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Demographic modelling helps track the rapid and recent divergence of a conifer species pair from Central Mexico

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Abstract

Secondary contact of recently diverged species may have several outcomes, ranging from rampant hybridization to reinforced reproductive isolation. In plants, selfing tolerance and disjunct reproductive phenology may lead to reproductive isolation at contact zones. However, they may also evolve under both allopatric or parapatric frameworks and originate from adaptive and/or neutral forces. Inferring the historical demography of diverging taxa is thus a crucial step to identify factors that may have led to putative reproductive isolation. We explored various competing demography-potheses to account for the rapid divergence of a fir species complex (*Abies flinckii–A. religiosa*) distributed in "sky-islands" across central Mexico (i.e., along the Trans-Mexican Volcanic Belt; TMVB). Despite co-occurring in two independent sympatric regions (west and centre), these taxa rarely interbreed because of disjunct reproductive phenologies. We genotyped 1147 single nucleotide polymorphisms, generated by GBS (genotyping by sequencing), across 23 populations, and compared multiple scenarios based on the geological history of the TMVB. The best-fitting model revealed one of the most rapid and complete speciation cases for a conifer species-pair, dating back to ~1.2 million years ago. Coupled with the lack of support for stepwise colonization, our coalescent inferences point to an early cessation of interspecific gene flow under parapatric speciation; ancestral gene flow during divergence was asymmetrical (mostly from western firs into *A. religiosa*) and exclusive to the most ancient (i.e., central) contact zone. Factors promoting rapid reproductive isolation should be explored in other slowly evolving species complexes as they may account for the large tropical and subtropical diversity.

KEYWORDS
Abies flinckii–A. religiosa complex, conifers, disjunct reproductive phenology, geographic isolation, phylogeography, Trans-Mexican Volcanic Belt

1 | INTRODUCTION

Speciation is a continuous process that should ultimately lead to reproductive isolation, which at its turn is determined, among others, by the spatial distribution of populations (Coyne & Orr, 2004; Gavrilets, 2003; Ravinet et al., 2017). For instance, when there are physical barriers that hamper gene flow among diverging taxa, the action of genetic drift and/or divergent selection may fix phenotypic and genetic characters related to reproductive isolation (Coyne & Orr, 2004; Feder et al., 2013; Mayr, 1963). Conversely, speciation is thought to lag when populations continue to exchange alleles, or when they meet after evolving in geographical isolation for a period of time (i.e., secondary contact; Bolnick & Fitzpatrick, 2007; Pinho & Hey, 2010).

The evolutionary outcome of a secondary contact zone depends in part on the degree of divergence between the interacting taxa. That is, recently diverged species are expected to almost freely exchange alleles, which may result in the formation of hybrid swarms (e.g., Wells et al., 2019). On the other hand, reproductive isolation may arise or be favoured in secondary contact zones of more anciently diverged species, which may lead to increased divergence in sympatric as compared to allopatric areas (e.g., Grossenbacher & Whittall, 2011; Rosser et al., 2019). In plants, this is often produced by changes in mating traits (Dobzhansky, 1940), which include variations in reproductive phenology, such as mismatches of pollen shedding and female receptivity (Fox, 2003; Hopkins, 2013); or self-fertilization tolerance, which leads to a reduced allocation to male reproduction (reviewed in Roux et al., 2020), and lowers the probability of heterospecific pollen deposition (Charlesworth & Pannell, 2001; Hamrick & Godt, 1996).

Various evolutionary processes can prevent interspecific gene flow in contact zones. For instance, shifts to self-fertilization can be promoted by stochastic or adaptive processes for ensuring the reproductive success of individuals during the colonization of a new habitat and prior to a secondary contact with a sister species (Busch et al., 2011; Pannell, 2015). Under such a context, reproductive traits may evolve through pleiotropic effects: for example, when alleles shaping the founders' reproductive phenology lead to local adaptation to the novel conditions (Thibert-Plante & Gavrilets, 2013). Alternatively, interspecific mating may cease during secondary contact when there is strong ecological divergence between parental species, or selection against hybrids (Gavrilets, 2014; Hopkins, 2013; Smadja & Butlin, 2011). Therefore, the inference of phylogeographical structure, historical demography and ancestral gene flow represents a crucial step for correctly identifying which processes might explain how interspecific gene flow breaks down and species diverge from each other (Harvey et al., 2019).

In this study, we tested several demographic scenarios to account for the diminished interspecific gene flow within a fir species-complex endemic to the mountains of central Mexico (i.e., the Trans-Mexican Volcanic Belt; TMVB). The TMVB is an east–west-oriented volcanic formation (Figure 1) that bears fir forests distributed in "sky-islands" between ~2000 and 3500 m.a.s.l. (Rzedowski, 2006; Velázquez et al., 2000). At least two species have been recognized in this region: *Abies flinckii* and *A. religiosa* (Farjon & Rushforth, 1989). They are respectively located in the western and central–eastern areas of this mountain range (Figure 1). Morphologically, they can be differentiated by stem height and diameter (larger in *A. religiosa*), colour and shape of female cones (wider and darker in *A. religiosa*), and needle length and apex form (longer needles with emarginated apex in *A. flinckii*; shorter needles with acute apex in *A. religiosa*; Cruz-Nicolás, Giles-Pérez, González-Linares, et al., 2020; Eckenwalder, 2009; Farjon & Rushforth, 1989; Shalisko, 2014). The distribution of these species overlaps in two contact zones: one in the centre and one in the western portion of the TMVB (respectively "C contact" and "W contact" in Figure 1), although taxa still partially segregate by altitude within these zones,
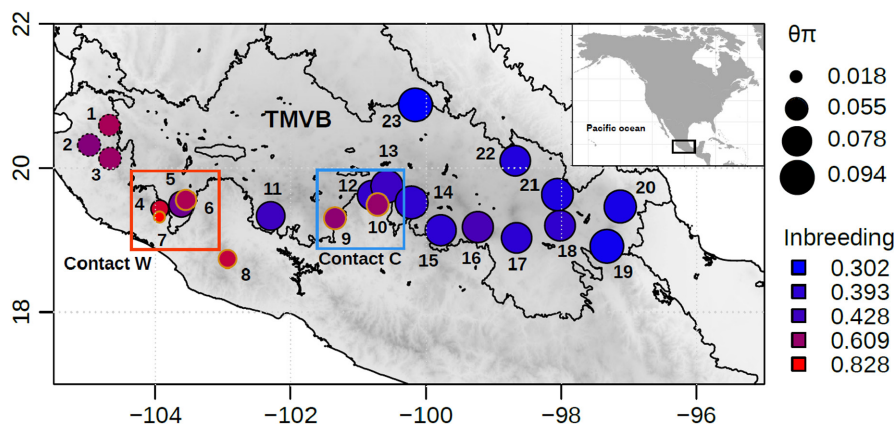
FIGURE 1 Geographical location of the 23 fir populations studied along the TMVB, in Central Mexico. Circles outlined in light brown and black correspond to *Abies flinckii* and *A. religiosa*, respectively; those with dotted margins are populations described as *A. jaliscana* (Vázquez-García et al., 2014). Circle size is proportional to the average number of pairwise differences between individuals (θ_{π}, Tajima, 1983), and colour denotes the average inbreeding coefficient (*f*, as estimated with popkin version 1.2.2; Ochoa & Storey, 2021). Blue and red squares indicate the approximate location of secondary contact zones between *A. flinckii* and *A. religiosa*.

with *A. religiosa* occurring at higher elevations (~2800–3500 m.a.s.l.) than *A. flinckii* (~1700–3000 m.a.s.l.; Rzedowski, 2006; Vázquez-García et al., 2014). Further complicating matters, the western-most populations of the TMVB are recognized by some authors as a different species: *A. jaliscana* (Vázquez-García et al., 2014). However, these populations are isolated and not implicated in any contact zone (see below).

While phenological data remain scarce for these firs, available records indicate that they all shed pollen between February and April (see Table S1). However, central and western populations of *A. religiosa* (i.e., those in sympatry with *A. flinckii*) release their pollen later, from April to June (Table S1), which may be diminishing interspecific gene flow. This is reflected in a clear morphological and genetic differentiation at "W contact," although differentiation at "C contact" is less clear (Cruz-Nicolás, Giles-Pérez, González-Linares, et al., 2020; Jaramillo-Correa et al., 2008; Shalisko, 2014). Reproductive phenology in conifers is highly inheritable (Howe et al., 2003), and has shown only small plastic responses to the environment (Ma et al., 2020; e.g., *Abies alba*, Vitasse et al., 2009). Therefore, phenological changes in sympatric *A. religiosa* stands may have evolved in situ, during secondary contact with *A. flinckii*. However, given that the geological history of the TMVB also provides the ideal scenario for stepwise colonization and founder effects, stochastic events at expansion fronts cannot be ruled out. To distinguish between these scenarios, the demographic background of these diverging species must be first investigated.

We evaluated the fit of several demographic scenarios on high-throughput genomic data with model-based coalescent analyses to disentangle which processes may account for the diminished interspecific gene flow between these central Mexican firs. Scenarios were based on the well-known geological and climatic history of the TMVB (Caballero-Miranda et al., 2010; Ferrari et al., 2012; see below). We begin by asking whether there is a relationship between range overlap and inbreeding, as self-fertilization may be favoured in sympatric zones (Wright et al., 2013). If that is the case, genetic differentiation should also be higher in sympatric relative to allopatric areas. Then, we reconstructed the historical demography of species divergence by testing competing colonization and range split hypotheses, and then further tested whether divergence occurred in the presence or absence of historical gene flow. Under parapatric divergence, selfing and/or phenological shifts should have evolved in situ, during secondary contact, and with a limited role of stochastic processes, which should have allowed interspecific gene flow in most regions of the genome (except those implicated in the phenological shift). On the other hand, under allopatric divergence, such changes should have evolved at colonization fronts and before secondary contact, which should have limited gene flow in most regions of the genome. Setting geologically based demographic inferences for inferring processes promoting rapid speciation helps us understand how species diversity arises and is maintained in the tropics and subtropics, particularly for reputedly slowly evolving lineages, like conifers.

2 | MATERIALS AND METHODS

We collected foliar tissue for 123 trees from 23 populations spanning most of the known fir distribution across the TMVB, including both contact zones (Figure 1). Geographical distance between samples varied within each contact zone. Tissue was collected following two altitudinal gradients at "W contact," and thus included *Abies flinckii* and *A. religiosa* individuals separated by <100 m. On the other hand, the "C contact" encompasses a continuous mountain chain where sampling sites are separated by more than 6 km (see Aguirre-Planter et al., 2000; Méndez-González et al., 2017 for additional sampling details). Genomic DNA was isolated with a CTAB 2 mini-prep protocol (Vázquez-Lobo, 1996); its quality was evaluated by agarose gel electrophoresis using a 1000-bp DNA marker as reference (Thermo Scientific™), and its concentration quantified with a Qubit 4 fluorometer (Thermo Scientific™).

