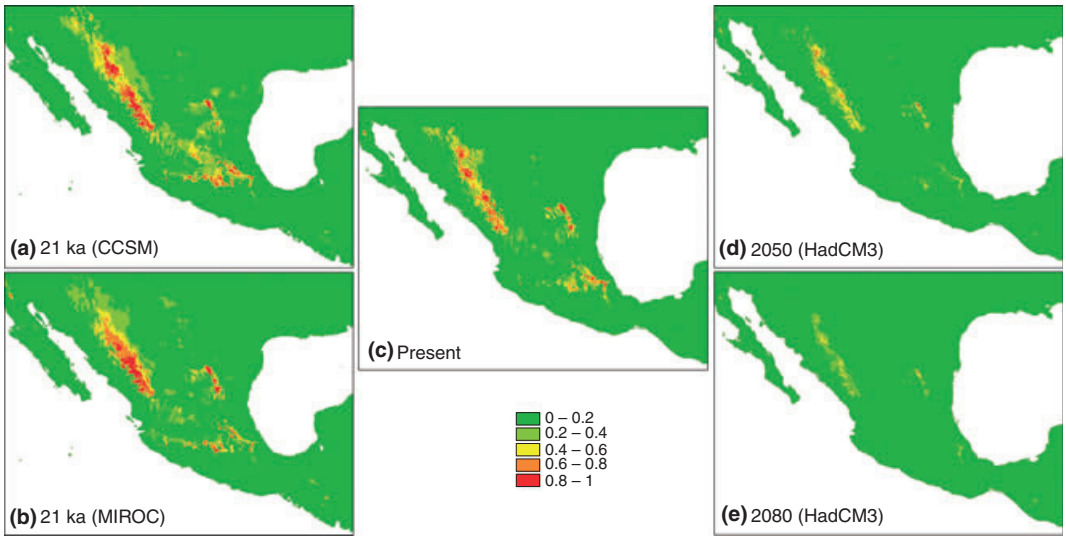

Fig. 5 Maps showing potential distribution as probability of occurrence (green, 0–0.2; red, 0.8–1.0) for *Pseudotsuga* in Mexico (a) at the Last Glacial Maximum (LGM; 21 thousand years before present (ka)) based on the CCSM model, (b) at the LGM based on the MIROC 3.2 model, (c) the present, and (d) the years 2050 and (e) 2080 based on the HadCM3 model. At the LGM, populations within regions may have been more continuous, but regions likely remained isolated. Further range contraction is predicted in the future.

Douglas-fir in Mexico, a result that may be important in explaining origins of other temperate taxa in Mexico. Mexican Douglas-fir populations show a high probability of having diverged from Rocky Mountain populations in the mid-Pleistocene (958 ka; Table 3). However, other Pleistocene divergence times are possible given the uncertainties in these estimates (90% HPD: 1.6 Ma–464 ka), in mutation rate estimates (Gugger *et al.*, 2010) and missing mtDNA data. For example, the error around mutation rate estimates could increase the two-locus divergence time estimate to 1.75 Ma (2.97 Ma–287 ka) or decrease it to 169 ka (846–82 ka). However, none of these estimates falls within the Miocene and most values fall within the Pleistocene. Other sources of error could be introduced in violating the assumptions of constant population size and no substructure within populations, although the IMa model has been shown to be robust to such violations (Strasburg & Rieseberg, 2010).

Mexican populations did not share any cpDNA haplotypes with USA and Canadian populations, and three mtDNA haplotypes were confined to Mexico (Fig. 2). For both mtDNA and cpDNA, Mexican haplotypes were more similar to Rocky Mountain haplotypes than coastal ones. In addition, the most common mtDNA haplotype in Mexico (M4) is also common in the central Rockies. The complete divergence of cpDNA haplotypes and divergence of some mtDNA haplotypes suggests that the shared mtDNA haplotype, M4, is ancestral variation that persists because of low mutation rates and incomplete lineage sorting. The different modes of inheritance and dispersal of mtDNA (maternal,

seed dispersed) and cpDNA (paternal, pollen- then seed-dispersed) could not explain this pattern because a fertile seed bearing a particular mtDNA haplotype could not disperse without also bringing its pollen donor's cpDNA haplotype. Moreover, divergence models without gene flow for each locus or both loci combined could not be rejected (Table 3). Mexican populations also differed from the Rocky Mountain populations in that mtDNA could not be amplified in nearly 60% of the samples (especially from central Mexico), presumably owing to major insertion–deletions or rearrangements at that locus.

