UNIVERSIDAD JUÁREZ DEL ESTADO DE DURANGO

UJED
Universidad Juárez
del Estado de Durango

PROGRAMA INSTITUCIONAL DE DOCTORADO EN CIENCIAS AGROPECUARIAS Y FORESTALES

Demografía, reproducción y diversidad genética de *Picea martinezii* Patterson y *Picea mexicana* Martínez, en un contexto de cambio climático

Tesis que presenta:

EDUARDO MENDOZA MAYA

Como requisito parcial para obtener el grado de

DOCTOR EN CIENCIAS AGROPECUARIAS Y FORESTALES

OPCIÓN TERMINAL: MANEJO, APROVECHAMIENTO Y CONSERVACIÓN DE RECURSOS NATURALES

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Los abajo firmantes, certifican que la tesis de doctorado **"Demografía, reproducción y diversidad genética de *Picea martinezii* Patterson y *Picea mexicana* Martínez, en un contexto de cambio climático"**, que se presenta como requisito parcial para la obtención del Grado de Doctor en Ciencias Agropecuarias y Forestales por parte de:

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Julio 2023

A mi familia

AGRADECIMIENTOS

Al Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT) por la beca de estudios para el desarrollo del presente trabajo de tesis.

Al Consejo Nacional de Humanidades, Ciencias y Tecnologías y la Comisión Nacional Forestal por el financiamiento al proyecto CONACYT-CONAFOR-292615 “Cambios demográficos, reproducción y estatus de poblaciones naturales de *Picea mexicana* Martínez y *Picea martinezii* Patterson en México: especies susceptibles a eventos de variabilidad climática”, del cual se derivaron los resultados que integran la presente tesis.

A la Universidad Juárez del Estado de Durango (UJED), el Programa Institucional de Doctorado en Ciencias Agropecuarias y Forestales (PIDCAF) y el Instituto de Silvicultura e Industria de la Madera (ISIMA) por la oportunidad para realizar mis estudios de doctorado y todas las facilidades para el desarrollo de mi proyecto de investigación.

A mi Comité de Tesis: Dr. J. Jesús Vargas Hernández, Dr. Cuauhtémoc Sáenz Romero, Dr. Christian A. Wehenkel, Dr. José Ciro Hernández Díaz y Dr. José Ángel Prieto Ruíz por la guía y todo el apoyo brindado.

A todos los colaboradores externos que contribuyeron a la obtención y análisis de datos: Dr. Carlos López Sánchez, Dr. Juan Pablo Jaramillo Correa, M.C. Gustavo Ibrahim Pérez Giles, Dr. Miguel Martínez Trujillo, Dra. María A. Beltrán Nambo, Biol. Fidel Anguiano Rodríguez, Dr. Abdul Mauricio Reyes, Dr. Miguel M. Correa Ramírez y Dr. Celestino Flores López. Un agradecimiento también a los compañeros de laboratorio por el apoyo en el trabajo de campo y laboratorio, así como a las personas de las comunidades y propiedades privadas donde se encuentran las poblaciones de *Picea martinezii* y *Picea mexicana*, por todas las facilidades para el acceso a las mismas y el apoyo en el trabajo de campo. Gracias a todas y a todos.

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RESUMEN GENERAL

Picea martinezii Patterson y *P. mexicana* Martínez son dos especies endémicas de México consideradas raras y en peligro de extinción. Aunque se conocen aspectos de su taxonomía, ecología y genética, aún se desconocen aspectos clave para determinar su estatus actual y las posibilidades de conservación *in situ* y *ex situ*. El presente trabajo se enfocó en conocer: *i*) la distribución natural completa y potencial (pasada, presente y futura); *ii*) el estatus reproductivo-demográfico; y *iii*) la diversidad genética y su asociación con caracteres reproductivos, el tamaño y estructura demográfica de las poblaciones en ambas especies. Se realizaron búsquedas de nuevos rodales y censos poblacionales completos, y se elaboraron modelos de distribución potencial para México y mundiales. Se evaluaron diferentes indicadores reproductivos asociados a la producción y germinación de semillas y se estimaron las estructuras demográficas de las poblaciones y el tamaño efectivo de población con base en la frecuencia de categorías diamétricas de los individuos en las poblaciones censadas. También se evaluó el riesgo de extinción de las poblaciones con datos genómicos, reproductivos y demográficos, incluyendo la exploración de las bases y estructura genética de los indicadores reproductivos más representativos, mediante asociaciones heterocigosidad-fitness (HFCs) y genotipo-fenotipo (GWAS). Se censaron 89,266 y 39,059 individuos totales respectivamente para *P. martinezii* y *P. mexicana*, incluyendo individuos de nuevos rodales. El hábitat potencial fue más abundante en el pasado sólo para *P. mexicana*; actualmente se restringe a las poblaciones y rodales naturales de ambas especies y desaparecerá en 2050 en un escenario pesimista de cambio climático (RCP8.5). Existen posibilidades de conservación *ex situ* para *P. martinezii* sólo en áreas con hábitat marginal en México, y para *P. mexicana* solo fuera del territorio mexicano. El tamaño de población se asoció con la aptitud reproductiva, cuya base genética mostró diferentes loci candidatos para cada especie y una mayor cantidad para *P. martinezii*. Ambas especies son genética, demográfica y reproductivamente viables, pero el riesgo de extinción es alto en las poblaciones de La Encantada y El Butano de *P. martinezii*.

Palabras clave: Análisis de Asociación de Genoma Completo (GWAS), cambio climático, conservación *in situ* y *ex situ*, Correlaciones Heterocigosidad-Fitness (HFCs), especies amenazadas, indicadores reproductivos.

GENERAL SUMMARY

Picea martinezii Patterson and *P. mexicana* Martínez are two endemic species of Mexico considered rare and endangered. Several aspects of their taxonomy, ecology and genetics are already known, however key aspects to determine their current status and the possibilities for *in situ* and *ex situ* conservation are still unknown. The present work focused on knowing: *i*) complete and potential natural distribution (past, present, and future); *ii*) reproductive-demographic status; and *iii*) genetic diversity and its association with reproductive traits, population size and demographic structure in both species. Searches for new stands and complete population censuses were conducted, and potential distribution models were developed for Mexico and worldwide. Different reproductive indicators associated with seed production and germination were evaluated and the demographic structures of the populations and the effective population size were estimated based on diametric categories of individuals the censused populations. Finally, the extinction risk of the populations was evaluated with genomic, reproductive and demographic data, including the exploration of the genetic basis and structure of the most representative reproductive indicators, through the analysis of Heterozygosity-Fitness Correlations (HFCs) and genotype-phenotype associations (GWAS). A total of 89,266 and 39,059 individuals were censused for *P. martinezii* and *P. mexicana*, respectively, including individuals from new stands. Potential habitat was more abundant in the past only for *P. mexicana*, it is currently restricted to natural populations and stands of both species, and will disappear by 2050 in a pessimistic climate change scenario (RCP8.5). There are *ex situ* conservation possibilities for *P. martinezii* only in areas with marginal habitat in Mexico, and for *P. mexicana* only outside of Mexico. Population size was associated with reproductive fitness, whose genetic basis showed different candidate loci for each species and a greater number for *P. martinezii*. Both species are genetically, demographically and reproductively viable, but the risk of extinction is high in La Encantada and El Butano populations of *P. martinezii*.

Key words: climate change, *in situ* and *ex situ* conservation, Genome Wide Association Analysis (GWAS), Heterozygosity-Fitness Correlations (HFCs), reproductive indicators, threatened species.

CAPÍTULO 1. INTRODUCCIÓN GENERAL

Derivado de las actividades antropogénicas, actualmente, a nivel mundial, se registran efectos negativos en todos los niveles de organización de la diversidad biológica (Williams *et al*. 2020). En el corto y mediano plazo se prevé que esto afecte la funcionalidad de los sistemas naturales, así como su capacidad para suministrar bienes y servicios (Cardinale *et al*. 2012). Aunque la tasa de extinción actual es similar a las ocurridas en forma natural en periodos geológicos pasados (Briggs 2017), la drástica reducción del tamaño poblacional y de la diversidad genética elevarán el riesgo de extinción de muchas especies (Briggs 2017; WWF 2022; Exposito-Alonso *et al*., 2022; Finn *et al*., 2023). No obstante, aún se desconocen los aspectos biológicos básicos de muchas especies amenazadas, necesarios para comprender su importancia en el ecosistema, así como para su manejo y conservación (IUCN 2022).

México posee gran riqueza biológica como resultado de su ubicación geográfica, orografía y variación ambiental (Marshall y Liebherr 2000; Salinas-Rodríguez *et al*. 2018). Respecto a la diversidad vegetal, en México se han identificado 23,314 especies de plantas vasculares, de las cuales 50 % son endémicas y 3,167 se consideran especies raras o de hábitat restringido (Villaseñor 2016; Salinas-Rodríguez *et al*. 2018); esto lo ubica, a nivel mundial, como el cuarto país con mayor diversidad vegetal y el segundo con más endemismos (Villaseñor 2016). En cuanto a la diversidad de especies arbóreas, en México se reconocen 4,331 especies (Wehenkel *et al*. 2017); sin embargo, de acuerdo con la NOM-059-SEMARNAT-2010, se estima que 4.2 % (987 especies) de la diversidad vegetal arriba citada se encuentra en alguna categoría de riesgo, dentro de las cuales se incluyen 535 especies endémicas y 167 especies arbóreas (SEMARNAT 2010).

El presente trabajo se enfocó en dos de las 43 especies de coníferas endémicas de México (Gernandt y Pérez-De la Rosa 2014): *Picea martinezii* Patterson y *P. mexicana* Martínez; ambas especies son relictos de la última glaciación y están enlistadas en la NOM-059 como especies en peligro de extinción (NOM-059-SEMARNAT-2010), debido a que cuentan con pocas poblaciones, las cuales además están fragmentadas y aisladas en refugios ecológicos específicos (Ledig *et al*. 2000a).