2.1 | Sequencing, assembly, SNP calling and genotyping

Following standard protocols for plants with large and complex genomes (Boudhrioua et al., 2017; Poland et al., 2012), genomic DNA (200 ng per sample) was double-digested with *Pst*I and *Msp*I restriction enzymes. Then, P1 adapters containing 4- to 8-bp-long individualized barcodes (differing from each other by at least 2 bp) were ligated to each sample. Equimolar quantities of DNA were pooled to elaborate GBS libraries after sizing with a blue Pippin prep machine (SAGE sciences). Each 96-plex library was single-end-sequenced on an Illumina HiSeq 2500 system. Both GBS library preparation and sequencing were performed at the "Plateforme d'analyses génomiques, Institut de Biologie Intégrative et des Systèmes" (IBIS, Université Laval, Québec, Canada).

Raw reads filtering, parameter optimization, contig assembly and single nucleotide polymorphism (SNP) calling were performed following the IPYRAD pipeline (Eaton & Overcast, 2020). Briefly, after demultiplexing, Illumina adapters and low-quality 3′ ends were trimmed (Phred score < 43). Only those reads that lacked low-quality bases and had a minimal length of 80 bp were kept. Then, a de novo assembly was performed using VSEARCH version 2.0.2 (Rognes et al., 2016), with parameters defined after optimization, using 11 replicate pair samples (see Material S1 for more details). We considered a clustering threshold of 0.90, a maximum number of 4 heterozygous sites per locus (i.e., contig) and a minimum statistical depth of 8 reads. However, only those loci exhibiting a single SNP were considered, as these are the least prone to be spurious polymorphisms (Table S2 and Figure S1). Such a stringent filter further diminishes linkgage disequilibrium (LD) bias between SNPs for population genomic analyses (Zheng et al., 2012). Singletons (i.e., mac = 2), indels, nonbiallelic SNPs and polymorphisms with a mindepth of 10× or less, and 100× or more were removed using VCFTOOLS version 0.1.14 (Danecek et al., 2011).

As an additional filter, retained SNPs were compared with those obtained from a sequenced *A. religiosa* megagametophyte (the haploid tissue surrounding conifer embryos). Those observed in both data sets ($n = 62$) were eliminated as they are probably the result of paralogue misalignment.

We finally constructed three data sets by excluding sites with 50%, 13% and 0% missing data, respectively, to identify potential bias in our inferences. These data sets were used to model some genomic diversity statistics as a function of missing data level, and to account for this factor during demographic model selection (Material S2). After verifying that missing data were not producing serious confounding effects (Table S3 and Figure S2), the 50% missing-data set was used for subsequent analyses.

2.2 | Inbreeding and genomic differentiation at contact zones

As a proxy to self-fertilization, we first estimated population inbreeding coefficients (*f*) from a kinship matrix constructed with the R package popkin version 1.2.2 (Ochoa & Storey, 2021), which accounts for complex genetic structures. For comparative purposes and because popkin might slightly overestimate inbreeding when populations are well structured (i.e., when populations have independently evolved, Ochoa & Storey, 2021), we also computed inbreeding coefficients (*F*) using VCFTOOLS version 0.1.14. Then, we calculated the ratio of heterozygous to segregating sites (Het/S), the average number of nucleotide differences (θ_π; Tajima, 1983), the nucleotide diversity (θ_w; Watterson, 1975), the observed heterozygosity (H_O), Tajima's *D* (Tajima, 1989) and pairwise F_{ST} (Hudson et al., 1992) using DNASP version 6.12.03 (Rozas et al., 2017), VCFTOOLS version 0.1.14 and POPGENOME version 2.7.1 (Pfeifer et al., 2014). Note that θ_π and θ_W could be downwardly biased as they were calculated using polymorphic sites only (Korunes & Samuk, 2021). Thus, to further explore genomic diversity, we visualized the site frequency spectrum of minor alleles for each inferred lineage (see Section 3).

Selfing species tend to reduce resource allocation to male function, which lowers pollen production and diminishes pollen-mediated gene flow (Charlesworth & Pannell, 2001; Hamrick & Godt, 1996; Roux et al., 2020). Therefore, if self-tolerance has evolved at contact zones, we expected less genetic diversity and both higher inbreeding and interspecific genetic differentiation (F_{ST}) in sympatric than in allopatric populations (Hopkins, 2013; Wright et al., 2013). Opposite results would be expected if reproductive barriers are absent or are inefficient for countering interspecific mating (e.g., Garner et al., 2018; Moran et al., 2018). Note that these patterns should be readily observed with genetic markers randomly distributed across an extremely large genome (~18 Gb; Mosca et al., 2019), as the likelihood of hitting genes associated with reproductive traits is very low; looking for and removing such genes was thus unnecessary. We further performed a Mantel test to account for potential bias in genetic differentiation estimates given by the geographical separation of samples within each contact zone. We estimated kinship between individual pairs with the R package popkin version 1.2.2, while pairwise geographical distances and Mantel tests were computed in the R package ade4 version 1.7–13 (Dray et al., 2018).

2.3 | Genetic structure

To infer population genetic structure, we conducted a principal components analysis (PCA) with SNPRELATE (Zheng et al., 2012), and a model-based assignment with ADMIXTURE version 1.3.0 (Alexander et al., 2009). For this last method, we performed 10 independent runs, each one with *K*-values ranging from one to eight; the *K*-value with the lowest averaged cross-validation error was chosen as the most likely. Since westernmost populations (i.e., populations 1–10) showed high inbreeding coefficients (see Results), we repeated ADMIXTURE analyses without these localities. We finally carried out a discriminant analysis of principal components (DAPC) for this reduced population set, with R package adegenet (Jombart, 2008), to identify more subtle structure patterns between and within clusters, including isolation by distance (Jombart et al., 2010). The most likely number of PCs was investigated with a cross-validation procedure also implemented in adegenet.

2.4 | Historical range shifts of firs in the TMVB

Interspecific gene flow may be mainly interrupted through the action of stochastic forces (e.g., Cutter, 2019; Pannell, 2015). To test this possibility, we fitted several divergence and demographic scenarios to the "observed" multisite frequency spectrum (MSFS). We conducted a hierarchical comparison of scenarios to simplify hypothesis testing. Hence, we first focused on evaluating three divergence scenarios at the species level, which respectively involved ancient colonization events (Figure S3a; Ha and Hb scenarios in Figure S4) and a more recent allopatric divergence (i.e., through range split; Figure S3b; Hc scenario in Figure S4). Then, the best-fitting scenario was used as a baseline to test four more complex hypotheses at the population/regional levels. These explored in more detail different range expansion histories within *A. religiosa*, considering stepwise colonization from one (i.e., Hc9 and Hc10 scenarios, Figure S5) or two source populations (i.e., Hc11 and Hc12, Figure S5), with the former supposing a stronger founder effect than the latter (Cutter, 2019; Pannell, 2015).

The geological history of the TMVB is characterized by the uplifting of four temporal and spatially localized magmatic arcs (Ferrari et al., 2012), together with some local topographic modifications, such as the formation of tectonic valleys in its western portion (Rosas-Elguera et al., 2003). Consistently, the demographic scenarios above considered colonization of younger arcs from locations at older arcs, and/or population splits between rising tectonic valleys (Material S3).

We derived "observed" MSFS with the script easySFS.py (Overcast, 2019) and fitted it to those produced by each demographic scenario. Scenarios were simulated in FASTSIMCOAL2

2.5 | Interspecific gene flow

Parapatric and allopatric divergence may lead to different interspecific gene flow patterns. The former would result in only early interspecific gene flow, while the latter would produce recent and limited interspecific gene flow. We thus compared the retained models above (no gene flow) with scenarios including ancient (i.e., shortly after species split) and recent interspecific gene flow (see Figure 2), recent gene flow exclusive within *A. religiosa* (RIM), or a combination of both RIM and ancient interspecific gene flow (see Figure S6). We expected recent migration events to be associated with mid- to late Pleistocene glacial periods, which should have favoured population downslope migration and expansion, and thus increase chances for secondary contact (Caballero-Miranda et al., 2010; Ramírez-Barahona & Eguiarte, 2013; see Figure S3c). All models allowed for asymmetric gene flow between neighbour species/genomic groups. Migration rate "priors" ranged from 0.00001 to 0.01, which helped account for nested migration scenarios (i.e., migration only between some population pairs).

To compare and choose the best-fitting gene flow scenario(s), we implemented the same strategy using wAICs. To assess fitting of the selected gene flow model to the observed genetic variation, we performed a likelihood ratio G-statistic test (CLR = $\log_{10}[CL_O/CL_E]$; Excoffier et al., 2013). Briefly, we used the maximum likelihood estimates of the best-fitted scenario to simulate 100 parametric bootstrapped MSFSs (bMSFSs). Then, we computed the composite likelihood and CRL for each bMSFS under the first three best scenarios. The *p*-value was estimated for each model as the fraction of simulated data sets with a CLR larger than or equal to the observed CLR. Hence, significant *p*-values would suggest that the model tested is poorly fitted. Likewise, we used each bMSFS to compute population parameter distributions and associated 95% confidence intervals. Divergence times were transformed into million years by assuming a generation time of 60 years (Sánchez-Velásquez et al., 1991).

3 | RESULTS

3.1 | GBS and bioinformatic analysis

Approximately 260 million reads of about 100 bp and a mean Phred score of 36 were obtained per sequencing lane. After SNP calling and filtering, 1147, 602 and 138 SNPs were respectively recovered when allowing for only one SNP per loci and 50%, 13% and 0% missing data. Each polymorphism had a mean depth sequencing of 52 across individuals. Linear mixed models indicated only a weak effect of missing data on genomic statistics, such as inbreeding coefficients (*f*) and observed heterozygosity (H_O) (see Material S2); thus, we used the SNP data set with 50% missing data for all remaining analyses.
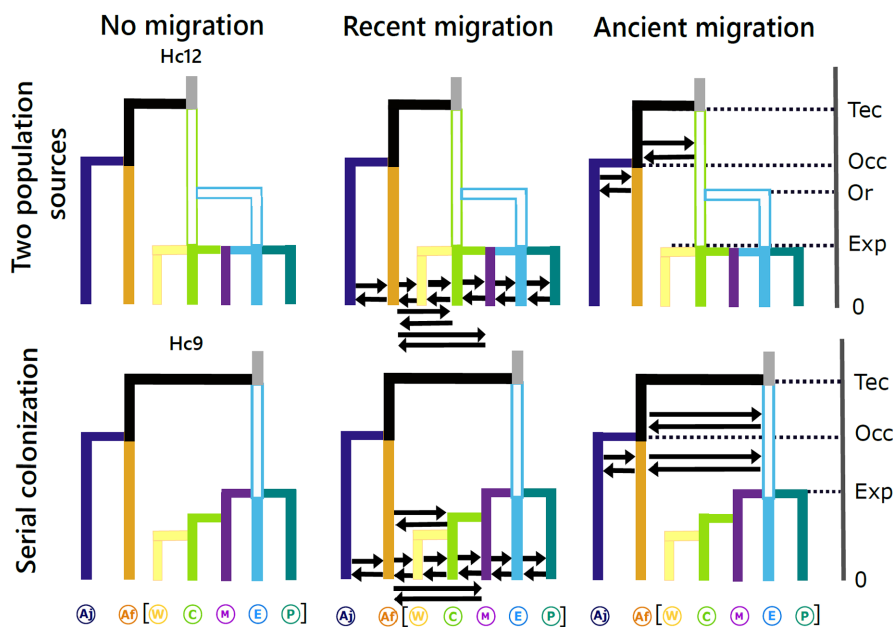
FIGURE 2 Demographic models tested for inferring divergence times, expansion and gene flow between fir species/populations in Central Mexico. Scenarios considered that *Abies religiosa* (i.e., the lineages enclosed in brackets) either colonized its range from a single (Hc9; bottom row) or two sources (Hc12; top row). Three migration hypotheses were then tested: No migration (left), recent gene flow (centre) and ancient gene flow (right). Time events were named as Tec (i.e., divergence associated with tectonism), Occ (i.e., divergence in the west of the TMVB), Or (i.e., divergence in the east of the TMVB) and exp (i.e., range expansion of *A. religiosa*). Colours match the inferred population ancestries, as in Figure 4. Ancestral C and E populations are illustrated with green and blue outlined branches, respectively. Further migration hypotheses tested in this study are shown in Figure S6.