Three lines of evidence support the hypothesis of Pleistocene colonization of Mexico and subsequent isolation from the Rocky Mountain variety: our Pleistocene divergence time estimates (Table 3), near-complete differentiation between Mexico and the Rockies (Fig. 2; Gugger *et al.*, 2010) and Pleistocene fossil evidence from southern Arizona (Gray, 1961; Martin, 1963). Continuous connectivity with populations in the Rockies from the Miocene until Pleistocene divergence is highly unlikely given the dynamic climate and general lack of fossils in the region (although conditions for fossil preservation are poor). This conclusion is consistent with early interpretations of the fossil record (Deevey, 1949; Dressler, 1954); however, it contrasts with more recent interpretations (Palacios-Chavez & Rzedowski, 1993) that *Pseudotsuga* arrived in southern Mexico in the early to middle Miocene (*c.* 20 Ma; Graham, 1999). Miocene pollen attributed to *Pseudotsuga* could have been *Larix*, *Pseudotsuga*, or both, which are plausible associates of the other temperate taxa found in the fossil bed (e.g. *Abies*, *Picea*, *Pinus*, *Alnus*, *Populus*, *Quercus* and *Salix*; Palacios-Chavez & Rzedowski, 1993). Given that *Larix* is not currently found in Mexico and our data suggest a Pleistocene arrival for *P. menziesii* in Mexico, that Miocene species is most likely extinct. However, we were unable to sample a very small, isolated, morphologically divergent population (Debreczy & Rácz, 1995) in the Sierra Madre del Sur of Oaxaca, which could be a relict population.

The colonization of Mexico could have occurred in multiple waves or there could have been one colonization with varying durations of isolation for central compared with northern populations. Within Mexico, populations are drawn from two cpDNA clades: one is exclusively Mexican and mostly to the south and east (C25–C32), and the other is primarily northern Mexican (C21–C24) with one related chlorotype found only in Arizona/New Mexico (C20; Gugger *et al.*, 2010). The southern/eastern clade, has several derived subclades that are restricted to populations in central Mexico (C26 and C30–C31). To some extent, the mtDNA parallel this pattern. Chihuahuan populations are different: M4 reaches farther south and east, and the southernmost populations failed to amplify. Moreover, central populations are more distinct morphologically from the Rocky Mountain variety than northern populations (Reyes-Hernández *et al.*, 2006). Thus the correlation of latitude and genetic diversity (not significant) is consistent with the long-term isolation of small populations in central Mexico, rather than successive dispersal bottlenecks.

A mid-Pleistocene colonization of Mexico would likely have been associated with southward escape from glaciation(s) at high latitudes and the expansion of more favorable cool, moist conditions in montane Mexico. Our divergence estimate coincides with the 'mid-Pleistocene revolution' when glacial cycles transitioned from 41 000 yr to deep 100 000 yr periods (*c.* 900 ka; Head & Gibbard, 2005). The divergence of an isolated population of MacGillivray's warbler (*Oporornis tolmiei*) in north-eastern Mexico from its primarily north-west North American range also occurred about this time (Milá *et al.*, 2000), supporting the contention that this transition could have promoted the colonization of cool temperate species into Mexico. Alternatively, colonization could have been associated with some of the later deep pre-Illinoisan glaciations that fall within the HPD intervals of our estimates (e.g. *c.* 650 ka; Richmond & Fullerton, 1986; Lisiecki & Raymo, 2005).

Late Pleistocene history

The post-colonization Pleistocene history of Douglas-fir in Mexico was likely complicated, consisting of long-term population isolation and repeated phases of expansion and contraction, possibly associated with climatic changes (Pennington *et al.*, 2000). Population structure identified by SAMOVA and UPGMA differed for each molecular marker and was not consistent with geographic regions defined in other studies (Fig. 4; Table S4). In each case, some groupings spanned multiple regions that are now separated by up to 500 km of arid lands uninhabited by Douglas-fir, whereas others were restricted to small areas within a region.

The subdivision of populations in the Sierra Madre Occidental was a consistent pattern found for all markers. Populations in Chihuahua and the bordering area of Durango were fixed or nearly-fixed for private mtDNA haplotypes and thus mtDNA SAMOVA identified them as separate groups from one another and the remaining Durangan populations. The cpDNA SAMOVA identified Chihuahuan and Durangan populations as two separate groups in the region, but the two common cpDNA haplotypes in the region formed opposing gradients with C21 most common in Chihuahua and C25 most common in Durango. Similarly, cpSSR UPGMA distinguished the southernmost population in Durango from the remaining Sierra Madre Occidental populations. Common cpSSR haplotypes (S11, S12, S15, S16) are shared in Durango and Chihuahua, but Durangan populations have many private

haplotypes. Finally, the Bayesian skyline analysis of cpDNA supports modest population expansion during the Wisconsinan glaciation (Fig. 3), and F_S for cpSSR suggests expansion only in the Sierra Madre Occidental (Table 1). The strong groupings found in maternally inherited mtDNA, shared haplotype compositions observed in paternally inherited cpDNA and cpSSR, and signature of demographic expansion in cpSSR suggest that Douglas-fir pollen spread genes across populations isolated from seed exchange. Some of the deep east-west canyons in the region (e.g. Río Fuerte and Río Culiacán basins) may function as barriers to seed dispersal. Wisconsinan population expansion in the Sierra Madre Occidental permitting north–south gene flow has also been observed in Chihuahua spruce (*Picea chihuahuana*; Jaramillo-Correa *et al.*, 2006), Chihuahua pine (*Pinus leiophylla*; Rodríguez-Banderas *et al.*, 2009), and southwestern white pine (*Pinus strobiformis*; Moreno-Letelier & Piñero, 2009) and population expansion has been observed in Mexican pine beetles (*Dendroctonus mexicanus*; Anducho-Reyes *et al.*, 2008).