Desde el descubrimiento de *P. mexicana* (Martínez 1961) y *P. martinezii* (Müller-Using y Alanís 1984), se han realizado estudios para determinar su estatus taxonómico (Taylor *et al*. 1994; Ledig *et al*. 2004; Jaramillo-Correa *et al*. 2006; Boullié *et al*. 2011; Lockwood *et al*. 2013), su demografía mediante muestreos parciales (Flores-López *et al*. 2005; Flores-López 2014), la diversidad y estructura genética con isoenzimas (Ledig *et al*. 2000b, 2002) y su capacidad reproductiva (Flores-López *et al*. 2005, 2012). Estos estudios han esclarecido su taxonomía y han mostrado que ambas especies tienen poblaciones pequeñas, con baja diversidad genética y con poca viabilidad reproductiva (Mendoza-Maya *et al*., 2015). Además, se ha estimado una alta susceptibilidad a escenarios climáticos futuros (Ledig *et al*., 2010).

Sin embargo, aún se desconoce la historia migratoria de esas especies y los cambios demográficos históricos asociados, así como su influencia sobre la actual distribución de la diversidad genética. De igual forma, se desconocen los factores genéticos subyacentes a su viabilidad reproductiva, así como los efectos de esta asociación genético-reproductiva sobre factores demográficos clave para la permanencia de las poblaciones de ambas especies, por lo que es poco claro si éstas han entrado en vórtices de extinción. Adicionalmente, permanece incompleta la búsqueda de nuevos rodales para determinar el nicho realizado de estas especies y su tamaño poblacional completo, así como las posibilidades de conservación *ex situ*, más allá de los límites de México.

Debido a sus distribuciones restringidas y sus tamaños poblacionales reducidos, *P. martinezii* y *P. mexicana* pueden servir como modelos para el estudio de fenómenos genéticos y ecológicos en especies forestales que pueden aportar información de utilidad principalmente para la conservación de estas y otras especies en situaciones similares, pero también para el manejo y aprovechamiento de otras especies forestales de importancia económica. El presente trabajo se enfocó en conocer: i) la distribución natural y potencial, ii) el estatus reproductivo-demográfico y iii) la diversidad genética y su asociación con caracteres reproductivos y la demografía en ambas especies.

1.1. Objetivos e hipótesis

1.1.1. Objetivo general

Determinar la distribución geográfica y la relación de la diversidad genética con la capacidad reproductiva y la estructura demográfica de *Picea martinezii* y *Picea mexicana*.

1.1.2. Objetivos particulares

- Estimar los tamaños poblacionales completos y la distribución geográfica potencial pasada, presente y futura de las dos especies mencionadas.
- Evaluar la aptitud reproductiva de los individuos en sus diferentes poblaciones.
- Determinar la diversidad genética con polimorfismos de un solo nucleótido (SNPs) y el vínculo con la aptitud reproductiva de los individuos y con el tamaño y la estructura demográfica de dichas poblaciones.

1.2. Hipótesis

- En el pasado, ambas especies tuvieron una distribución más amplia en México; en el presente, el hábitat adecuado se encuentra en sitios fuera de su distribución natural, en donde es posible realizar conservación *ex situ*.
- El tamaño de población se asocia de manera positiva con el nivel de diversidad genética y la aptitud reproductiva de los individuos.
- Las poblaciones con menor aptitud reproductiva son aquellas en las que los individuos portan mayores frecuencias de alelos deletéreos que incrementan la depresión endogámica.

1.3. Organización de la tesis

El presente documento se redactó de acuerdo con los lineamientos establecidos por el Programa Institucional de Doctorado en Ciencias Agropecuarias y Forestales, de la Universidad Juárez del Estado de Durango (PIDCAF-UJED), para la modalidad de tesis por artículos científicos, como una compilación de artículos publicados o por publicar en revistas científicas. El documento se divide en cinco capítulos. El primer capítulo, titulado “Introducción General”, aborda el panorama general sobre el cual se basa esta tesis, incluyendo los objetivos generales y particulares, así como las hipótesis y una breve descripción taxonómica y del hábitat de las especies bajo estudio. El segundo capítulo titulado “Assisted migration and the rare endemic plant species: the case of two endangered Mexican spruces”, aborda el objetivo específico No. 1 de la tesis referido a los censos poblacionales y las distribuciones geográficas potenciales pasadas, presentes y futuras, así como las posibilidades de conservación *in situ* y *ex situ* para ambas especies, con base en los requerimientos de hábitat. El tercer capítulo titulado “Indicadores reproductivos de *Picea martinezii* T.F. Patterson y *Picea mexicana* Martínez” aborda el objetivo No. 2 referido al estatus reproductivo de los individuos en las poblaciones, con un énfasis en la metodología del análisis reproductivo y el estatus general de las especies. El cuarto capítulo titulado “Genomic and evolutionary drivers of reproductive fitness in two endangered forest trees” aborda el objetivo No. 3, referente a la relación que existe entre el tamaño de las poblaciones con la diversidad genética y la aptitud reproductiva de las especies. El quinto capítulo titulado “Consideraciones finales y recomendaciones” provee algunas observaciones y recomendaciones derivadas de los resultados de este trabajo, para la conservación de las especies bajo estudio. Finalmente, el sexto capítulo titulado “Conclusiones generales” contiene las conclusiones generales de este trabajo de tesis.

1.4. Revisión de literatura

1.4.1. Descripción taxonómica de las especies

Picea martinezii es un árbol de 25 a 40 m de altura con copas irregulares abiertas o cónico-columnares, fustes con DAP (diámetro a 1.3 m de altura) de hasta 1 m, y follaje perennifolio verde brillante o verde oscuro (Fig. 1.1-A). La corteza es delgada, escamosa y grisácea. Las ramas son espaciadas; las inferiores colgantes, las superiores ascendentes, con ramillas glabras. Hojas linear-aciculares acomodadas hacia todos lados de la ramilla, de 11 a 35 mm de largo, 1.0 a 2.0 mm de ancho, planas, con puntas afiladas dirigidas hacia arriba, con cuatro a diez filas de estomas en cada cara; sin canales resiníferos. Conos masculinos sub-terminales en grupos de tres, ovales, de 15 mm de largo por 10 mm de ancho; polen oval con dos grandes vejigas áreas. Conos femeninos terminales o laterales de 80 a 180 mm de largo, 30 a 62 mm de ancho, oblongo-cilíndricos, de color marrón a marrón-rojizo; escamas lisas con el ápice redondeado, de 19 a 30 mm de largo y 18-25 mm de ancho; bráctea de 4 mm de largo, color café, oblonga, con ápice redondeado. Las semillas son color café, fusiformes, ligeramente aplanadas, de 4 a 8 mm de largo, ápice redondeado, base aguda, y alas de 12 a 23 mm de largo (Patterson 1988, González-Elizondo 2022).

Los nombres comunes con que se conoce a *P. martinezii* son: picea, pinabete, pinabete de Nuevo León, Martínez spruce (CONABIO-CONAMP 2009).

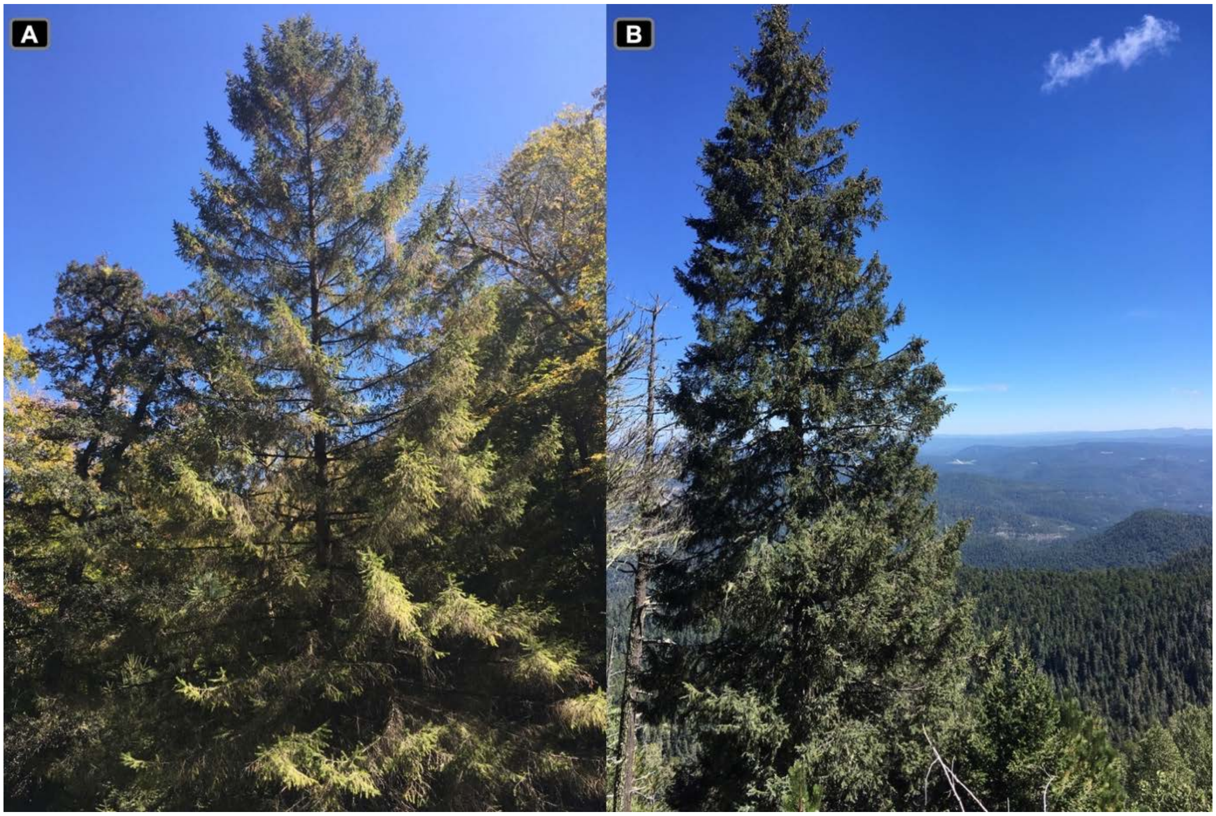

Figura 1.1. *Picea martinezii* Patterson (A) y *Picea mexicana* Martínez (B) en su hábitat natural. Fotografías tomadas en las poblaciones de Agua Fría, Nuevo León (2020) y en el Cerro El Mohinora, Chihuahua (2019), respectivamente.