3.2 | Inbreeding coefficients and genomic diversity

Mean inbreeding coefficients calculated with popkin version 1.2.2 (f) were higher for *Abies flinckii* (f = 0.592–0.828) and *A. jaliscana* (f = 0.575–0.648) than for *A. religiosa* (f = 0.302–0.733; t-statistic = −9.214, p = 2.3e^{-15}; Figure 1; Figure S2). Such values were significantly higher at "W contact" (t-statistic = 5.949, p = 3.2e^{-08}; Figure 1) than for the remaining populations, including those at "C contact". Mean inbreeding, as estimated with VCFTOOLS version 0.1.14 (F), was significantly lower overall (Table S5); however, estimates had the same significant trends across populations and species as those observed with f (Adjusted r^2 =.997, F-statistic = 3.983e^4, p = 2.2e^{-6}). Consistent with inbreeding estimates, lower Het/S ratios (t = 3.570, p =.001918), H_O (t = 9.214, p = 2.3e^{-15}), θ_π (t = 4.180, p =.000462) and θ_w values (t = 4.571, p =.000185) were observed for western firs than for *A. religiosa* (Figures 1 and 3a; Table S5). Again, these estimates were significantly lower at "W contact" than at "C contact" or all allopatric stands (t-statistic$_{Het/S}$ =−2.789, p =.0113; t-statistic$_{HO}$ =−5.949, p = 3.2e-08; t-statistic$_{\theta w}$ =−3.317, p =.00344; t-statistic$_{\theta \pi}$ = −3.514, p =.00218; Figures 1 and 3a; Table S5). Furthermore, *A. flinckii* and *A. jaliscana* showed positive Tajima's *D*, while *A. religiosa* had negative estimates at all populations, except those at 'W contact," which were positive (Table S5). Visual inspection of the minor alleles SFSs pointed in the same direction, with a relatively major number of intermediate frequency alleles in *A. flinckii* and *A. religiosa* from "W contact" than in eastern or central populations of the last species (Figure S7).

Higher pairwise F_{ST} were observed between than within species (t-statistic = −27.23, p < 2e^{-16}; Figure 3b), except for *A. flinckii* (F_{ST} =0.330;

SD = 0.148). Sympatric population pairs of *A. flinckii* and *A. religiosa* within "W contact" had higher F_{ST} than allopatric pairs (F_{ST} =0.597 vs. 0.508; t-statistic = 2.599, p =.011), while population pairs within "C contact" had virtually identical F_{ST} as allopatric pairs (F_{ST} =0.491, SD = 0.047; Figure 3b), which suggest less interspecific gene flow in the former contact area. Mantel tests revealed a nonsignificant relationship between geographical and kinship distances for both central (r =−.376, p =.999) and western (r =−.063, p =.839) contact zones (Figure S8).

3.3 | Genetic structure

The first two axes of the PCA respectively explained 7.7% and 6.3% of the genomic variance, and separated individuals according to taxonomy, including those described as *A. jaliscana* (Figure S9a). ADMIXTURE mirrored these results, with a retained K value of 3 (Figure 4c). However, further genomic structure was revealed for higher K values (Figure S9c). For K = 4, *A. religiosa* populations from "W contact" were separated in an independent cluster (i.e., W group). For K = 5, the rest of *A. religiosa* individuals were divided along an east-to-centre genetic cline (E and C groups, respectively), with some admixture observed in populations 12, 13 and 14 (grouped hereafter in the "M" group for within-species analyses, Figure 4c). Unexpectedly, the northernmost population of this species (i.e., Pop 23) was clustered with populations from "C contact" (Figure 4a). Finally, for K = 6, the two easternmost populations (i.e., Pops 19 and 20) were separated from the rest (P group); some individuals from populations 21 and 22 further showed some ancestry from this group (Figure 4c).
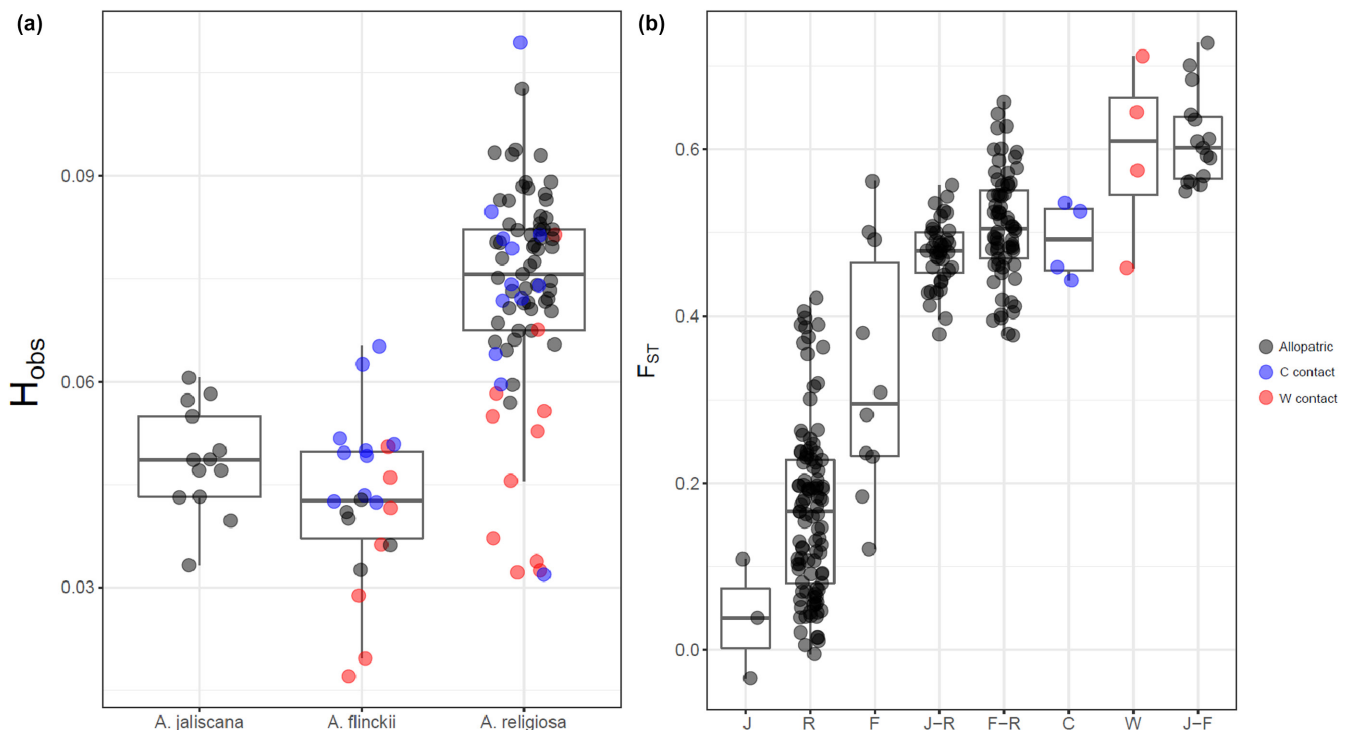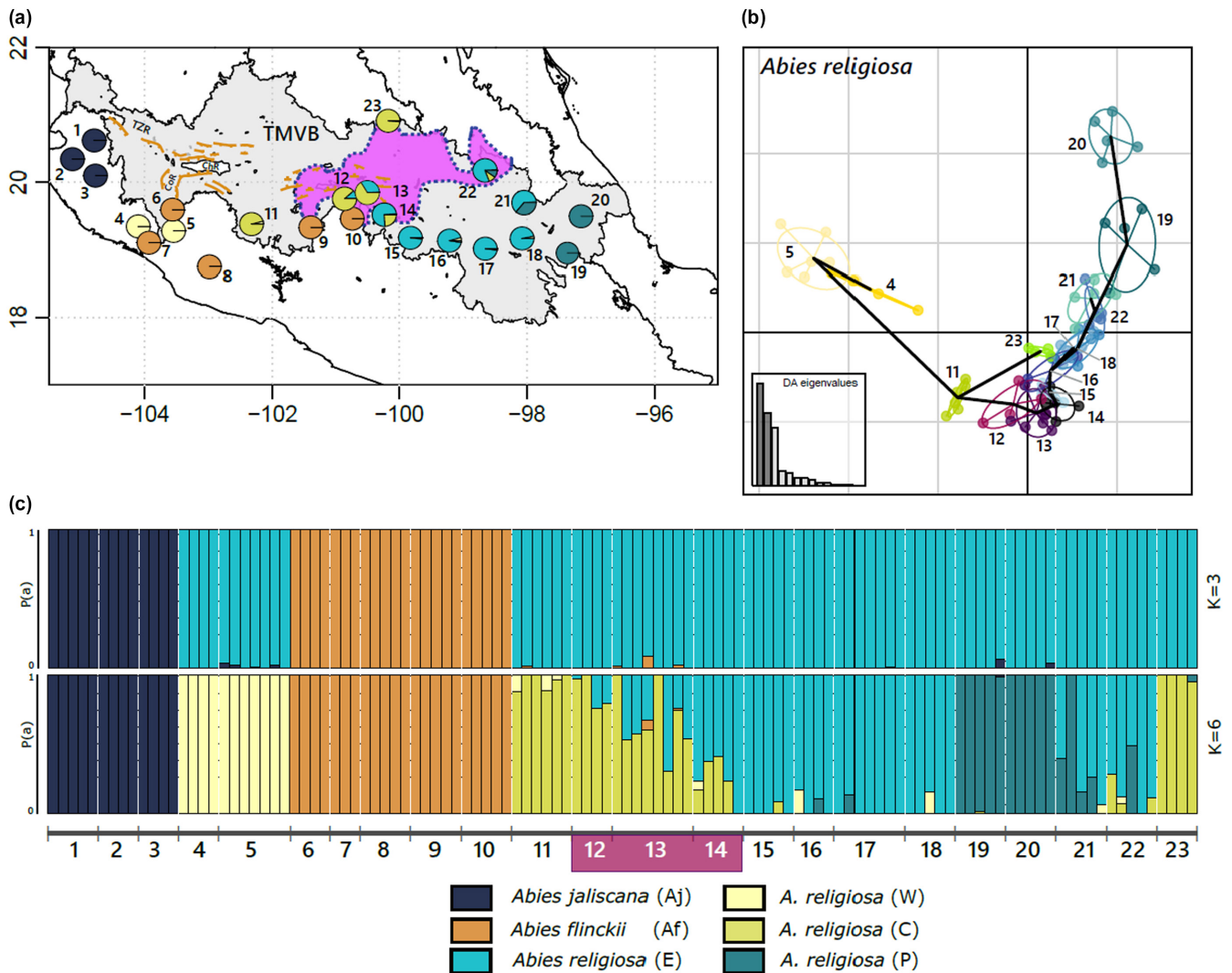

FIGURE 3 Boxplots of observed heterozygosity (H_O) per species (a) and pairwise F_{ST} (Hudson et al., 1992) (b) between conspecific (J, *Abies jaliscana*; R, *A. religiosa*; F, *A. flinckii*) and interspecific fir populations (J-R, F-R, J-F) in Central Mexico. Colo points highlight H_O (a) and interspecific pairwise F_{ST} values (b) within W (red) and C (blue) contact zones, respectively.