Other populations throughout Mexico are better described by recent histories of stability and isolation. In the Sierra Madre Oriental and central Mexico, no significant signature of population expansion or contraction was observed (Table 1). Despite sharing some haplotypes with other regions (Fig. 2), each retains a unique set of private haplotypes (e.g. C27, C29, C32, S10 in Sierra Madre Oriental). The isolated Cerro Pingüica, Querétaro population is fixed for a derived cpDNA subclade (C30, C31). Further south, the Estanzuela, Hidalgo population is fixed for C26, which it shares with the Villa Real, Tlaxcala population. These populations may have last been connected during a severe glaciation recorded in the region *c.* 195 ka (Vázquez-Selem & Heine, 2004). Mountain glaciers reached over 1000 m lower in than present and climate was cooler and moister.

Ecological niche models suggest that regions were not connected during the LGM (Fig. 5). Possible connections among regions are apparent under one of the two models at the LGM, but these models clearly overestimate distributions in the present. Nonetheless, these models suggest that populations within regions may have been more continuous and that corridors among regions along the Sierra Madre Occidental, Sierra Madre Oriental and Trans-Mexican Volcanic Belt could have opened during some Pleistocene glaciations and closed during interglacials. Periodic corridors might explain the complex pattern of haplotype sharing throughout Mexico.

Taken together, evidence of Holocene contraction from ecological niche models (Fig. 4) and molecular signatures of Wisconsinan population expansion (Fig. 3) suggest that Douglas-fir has responded to glacial cycles in phases of isolation and recontact along mountain corridors. Other temperate taxa may have followed similar histories. The strong neutral molecular differentiation and history of isolation observed here, coupled with the diverse Mexican environment, further suggests that functional genomic variation may be strongly differentiated (in contrast to studies in coastal Douglas-fir (Eckert *et al.*, 2009)). This is an important area for future research (Neale & Ingvarsson, 2008).

Taxonomic implications

Mexican Douglas-fir represents at least one evolutionarily significant unit (Ryder, 1986; Crandall *et al.*, 2000) and might be treated best as a third variety (Earle, 2009). There is strong evidence for molecular genetic differentiation (Fig. 2; Gugger *et al.*, 2010) and long-term isolation without gene flow from the Rocky Mountain variety (Table 3). This divergence (958 ka) is of similar magnitude to the divergence time estimated between coastal and Rocky Mountain varieties: roughly half as long when considering both loci (2.11 Ma) or twice as long when considering only cpDNA (491 ka; Gugger *et al.*, 2010). Leaf and cone morphology of central Mexican populations also differs from the Rocky Mountain variety, although these traits may vary clinally (Reyes-Hernández *et al.*, 2005, 2006). Finally, Mexican Douglas-fir exhibits phenological and ecological differentiation with earlier budburst and later budset associated with the generally warmer climate (Acevedo-Rodríguez *et al.*, 2006).

The lack of deep genetic divergences within Mexico and shared variation across regions (Figs 2 and 4; Table S4) argue against the hypothesis of multiple species within Mexico (Flous, 1934a,b; Martínez, 1949). Morphological and phenological studies also fail to support the multiple species hypothesis (Vargas-Hernández *et al.*, 2004; Reyes-Hernández *et al.*, 2005, 2006; Acevedo-Rodríguez *et al.*, 2006).

Implications for conservation

Although Mexican Douglas-fir once must have formed the leading edge as it colonized Mexico, those populations have long since been isolated and sit at the subtropical rear edge of a wide-ranging temperate species. Hampe & Petit (2005) suggest that such rear-edge populations far from the dynamic northern range limit are an important genetic resource because they contain much of the neutral and adaptive diversity within species, form reserves under changing climates, and are a principal site of speciation.

Mexican Douglas-fir has high haplotype diversity for all markers investigated here (Tables 1 and 2) and populations are strongly differentiated at mtDNA (G_{ST} = 0.77), cpDNA (G_{ST} = 0.56), and cpSSR (G_{ST} = 0.16) sequence markers. About 40% of all mtDNA and cpDNA haplotypes observed across the range of Douglas-fir are found in Mexico and all but one are endemic (Gugger *et al.*, 2010).

The high differentiation and overall diversity of Douglas-fir rear-edge populations in Mexico (Tables 1 and 2) is similar to other rear-edge populations – a case where conservation of many local populations is recommended (Hampe & Petit, 2005). Our results indicate that conservation efforts for Douglas-fir and other Madrean 'sky island' species might target many isolated populations, while still protecting the highest diversity regions (Frankham *et al.*, 2002) such as in the Sierra Madre Occidental. Proposals to cross inbred populations regionally (Cruz-Nicolás *et al.*, 2008) or to move populations long distances (Vargas-Hernández *et al.*, 2004) should consider the unique local genetic compositions of species.