Picea mexicana es un árbol que alcanza hasta 34 m de altura con copas cónicas a cónico-columnares formadas por ramas verticiladas, ascendentes o extendidas, fustes de hasta 0.9 m de DAP y follaje verde grisáceo a glauco (verde azuloso) (Fig. 1.1-B). La corteza es poco rugosa, con placas grisáceo-blanquecinas; la corteza interior de color amarillo a café grisáceo. Las ramillas son de color amarillo opaco, pubescentes. Las hojas están acomodadas hacia todos lados de la ramilla, linear-aciculares, rígidas, de 15 a 36 mm de largo y 10 a 20 mm de ancho, cuadrangulares, con punta punzante; con tres o cuatro filas de estomas en cada cara, sin canales resiníferos. Conos masculinos sub-terminales, en grupos de tres, ovales de 15 mm de largo por 10 mm de ancho; anteras de 2.5 mm que se abren por hendeduras longitudinales; polen oval con dos grandes vejigas áreas. Conos femeninos terminales o laterales colgantes, cilíndricos a oblongo-cilíndricos, de 50 a 80 mm de largo, color amarillento a marrón-amarillento; escamas de 14 mm de largo y 10 a 12 mm de ancho, bráctea dorsal elíptica, acuminada, de 5.5 a 6.0 mm. Semillas de 3.5 a 5.0 mm de largo y 2 a 3 mm de ancho, ovoides y pardas (Martínez 1961; González-Elizondo 2022).

Los nombres comunes con que se conoce a *P. mexicana* son: ciprés (Martínez 1963), abeto, Mexican spruce, picea, pícea mexicana, pinabete o pinabete mexicano (González-Elizondo 2022).

1.4.2. Hábitat de las especies

Picea martinezii se localiza en cuatro localidades al norte de la Sierra Madre Oriental en el estado de Nuevo León: El Butano, Agua Fría, Agua de Alardín (También conocida como Agua Lardín) y La Encantada (Cuadro 1.1 y Fig. 1.2). Las cuatro poblaciones se ubican en sitios específicos localizados cerca de arroyos, barrancas y acantilados en las laderas norte de las montañas, a elevaciones que van de los 1,800 a 2,500 m (Ledig *et al*. 2000a). *Picea mexicana* se ubica en las tres montañas más elevadas en el norte de la Sierra Madre Occidental (Cerro El Mohinora, Chihuahua) y la Sierra Madre Oriental (Sierra La Marta y Sierra el Coahuilón, Coahuila) (Cuadro 1.1 y Fig. 1.2). Las tres poblaciones conocidas de *P. mexicana* se ubican en las laderas norte de las secciones superiores de las montañas, en elevaciones de 3,000 a 3,600 m (Ledig *et al*. 2000a).

Los ecosistemas en que subsisten las poblaciones naturales de *Picea martinezii* y *Picea mexicana* son el bosque mesófilo de montaña o bosque mixto y el bosque de ayarín o pinabete, respectivamente (Ledig *et al*. 2000a; Valdez-Tamez *et al*. 2003; Rzedowski 2006).

Cuadro 1.1. Poblaciones conocidas de *Picea martinezii* Patterson y *Picea mexicana* Martínez en México.

Población	Localidad	Municipio[1]	Elevación (m)
		Picea martinezii	
Agua de Alardín	Propiedad privada: Agua de Alardín	Aramberri, NL	2,120
Agua Fría	Propiedad privada: Agua Fría	Aramberri, NL	1,820
El Butano	Ejido La Trininad	Montemorelos, NL	2,180
La Encantada	Ejido La Encantada	Zaragoza, NL	2,515
		Picea mexicana	
El Mohinora	Ejido El Tule y Portugal	Guadalupe y Calvo, Chih.	3,113
La Marta	Propiedad privada: Felipe de la Peña	Rayones, NL	3,494
El Coahuilón	Ejido Nuncio	Arteaga, Coah.	3,528

[1] NL = Nuevo León, Chih. = Chihuahua, Coah. = Coahuila.

Nota: Agua de Alardín es también conocida como Agua Lardín.

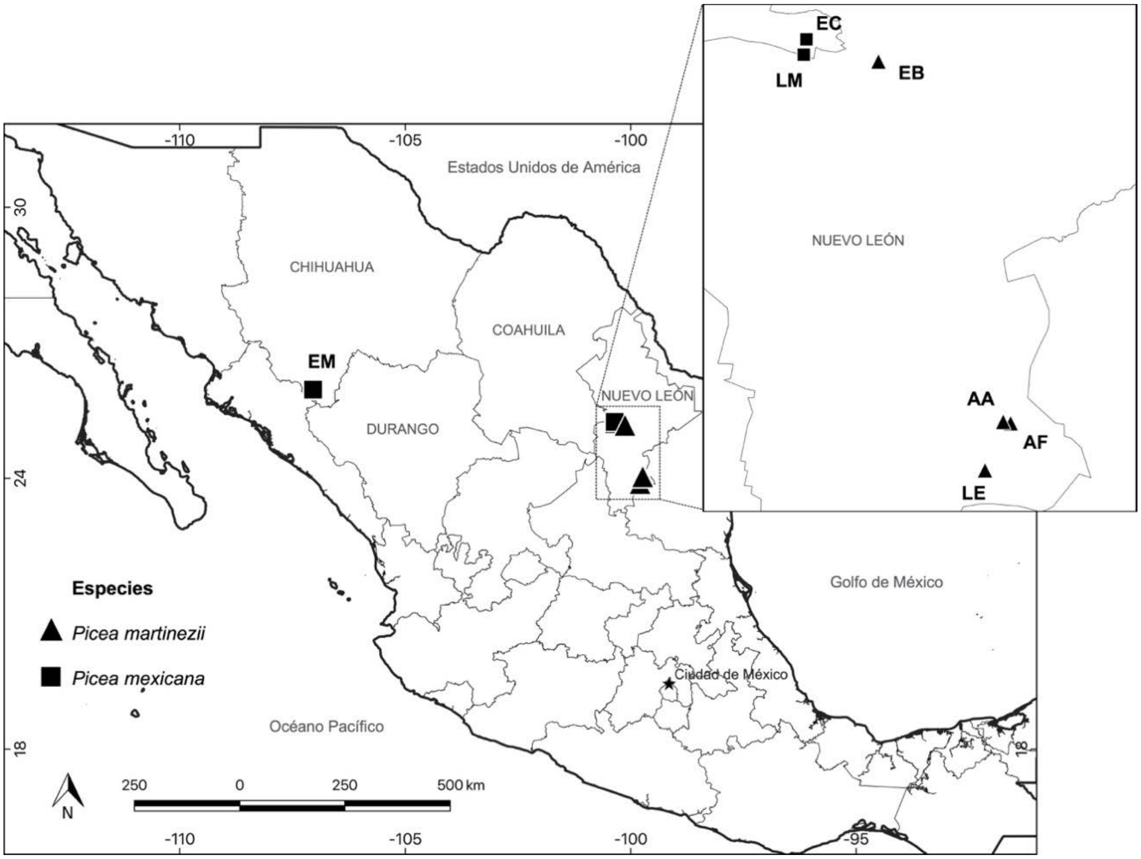

Figura 1.2. Mapa de la localización de las poblaciones de *Picea martinezii* Patterson y *Picea mexicana* Martínez. Nota: EM = El Mohinora, LM = La Marta, EC = El Coahuilón, EB = El Butano, AF = Agua Fría, AL = Agua Lardín, LE = La Encantada.

De acuerdo con las descripciones de Rzedowski (2006) y CONABIO (2008), el bosque mesófilo de montaña se distribuye en elevaciones entre los 800 y 2,200 m y está constituido por árboles en su mayoría perennifolios, que forman bosques ubicados al barlovento de los macizos montañosos, donde se condensan las nubes y se forman neblinas, así como en barrancas y laderas muy húmedas y sombreadas, por lo que se encuentran protegidas de la insolación durante la mayor parte del año.

El rango altitudinal de presencia de *Picea martinezii* la ubica en el estrato superior del bosque mesófilo de montaña, que corresponde al subtipo de los bosques de pino, encino y liquidámbar. Por otra parte, los bosques de ayarín o pinabete corresponden al subtipo de bosques de coníferas que a su vez son una sub-clasificación de los bosques templados subhúmedos. Los bosques de ayarín se caracterizan por su vegetación perennifolia dominada por los géneros *Abies*, *Pseudotsuga* y *Picea* y por su ubicación restringida a sitios sombríos y húmedos, preferentemente en laderas de cañadas y barrancas o valles muy protegidos, en las partes altas de las montañas donde forman comunidades muy reducidas en elevaciones entre los 2,000 y 3,400 m.

El rango altitudinal de presencia de *Picea mexicana* la ubica en el estrato superior de los bosques de ayarín, en lo que constituye las zonas subalpinas (Ledig *et al*. 2000a). Las condiciones climáticas que requieren ambos tipos de ecosistemas se presentan en zonas restringidas del territorio mexicano, lo que los remite a distribuciones limitadas y fragmentadas (Rzedowski 2006).

1.5. Literatura citada

Bouillé, M; Senneville, S; Bousquet, J. 2011. Discordant mtDNA and cpDNA phylogenies indicate geographic speciation and reticulation as driving factors for the diversification of the genus *Picea*. Tree Genetics and Genomes 7(3):469-484. DOI: https://doi.org/10.1007/s11295-010-0349-z.

Briggs, JC. 2017. Emergence of a sixth mass extinction? Biological Journal of the Linnean Society 122(2):243-248. DOI: https://doi.org/10.1093/biolinnean/blx063.

Cardinale, BJ; Duffy, JE; Gonzalez, A; Hooper, DU; Perrings, C; Venail, P; Narwani, A; Mace, GM; Tilman, D; Wardle, DA. 2012. Biodiversity loss and its impact on humanity. Nature 486(7401):59-67. DOI: https://doi.org/10.1038/nature11148.

CONABIO. 2008. Capital natural de México, vol. I: Conocimiento actual de la biodiversidad. México, D. F., Comisión Nacional para el Conocimiento y Uso de la Biodiversidad.

CONABIO-CONANP. 2009. Pinabete de Nuevo León (*Picea martinezii*). Fichas de especies mexicanas. México, D. F., Comisión Nacional para el Conocimiento y Uso de la Biodiversidad y Comisión Nacional de Áreas Naturales Protegidas.

Exposito-Alonso, M; Booker, TR; Czech, L; Gillespie, L; Hateley, S; Kyriazis, CC; Lang, PLM; Leventhal, L; Nogues-Bravo, D; Pagowski, V; Ruffley, M; Spence, JP; Toro Arana, SE; Weiß, CL; Zess, E. 2022. Genetic diversity loss in the Anthropocene. Science 377(6613):1431-1435. DOI: https://doi.org/10.1126/science.abn5642.