FIGURE 4 Population genetic structure of fir populations along the TMVB, in Central Mexico. (a) Geographical distribution of genetic ancestries when assuming $K = 6$ in ADMIXTURE (depicted in c). Brown lines illustrate the main tectonic valleys in the region (TZR, Tepic-Zoacalco rift; CoR, Colima rift; ChR, Chapala rift; Rosas-Elguera et al., 2003). The purple region is the active volcanic landscape between 7.5 and 3 Ma (Ferrari et al., 2012), where interspecific gene flow was probably interrupted (see Discussion). (b) Discriminant analysis of principal components (DAPC) for *Abies religiosa* populations. Black lines represent a minimum spanning tree into two-dimensional space for illustrating genomic distance between populations. (c) Clustering of fir individuals as inferred with ADMIXTURE when assuming $K = 3$ and $K = 6$. Ancestries for *A. religiosa* were named as W (west), C (centre), E (east) and P (Perote), according to their geographical situation on the TMVB. Importantly, both W and C genetic groups are respectively placed within W and C contact zones, which are the capital letters used for these lineages and their distribution. The purple rectangle below the graph denotes admixed populations with C and E ancestries; these stands were grouped in the M cluster for analyses shown in Figures 2 and 5.

After excluding the highly inbred western populations (i.e., *A. jaliscana*, *A. flinckii* and W stands of *A. religiosa*), no genetic structure was observed with ADMIXTURE (Figure S9e). However, DAPC analyses did show a similar intraspecific genetic structure for *A. religiosa* to the one previously observed for the whole data set with ADMIXTURE, supporting both P and W clusters, and the genetic cline between E and C groups (Figure 4b).

3.4 | Inter- and intraspecific divergence

At the species level, the best-fitting model (according to likelihood and wAIC estimations; Table S6) was Hc, which consisted of a recent

species divergence that coincided with the uplift of tectonic valleys (Figure S4c). Similar results were obtained when using data sets with less missing data, and after representing *A. religiosa* by just the C or E genetic pools (Table S6); thus, population substructure or missing data should not be affecting model choice at this level (Table S6).

Model Hc was then used as a baseline for testing four more complex demographic hypotheses (Figure S5), and to address population divergence after range expansion within *A. religiosa*. According to AIC and wAIC, scenarios Hc9 and Hc12 were equally likely for explaining within-species genetic structure (Table S7). The former comprised an east–west stepwise colonization (i.e., Figure S5a), while the latter included the initial divergence of two gene pools (C and E), followed by their expansion and secondary contact (i.e., Figure S5d). These

two scenarios were retained to test for interspecific gene flow at different times.

3.5 | Incorporating gene flow and estimating parameters

The highest wAIC was obtained when ancient interspecific gene flow (i.e., shortly after species split) was incorporated into scenario Hc12 (two ancestral gene pools within *A. religiosa*; Figures 2 and 5; Table 1), which further refutes the stepwise colonization hypothesis. According to this scenario, the divergence between *A. religiosa* and the western firs occurred ~1.26 million years ago (Ma; 95% confidence interval [CI]: 1.25–1.27 Ma) and was followed by the split

of E and C genomic pools within *A. religiosa*, which was dated to ~1.06 Ma (95% CI: 1.03–1.08 Ma). The divergence between *A. flinckii* and *A. jaliscana* was estimated to ~0.402 Ma (95% CI: 0.37–0.43 Ma; Table 2; Figure 5). Within *A. religiosa*, the range expansion of E and C genetic pools, and the differentiation of W and P groups, would have taken place ~0.218 Ma (95% CI: 0.214–0.221 Ma; Table 2; Figure 5).

Inferred ancestral effective population sizes (N_e) were large in all cases, except for the ancestor of western firs (Table 2; Figure 5), which showed a severe decline shortly after divergence. Modern N_e (after accounting for inbreeding) for *A. flinckii* and *A. jaliscana* suggested a subtle recovery after that initial collapse. On the other hand, ancestral *A. religiosa* genetic pools had large N_e values, although estimates for modern lineages suggest recent population declines; such modern N_e values are all in the same order of magnitude as those
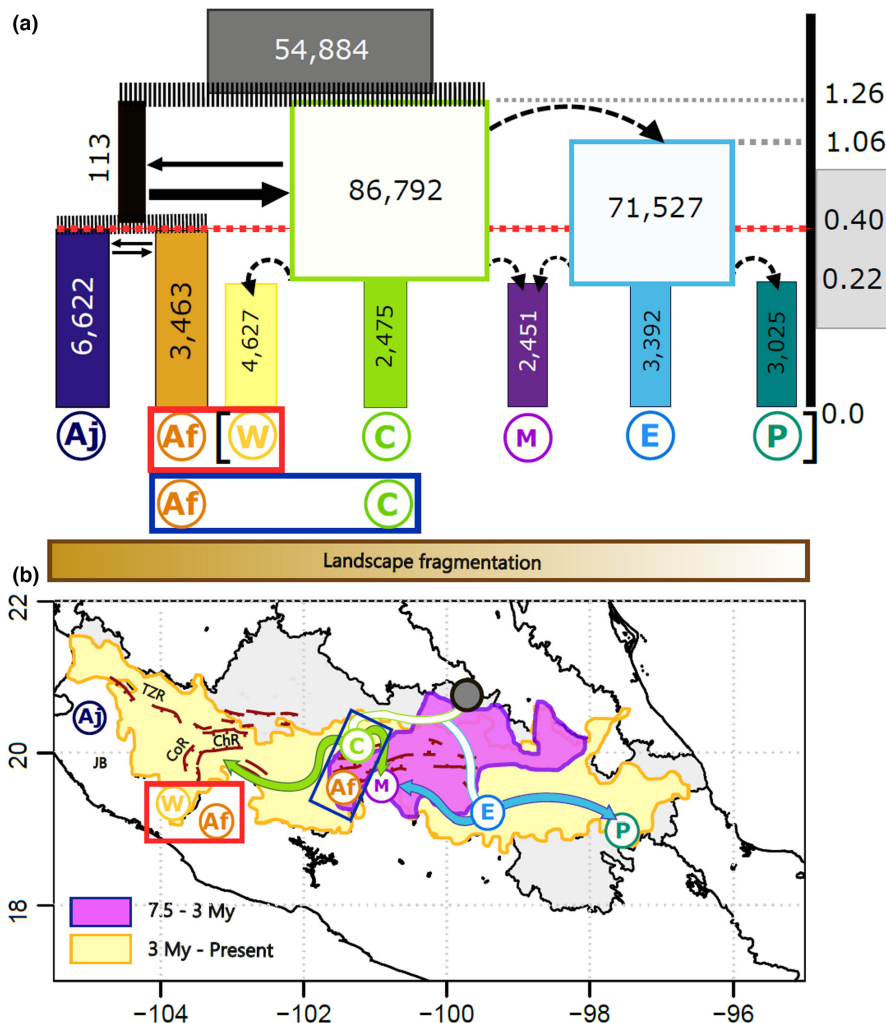
FIGURE 5 Graphical representation of the best-fitting divergence and demographic scenario for firs in Central Mexico. (a) Divergence (barcoded lines) and colonization (dotted rounded arrows) times (see timescale in million years on the right; mid-Pleistocene in grey), effective population sizes (N_e; proportional to rectangle size) and gene flow rate between lineages (proportional to continuous horizontal line weight). Red dotted line shows the proposed time for gene flow interruption between *Abies religiosa* and *A. flinckii* (see Discussion). Colours and lineages are the same as in Figure 4. Ancestral C and E populations are shown with green and blue outlined rectangles, respectively. *A. religiosa* lineages are enclosed in brackets. Tones of brown in the bottom horizontal bar correspond to a more fragmented volcanic landscape (dark), and a more continuous landscape (light). (b) Geographical illustration of events depicted in (a), including the putative location of source populations (circles) and dispersal routes (arrows). The two most recent magmatic arcs are represented as purple and yellow polygons (based on Ferrari et al., 2012). The main tectonic valleys (e.g., TZR, Tepic-Zoacalco rift; CoR, Colima rift; Chapala rift) are shown as dark lines (based on Rosas-Elguera et al., 2003). The approximate location of secondary contact zones is indicated by the red (W) and blue (C) rectangles.

TABLE 1 Likelihood-based model choice for 10 demographic scenarios of fir divergence in Central Mexico

Process	Migration	LogLikelihood	N_{Params}	Δlhood	AIC	ΔAIC	wAIC
2 pop sources	RIM	−809.999	24	116.361	3778.183	128.807	$1.07e^{-28}$
Serial colonization	A-RIM	−789.706	26	96.068	3688.730	39.355	$2.84e^{-09}$
Serial colonization	RIM	−788.964	22	95.326	3677.313	27.938	$8.56e^{-07}$
2 pop sources	A-RIM	−786.342	28	92.704	3677.239	27.863	$8.89e^{-07}$
2 pop sources	No	−789.946	16	96.308	3669.836	20.460	$3.60e^{-05}$
Serial colonization	No	−790.665	14	97.027	3669.147	19.771	$5.08e^{-05}$
Serial colonization	Ancient	−788.346	19	94.708	3668.467	19.092	$7.14e^{-05}$
2 pop sources	Recent	−782.682	32	89.044	3668.384	19.008	$7.44e^{-05}$
Serial colonization	Recent	−782.279	30	88.641	3662.528	13.152	0.00139107
2 pop sources	**Ancient**	**−783.766**	**20**	**90.128**	**3649.376**	**0**	**0.99837453**

Note: Models consisted of either a serial colonization from one source or a range shift from two source populations. Five migration patterns were further tested: no-migration (No), ancient gene flow (Ancient), recent gene flow (Recent), recent intraspecific migration within *Abies religiosa* (RIM) or ancient interspecific migration with RIM (A-RIM; see Figure 2; Figure S6). The best-fitting model based on Akaike weight (wAIC) is shown in bold.

TABLE 2 Estimated population parameters that maximize the likelihood function of the best-fitting demographic model for fir divergence in Central Mexico (Figure 5)

Parameter	Upper	Mean	Lower
A. jaliscana (J)	6998	6622	6246
A. flinckii (F)	3731	3463	3196
A. religiosa (W)	4864	4627	4390
A. religiosa (C)	3714	2475	1236
A. religiosa (M)	2527	2451	2374
A. religiosa (E)	3469	3392	3315
A. religiosa (P)	3091	3025	2958
Ancient-West	89,457	86,792	84,128
Ancient-East	77,715	71,527	65,339
F-J ancestral pop	125	119	112
Fir ancestral pop	59,243	54,884	50,525
Admixture	0.207	0.163	0.119
Tec	1.271	1.26	1.249
Or	1.082	1.056	1.03
Occ	0.43	0.402	0.373
Exp	0.221	0.218	0.214
$M_{F \leftarrow C}$[a]	$1.27e^{-04}$	$1.02e^{-04}$	$0.76e^{-04}$
$M_{C \leftarrow F}$[a]	$92.92e^{-04}$	$86.11e^{-04}$	$79.31e^{-04}$
$M_{J \leftarrow F}$[a]	$12.30e^{-04}$	$8.08e^{-04}$	$3.88e^{-04}$
$M_{F \leftarrow J}$[a]	$4.54e^{-04}$	$3.19e^{-04}$	$1.85e^{-04}$

Note: Upper and lower 95% confidence intervals estimated after computing parameters from 100 bootstrapped MSFSs. M, migration rate. Divergence times (Tec, Or, Occ, Exp) were transformed to million years by assuming a generation time of 60 years (Sánchez-Velásquez et al., 1991).