Models project range contractions for pine and oak species across Mexico by up to 60% (Gómez-Mendoza & Arriaga, 2007) under projected warmer and drier conditions (Liverman & O'Brien, 1991). For Douglas-fir, ecological niche models also suggest population decline under projected future climates (Fig. 5), consistent with the observed sensitivity of Mexican Douglas-fir growth rates to precipitation and maximum temperature (González-Elizonado *et al.*, 2005). With temperatures already increasing (Pavia *et al.*, 2009), careful conservation measures will be necessary to protect the unique biodiversity of Douglas-fir and other temperate species in Mexico.

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References

Acevedo-Rodríguez R, Vargas-Hernández JJ, López-Upton J, Mendoza JV. 2006. Effect of geographic origin and nutrition on shoot phenology of Mexican Douglas-Fir (*Pseudotsuga* spp.) seedlings. *Agrociencia* **40**: 125–137.

Anducho-Reyes MA, Cognato AI, Hayes JL, Zúñiga G. 2008. Phylogeography of the bark beetle *Dendroctonus mexicanus* Hopkins (Coleoptera: Curculionidae: Scolytinae). *Molecular Phylogenetics and Evolution* **49**: 930–940.

Barnosky CW. 1985. Late Quaternary vegetation near Battle Ground Lake, southern Puget Trough, Washington. *Geological Society of America Bulletin* **96**: 263–271.

Birky WC Jr. 2001. The inheritance of genes in mitochondria and chloroplasts: laws, mechanisms, and models. *Annual Review of Genetics* **35**: 125–148.

Braconnot P, Otto-Bliesner B, Harrison S, Joussaume S, Peterchmitt J-Y, Abe-Ouchi A, Crucifix M, Driesschaert E, Fichefet T, Hewitt CD *et al.* 2007. Results of PMIP2 coupled simulations of the mid-Holocene and Last Glacial Maximum–Part 1: experiments and large-scale features. *Climate of the Past* **3**: 261–277.

Braukmann TWA, Kuzmina M, Stefanović S. 2009. Loss of all plastid *ndh* genes in Gnetales and conifers: extent and evolutionary significance for the seed plant phylogeny. *Current Genetics* **55**: 323–337.

Brown RB. 1985. A summary of late-Quaternary pollen records from Mexico west of the Isthmus of Tehuantepec. In: Bryant VM, Holloway RG, eds. *Pollen records of Late-Quaternary North American sediments*. Dallas, TX, USA: American Association of Stratigraphic Palynologists Foundation, 71–93.

Chang CS, Kim H, Park TY, Maunder M. 2004. Low levels of genetic variation among southern peripheral populations of the threatened herb, *Leontice microrhyncha* (Berberidaceae) in Korea. *Biological Conservation* **119**: 387–396.

Clement M, Posada D, Crandall KA. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* **9**: 1657–1660.

Collins WD, Bitz CM, Blackmon ML, Bonan GB, Bretherton CS, Carton JA, Chang P, Doney SC, Hack JJ, Henderson TB *et al.* 2006. The community climate system model version 3 (CCSM3). *Journal of Climate* **19**: 2122–2143.

Cortés-Palomec AC, McCauley RA, Oyama K. 2008. Isolation, characterization and cross-amplification of polymorphic microsatellite loci in *Laelia speciosa* (Orchidaceae). *Molecular Ecology Resources* **8**: 135–138.

Crandall KA, Bininda-Emonds ORP, Mace GM, Wayne RK. 2000. Considering evolutionary processes in conservation biology. *Trends in Ecology and Evolution* **15**: 290–295.

Critchfield WB. 1984. Impact of the Pleistocene on the genetic structure of North American conifers. In: Lanner RM, ed. *Proceedings of the eighth North American forest biology workshop*. Logan, UT, USA: Utah State University, 70–118.

Cruz-Nicolás J, Vargas-Hernández JJ, Ramírez-Vallejo P, López-Upton J. 2008. Mating pattern in natural populations of *Pseudotsuga menziesii* (Mirb.) Franco in Mexico. *Agrociencia* **42**: 367–378.

Davis MB. 1981. Quaternary history and the stability of forest communities. In: West DC, Shugart HH, Botkin DB, eds. *Forest succession: concepts and application*. New York, NY, USA: Springer-Verlag, 132–153.

Debreczy Z, Rácz I. 1995. New species and varieties of conifers from Mexico. *Phytologia* **78**: 217–243.

Deevey ES Jr. 1949. Biogeography of the Pleistocene. *Geological Society of America Bulletin* **60**: 1315–1416.

Dressler RL. 1954. Some floristic relationships between Mexico and the United States. *Rhodora* **56**: 81–96.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology* **7**: 214–221.