Finn, C; Grattarola, F; Pincheira-Donoso, D. 2023. More losers than winners: investigating Anthropocene defaunation through the diversity of population trends. Biological Reviews . DOI: https://doi.org/10.1111/brv.12974.

Flores-López, C. 2014. Líneas para la conservación de los recursos genéticos de *Picea mexicana* Martínez y *Picea martinezii* Patterson. Universidad de Pinar del Río «Hermanos Saíz Montes de Oca», Cuba. 113 p.

Flores-López, C; Geada-López, CG; López-Upton, CJ; López-Ramírez, E. 2012. Reproductive indicators in natural populations of *Picea martinezii* T. F. Patterson. Revista Forestal Baracoa 31(2):49-58.

Flores-López, C; López-Upton, J; Vargas-Hernández, JJ. 2005. Reproductive indicators in natural populations of *Picea mexicana* Martínez. Agrociencia 39(001):117-126.

Gernandt, DS; Pérez-De La Rosa, JA. 2014. Biodiversity of Pinophyta (conifers) in Mexico. Revista Mexicana de Biodiversidad 85(SUPPL.):126-133. DOI: https://doi.org/10.7550/rmb.32195.

González-Elizondo, MS. 2022. Taxonomía de las piceas mexicanas. *In* González-Elizondo, MS; Wehenkel, C (eds.). Las Piceas (*Picea*, Pinaceae) de México. Comisión Nacional Forestal. México. p. 4-15.

IUCN (International Union for Conservation of Nature). 2022. The IUCN Red List of Threatened Species. Version 2020-3. https://www.iucnredlist.org. Accesado el 27 de mayo de 2022.

Jaramillo-Correa, JP; Beaulieu, J; Ledig, FT; Bousquet, J. 2006. Decoupled mitochondrial and chloroplast DNA population structure reveals Holocene collapse and population isolation in a threatened Mexican-endemic conifer. Molecular Ecology 15(10):2787-2800. DOI: https://doi.org/10.1111/j.1365-294X.2006.02974.x.

Ledig, FT; Mapula-Larreta, M; Bermejo-Velázquez, B; Reyes-Hernández, V; Flores-López, C; Capó-Arteaga, MA. 2000a. Locations of endangered spruce populations in Mexico and the demography of *Picea chihuahuana*. Madroño 47(2):71-88. DOI: https://doi.org/http://www.jstor.org/stable/41425349.

Ledig, FT; Bermejo-Velázquez, B; Hodgskiss, PD; Johnson, DR; Flores-López, C; Jacob-Cervantes, V. 2000b. The mating system and genic diversity in Martínez spruce, an extremely rare endemic of México's Sierra Madre Oriental: an example of facultative selfing and survival in interglacial refugia. Canadian Journal of Forest Research 30(7):1156-1164. DOI: https://doi.org/10.1139/x00-052.

Ledig, FT; Hodgskiss, PD; Jacob-Cervantes, V. 2002. Genetic diversity, mating system, and conservation of a Mexican subalpine relict, *Picea mexicana* Martínez. Conservation Genetics 3(2):113-122. DOI: https://doi.org/10.1023/A:1015297621884.

Ledig, FT; Hodgskiss, PD; Krutovskii, K V.; Neale, DB; Eguiluz-Piedra, T. 2004. Relationships among the Spruces (*Picea*, Pinaceae) of Southwestern North America. Systematic Botany 29(2):275-295. DOI: https://doi.org/10.1600/036364404774195485.

Ledig, FT; Rehfeldt, GE; Sáenz-Romero, C; Flóres-López, C. 2010. Projections of suitable habitat for rare species under global warming scenarios. American Journal of Botany 97:970-987. https://doi.org/10.3732/ajb.0900329.

Lockwood, JD; Aleksić, JM; Zou, J; Wang, J; Liu, J; Renner, SS. 2013. A new phylogeny for the genus *Picea* from plastid, mitochondrial, and nuclear sequences. Molecular Phylogenetics and Evolution 69(3):717-727. DOI: https://doi.org/10.1016/j.ympev.2013.07.004.

Marshall, CJ; Liebherr, JK. 2000. Cladistic biogeography of the Mexican transition zone. Journal of Biogeography 27(1):203-216. DOI: https://doi.org/10.1046/j.1365-2699.2000.00388.x.

Martínez, M. 1961. Una nueva especie de *Picea* en México. Anales del Instituto de Biología UNAM 32:137-142.

Mendoza-Maya, E; Espino-Espino, J; Quiñones-Pérez, CZ; Flores-López, C; Wehenkel, C; Vargas-Hernández, JJ; Sáenz-Romero, C. 2015. Proposal for conservation of three endangered species of Mexican spruce. Revista Fitotecnia Mexicana 38(3):235-247.

Müller-Using, B; Alanis, G. 1984. New records of Chihuahua spruce (*Picea chihuahuana* Martínez) in Nuevo León proposed for the legal protection of two areas of special ecological interest. In Reunión Regional de Ecología Norte, 25, 26 y 27 de abril 1984, Monterrey, N.L. SEDUE - Secretaría de Desarrollo Urbano y Ecología, Subsecretaría de Ecología. Secretaría de Desarrollo Urbano y Ecología (ed.). Monterrey, N.L., SEDUE. p. 130-132.

Patterson, TF. 1988. A new species of *Picea* (Pinaceae) from Nuevo León, México. SIDA, Contributions to Botany 13(2):131-135. DOI: https://doi.org/https://www.jstor.org/stable/41966770.

Rzedowski, J. 2006. Vegetación de México. México, D. F., Comisión Nacional para el Conocimiento y Uso de la Biodiversidad. 504 p.

Salinas-Rodríguez, MM; Sajama, MJ; Gutiérrez-Ortega, JS; Ortega-Baes, P; Estrada-Castillón, AE. 2018. Identification of endemic vascular plant species hotspots and the effectiveness of the protected areas for their conservation in Sierra Madre Oriental, Mexico. Journal for Nature Conservation 46(January):6-27. DOI: https://doi.org/10.1016/j.jnc.2018.08.012.

SEMARNAT (Secretaría de Medio Ambiente y Resursos Naturales). 2010. NORMA Oficial Mexicana NOM-059-SEMARNAT-2010, 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. D. O. F. 30 de diciembre de 2010. México. 77 p. Disponible en URL: https://www.gob.mx/profepa/documentos/norma-oficial-mexicana-nom-059-semarnat-2010. Accesado el 15 de mayo de 2022.

Taylor, RJ; Patterson, TF; Harrod, RJ. 1994. Systematics of Mexican spruce-revisited. Systematic Botany 19(1):47-59. DOI: https://doi.org/10.2307/2419711.

Valdez-Tamez, V; Foroughbakhch, RP; Alanís, GF. 2003. Distribución relictual del bosque mesófilo de montaña en el noreste de México. Ciencia UANL VI(3):360-365.

Villaseñor, JL. 2016. Checklist of the native vascular plants of Mexico. Revista Mexicana de Biodiversidad 87(3):559-902. DOI: https://doi.org/10.1016/j.rmb.2016.06.017.

Wehenkel, C; Mariscal-Lucero, S del R; Jaramillo-Correa, JP; López-Sánchez, CA; Vargas-Hernández, JJ; Sáenz-Romero, C. 2017. Genetic diversity and conservation of Mexican forest trees. In Ahuja, MR; Jain, SM (eds.). Cham, Springer International Publishing, vol.17, (Sustainable Development and Biodiversity). p. 35-68. DOI: https://doi.org/10.1007/978-3-319-66426-2_2.

Williams, SE; Hobday, AJ; Falconi, L; Hero, JM; Holbrook, NJ; Capon, S; Bond, NR; Ling, SD; Hughes, L. 2020. Research priorities for natural ecosystems in a changing global climate. Global Change Biology 26(2):410-416. DOI: https://doi.org/10.1111/gcb.14856.

WWF. 2022. Living Planet Report 2022 – Building a naturepositive society. Almond REA, Grooten M, Juffe Bignoli D, Petersen T (eds). Gland, Switzerland, World Wildlife Fund. Disponible en URL: https://wwflpr.awsassets.panda.org/downloads/lpr_2022_full_report.pdf. Accesado el 27 de mayo de 2023.

CAPÍTULO 2: ASSISTED MIGRATION AND THE RARE ENDEMIC PLANT SPECIES: THE CASE OF TWO ENDANGERED MEXICAN SPRUCES

Artículo publicado en la revista PeerJ 10:e13812, DOI 10.7717/peerj.13812

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Abstract

Background: In the projected climate change scenarios, assisted migration might play an important role in the ex situ conservation of the threatened plant species, by translocate them to similar suitable habitats outside their native distributions. However, it is unclear if such habitats will be available for the Rare Endemic Plant Species (REPS), because of their very restricted habitats. The aims of this study were to perform a population size assessment for the REPS *Picea martinezii* Patterson and *Picea mexicana* Martínez, and to evaluate the potential species distributions and their possibilities for assisted migration inside México and worldwide.

Methods: We performed demographic censuses, field surveys in search for new stands, and developed distribution models for Last Glacial Maximum (22,000 years ago), Middle Holocene (6,000 years ago), current (1961–1990) and future (2050 and 2070) periods, for the whole Mexican territory (considering climatic, soil, geologic and topographic variables) and for all global land areas (based only on climate).

Results: Our censuses showed populations of 89,266 and 39,059 individuals for *P. martinezii* and *P. mexicana*, respectively, including known populations and new stands. Projections for México indicated somewhat larger suitable areas in the past, now res tricted to the known populations and new stands, where they will disappear by 2050 in

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a pessimistic climatic scenario, and scarce marginal areas (p = 0.5–0.79) remaining only for *P. martinezii* by 2070. Worldwide projections (based only on climate variables) revealed few marginal areas in 2050 only in México for *P. martinezii*, and several large areas (p ≥ 0.5) for *P. mexicana* around the world (all outside México), especially on the Himalayas in India and the Chungyang mountains in Taiwan with highly suitable (p ≥ 0.8) climate habitats in current and future (2050) conditions. However, those suitable areas are currently inhabited by other endemic spruces: *Picea smithiana* (Wall.) Boiss and *Picea morrisonicola* Hayata, respectively.