[a]Arrows indicate the direction of gene flow.

estimated for the western firs. Other than occurring early after species divergence, gene flow was apparently highly asymmetrical, with most migration taking place from western firs into *A. religiosa* (i.e., C genetic group). Gene flow was also higher from *A. flinckii* into *A. jaliscana* than in the opposite direction (Table 2; Figure 5).

The fitting of the best three demographic scenarios (Table 1) was assessed using the G-statistic test. The *p* values were nonsignificant for all cases, which indicated that all scenarios explained the observed genetic data relatively well (Figure S10). However, the mean of the CLR distribution for the ancient interspecific gene flow scenario was the closest to zero (i.e., CLR = 0 when CL_O and CL_E are the same), which suggests that the selected model still has the best performance (Figure S10).

4 | DISCUSSION

Disentangling the evolutionary processes underlying species differentiation and their drivers is a major challenge, especially for nonmodel systems such as conifers (Campbell et al., 2018). Here, we addressed such a challenge using a rapidly diverging fir species complex from central Mexico that exhibit dissimilar pollen phenologies exclusively at their contact zones. We compared patterns of genomic variation between these zones and allopatric regions, and inferred their past demography. Our best supported model suggests a rapid species differentiation, with gene flow occurring only shortly after divergence and only at the oldest of contact zones ("C contact"). This model further rejects the stepwise colonization of newly formed habitats, which suggests that the interruption of interspecific gene flow, probably mediated by changes in pollen phenology, occurred shortly after species divergence, and that it is not the outcome of more recent stochastic events.

4.1 | Rapid species differentiation and the evolution of inbreeding tolerance in western firs

Our approximation, with a larger number of markers and a more complete battery of analyses than in previous studies with Mexican firs (e.g., Aguirre-Planter et al., 2000; Jaramillo-Correa et al., 2008), revealed that these species are composed of well-defined genetic clusters with virtually no current gene flow, even at secondary contact

zones (Figures 4c and 5a). Such a structure coincides with the most recent taxonomic description of species (Figures 1 and 4c), including the separation of the westernmost populations, as *Abies jaliscana* (Vázquez-García et al., 2014). According to our most likely demographic scenario, these species diverged and probably attained some degree of reproductive isolation in a shorter period (~1.26 million years, Table 2; Figure 5a) than most conifer complexes, which still thoroughly interbreed at contact zones despite a much ancient divergence (i.e., ~20–5 Ma; Balao et al., 2020; Jin et al., 2021; Shen et al., 2019).

The TMVB currently bears isolated fir forests forming sky-islands, which should be promoting rapid allopatric divergence of species/populations (Mastretta-Yanes et al., 2015; Ramírez-Barahona & Eguiarte, 2013). The formation of this mountain chain started in the early Miocene and included the uplift of four main volcanic arcs (Ferrari et al., 2012). More recent topographic changes, mostly driven by the formation of large tectonic valleys since the late Pliocene, produced a highly fragmented landscape in its western portion (Rosas-Elguera et al., 2003; Figures 4a and 5b). Accordingly, fir populations from this region are smaller and much less dense than their central/eastern counterparts (e.g., 100–508 trees per ha in the west vs. over 3000 trees per ha in the east; Cuevas-Guzmán et al., 2011; Pineda-López et al., 2013). This is reflected in the SFS of minor alleles (Figure S7), Tajima's D (Table S5) and the long-term reduced N_e values estimated for all populations/species from this region (i.e., *A. jaliscana*, *A. flinckii* and the W lineage of *A. religiosa*; Table 2, Figure 5a); it further agrees with the limited historical pollen flow inferred herein, which only occurred soon after the western species split (Table 2, Figure 5a).

Inbreeding coefficients (*F*) revealed the same pattern. They were not only higher for western than for central and eastern populations (Figure 1), but also mirrored those observed for other conifers with restricted distributions such as *Larix lyallii* (Vance, 2019) and *Pseudotaxus chienii* (Liu et al., 2021). Such a contrasting distribution of inbreeding coefficients between western and eastern/central taxa remained unchanged when using data sets with other missing data thresholds (Material S2) and/or statistical approaches (Table S5), including popkin version 1.2.2, a method reputed to better reflect actual inbreeding than other method-of-moments estimators (Ochoa & Storey, 2021). However, even if the strict filters used herein did bias inbreeding estimates upwards (Material S2), high inbreeding and reduced genetic diversity are not new for western firs; they have been previously reported based on resequenced nuclear genes and simple sequence repteats (Cruz-Nicolás, Giles-Pérez, González-Linares, et al., 2020; Cruz-Nicolás, Giles-Pérez, Lira-Noriega, et al., 2020), plastid markers (Jaramillo-Correa et al., 2008) and even allozymes (Aguirre-Planter et al., 2000). These values point to an eventual transition from outcrossing to selfing in western firs, a mechanism recurrently reported in small conifer populations (Ledig et al., 1997; Robledo-Arnuncio et al., 2004), and often invoked as a driver of speciation in plants (Cutter, 2019; Hopkins, 2013; Wright et al., 2013).

Interestingly, the most likely demographic scenario indicates that interspecific gene flow ceased before secondary contact in the western region (i.e., before the divergence of the "W genetic pool";

Figure 5a). In other words, it suggests an allopatric speciation model, where inbreeding seems to be the result of the long-term isolation of small populations (e.g., Restoux et al., 2008; Robledo-Arnuncio et al., 2004), instead of a mechanism that drove species divergence (Figure 3b). The absence of a correlation between the geographical distance and the interspecific differentiation within this contact zone also points in this direction (Figure S9).

Increased selfing is expected to reduce fitness through inbreeding depression (Charlesworth & Charlesworth, 1987; Lynch et al., 1995). However, it also allows purging of highly deleterious variants in homozygous offspring (Williams & Savolainen, 1996). Conifers are reputed for their strong selective regimes at early life stages, particularly in small populations (e.g., Ferriol et al., 2011; Restoux et al., 2008; Sorensen, 2001); this has often led to inbreeding tolerance and allowed the long-term survival of extremely genetically pauperized taxa (e.g., *Pinus pinea*; Jaramillo-Correa et al., 2020). A previous analysis of 11 nuclear genes in the same Mexican firs studied herein suggests that western populations are indeed more efficient in purging highly deleterious alleles than their central–eastern counterparts (Cruz-Nicolás, Giles-Pérez, Lira-Noriega, et al., 2020). However, given that chronically small populations (like those in the west) are also prone to accumulate weakly deleterious variants at high frequencies, which diminishes adaptive potential (Lanfear et al., 2014; Ohta, 1992), future studies for correctly disentangling the distribution of fitness effects and estimating adaptive potential are necessary (e.g., Chen et al., 2021).

4.2 | Reproductive isolation evolved under a parapatric model at "C contact"

Our demographic simulations support that interspecific gene flow only occurred shortly after species divergence (i.e., Figure 5a), probably at the centre of the TMVB, an ancient and stable geological region where both lineages are currently distributed (i.e., the purple polygon in Figure 4a); it then ceased before *A. religiosa* expanded westwards (i.e., red pointed line, Figure 5a). Moreover, inbreeding estimates at "C contact" suggest that other processes than shift to self-fertilization are preventing interspecific gene flow. This is further supported by the rejection of all stepwise colonization scenarios (Table 1; Table S6), which together with the larger ancestral N_e of central populations, point again to a minor role of stochastic processes during interspecific gene flow interruption.

Why most closely related conifers have not developed effective reproductive barriers, while *A. religiosa*–*A. flinckii* apparently achieved it at such a rapid pace (~1.26 million years; Figure 5) is still an unanswered question. Reproductive isolation can quickly develop because of short-term genomic shuffling or rapid changes at mating traits, but this is apparently rare in conifers (e.g., Guo et al., 2009; Momigliano et al., 2017; Pavy et al., 2017). To our knowledge, only *Juniperus phoenicea*–*J. turbinata* share certain similarities with our study system, with phenological differences preventing interspecific mating at natural contact zones (Arista et al., 1997).

Under parapatric speciation, reduction of interspecific gene flow, and thus speciation, are mainly driven by selection (Coyne & Orr, 2004; Gavrilets, 2014; Smadja & Butlin, 2011). Identifying which pressure(s) underlie such changes in Mexican firs remains unexplored and hence can be an interesting avenue for future research. In the simplest scenario, mating traits, probably related to pollen shedding, may quickly evolve through epistatic/pleiotropic effects during ecological divergence (Hendry et al., 2007). However, such processes usually involve strong ecological differentiation between parental species and, although segregating by altitude, the firs studied herein do not have significantly different ecological niches (Cruz-Nicolás, Giles-Pérez, Lira-Noriega, et al., 2020; Martínez-Méndez et al., 2016).

Alternatively, interspecific gene flow may be selectively interrupted when hybrid offspring have lower fitness than parental taxa (Hopkins, 2013; Rahmé et al., 2009; Servedio & Noor, 2003). It is not possible to estimate the cost of hybridization with the available data for these Mexican firs, which would require controlled cross-pollination experiments and coding genomic information, but we could speculate on possible hypotheses to test in future studies. For instance, our results (Table 2, Figure 5a), and those of previous theoretical and empirical studies in these and other taxa, suggest that contrasting amounts of genetic load should be expected between species (Chen et al., 2017; Cruz-Nicolás, Giles-Pérez, Lira-Noriega, et al., 2020; Ohta, 1992). Such a load would be more easily exposed in recombinant hybrids (i.e., F_2, F_3, ... F_n) and backcrosses involving the parental species with the smallest N_e (i.e., *A. flinckii*) than in the opposite direction, which may result in reduced hybrid fitness (i.e., "hybrid load"; Moran et al., 2020; e.g., Christe et al., 2017; Fenster & Galloway, 2000; Hamilton et al., 2013; Moran et al., 2018), and explain the observed asymmetrical introgression from *A. flinckii* into *A. religiosa* (see also Pickup et al., 2019). Selection could also be favouring reproductive isolation to alleviate competition driven by interspecific pollen–style interactions (Rahmé et al., 2009; Rieseberg & Blackman, 2010). An example in conifers is that of *Pinus parviflora* var. *pentaphylla* and *P. pumila*, which are partly isolated by stronger cross-incompatibilities in the former taxa, which also results in asymmetrical gene flow because of differential fertilization success between species (Ito et al., 2008). It would be interesting to verify whether *A. flinckii* and *A. religiosa* also bear such incompatibilities.