Drummond AJ, Rambaut A. 2009. *Tracer 1.5*. [WWW document].URL http://tree.bio.ed.ac.uk/software/tracer/ [accessed on 24 February 2010].

Drummond AJ, Rambaut A, Shapiro B, Pybus OG. 2005. Bayesian coalescent inference of past population dynamics from molecular sequences. *Molecular Biology and Evolution* **22**: 1185–1192.

Duff RJ, Nickrent DL. 1997. Characterization of mitochondrial small-subunit ribosomal RNAs from holoparasitic plants. *Journal of Molecular Evolution* **45**: 631–639.

Duff RJ, Nickrent DL. 1999. Phylogenetic relationships of land plants using mitochondrial small-subunit rDNA sequences. *American Journal of Botany* **86**: 372–386.

Dupanloup I, Schneider S, Excoffier L. 2002. A simulated annealing approach to define the genetic structure of populations. *Molecular Ecology* **11**: 2571–2581.

Earle CJ. 2009. The gymnosperm database: *Pseudotsuga lindleyana*. URL http://www.conifers.org/pi/ps/lindleyana.htm.

Eckert AJ, Bower AD, Wegrzyn JL, Pande B, Jermstad KD, Krutovsky KV, St. Clair JB, Neale DB. 2009. Association genetics of coastal Douglas-fir (*Pseudotsuga menziesii* var. *menziesii*, Pinaceae). I. Cold hardiness related traits. *Genetics* **182**: 1289–1302.

Excoffier L, Laval G, Schneider S. 2005. Arlequin version 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1**: 47–50.

Farjon A, Page CN. 1999. *Conifers: status survey and conservation action plan*. Gland, Switzerland: IUCN/SSC Conifer Specialist Group.

Flous F. 1934a. Deux espèces nouvelles de *Pseudotsuga* Américains. *Bulletin de la Société d'histoire naturelle de Toulouse* **66**: 211–224.

Flous F. 1934b. Diagnoses d'espèces et variétés nouvelles de *Pseudotsuga* Américains. *Bulletin de la Société d'histoire naturelle de Toulouse* **66**: 329–346.

Frankham R, Ballou JD, Briscoe DA. 2002. *Introduction to conservation genetics*. Cambridge, UK: Cambridge University Press.

Fu Y-X. 1997. Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics* **147**: 915–925.

Gernandt DS, Liston A. 1999. Internal transcribed spacer region evolution in *Larix* and *Pseudotsuga* (Pinaceae). *American Journal of Botany* **86**: 711–723.

Goldstein DB, Ruiz-Linares A, Cavalli-Sforza LL, Feldman MW. 1995a. Genetic absolute dating based on microsatellites and the origin of modern humans. *Proceedings of the National Academy of Sciences, USA* **92**: 6723–6727.

Goldstein DB, Ruiz-Linares A, Cavalli-Sforza LL, Feldman MW. 1995b. An evaluation of genetic distances for use with microsatellite loci. *Genetics* **139**: 463–471.

Gómez-Mendoza L, Arriaga L. 2007. Modeling the effect of climate change on the distribution of oak and pine species of Mexico. *Conservation Biology* **21**: 1545–1555.

González-Elizonado M, Jurado E, Návar J, González-Elizonado MS, Villanueva J, Aguirre O, Jiménez J. 2005. Tree-rings and climate relationships for Douglas-fir chronologies from the Sierra Madre Occidental, Mexico: a 1681–2001 rain reconstruction. *Forest Ecology and Management* **213**: 39–53.

Graham A. 1999. The Tertiary history of the northern temperate element in the northern Latin American biota. *American Journal of Botany* **86**: 32–38.

Gray J. 1961. Early Pleistocene paleoclimatic record from Sonoran Desert, Arizona. *Science* **133**: 38–39.

Gugger PF, McLachlan JS, Manos PS, Clark JS. 2008. Inferring long-distance dispersal and topographic barriers during post-glacial colonization from the genetic structure of red maple (*Acer rubrum* L.) in New England. *Journal of Biogeography* **35**: 1665–1673.

Gugger PF, Sugita S. 2010. Glacial populations and postglacial migration of Douglas-fir based on fossil pollen and macrofossil evidence. *Quaternary Science Reviews* **29**: 2052–2070.

Gugger PF, Sugita S, Cavender-Bares J. 2010. Phylogeography of Douglas-fir based on mitochondrial and chloroplast DNA sequences: testing hypotheses from the fossil record. *Molecular Ecology* **19**: 1877–1897.

Hampe A, Bairlein F. 2000. Modified dispersal-related traits in disjunct populations of bird-dispersed *Frangula alnus* (Rhamnaceae): a result of its Quaternary distribution shifts? *Ecography* **23**: 603–613.

Hampe A, Petit RJ. 2005. Conserving biodiversity under climate change: the rear edge matters. *Ecology Letters* **8**: 461–467.

Hasegawa MH, Kishino H, Yano T. 1985. Dating of the human-ape splitting by a molecular clock of mitochondrial DNA. *Journal of Molecular Evolution* **22**: 160–174.