Conclusions: Assisted migration would only be an option for *P. martinezii* on scarce marginal sites in México, and the possibilities for *P. mexicana* would be continental and transcontinental translocations. This rises two possible issues for future ex situ conservation programs: the first is related to whether or not consider assisted migration to marginal sites which do not cover the main habitat requirements for the species; the second is related to which species (the local or the foreign) should be prioritized for conservation when suitable habitat is found elsewhere but is inhabited by other endemic species. This highlights the necessity to discuss new policies, guidelines and mechanisms of international cooperation to deal with the expected high species extinction rates, linked to projected climate change.

Keywords: conservation of threatened species, climate change, narrow endemics, paleodistributions, *Picea martinezii*, *Picea mexicana*, species distribution modelling.

2.1. Introduction

To know the species distributions is fundamental for their conservation. Certainly, understanding the species' niche requirements and habitat specificity is essential to define the possibilities for management in the context of climate change. As linked ecological properties, niche requirements and habitat specificity influence geographical ranges of taxa (Crain et al., 2015) and ultimately, it determines commonness, endemism and rarity. This last ecological property, shared by 36.5% of the global plant diversity (Enquist et al., 2019), needs special attention because involves taxa with strong influence on ecosystem services (Mouillot et al., 2013) and high vulnerability to extinction (Işik, 2011).

Assisted migration is an ex situ conservation approach that emerged as a response to the imminent decoupling between climate and species in natural reserves (Peters & Darling, 1985). Pedlar et al. (2012) distinguished two types: forestry assisted migration, the objective of which is to maintain forest health and productivity; and species rescue assisted migration, whose goal is to avoid extinctions of threatened species.

Rescue assisted migration, the approach considered in this study (hereafter named only assisted migration), has been recognized as a viable method to conserve vulnerable species, by translocating them to similar suitable habitats outside their native ranges, where they can reproduce and compete successfully (McLane & Aitken, 2012). However, this adaptation strategy continues to be debated, pointing to the potential risk that translocated species could become invasive or could serve as vectors of new pests and diseases (Schwartz et al., 2012; Simler et al., 2019; Butt et al., 2021).

Additionally, if assisted migration becomes a regular ex situ conservation strategy, some questions remain unanswered regarding the Rare Endemic Plant Species (REPS): (i) Is it possible to find potential habitats outside the natural range of REPS, considering their high habitat specificity? (ii) If new suitable environments are found, is the area large enough to establish populations of a minimum viable size, able to survive in the long-term? (iii) Furthermore, what can be done if a new suitable habitat is found elsewhere, but is already occupied by other REPS? We do not have the answers to these questions, mainly because field studies of assisted migration with REPS are scarce (Butt et al., 2021).

Species Distribution Modelling (SDM), based on empirical associations between species occurrences and environmental variables, has become an important tool to understand current species distributions and for designing management and conservation strategies (López-Tirado & Hidalgo, 2016; Bosso et al., 2017; Ongaro et al., 2018; Sofaer et al., 2019), including assisted migration (McLane & Aitken, 2012). Regarding the rare endemic species, SDM has been improved by a variety of algorithms and methodologies for model construction, in addition to the incorporation of environmental factors with different scales of influence on species distributions (e.g. Pearson et al., 2007; Williams et al., 2009; Patsiou et al., 2014; McCune, 2016; Mi et al., 2017; Feng et al., 2022).

Previous studies have proposed systematic decision-making guides for assisted migration (Pérez et al., 2012), have evaluated the need and potential for assisted migration in different taxa through the SDM approach (Hällfors et al., 2016) and have used SDM to delimit current distributions of rare species at local or regional scales (e.g. McCune, 2016), or to evaluate future impacts of climate change, mainly by using climatic variables (Ledig et al., 2010; Pinedo-Alvarez et al., 2019). Nevertheless, studies on the potential value of assisted migration for REPS, considering the most complete sets of environmental variables of recognized influence on species distributions (e.g. Penteriani et al., 2019; Barrio-Anta et al., 2020; López-Sánchez et al., 2021), or global habitat searches using climatic variables, are scarce.

In this study we explore the potential of assisted migration as a tool for the conservation of two Mexican REPS (both at the category of endangered; IUCN, 2021): Picea martinezii Patterson and Picea mexicana Martínez. These species are relicts of the last glacial age, confined to very specific habitats, with scattered, fragmented and few isolated populations (Ledig et al., 2000a). In addition to other threats distinctive of the rare endemic species (Işik, 2011; Cogoni et al., 2019), such as their low genetic diversity (Ledig et al., 2000b; Ledig, Hodgskiss & Jacob-Cervantes, 2002) and decreased reproductive fitness (Flores- López, López-Upton & Vargas-Hernández, 2005; Flores-López et al., 2012), these spruces are threatened by climate change, as indicated by projected alterations in temperature and rain regimes on Mexican temperate forests (Sáenz-Romero et al., 2010), particularly in sites where these species thrive (Ledig et al., 2010), which could increase tree mortality through hotter-drought events (Hammond et al., 2022).

Since the discovery of P. mexicana (Martínez, 1961) and P. martinezii (Müller-Using & Alanis, 1984; Patterson, 1988), a total of seven populations had been roughly documented, but the population sizes, the exact extent and spatial distribution of these populations remain relatively unknown, and exploration to discover new stands had not been completed. Both spruces represent proper models for testing the viability of assisted migration in the ex situ conservation of rare species with restricted habitat.

We focused our study on three aspects of these REPS: (i) the exploration of potential new stands and performance of a population size assessment as a starting point for future demographic evaluations; (ii) construction of models describing the potential past and current distributions of these species; and (iii) identification of areas outside their current ranges (inside México and worldwide) with probability of harboring suitable habitats for future assisted migration, by modelling future distributions. Our hypothesis is that the suitable climatic habitat of P. martinezii and P. mexicana can be found elsewhere outside their current ranges and hence, assisted migration is a viable tool for the ex situ conservation of both species, considering future projections of climatic change.

2.2. Materials and methods

2.2.1. Study area, exploration of new stands and species distribution data

The study area is located in the Sierra Madre Oriental (SMOr) and Sierra Madre Occidental (SMOc), two parallel mountain ranges that cross northern México from north to south; SMOr alongside the Gulf of México, SMOc alongside the Pacific Ocean. Both mountain ranges are connected east-west by the Trans-Mexican Neovolcanic Axis (TMNVA) in central México (Fig. 2.1). *Picea martinezii* is only located in the northern SMOr, in four populations: El Butano, Agua de Alardín, Agua Fría and La Encantada, state of Nuevo León (Table 2.1, Fig. 2.1). These populations are generally found on north-facing slopes, near creeks, ravines or cliffs in the montane cloud forests at elevations ranging from 1,800 to 2,500 m (Ledig et al., 2000a). There are only three documented populations of *Picea mexicana* on the north-facing slopes of the highest peaks of the northern SMOc (one single population: El Mohinora, state of Chihuahua) and on SMOr (two populations: La Marta and El Coahuilón, state of Coahuila), in the conifer forests of the subalpine zones in elevations ranging from 3,000 to 3,600 m (Table 2.1, Fig. 2.1) (Ledig et al., 2000a). Detailed descriptions of the Mexican montane cloud and subalpine forest vegetation types are provided by Rzedowski (2006).

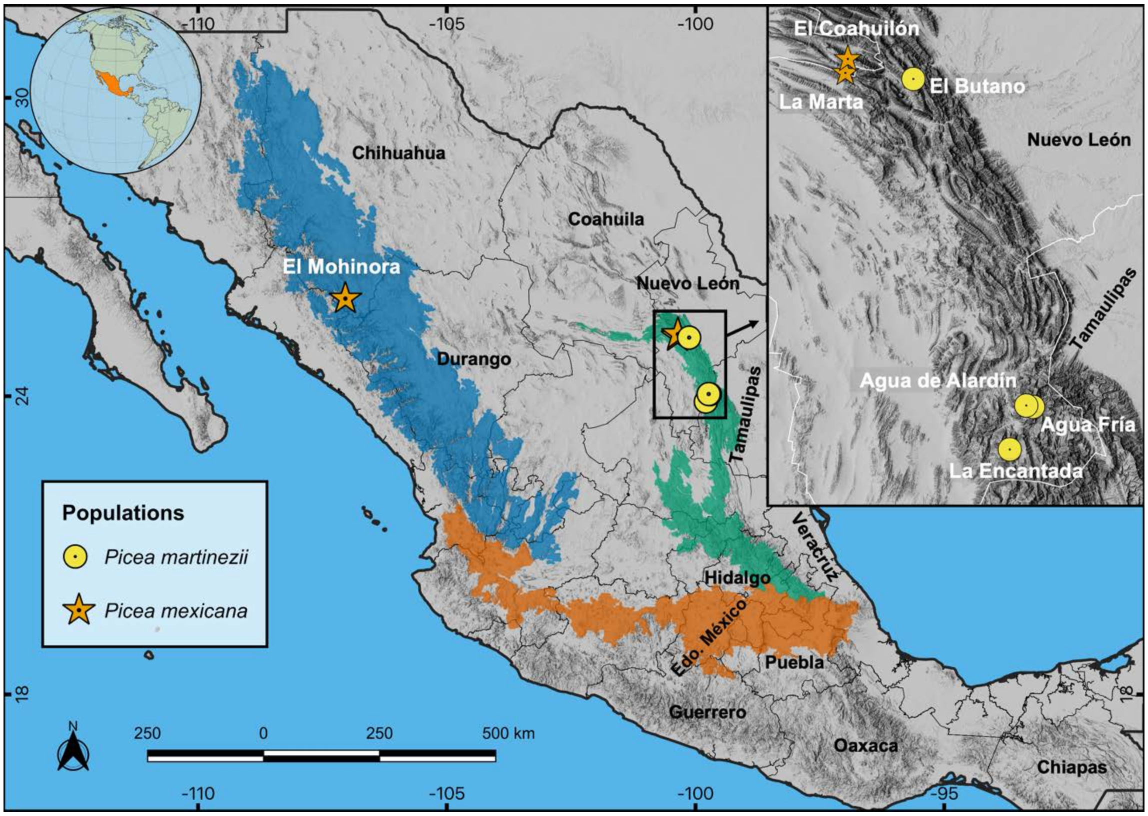

Figure 2.1. Locations of the four known contemporary populations of *Picea martinezii* (circle symbols) and the three known populations of *Picea mexicana* (star symbols). Prominent geographic regions of the Sierra Madre Occidental (SMOc, dark blue), Sierra Madre Oriental (SMOr, bluish green) and Trans-Mexican Neovolcanic Axis (TMNVA, vermilion) are shown. Note: The population of Agua de Alardín is also known as Agua Lardín. The SMOc, SMOr and TMNVA provinces file shapes were taken from Morrone, Escalante & Rodríguez-Tapia (2017), and the topographic base map for all figures was taken from ESRI (2018).