4.3 | *Abies religiosa* and intraspecific diversification in tropical mountain systems

Other than rapid species divergence, our demographic models suggest that *A. religiosa* expanded from the centre of the TMVB towards both the east and the west during the late Pleistocene (~218,000 years ago, Figure 5b). Such an expansion corresponds to the last formation phase of the TMVB, which included uplift of the large stratovolcanoes (Ferrari et al., 2012) that now provide suitable habitats for fir forests (Rzedowski, 2006; Velázquez et al., 2000). Surprisingly, our most likely demographic scenario suggested a population decrease after that initial expansion for all modern *A. religiosa* lineages (Figure 5). However, lineage-specific SFSs of minor alleles and Tajima's *D* values pointed to population expansion in all cases, except for the W genomic group (Figure S7 and Table S5). Spurious population decreases can be inferred with coalescent approaches when demes within lineages/populations fit a *n-island* model with relatively limited gene flow (Chikhi et al., 2010), and it is likely that the sky-islands from central Mexico fit such a model (Mastretta-Yanes et al., 2018; Uscanga et al., 2021). Indeed, the observed genetic structure within *A. religiosa* (Figure 4b,c) might be the result of limited gene flow during the late Pleistocene (Mastretta-Yanes et al., 2015; Ramírez-Barahona & Eguiarte, 2013), although palynological data and niche projections into the Last Glacial Maximum suggest that gene flow among lineages was likely (Caballero-Miranda et al., 2010; Cruz-Nicolás, Giles-Pérez, Lira-Noriega, et al., 2020). A phylogeographical study at finer geographical scale is thus necessary to explore the historical demography of this species in more detail.

Finally, it would be worth testing if the main role of geological and topographic features in promoting rapid genetic divergence also applies for other subtropical and tropical conifers. Indeed, the possibility that interspecific gene flow can quickly cease between species prone to long-distance dispersal and with reputed permeable reproductive barriers, such as conifers, could explain their large species diversity in subtropical and tropical regions of the world (Gernandt & Pérez-de la Rosa, 2014; Rahbek et al., 2019; e.g., Moreno-Letelier et al., 2014; Peláez et al., 2020).

CONFLICT OF INTEREST
The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT
Demultiplexed sequencing data, including those samples previously analyzed in a phylogenetic survey (i.e., 10 samples, Cruz-Nicolas et al., 2021), were deposited in NCBI with the Bioproject ID: PRJNA856692; while filtered variant files used for population genomic analyses, code and pipelines are hosted on Dryad Repository at https://doi.org/10.5061/dryad.0rxwdbs3h and on GitHub at https://github.com/TavoIbrahim/GBS_Abies. Detailed SNP calling procedure can be found in the Supplemental material.

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REFERENCES
Aguirre-Planter, E., Furnier, G. R., & Eguiarte, L. E. (2000). Low levels of genetic variation within and high levels of genetic differentiation among populations of species of *Abies* from southern Mexico and Guatemala. *American Journal of Botany*, 87(3), 362–371. https://doi.org/10.2307/2656632

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, 19(9), 1655–1664. https://doi.org/10.1101/gr.094052.109

Arista, M., Ortiz, P. L., & Talavera, S. (1997). Reproductive isolation of two sympatric subspecies of *Juniperus phoenicea* (Cupressaceae) in southern Spain. *Plant Systematics and Evolution*, 208(3), 225–237. https://doi.org/10.1007/BF00985443

Balao, F., Lorenzo, M. T., Sánchez-Robles, J. M., Paun, O., García-Castaño, J. L., & Terrab, A. (2020). Early diversification and permeable species boundaries in the Mediterranean firs. *Annals of Botany*, 125(3), 495–507. https://doi.org/10.1093/aob/mcz186

Blischak, P. D., Barker, M. S., & Gutenkunst, R. N. (2020). Inferring the demographic history of inbred species from genome-wide SNP frequency data. *Molecular Biology and Evolution*, 37(7), 2124–2136. https://doi.org/10.1093/molbev/msaa042

Bolnick, D. I., & Fitzpatrick, B. M. (2007). Sympatric speciation: Models and empirical evidence. *Annual Review of Ecology, Evolution, and Systematics*, 38, 459–487. https://doi.org/10.1146/annurev.ecolsys.38.091206.095804

Boudhrioua, C., Bastien, M., Légaré, G., Pomerleau, S., St-Cyr, J., Boyle, B., & Belzile, F. (2017). Genotyping-by-sequencing in potato. In S. Kumar Chakrabarti, C. Xie, & J. Kumar Tiwari (Eds.), *The potato genome. Compendium of plant genomes* (pp. 283–296). Springer International Publishing. https://doi.org/10.1007/978-3-319-66135-3_15

Burnham, K., & Anderson, D. (2002). *Model selection and multi-model inference. A practical information-theoretic approach* (2nd ed.). Springer-Verlag.

Busch, J. W., Joly, S., & Schoen, D. J. (2011). Demographic signatures accompanying the evolution of selfing in *Leavenworthia alabamica*. *Molecular Biology and Evolution*, 28(5), 1717–1729. https://doi.org/10.1093/molbev/msq352

Caballero-Miranda, M., Lozano-García, S., Vázquez-Selem, L., & Ortega, B. (2010). Evidencias de cambio climático y ambiental en registros glaciales y en cuencas lacustres del centro de México durante el Último Máximo Glacial. *Boletín de la Sociedad Geológica Mexicana*, 62(3), 359–377.

Campbell, C. R., Poelstra, J. W., & Yoder, A. D. (2018). What is speciation genomics? The roles of ecology, gene flow, and genomic architecture in the formation of species. *Biological Journal of the Linnean Society*, 124(4), 561–583. https://doi.org/10.1093/biolinnean/bly063

Charlesworth, D., & Charlesworth, B. (1987). Inbreeding depression and its evolutionary consequences. *Annual Review of Ecology and Systematics*, 18(1), 237–268. https://doi.org/10.1146/annurev.es.18.110187.001321

Charlesworth, D., & Pannell, J. R. (2001). Mating systems and population genetic structure in the light of coalescent theory. In J. Silvertown & J. Antonovics (Eds.), *Integrating ecology and evolution in a spatial context* (pp. 73–95). British Ecological Society.

Chen, J., Bataillon, T., Glémin, S., & Lascoux, M. (2021). What does the distribution of fitness effects of new mutations reflect? Insights from plants. *New Phytologist*, 233, 1613–1619. https://doi.org/10.1111/nph.17826

Chen, J., Glémin, S., & Lascoux, M. (2017). Genetic diversity and the efficacy of purifying selection across plant and animal species. *Molecular Biology and Evolution*, 34(6), 1417–1428. https://doi.org/10.1093/molbev/msx088

Chikhi, L., Sousa, V. C., Luisi, P., Goossens, B., & Beaumont, M. A. (2010). The confounding effects of population structure, genetic diversity and the sampling scheme on the detection and quantification of population size changes. *Genetics*, 186(3), 983–995. https://doi.org/10.1534/genetics.110.118661

Christe, C., Stölting, K. N., Paris, M., Fraïsse, C., Bierne, N., & Lexer, C. (2017). Adaptive evolution and segregating load contribute to the genomic landscape of divergence in two tree species connected by episodic gene flow. *Molecular Ecology*, 26(1), 59–76. https://doi.org/10.1111/mec.13765

Coyne, J. A., & Orr, H. A. (2004). *Speciation*. Sinauer Associates.

Cruz-Nicolás, J., Giles-Pérez, G., González-Linares, E., Múgica-Gallart, J., Lira-Noriega, A., Gernandt, D. S., Eguiarte, L. E., & Jaramillo-Correa, J. P. (2020). Contrasting evolutionary processes drive morphological and genetic differentiation in a subtropical fir (*Abies*, Pinaceae) species complex. *Botanical Journal of the Linnean Society*, 192(2), 401–420. https://doi.org/10.1093/botlinnean/boz077

Cruz-Nicolás, J., Giles-Pérez, G. I., Lira-Noriega, A., Martínez-Méndez, N., Aguirre-Planter, E., Eguiarte, L. E., & Jaramillo-Correa, J. P. (2020). Using niche centrality within the scope of the nearly neutral theory of evolution to predict genetic diversity in a tropical conifer species-pair. *Journal of Biogeography*, 47(12), 2755–2772. https://doi.org/10.1111/jbi.13979

Cruz-Nicolás, J., Villarruel-Arroyo, A., Gernandt, D. S., Fonseca, R. M., Aguirre-Planter, E., Eguiarte, L. E., & Jaramillo-Correa, J. P. (2021). Non-adaptive evolutionary processes governed the diversification of a temperate conifer lineage after its migration into the tropics. *Molecular Phylogenetics and Evolution*, 160, 107125. https://doi.org/10.1016/j.ympev.2021.107125

Cuevas-Guzmán, R., Cisneros-Lepe, E. A., Jardel-Peláez, E. J., Sánchez-Rodríguez, E. V., Guzmán-Hernández, L., Núñez-López, N. M., & Rodríguez-Guerrero, C. (2011). Análisis estructural y de diversidad en los bosques de *Abies* de Jalisco, México. *Revista Mexicana de Biodiversidad*, 82(4), 1219–1233.

Cutter, A. D. (2019). Reproductive transitions in plants and animals: Selfing syndrome, sexual selection and speciation. *New Phytologist*, *224*(3), 1080–1094. https://doi.org/10.1111/nph.16075

Danecek, P., Auton, A., Abecasis, G., Albers, C. A., Banks, E., DePristo, M. A., Handsaker, R. E., Lunter, G., Marth, G. T., Sherry, S. T., McVean, G., Durbin, R., & 1000 Genomes Project Analysis Group. (2011). The variant call format and VCFtools. *Bioinformatics*, *27*(15), 2156–2158. https://doi.org/10.1093/bioinformatics/btr330

Dobzhansky, T. (1940). Speciation as a stage in evolutionary divergence. *The American Naturalist*, *74*(753), 312–321. https://doi.org/10.1086/280899

Dray, S., Dufour, A.-B., & Thioulouse, J. (2018). Package "ade4". https://doi.org/10.18637/jss.v022.i04

Eaton, D. A., & Overcast, I. (2020). Ipyrad: Interactive assembly and analysis of RADseq datasets. *Bioinformatics*, *36*(8), 2592–2594. https://doi.org/10.1093/bioinformatics/btz966

Eckenwalder, J. E. (2009). *Conifers of the world: The complete reference*. Timber Press.

Excoffier, L., Dupanloup, I., Huerta-Sánchez, E., Sousa, V. C., & Foll, M. (2013). Robust demographic inference from genomic and SNP data. *PLoS Genetics*, *9*(10), e1003905. https://doi.org/10.1371/journal.pgen.1003905

Farjon, A., & Rushforth, K. D. (1989). A classification of *Abies* Miller (Pinaceae). *Notes from the Royal Botanic Garden, Edinburgh*, *46*(1), 59–79.

Feder, J. L., Flaxman, S. M., Egan, S. P., Comeault, A. A., & Nosil, P. (2013). Geographic mode of speciation and genomic divergence. *Annual Review of Ecology, Evolution and Systematics*, *44*, 73–97. https://doi.org/10.1146/annurev-ecolsys-110512-135825

Fenster, C. B., & Galloway, L. F. (2000). Population differentiation in an annual legume: Genetic architecture. *Evolution*, *54*(4), 1157–1172. https://doi.org/10.1111/j.0014-3820.2000.tb00551.x

Ferrari, L., Orozco-Esquivel, T., Manea, V., & Manea, M. (2012). The dynamic history of the Trans-Mexican Volcanic Belt and the Mexico subduction zone. *Tectonophysics*, *522*, 122–149. https://doi.org/10.1016/j.tecto.2011.09.018

Ferriol, M., Pichot, C., & Lefevre, F. (2011). Variation of selfing rate and inbreeding depression among individuals and across generations within an admixed *Cedrus* population. *Heredity*, *106*(1), 146–157. https://doi.org/10.1038/hdy.2010.45

Fox, G. A. (2003). Assortative mating and plant phenology: Evolutionary and practical consequences. *Evolutionary Ecology Research*, *5*(1), 1–18.