Hasumi H, Emori S. 2004. *K-1 coupled GCM (MIROC) description*. Tokyo, Japan: Center for Climate System Research, University of Tokyo.

Head MJ, Gibbard PL. 2005. Early-Middle Pleistocene transitions: an overview and recommendation for the defining boundary. *Geological Society of London Special Publications* **247**: 1–18.

Hermann RK. 1985. *The genus Pseudotsuga: ancestral history and past distribution*. Corvallis, OR, USA: Forest Research Laboratory, Oregon State University.

Hermann RK, Lavender DP. 1990. Douglas-fir. In: Burns RM, Honkala BH, eds. *Silvics of North America: conifers*. Washington, DC, USA: USDA Forest Service, 1080–1108.

Hewitt G. 2000. The genetic legacy of the Quaternary ice ages. *Nature* **405**: 907–913.

Hey J, Nielson R. 2007. Integration within the Felsenstein equation for improved Markov chain Monte Carlo methods in population genetics. *Proceedings of the National Academy of Sciences, USA* **104**: 2785–2790.

Hijmans RJ, Graham CH. 2006. The ability of climate envelope models to predict the effect of climate change on species distributions. *Global Change Biology* **12**: 2272–2281.

Hudson RR. 1990. Gene genealogies and the coalescent process. *Oxford Surveys in Evolutionary Biology* **7**: 1–44.

Huntley B, Birks HJB. 1983. *An atlas of past and present pollen maps for Europe: 0–13000 years ago*. Cambridge, UK: Cambridge University Press.

Hurlbert SH. 1971. The nonconcept of species diversity: a critique and alternative parameters. *Ecology* **52**: 577–586.

Jaramillo-Correa JP, Beaulieu J, Bousquet J. 2004. Variation in mitochondrial DNA reveals multiple distant glacial refugia in black spruce (*Picea mariana*), a transcontinental North American conifer. *Molecular Ecology* **13**: 2735–2747.

Jaramillo-Correa JP, Beaulieu J, Ledig FT, Bousqet J. 2006. Decoupled mitochondrial and chloroplast DNA population structure reveals Holocene collapse and population isolation in a threatened Mexican-endemic conifer. *Molecular Ecology* **15**: 2787–2800.

Juárez-Agis A, López-Upton J, Vargas-Hernández JJ, Saénz-Romero C. 2006. Geographic variation in germination and initial seedling growth of *Pseudotsuga menziesii* of Mexico. *Agrociencia* **40**: 783–792.

Li P, Adams WT. 1989. Range-wide patterns of allozyme variation in Douglas-fir (*Pseudotsuga menziesii*). *Canadian Journal of Forest Research* **19**: 149–161.

Librado P, Rozas J. 2009. DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* **25**: 1451–1452.

Lisiecki LE, Raymo ME. 2005. A Pliocene–Pleistocene stack of 57 globally distributed benthic $\delta^{18}O$ records. *Paleooceanography* **20**: PA1003.

Little EL Jr. 1952. The genus *Pseudotsuga* (Douglas-fir) in North America. *Leaflets Western Botany* **6**: 181–198.

Liverman DM, O'Brien KL. 1991. Global warming and climate change in Mexico. *Global Environmental Change* **1**: 351–364.

Lynch M, Crease TJ. 1990. The analysis of population survey data on DNA sequence variation. *Molecular Biology and Evolution* **7**: 377–394.

Mápula-Larreta M, López-Upton J, Vargas-Hernández JJ, Hernández-Livera A. 2007. Reproductive indicators in natural populations of Douglas-fir in Mexico. *Biodiversity and Conservation* **16**: 727–742.

Marshall KA, Neale DB. 1992. The inheritance of mitochondrial DNA in Douglas-fir (*Pseudotsuga menziesii*). *Canadian Journal of Forest Research* **22**: 73–75.

Martin PS. 1963. Geochronology of pluvial Lake Cochise, southern Arizona. II. Pollen analysis of a 42-meter core. *Ecology* **44**: 436–444.

Martin PR, McKay JK. 2004. Latitudinal variation in genetic divergence of populations and the potential for speciation. *Evolution* **58**: 938–945.

Martínez M. 1949. Las Pseudotsugas de México. *Anales del Instituto de Biología* **20**: 129–184.

Milá B, Girman DJ, Kimura M, Smith TB. 2000. Genetic evidence for the effect of a postglacial population expansion on the phylogeography of a North American songbird. *Proceedings of the Royal Society B* **267**: 1033–1040.

Moreno-Letelier A, Piñero D. 2009. Phylogeographic structure of *Pinus strobiformis* Engelm. across the Chihuahuan Desert filter-barrier. *Journal of Biogeography* **36**: 121–131.

Nakicenovic N, Swart R. 2000. *Special report on emissions scenarios*. Cambridge, UK: Cambridge University Press.