The distribution models were based on presence/absence data. In order to explore the potential existence of new stands and get more presence records for SDM, high-elevation, moist, cold, north exposure sites were surveyed in the surroundings of the known populations (at distances of up to 39 km). The potential sites were identified from maps of suitable climatic habitat for P. martinezii and *P. mexicana*, projected under contemporary climate (average 1961–1990) provided by Ledig et al. (2010), as well as from unconfirmed oral testimonies given by local foresters and landowners; this allowed us to get 46 to 50 presence records of P. martinezii and P. mexicana, respectively (Tables 2.1, S2.1, S2.2 and S2.3). All these presences were recorded in the center and periphery of the known populations and new stands, during the population size assessments that considered all individuals taller than 30 cm (including recruitment, saplings and trees), which were carried out as part of this study in 2018 and 2019 (Table

Table 2.1. Populations of *Picea martinezii* and *Picea mexicana* used to characterize the abiotic niches and for modelling distributions of both species.

Population or stand	**Code**	**Municipality and State**^{a}	**Elevation (m)**	**Coordinates (decimals)**	**Status (number of individuals**^{b}**)**	**Area (ha)**
Picea martinezii						
El Butano	EB	Montemorelos, NL	2,180	25.178N -100.126W	Known (1,253)	23.0
Agua de Alardín [c]	AL	Aramberri, NL	2,120	24.042N -99.734W	Known (84,498)	74.3
Agua Fría	AF	Aramberri, NL	1,820	24.038N -99.710W	Known (2,769)	53.8
La Encantada[d]	LE	Zaragoza, NL	2,515	23.890N -99.791W	Known (712)	5.2
La Encantada 2	LE-2	Zaragoza, NL	2,378	23.890N -99.778W	New (12)	0.1
Zaragoza	ZA	Zaragoza, NL	2,483	23.890N -99.773W	New (22)	1.0
Total					(89,266)	157.4
Picea mexicana						
El Mohinora	EM	Guadalupe y Calvo, Chi.	3,113	25.961N -107.042W	Known (11,383)	33.0
El Coahuilón	EC	Arteaga, Coah.	3,528	25.247N -100.354W	Known (2,253)	49.0
La Marta	LM	Arteaga, Coah.	3,494	25.198N -100.364W	Known (17,728)	41.6
La Marta 2	LM-2	Arteaga, Coah.	3,393	25.213N -100.413W	New (6,300)	24.0
La Marta 3	LM-3	Arteaga, Coah.	3,475	25.205N -100.386W	New (35)	6.5
La Marta 4	LM-4	Arteaga, Coah.	3,364	25.203N -100.369W	New (50)	1.7
La Marta 5	LM-5	Arteaga, Coah.	3,096	25.207N -100.369W	New (60)	1.0
El Mohinora 2	EM-2	Guadalupe y Calvo, Chi.	3,139	25.957N -107.029W	New (1,250)	16.3
Total					(39,059)	173.1

Notes:

[a] NL = Nuevo León; Coah. = Coahuila (both in the Sierra Madre Oriental); Chi. = Chihuahua (in the Sierra Madre Occidental).

[b] Including trees, saplings and natural regeneration (heights > 30 cm).

[c] Agua de Alardín (also known as Agua Alardín) = Cañada El Puerto (I, II, III) in Ledig et al. (2000a).

[d] La Encantada = La Tinaja in Ledig et al. (2000a).

2.1). Absences records (22,004 to 32,571) were sampled from the sites listed in the Mexican National Forest and Soil Inventory (MexFI), developed by the Mexican National Forest Commission (CONAFOR, 2009) (Table S2.3). Model projections for mapping the distribution of suitable areas for both tree species were performed for the whole Mexican territory and for all the world land areas (excluding Antarctica).

2.2.2. Environmental variables and species distribution modelling

Forty two environmental variables of different classes: climate, topography, soil and geology (e.g. succesfully used by Penteriani et al., 2019; Barrio-Anta et al., 2020; López- Sánchez et al., 2021) were considered possible predictors of the distribution of

P. martinezii and *P. mexicana*. Gridded data of 19 climatic variables (1961–1990 reference period) were retrieved at 30 arc-second resolution from WorldClim dataset (Hijmans et al., 2005, available at URL: https://worldclim.org); data of 14 soil variables were obtained from the SoilGrids250m at 250 m × 250 m resolution (available at URL: https://www.soilgrids.org), a repository of the spatial distribution of soil properties across the globe (Hengl et al., 2017). Data on two geological (30 arc-second resolution) and seven topographic variables (250 m × 250 m resolution) were obtained from digital models provided by the Mexican National Institute of Statistics, Geography and Informatics (INEGI) (available at URL: http://www.inegi.mx) (Table S4). Presences records were interleaved with each raster layer of the analyzed environmental variables, which were resampled at 30 arc-second cell resolution with the nearest neighbor method. Then, mean values of the environmental variables were extracted from pixels holding the presence records.

The varying methodologies for SDM may influence the final model metrics and projections, and the need to evaluate such methods in this kind of projects has previously been recommended (e.g. Pearson et al., 2006; Qiao, Soberón & Peterson, 2015). Regarding the rare species with narrow distributions, some methods have shown better results (Mi et al., 2017). Distribution models for *P. martinezii* and *P. mexicana* were constructed with the non-parametric regression Random Forest (RF) algorithm including cross validation, based on its higher performance than other methods (including MaxEnt) to predict the rare species distributions (Mi et al., 2017), and following a similar methodology as Penteriani et al. (2019), Castaño-Santamaría et al. (2019), and Barrio-Anta et al. (2020). In brief, RF constructs a set of regression and classification trees using different independent variables randomly selected from the complete data set (Breiman, 2001; Deschamps et al., 2012). To include only the main predictors shaping the species distributions (Hall, 1999), collinearity between variables was evaluated before model construction. This was performed with the open source WEKA software (Hall et al., 2009), using the wrapper methodology (Zhiwei & Xinghua, 2010) which selects the best ranked variables through the Variable Importance Measure (VIM) function (see the Results section and Table 2.2 for the list of selected variables included in final models). Variables of different scales were

Table 2.2. Environmental variables of greatest importance in the *Picea martinezii* and *Picea mexicana* distribution models for México and the world at 30 arc-second, as indicated by the percentage of normalized importance of the Variable Importance Measure (VIM) function.

Class	**Variable**	**Description**	**Normalized importance**
Picea martinezii model for Mexico			
Topography	PRC	Profile curvature	41.89
Soil	SC	Soil organic carbon content (g kg-1)	32.46
Climate	Bio_01	Annual mean temperature (ºC)	25.65
Picea martinezii global model			
Climate	Bio_02	Mean diurnal range (mean of monthly (max temp - min temp)) (ºC)	22.90
Climate	Bio_13	Precipitation of wettest month (mm)	17.85
Climate	Bio_04	Temperature seasonality (standard deviation *100) (ºC)	17.57
Climate	Bio_07	Temperature annual range (BIO5-BIO6) (ºC)	17.48
Climate	Bio_08	Mean temperature of wettest quarter (ºC)	15.28
Climate	Bio_19	Precipitation of coldest quarter (mm)	8.93
Picea mexicana model for Mexico			
Climate	Bio_06	Min temperature of coldest month (ºC)	18.27
Topography	WI	Wetness index	13.89
Soil	SC	Soil organic carbon content (g kg-1)	13.75
Soil	BD	Bulk density of the fine earth fraction (< 2mm) (kg m^{-3})	12.71
Topography	ASP	Aspect (º)	11.89
Climate	Bio_09	Mean temperature of driest quarter (ºC)	11.77
Climate	Bio_17	Precipitation of driest quarter (mm)	9.83
Geology	Geo	Geological units	7.90
Picea mexicana global model			
Climate	Bio_04	Temperature seasonality (standard deviation *100) (ºC)	17.72
Climate	Bio_16	Precipitation of wettest quarter (mm)	15.73
Climate	Bio_08	Mean temperature of wettest quarter (ºC)	14.49
Climate	Bio_11	Mean temperature of coldest quarter (ºC)	14.09
Climate	Bio_18	Precipitation of warmest quarter (mm)	13.59
Climate	Bio_01	Annual mean temperature (ºC)	13.57
Climate	Bio_14	Precipitation of driest month (mm)	10.82

normalized following the methodology of Castaño-Santamaría et al. (2019) to make them comparable, and VIM values were expressed adding up to a unitary value (normalized importance), which can also be expressed in percentage (Table 2.2).

Spatially continuous maps were generated by applying the final models to environmental spatial variables resampled to a 30 arc-second resolution.

We used the k-fold cross validation approach (k-fold = 10) to test the precision (repeated 10 times) of the RF classifier on unseen data. This was done by dividing the data set into k subsets and using one subset as the test set and the other k-1 subsets as the training set, each time the model was applied. The accuracy of the model predictions was evaluated with the confusion matrix that shows the four-way classification of a sampled point. From this last evaluation, we calculated the following model metrics, widely used in SDM studies (Freeman & Moisen, 2008; Penteriani et al., 2019): (i) the Area Under the Receiver Operating Characteristic Curve (AUC); (ii) the Overall Accuracy (OA); (iii) Matthews Correlation Coefficient (MCC); (iv) the True Skill Statistic (TSS), (v) Cohen's Kappa, (vi) Sensitivity; and (vii) Specificity.

To map the species distributions, thresholds for the probability of presence ($PoP_{threshold}$) were selected for each species by combining two approaches: (i) the method that minimizes the difference between the absolute values of sensitivity and specificity (Jiménez-Valverde & Lobo, 2007); and (ii) the method that requires an appropriate fixed specificity (Freeman & Moisen, 2008), in this case, based on proper probability values around the thresholds obtained with the first approach (see the Results section and Table S2.5). The last approach mentioned has been recommended particularly for rare species when it is important to include all possible populations in planning. Both approaches for threshold selection were based on the evaluation of models constructed for current conditions for México (see next section) and the real species presences/absences (Table S2.5). The final $PoP_{threshold}$ values were used to map the two species distributions in all projections in the hyperspace.