Garner, A. G., Goulet, B. E., Farnitano, M. C., Molina-Henao, Y. F., & Hopkins, R. (2018). Genomic signatures of reinforcement. *Genes*, *9*(4), 191. https://doi.org/10.3390/genes9040191

Gavrilets, S. (2003). Perspective: Models of speciation: What have we learned in 40 years? *Evolution*, *57*(10), 2197–2215. https://doi.org/10.1111/j.0014-3820.2003.tb00233.x

Gavrilets, S. (2014). Models of speciation: Where are we now? *Journal of Heredity*, *105*(S1), 743–755. https://doi.org/10.1093/jhered/esu045

Gernandt, D. S., & Pérez-de la Rosa, J. A. (2014). Biodiversity of Pinophyta (conifers) in Mexico. *Revista Mexicana de Biodiversidad*, *85*, S126–S133. https://doi.org/10.7550/rmb.32195

Grossenbacher, D. L., & Whittall, J. B. (2011). Increased floral divergence in sympatric monkeyflowers. *Evolution: International Journal of Organic Evolution*, *65*(9), 2712–2718. https://doi.org/10.1111/j.1558-5646.2011.01306.x

Guo, Y. L., Bechsgaard, J. S., Slotte, T., Neuffer, B., Lascoux, M., Weigel, D., & Schierup, M. H. (2009). Recent speciation of *Capsella rubella* from *Capsella grandiflora*, associated with loss of self-incompatibility and an extreme bottleneck. *Proceedings of the National Academy of Sciences*, *106*(13), 5246–5251. https://doi.org/10.1073/pnas.0808012106

Hamilton, J. A., Lexer, C., & Aitken, S. N. (2013). Genomic and phenotypic architecture of a spruce hybrid zone (*Picea sitchensis* x *P. glauca*). *Molecular Ecology*, *22*(3), 827–841. https://doi.org/10.1111/mec.12007

Hamrick, J. L., & Godt, M. W. (1996). Effects of life history traits on genetic diversity in plant species. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *351*(1345), 1291–1298. https://doi.org/10.1098/rstb.1996.0112

Harvey, M. G., Singhal, S., & Rabosky, D. L. (2019). Beyond reproductive isolation: Demographic controls on the speciation process. *Annual Review of Ecology, Evolution, and Systematics*, *50*, 75–95. https://doi.org/10.1146/annurev-ecolsys-110218-024701

Hendry, A. P., Nosil, P., & Rieseberg, L. H. (2007). The speed of ecological speciation. *Functional Ecology*, *21*(3), 455–464. https://doi.org/10.1111/j.1365-2435.2007.01240.x

Hopkins, R. (2013). Reinforcement in plants. *New Phytologist*, *197*(4), 1095–1103. https://doi.org/10.1111/nph.12119

Howe, G. T., Aitken, S. N., Neale, D. B., Jermstad, K. D., Wheeler, N. C., & Chen, T. H. (2003). From genotype to phenotype: Unraveling the complexities of cold adaptation in forest trees. *Canadian Journal of Botany*, *81*(12), 1247–1266. https://doi.org/10.1139/b03-141

Hudson, R. R., Slatkin, M., & Maddison, W. P. (1992). Estimation of levels of gene flow from DNA sequence data. *Genetics*, *132*(2), 583–589.

Ito, M., Suyama, Y., Ohsawa, T. A., & Watano, Y. (2008). Airborne-pollen pool and mating pattern in a hybrid zone between *Pinus pumila* and *P. parviflora* var. *pentaphylla*. *Molecular Ecology*, *17*(23), 5092–5103. https://doi.org/10.1111/j.1365-294X.2008.03966.x

Jaramillo-Correa, J. P., Aguirre-Planter, E., Khasa, D. P., Eguiarte, L. E., Pinero, D., Furnier, G. R., & Bousquet, J. (2008). Ancestry and divergence of subtropical montane forest isolates: Molecular biogeography of the genus *Abies* (Pinaceae) in southern Mexico and Guatemala. *Molecular Ecology*, *17*(10), 2476–2490. https://doi.org/10.1111/j.1365-294X.2008.03762.x

Jaramillo-Correa, J. P., Bagnoli, F., Grivet, D., Fady, B., Aravanopoulos, F. A., Vendramin, G. G., & González-Martínez, S. C. (2020). Evolutionary rate and genetic load in an emblematic Mediterranean tree following an ancient and prolonged population collapse. *Molecular Ecology*, *29*(24), 4797–4811. https://doi.org/10.1111/mec.15684

Jin, W. T., Gernandt, D. S., Wehenkel, C., Xia, X. M., Wei, X. X., & Wang, X. Q. (2021). Phylogenomic and ecological analyses reveal the spatiotemporal evolution of global pines. *Proceedings of the National Academy of Sciences*, *118*(20), e2022302118. https://doi.org/10.1073/pnas.2022302118

Jombart, T. (2008). Adegenet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, *24*(11), 1403–1405. https://doi.org/10.1093/bioinformatics/btn129

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: A new method for the analysis of genetically structured populations. *BMC Genetics*, *11*, 94. https://doi.org/10.1186/1471-2156-11-94

Korunes, K. L., & Samuk, K. (2021). Pixy: Unbiased estimation of nucleotide diversity and divergence in the presence of missing data. *Molecular Ecology Resources*, *21*(4), 1359–1368. https://doi.org/10.1111/1755-0998.13326

Lanfear, R., Kokko, H., & Eyre-Walker, A. (2014). Population size and the rate of evolution. *Trends in Ecology & Evolution*, *29*(1), 33–41. https://doi.org/10.1016/j.tree.2013.09.009

Ledig, F. T., Jacob-Cervantes, V., Hodgskiss, P. D., & Eguiluz-Piedra, T. (1997). Recent evolution and divergence among populations of a rare Mexican endemic, Chihuahua spruce, following Holocene climatic warming. *Evolution*, *51*(6), 1815–1827. https://doi.org/10.1111/j.1558-5646.1997.tb05105.x

Liu, L., Wang, Z., Su, Y., & Wang, T. (2021). Population transcriptomic sequencing reveals allopatric divergence and local adaptation in

Pseudotaxus chienii (Taxaceae). *BMC Genomics*, *22*(1), 1–21. https://doi.org/10.1186/s12864-021-07682-3

Lynch, M., Conery, J., & Burger, R. (1995). Mutation accumulation and the extinction of small populations. *The American Naturalist*, *146*(4), 489–518. https://doi.org/10.1086/285812

Ma, J. J., Liu, S. W., Han, F. X., Li, W., Li, Y., & Niu, S. H. (2020). Comparative transcriptome analyses reveal two distinct transcriptional modules associated with pollen shedding time in pine. *BMC Genomics*, *21*(1), 1–14. https://doi.org/10.1186/s12864-020-06880-9

Martínez-Méndez, N., Aguirre-Planter, E., Eguiarte, L. E., & Jaramillo-Correa, J. P. (2016). Modelado de nicho ecológico de las especies del género *Abies* (Pinaceae) en México: Algunas implicaciones taxonómicas y para la conservación. *Botanical Sciences*, *94*(1), 5–24. https://doi.org/10.17129/botsci.508

Mastretta-Yanes, A., Moreno-Letelier, A., Piñero, D., Jorgensen, T. H., & Emerson, B. C. (2015). Biodiversity in the Mexican highlands and the interaction of geology, geography and climate within the Trans-Mexican Volcanic Belt. *Journal of Biogeography*, *42*(9), 1586–1600. https://doi.org/10.1111/jbi.12546

Mastretta-Yanes, A., Xue, A. T., Moreno-Letelier, A., Jorgensen, T. H., Alvarez, N., Pinero, D., & Emerson, B. C. (2018). Long-term in situ persistence of biodiversity in tropical sky islands revealed by landscape genomics. *Molecular Ecology*, *27*(2), 432–448. https://doi.org/10.1111/mec.14461

Mayr, E. (1963). *Animal species and evolution*. The Belknap Press of Harvard University Press.

Méndez-González, I. D., Jardón-Barbolla, L., & Jaramillo-Correa, J. P. (2017). Differential landscape effects on the fine-scale genetic structure of populations of a montane conifer from Central Mexico. *Tree Genetics & Genomes*, *13*(1), 30. https://doi.org/10.1007/s11295-017-1112-5

Momigliano, P., Jokinen, H., Fraimout, A., Florin, A. B., Norkko, A., & Merilä, J. (2017). Extraordinarily rapid speciation in a marine fish. *Proceedings of the National Academy of Sciences*, *114*(23), 6074–6079. https://doi.org/10.1073/pnas.1615109114

Moran, B. M., Payne, C., Langdon, Q., Powell, D. L., Brandvain, Y., & Schumer, M. (2020). *The genetic consequences of hybridization*. *arXiv:2012.04077*.

Moran, R. L., Zhou, M., Catchen, J. M., & Fuller, R. C. (2018). Hybridization and postzygotic isolation promote reinforcement of male mating preferences in a diverse group of fishes with traditional sex roles. *Ecology and Evolution*, *8*(18), 9282–9294. https://doi.org/10.1002/ece3.4434

Moreno-Letelier, A., Mastretta-Yanes, A., & Barraclough, T. G. (2014). Late Miocene lineage divergence and ecological differentiation of rare endemic *Juniperus blancoi*: Clues for the diversification of north American conifers. *New Phytologist*, *203*(1), 335–347. https://doi.org/10.1111/nph.12761

Mosca, E., Cruz, F., Gómez-Garrido, J., Bianco, L., Rellstab, C., Brodbeck, S., Csilléry, K., Fady, B., Fladung, M., Fussi, B., Gömöry, D., González-Martínez, S. C., Grivet, D., Gut, M., Hansen, O. K., Heer, K., Kaya, Z., Krutovsk, K. V., & Neale, D. B. (2019). A reference genome sequence for the European silver fir (*Abies alba* Mill.): A community-generated genomic resource. *G3: Genes, Genomes Genetics*, *9*(7), 2039–2049. https://doi.org/10.1534/g3.119.400083

Ochoa, A., & Storey, J. D. (2021). Estimating F_{ST} and kinship for arbitrary population structures. *PLoS Genetics*, *17*(1), e1009241. https://doi.org/10.1371/journal.pgen.1009241

Ohta, T. (1992). The nearly neutral theory of molecular evolution. *Annual Review of Ecology and Systematics*, *23*(1), 263–286. https://doi.org/10.1146/annurev.es.23.110192.001403

Overcast, I. (2019). *easySFS [Bioinformatic tool]*. https://github.com/isaacovercast/easySFS

Pannell, J. R. (2015). Evolution of the mating system in colonizing plants. *Molecular Ecology*, *24*(9), 2018–2037. https://doi.org/10.1111/mec.13087