Navascués M, Vaxevanidou Z, González-Martínez SC, Climent J, Gil L, Emerson BC. 2006. Chloroplast microsatellites reveal colonization and metapopulation dynamics in the Canary Island pine. *Molecular Ecology* **15**: 2691–2698.

Neale DB, Ingvarsson PK. 2008. Population, quantitative and comparative genomics of adaptation in forest trees. *Current Opinion in Plant Biology* **11**: 149–155.

Neale DB, Wheeler NC, Allard RW. 1986. Paternal inheritance of chloroplast DNA in Douglas-fir. *Canadian Journal of Forest Research* **16**: 1152–1154.

Nei M. 1973. Analysis of gene diversity in subdivided populations. *Proceedings of the National Academy of Sciences, USA* **70**: 3321–3323.

Nei M. 1987. *Molecular evolutionary genetics*. New York, NY, USA: Columbia University Press.

Nielson R, Wakeley JW. 2001. Distinguishing migration from isolation: a Markov chain Monte Carlo approach. *Genetics* **158**: 885–896.

Norma Oficial Mexicana. 1994. *NOM-059-ECOL-1994: Que determina las espeies y subespecies de flora y fauna silvestres terrestres y acuáticas en penligro de extinción, amenazadas, raras y las sujetas a protección especial, y que establece especificaciones para su protección*. Mexico City, DF, México: Secretaría de Desarrollo Social.

Norma Oficial Mexicana. 2001. *NOM-059-SEMARNAT-2001: Protección ambiental – especies nativas de México de flora y fauna silvestres – categorías de riesgo y especificaciones para su inclusión, exclusión o cambio – lista de especies en riesgo*. Mexico City, DF, México: Secretaría de Medio Ambiente y Recursos Naturales.

Ohta T, Kimura M. 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. *Genetical Research* **22**: 201–204.

Palacios-Chavez R, Rzedowski J. 1993. Estudio palinológico de las floras fósiles del Mioceno inferior y principios del Mioceno medio de la región de Pichucalco, Chiapas, México. *Acta Botánica Mexicana* **24**: 1–96.

Palmer JD, Herbon LA. 1988. Plant mitochondrial DNA evolves rapidly in structure, but slowly in sequence. *Journal of Molecular Evolution* **28**: 87–97.

Parisod C, Joost S. 2010. Divergent selection in trailing- versus leading-edge populations of *Biscutella laevigata*. *Annals of Botany* **105**: 655–660.

Pavia EG, Graef F, Reyes J. 2009. Annual and seasonal surface air temperature trends in Mexico. *International Journal of Climatology* **29**: 1324–1329.

Pennington RT, Prado DE, Pendry CA. 2000. Neotropical seasonally dry forests and Quaternary vegetation changes. *Journal of Biogeography* **27**: 261–273.

Perry JP Jr, Graham A, Richardson DM. 1998. The history of pines in Mexico and Central America. In: Richardson DM, ed. *Ecology and Biogeography of Pinus*. Cambridge, UK: Cambridge University Press, 137–149.

Petit RJ, Aguinagalde I, de Beaulieu JL, Bittkau C, Brewer S, Cheddadi R, Ennos RA, Fineschi S, Grivet D, Lascoux M *et al.* 2003. Glacial refugia: hotspots but not melting pots of genetic diversity. *Science* **300**: 1563–1565.

Petit RJ, Duminil J, Fineschi S, Hampe A, Salvini D, Vendramin GG. 2005. Comparative organization of chloroplast, mitochondrial, and nuclear diversity in plant populations. *Molecular Ecology* **14**: 689–701.

Petit RJ, Mousadik AE, Pons O. 1998. Identifying populations for conservation on the basis of genetic markers. *Conservation Biology* **12**: 844–855.

Petit RJ, Pineau E, Demesure B, Bacilieri R, Ducousso A, Kremer A. 1997. Chloroplast DNA footprints of postglacial recolonization by oaks. *Proceedings of the National Academy of Sciences, USA* **94**: 9996–10001.

Phillips SJ, Anderson RP, Schapire RE. 2006. Maximum entropy modeling of species geographic distributions. *Ecological Modelling* **190**: 231–259.

Pons O, Petit RJ. 1995. Estimation, variance and optimal sampling of gene diversity. I. Haploid locus. *Theoretical and Applied Genetics* **90**: 462–470.

Pons O, Petit RJ. 1996. Measuring and testing genetic differentiation with ordered versus unordered alleles. *Genetics* **144**: 1237–1245.

Reyes-Hernández VJ, Vargas-Hernández JJ, López-Upton J, Vaquera-Huerta H. 2005. Variación morfológica y anatómica en poblaciones mexicanas de *Pseudotsuga* (Pinaceae). *Acta Botánica Mexicana* **70**: 47–67.

Reyes-Hernández VJ, Vargas-Hernández JJ, López-Upton J, Vaquera-Huerta H. 2006. Phenotypic similarity among Mexican populations of *Pseudotsuga* Carr. *Agrociencia* **40**: 545–556.