2.2.3. Past, contemporary and future distributions: projections for Mexico

The fitted models were projected onto spatial projections of the most important environmental variables (Table 2.2) at a 30 arc-second resolution, for the current conditions (1961–1990 reference period) to estimate the contemporary potential distributions of these species. Additionally, the following projections were performed using the Community Atmospheric Model scenario version 4 (CCSM4): (1) to the paleoclimate data of the Last Glacial Maximum (LGM, ~22,000 thousand years ago = 22 ka) and the Middle Holocene (MH, ~6 ka); and (2) to the future periods centered on years 2050 and 2070 under two different Representative Concentration Pathways (RCPs) (IPCC, 2013): a moderate scenario (RCP 4.5), which assumes a total radiative forcing stabilized at 4.5 Wm2 by 2100; and, a pessimistic scenario (RCP 8.5) which considers a higher radiative forcing of 8.5 Wm2 by 2100. Data sets for current climatic conditions were obtained from WorldClim version 1.4 (Hijmans et al., 2005, available at URL: http://www.worldclim.com) and data of the CCSM4 from the National Center for Atmospheric Research (available at URL: https:// www.cesm.ucar.edu/models/ccsm4.0/). The equivalence in surface area considered for each projected pixel of suitable habitat was 0.7 km^2 for latitudes corresponding to the Mexican territory (between 15º to 31º LN).

2.2.4. Contemporary and future distributions: global projections

We also estimated the potential contemporary (1961–1990 reference period) and future (period centered in 2050) distributions of the suitable climate habitats at a global scale, following the previously described methodology for model construction, but considering only the 19 climatic variables available in WorldClim (Tables S2.4 and 2.2). The resolution of the climatic data was 30 arc-second, which is the highest uniform resolution available for the entire world. Projections to the future period were performed using the CCSM4. We intended global cautionary projections for the two studied tree species, by considering the most pessimistic climatic scenario (RCP8.5) in an intermediate future period. All distribution maps were created in QGIS v.3.16.1 (QGIS Development Team, 2020).

2.3. Results

2.3.1. Demographic census, area extent and exploration of new populations

Population size assessments showed 89,266 *P. martinezii* individuals (including recruitment, saplings and trees) distributed in a total area of 157.4 ha, and 39,059 *P. mexicana* individuals in a total area of 173.1 ha (Table 2.1). The largest *P. martinezii* population was Agua de Alardín with 84,498 individuals covering an area of 74.3 ha, and the smallest was La Encantada with 712 individuals and 5.2 ha. The largest population of *P. mexicana* was La Marta, with 17,728 individuals covering an area of 41.6 ha; the smallest was El Coahuilón, with 2,253 individuals scattered in an area of 49 ha (Table 2.1).

On the other hand, seven new (previously unreported) stands were discovered and explored, with numbers of individuals per stand ranging from 12 to 6,300 and areas between 0.1 and 24 ha. All new stands were located close (0.5 to 5.0 km) and at similar elevations to the previously known populations in both the Sierra Madre Oriental (SMOr) and Sierra Madre Occidental (SMOc) (Table 2.1).

2.3.2. Species distribution modelling and model assessment

The normalized importance scores of the VIM function, selected three variables as the most important predictors for *P. martinezii*, and eight variables for *P. mexicana* in the models for México (Table 2.2). In the global models, the same importance analysis selected six and seven climatic variables for *P. martinezii* and *P. mexicana*, respectively (Table 2.2).

According to the model metrics AUC, OA, MCC, TSS, Kappa, Sensitivity and Specificity with values ≥0.9, the t goodness-of-fit were highly accurate (Table S2.3). The $PoP_{threshold}$ values for *P. martinezii* and *P. mexicana* were 0.73 and 0.83, respectively, based on the sensitivity-specificity balance approach. With a probability value of 0.8 or more, the correct presence prediction was 100% for *P. martinezii* and more than 55% for *P. mexicana* (Table S2.5). Hence, based on the fixed specificity approach, all the performed projections in the hyperspace considered a $PoP_{threshold}$ of 0.8 to denote the presence (above this value) or absence (under the value) of the highly suitable habitat. However, to show the area holding less suitable habitat, a minimum probability of presence of 0.5 was considered too. Finally, two categories of presence were used for displaying the results: 0.5–0.79 = intermediate and 0.8–1.0 = high.

2.3.3. Mapping suitable abiotic niche: projections for Mexico and the world

Projections of suitable habitat during the Last Glacial Maximum (LGM) and the Middle Holocene (MH) indicate overall a very small and fragmented distribution for both species. In particular for *P. martinezii*, it was projected only 15.4 km^2 (probability ≥ 0.5) during the LGM, and 12.6 km^2 (none of them with $p \geq 0.8$) for the MH (Fig. 2.2); all those pixels were found highly scattered, mainly at the Trans Mexican Neovolcanic Axis (Central México) and at the Sierra Madre Oriental, close to the contemporary distribution (near the border of Nuevo León and Tamaulipas states) (Figs. 2.3A and 2.3B). *Picea mexicana* had a maximum of 423.5 km^2 of suitable habitat ($p \geq 0.5$) during the LGM (Fig. 2.2), mostly at the Trans-Mexican Neovolcanic Axis, with an important area at northwest of Veracruz state, Sierra Madre Oriental (Fig. 23C, a, b, c), an area where the species is completely absent today. It is interesting to notice that suitable habitat of *P. mexicana* was absent at the Sierra Madre Occidental during the LGM (Fig. 2.3C), and then was present during the MH (Fig. 2.3D, a-d), around the place where the contemporary population of El Mohinora is located (Fig. 2.3D, c).

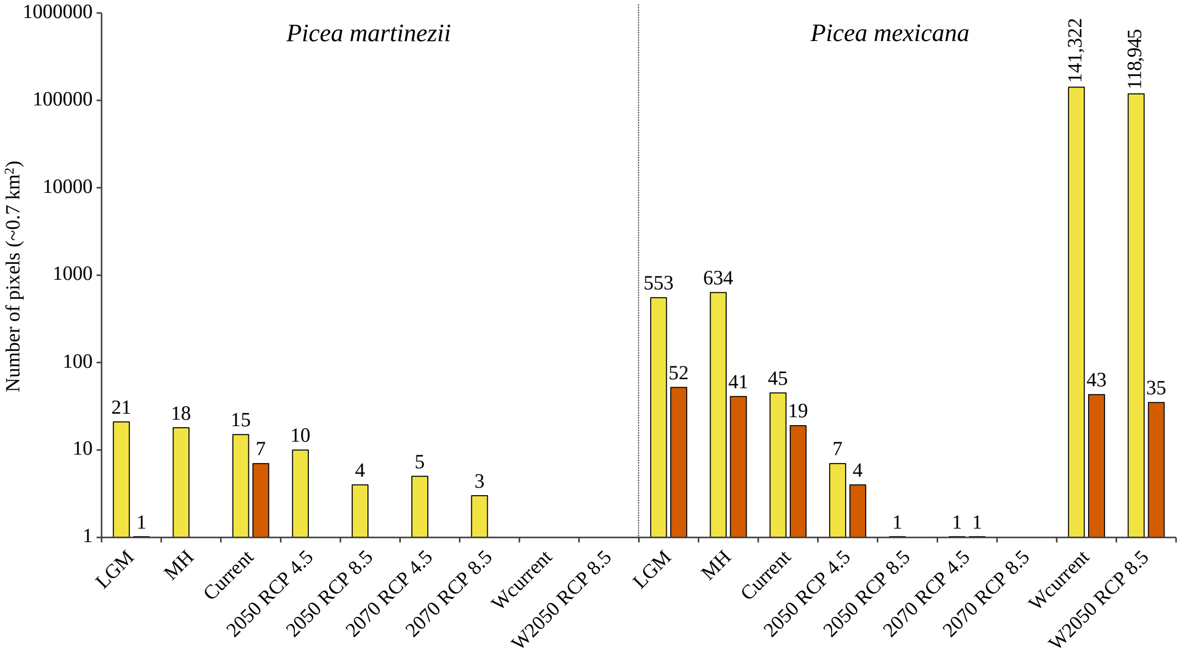

Figure 2.2. Projected area of suitable habitat for *P. martinezii* and *P. mexicana* in Mexico and the world at different times. Projections for Mexico, made after modeling considering climate, topographic, soil and geological variables, included the Last Glacial Maximum (LGM, ~22 thousand years ago = ka), Middle Holocene (MH, ~6 ka), current (1961-1990 period), and future time (decades 2050 and 2070, both with RCP 4.5 and RCP 8.5). Worldwide projections, made after modeling using only climate variables, included the 1961-1990 period (Wcurrent) and the decade 2050-2060 with RCP 8.5 (W2050 RCP 8.5). Pixel counts for global projections excluded predicted areas for Mexico in this Figure. All periods show projections made at 30-arc second resolution and pixels with probabilities of occurrence of 0.5-0.79 (yellow) and 0.8-1.0 (vermilion). Numbers above the bars indicate the projected number of pixels.