Pavy, N., Lamothe, M., Pelgas, B., Gagnon, F., Birol, I., Bohlmann, J., Mackay, J., Isabel, N., & Bousquet, J. (2017). A high-resolution reference genetic map positioning 8.8 K genes for the conifer white spruce: Structural genomics implications and correspondence with physical distance. *The Plant Journal*, *90*(1), 189–203. https://doi.org/10.1111/tpj.13478

Peláez, P., Ortiz-Martínez, A., Figueroa-Corona, L., Montes, J. R., & Gernandt, D. S. (2020). Population structure, diversifying selection, and local adaptation in *Pinus patula*. *American Journal of Botany*, *107*(11), 1555–1566. https://doi.org/10.1002/ajb2.1566

Pfeifer, B., Wittelsbürger, U., Ramos-Onsins, S. E., & Lercher, M. J. (2014). PopGenome: An efficient swiss army knife for population genomic analyses in R. *Molecular Biology and Evolution*, *31*(7), 1929–1936. https://doi.org/10.1093/molbev/msu136

Pickup, M., Brandvain, Y., Fraïsse, C., Yakimowski, S., Barton, N. H., Dixit, T., Lexer, C., Cereghetti, E., & Field, D. L. (2019). Mating system variation in hybrid zones: Facilitation, barriers and asymmetries to gene flow. *New Phytologist*, *224*(3), 1035–1047. https://doi.org/10.1111/nph.16180

Pineda-López, M. D. R., Ortega-Solis, R., Sánchez-Velásquez, L. R., Ortiz-Ceballos, G., & Vázquez-Domínguez, G. (2013). Estructura poblacional de *Abies religiosa* (Kunth) Schltdl. et Cham., En el Ejido el Conejo del Parque Nacional Cofre de Perote, Veracruz, México. *Revista Chapingo Serie Ciencias Forestales y del Ambiente*, *19*(3), 375–385. https://doi.org/10.5154/r.rchscfa.2012.11.058

Pinho, C., & Hey, J. (2010). Divergence with gene flow: Models and data. *Annual Review of Ecology, Evolution, and Systematics*, *41*, 215–230. https://doi.org/10.1146/annurev-ecolsys-102209-144644

Poland, J. A., Brown, P. J., Sorrells, M. E., & Jannink, J. L. (2012). Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. *PLoS One*, *7*(2), e32253. https://doi.org/10.1371/journal.pone.0032253

Rahbek, C., Borregaard, M. K., Colwell, R. K., Dalsgaard, B. O., Holt, B. G., Morueta-Holme, N., Nogues-Bravo, D., Whittaker, R. J., & Fjeldså, J. (2019). Humboldt's enigma: What causes global patterns of mountain biodiversity? *Science*, *365*(6458), 1108–1113. https://doi.org/10.1126/science.aax0149

Rahmé, J., Widmer, A., & Karrenberg, S. (2009). Pollen competition as an asymmetric reproductive barrier between two closely related *Silene* species. *Journal of Evolutionary Biology*, *22*(9), 1937–1943. https://doi.org/10.1111/j.1420-9101.2009.01803.x

Ramírez-Barahona, S., & Eguiarte, L. E. (2013). The role of glacial cycles in promoting genetic diversity in the neotropics: The case of cloud forests during the last glacial maximum. *Ecology and Evolution*, *3*(3), 725–738. https://doi.org/10.1002/ece3.483

Ravinet, M., Faria, R., Butlin, R. K., Galindo, J., Bierne, N., Rafajlović, M., Noor, M. A. F., Mehlig, B., & Westram, A. M. (2017). Interpreting the genomic landscape of speciation: A road map for finding barriers to gene flow. *Journal of Evolutionary Biology*, *30*(8), 1450–1477. https://doi.org/10.1111/jeb.13047

Restoux, G., Silva, D. E., Sagnard, F., Torre, F., Klein, E., & Fady, B. (2008). Life at the margin: The mating system of Mediterranean conifers. *Web Ecology*, *8*(1), 94–102. https://doi.org/10.5194/we-8-94-2008

Rieseberg, L. H., & Blackman, B. K. (2010). Speciation genes in plants. *Annals of Botany*, *106*(3), 439–455. https://doi.org/10.1093/aob/mcq126

Robledo-Arnuncio, J. J., Alia, R., & Gil, L. (2004). Increased selfing and correlated paternity in a small population of a predominantly outcrossing conifer, *Pinus sylvestris*. *Molecular Ecology*, *13*(9), 2567–2577. https://doi.org/10.1111/j.1365-294X.2004.02251.x

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: A versatile open-source tool for metagenomics. *PeerJ*, *4*, e2584. https://doi.org/10.7717/peerj.2584

Rosas-Elguera, J., Alva-Valdivia, L. M., Goguitchaichvili, A., Urrutia-Fucugauchi, J., Ortega-Rivera, M. A., Prieto, J. C. S., & Lee, J. K.

(2003). Counterclockwise rotation of the Michoacan block: Implications for the tectonics of western Mexico. *International Geology Review*, 45(9), 814–826. https://doi.org/10.2747/0020-6814.45.9.814

Rosser, N., Queste, L. M., Cama, B., Edelman, N. B., Mann, F., Mori Pezo, R., Morris, J., Segami, C., Velado, P., Schulz, S., Mallet, J. L. B., & Dasmahapatra, K. K. (2019). Geographic contrasts between pre-and postzygotic barriers are consistent with reinforcement in *Heliconius* butterflies. *Evolution*, 73(9), 1821–1838. https://doi.org/10.1111/evo.13804

Roux, C., Mullon, C., Neuenschwander, S., & Pannell, J. R. (2020). Sex allocation, inbreeding, and measures of population differentiation in hermaphroditic metapopulations. *bioRxiv*. https://doi.org/10.1101/2020.05.08.080929

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J. C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S. E., & Sánchez-Gracia, A. (2017). DnaSP 6: DNA sequence polymorphism analysis of large datasets. *Molecular Biology and Evolution*, 34, 3299–3302. https://doi.org/10.1093/molbev/msx248

Rzedowski, J. (2006). *Vegetación de México* [1a Edición digital, Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. México]. https://www.biodiversidad.gob.mx/publicaciones/librosDig/pdf/VegetacionMx_Cont.pdf

Sánchez-Velásquez, L. R., Pineda-López, M. D. R., & Hernández-Martínez, A. (1991). Distribución y estructura de la población de *Abies religiosa* (HBK) Schl. et Cham., en el Cofre de Perote, Estado de Veracruz, México. *Acta Botánica Mexicana*, 16, 45–55. https://doi.org/10.21829/abm16.1991.625

Servedio, M. R., & Noor, M. A. (2003). The role of reinforcement in speciation: Theory and data. *Annual Review of Ecology, Evolution, and Systematics*, 34(1), 339–364. https://doi.org/10.1146/annurev.ecolsys.34.011802.132412

Shalisko, V. (2014). *Morfometría y rasgos estructurales de las especies de oyamel en el Occidente de México*. Presented at the Foro para el conocimiento y conservación de los oyametales del occidente de México. Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara. Event information. https://www.udg.mx/es/convocatorias/foro-para-el-conocimiento-y-conservacion-de-los-oyametales-del-occidente-de-mexico

Shen, T. T., Ran, J. H., & Wang, X. Q. (2019). Phylogenomics disentangles the evolutionary history of spruces (*Picea*) in the Qinghai-Tibetan plateau: Implications for the design of population genetic studies and species delimitation of conifers. *Molecular Phylogenetics and Evolution*, 141, 106612. https://doi.org/10.1016/j.ympev.2019.106612

Smadja, C. M., & Butlin, R. K. (2011). A framework for comparing processes of speciation in the presence of gene flow. *Molecular Ecology*, 20(24), 5123–5140. https://doi.org/10.1111/j.1365-294.2011.05350.x

Sorensen, F. C. (2001). Effect of population outcrossing rate on inbreeding depression in *Pinus contorta* var. *murrayana* seedlings. *Scandinavian Journal of Forest Research*, 16(5), 391–403. https://doi.org/10.1080/02827580152632784

Tajima, F. (1983). Evolutionary relationship of DNA sequences in finite populations. *Genetics*, 105(2), 473–460.

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics*, 123(3), 585–595.

Thibert-Plante, X., & Gavrilets, S. (2013). Evolution of mate choice and the so-called magic traits in ecological speciation. *Ecology Letters*, 16(8), 1004–1013. https://doi.org/10.1111/ele.12131

Uscanga, A., López, H., Piñero, D., Emerson, B. C., & Mastretta-Yanes, A. (2021). Evaluating species origins within tropical sky-islands arthropod communities. *Journal of Biogeography*, 48(9), 2199–2210. https://doi.org/10.1111/jbi.14144

Vance, M. (2019). Population genomics of a timberline conifer, subalpine larch (*Larix lyallii* Parl.), Canada (PhD thesis), University of Victoria, Canada. https://dspace.library.uvic.ca/handle/1828/11433

Vázquez-García, J. A., Shalisko, V., Cuevas-Guzmán, R., Muñiz-Castro, M. A., & Mantilla-Blandón, M. R. (2014). *Abies jaliscana* (Pinaceae): A new combination in section Grandis and a key to the species of *Abies* in western Mexico. *Phytotaxa*, 183(1), 27–36. https://doi.org/10.11646/phytotaxa.183.1.3

Vázquez-Lobo, A. (1996). *Filogenia de hongos endófitos del género Pinus L: implementación de técnicas moleculares y resultados preliminares (BSc thesis). Facultad de Ciencias*, Universidad Nacional Autónoma de México, México. Universidad Nacional Autónoma de Méxic. https://tesiunam.dgb.unam.mx/F?func=find-b-0&local_base=TES01

Velázquez, A., Toledo, V. M., & Luna, I. (2000). Mexican temperate vegetation. In M. G. Barbour & W. D. Billings (Eds.), *North American terrestrial vegetation* (2nd ed., pp. 573–592). Cambridge University Press.

Vitasse, Y., Delzon, S., Bresson, C. C., Michalet, R., & Kremer, A. (2009). Altitudinal differentiation in growth and phenology among populations of temperate-zone tree species growing in a common garden. *Canadian Journal of Forest Research*, 39(7), 1259–1269. https://doi.org/10.1139/X09-054

Watterson, G. A. (1975). On the number of segregating sites in genetical models without recombination. *Theoretical Population Biology*, 7(2), 256–276.

Wells, C. P., Lavretsky, P., Sorenson, M. D., Peters, J. L., DaCosta, J. M., Turnbull, S., Uyehara, K. J., Malachowski, C. P., Dugger, B. D., Eadie, J. M., & Engilis, A., Jr. (2019). Persistence of an endangered native duck, feral mallards, and multiple hybrid swarms across the main Hawaiian islands. *Molecular Ecology*, 28(24), 5203–5216. https://doi.org/10.1111/mec.15286

Williams, C. G., & Savolainen, O. (1996). Inbreeding depression in conifers: Implications for breeding strategy. *Forest Science*, 42(1), 102–117. https://doi.org/10.1093/forestscience/42.1.102

Wright, S. I., Kalisz, S., & Slotte, T. (2013). Evolutionary consequences of self-fertilization in plants. *Proceedings of the Royal Society B: Biological Sciences*, 280(1760), 20130133. https://doi.org/10.1098/rspb.2013.0133

Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics*, 28(24), 3326–3328. https://doi.org/10.1093/bioinformatics/bts606

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