Richmond GM, Fullerton DS. 1986. Summation of Quaternary glaciations in the United States of America. *Quaternary Science Reviews* **5**: 183–196.

Rodríguez-Banderas A, Vargas-Mendoza CF, Buonamici A, Vendramin GG. 2009. Genetic diversity and phylogeographic analysis of *Pinus leiophylla*: a post-glacial range expansion. *Journal of Biogeography* **36**: 1807–1820.

Ryder OA. 1986. Species conservation and systematics: the dilemma of subspecies. *Trends in Ecology and Evolution* **1**: 9–10.

Schorn HE. 1994. A preliminary discussion of fossil larches (*Larix*, Pinaceae) from the Arctic. *Quaternary International* **22–23**: 173–183.

Slatkin M. 1995. A measure of population subdivision based on microsatellite allele frequencies. *Genetics* **139**: 457–462.

Sneath PHA, Sokal RR. 1973. *Numerical taxonomy: the principles and practice of numerical classification*. San Francisco, CA, USA: Freeman.

Strasburg JL, Rieseberg LH. 2010. How robust are 'isolation with migration' analyses to violations of the IM model? A simulation study. *Molecular Biology and Evolution* **27**: 297–310.

Sugita S. 1993. A model of pollen source area for an entire lake surface. *Quaternary Research* **39**: 239–244.

Swofford DL. 2003. *PAUP*: phylogenetic analysis using parsimony (*and other methods)*. Sunderland, MA, USA: Sinauer Associates.

Tajima F. 1983. Evolutionary relationship of DNA sequences in finite populations. *Genetics* **105**: 437–460.

Takezaki N, Nei M, Tamura K. 2009. POPTREE2: Software for constructing population trees from allele frequency data and computing other statistics with Windows interface. *Molecular Biology and Evolution* **27**: 747–752.

Vargas-Hernández JJ, López-Upton J, Reyes-Hernández VJ, Domínguez-Alvarez A, Mápula-Larreta M. 2004. Natural populations of Douglas-fir in Mexico: current status and needs for conservation. In: Beaulieu J, ed. *Silviculture and the conservation of genetic resources for sustainable forest management: proceedings of the symposium of the North American forest commission, forest genetic resources and silviculture working groups, and the International Union of Forest Research Organizations (IUFRO)*. Quebec City, QC, Canada, 26–36.

Vázquez-Selem L, Heine K. 2004. Late Quaternary glaciation of Mexico. In: Ehlers J, Gibbard PL, eds. *Quaternary glaciations extent and chronology: Part III: South America, Asia, Africa, Australasia, Antarctica*. Amsterdam, the Netherlands: Elsevier, 233–242.

Velasco-García MV, López-Upton J, Angeles-Pérez G, Vargas-Hernández JJ, Guerra-de la Cruz V. 2007. *Pseudotsuga menziesii* seed dispersion in populations of central Mexico. *Agrociencia* **41**: 121–131.

Vendramin GG, Anzidei M, Madaghiele A, Bucci G. 1998. Distribution of genetic diversity in *Pinus pinaster* Ait. as revealed by chloroplast microsatellites. *Theoretical and Applied Genetics* **97**: 456–463.

Vendramin GG, Lelli L, Rossi P, Morgante M. 1996. A set of primers for the amplification of 20 chloroplast microsatellites in Pinaceae. *Molecular Ecology* **5**: 595–598.

Viard F, El-Kassaby YA, Ritland K. 2001. Diversity and genetic structure in populations of *Pseudotsuga menziesii* (Pinaceae) at chloroplast microsatellite loci. *Genome* **44**: 336–344.

Wakasugi T, Tsudzuki J, Ito S, Nakashima T, Tsudzuki T, Sugiura M. 1994. Loss of all *ndh* genes as determined by sequencing the entire chloroplast genome of the black pine *Pinus thunbergii*. *Proceedings of the National Academy of Sciences, USA* **91**: 9794–9798.

Wallace AR. 1876. *The geographical distribution of animals with a study of the relations of living and extinct faunas as elucidating the past changes of Earth's surface*. New York, NY, USA: Harper.

Supporting Information

Additional supporting information may be found in the online version of this article.

Fig. S1 Posterior distributions for divergence time parameter (t) for the run with the highest effective sample size (ESS) under the full IMa model and a reduced model, where $m_1 = m_2 = 0$.

Table S1 Sampling site information and haplotype frequencies for each marker and each population

Table S2 Mitotype and chlorotype definitions in terms of *V7*, *nad7i1*, *rps7-trnL* and *rps15-psaC* haplotypes reported to GenBank (accessions in parentheses)

Table S3 Definitions of chloroplast DNA microsatellite (cpSSR) haplotypes based on fragment lengths of each cpSSR marker and binary coding used to calculate F_S (Table 1)

Table S4 SAMOVA tables for groupings that gave the highest F_{CT} for mitochondrial DNA (mtDNA) and chloroplast DNA (cpDNA) sequence data

Table S5 Training gain values when climatic variables were used in isolation for each model

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