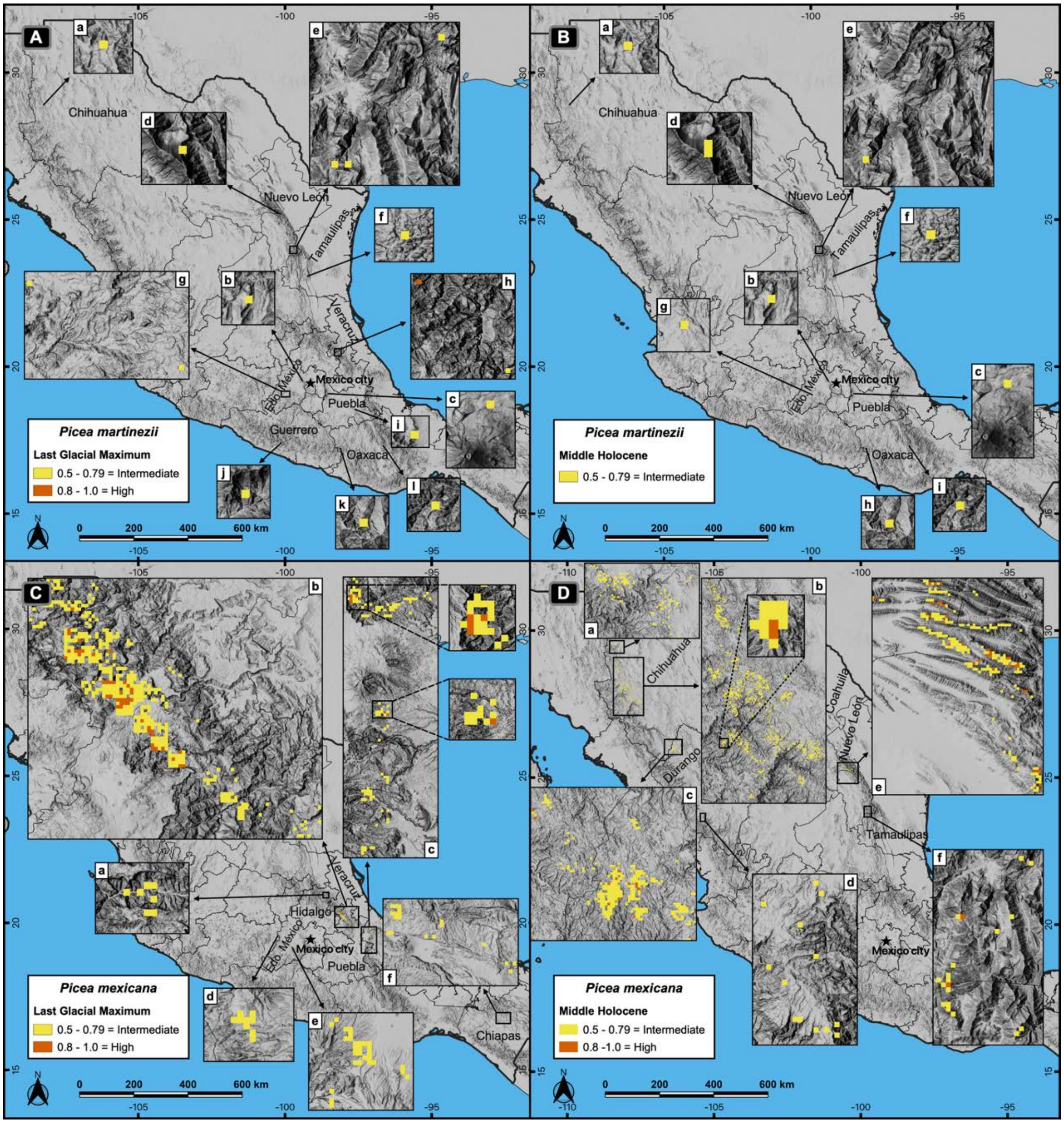

Figure 2.3. Potential distribution of the habitat for *Picea martinezii* and *P. mexicana* in the Last Glacial Maximum (~22 thousand years ago = ka) (A and C) and the Middle Holocene (~6 ka) (B and D). The Last Glacial Maximum projection for *P. martinezii* shows the less suitable habitat on the Mexican territory (A, panels a to l), with only one pixel holding the highly suitable habitat in the northwest of Veracruz state (A, panel h); the Middle Holocene projection shows the distribution of the less suitable areas (B panels a to i). For *P. mexicana*, the Last Glacial Maximum projection shows suitable areas in the center of Mexico at northeast of Hidalgo state (C, panel a), borders of Puebla and Hidalgo states (C, panel b), and west of Veracruz state (C, panel c), with some less suitable areas in Estado de México (C panels d and e) and Chiapas states (C, panel f); the Middle Holocene projection shows suitable areas in northern Mexico, at north (D, panel a) and center (D panel b) of Chihuahua, borders of Chihuahua and Durango states (D panel c), south of Durango (D panel d), borders of Coahuila and Nuevo León states (D panel e), and south of Nuevo León state (D panel f).

Contemporary projections (reference period 1961–1990) of highly suitable habitat distribution predicted perfectly all the actual populations for both species (Figs. 2.4A and 2.4B, a, b), which represent an area of 4.9 km^2 for *P. martinezii* and 13.3 km^2 for *P. mexicana* (Fig. 2.2). Besides, the predicted highly suitable areas outside the current distribution were totally absent for both species. For *P. martinezii*, there were very few scattered isolated pixels (all with probability 0.5 to 0.79) at northwest of Chihuahua state (Fig. 2.4A, c), along the TMNVA (Fig. 2.4A, d, e, f), and at the Sierra Madre del Sur (states of Guerrero, Oaxaca and Chiapas; Fig. 2.4A, g, h, i). For *P. mexicana*, there were even less regions with predicted pixels outside its natural distribution in the SMOc and SMOr (all of them with probabilities 0.5 to 0.79; Fig. 2.4B, c, d).

Projections of suitable habitat to the future within México, under climatic change scenarios, indicate a severe reduction for both species' habitats (Figs. 2.2 and 2.5). Suitable habitat ($p \geq 0.5$) predicted for *P. martinezii* drops from a total of 15.4 km^2 at present, to only 7.0 and 2.8 km^2 for 2050, scenarios RCP 4.5 and 8.5, respectively. For year 2070, the drop is even further, to have just 3.5 and 2.1 km^2 for scenarios RCP 4.5 and 8.5, respectively (Fig. 2.2). And still worse: by 2050, the highly suitable habitat ($p \geq 0.8$) disappears completely at and nearby all the current contemporary *P. martinezii* populations (Fig. 2.5A). For *P. mexicana*, projections show an even worse situation, with 0.7 km^2 (a single pixel) for year 2050 RCP 8.5 and 1.4 km^2 for year 2070 RCP 4.5; there is not a single pixel predicted for 2070 RCP 8.5 inside México (Figs. 2.2, 2.5C and 52.D).

Worldwide projections for *P. martinezii*, show similar and extremely grim scenarios, either for contemporary climate and for 2050 RCP 8.5: there is not a single pixel available outside México, but some areas in the Mexican territory, few of which will remain in the future (Figs. 2.2 and 2.6).

Worldwide projections for *P. mexicana* show a quite different and complex picture outside México: a total of about 141,000 pixels appears suitable for contemporary climate, and nearly 119,000 pixels for year 2050 RCP 8.5 (Figs. 2.2 and 2.7). Thus, despite the drastic contraction or even vanishing of suitable area for

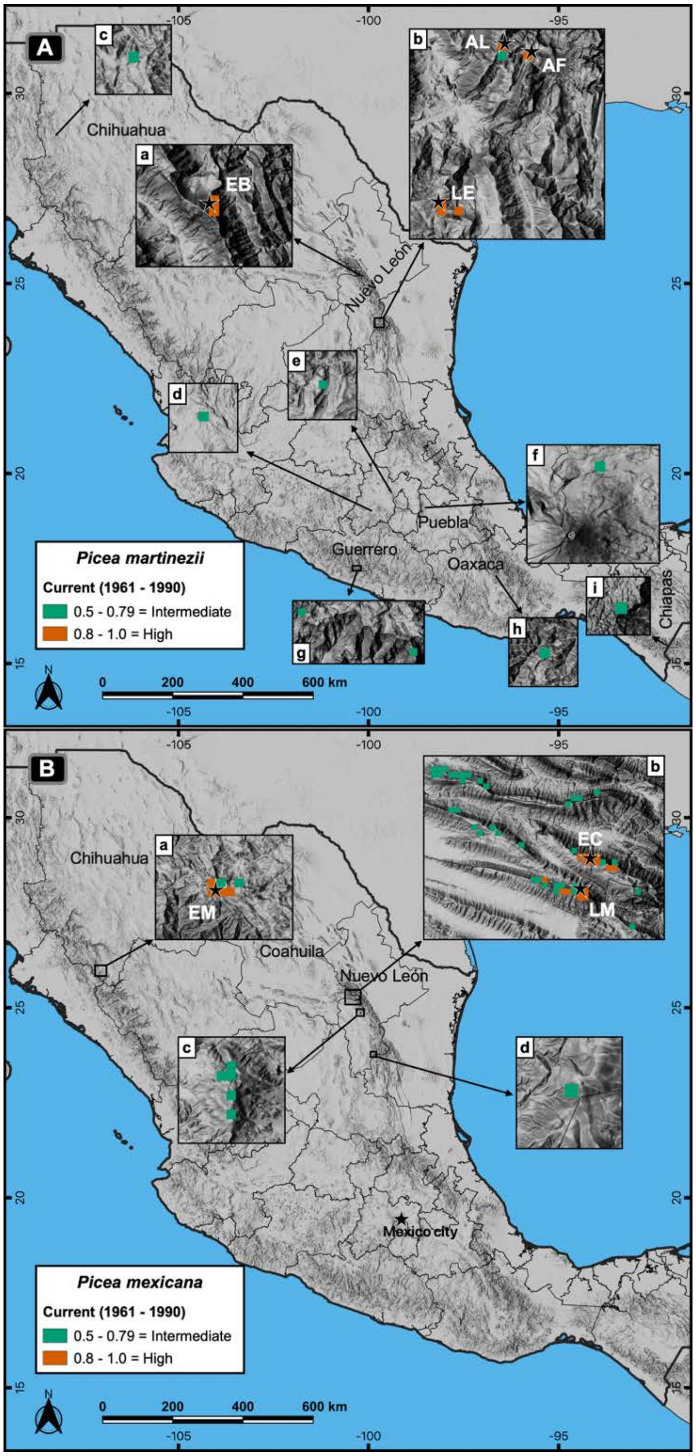

Figure 2.4. Actual and potential distribution of the suitable habitat in current conditions (1961-1990 reference period) for *P. martinezii* (A) and *P. mexicana* (B). Projection for *P. martinezii* shows the highly suitable habitat (probability ≥ 0.8) restricted to the natural populations (EB = El Butano, AL = Agua de Alardín, AF = Agua Fría, and LE = La Encantada) in the center west (A-a) and southeast (A-b) of Nuevo León state, and less suitable areas (probability 0.5 to 0.79) spread in northern (A-c) and central (A-d to A-i) Mexico. Projection for *P. mexicana* shows the presence of the highly suitable habitat restricted to the natural populations (EM = El Mohinora, EC = El Coahuilón, and LM = La Marta) (B-a and B-b), and less suitable areas near the populations at center (B-c) and south (B-d) of Nuevo León state.

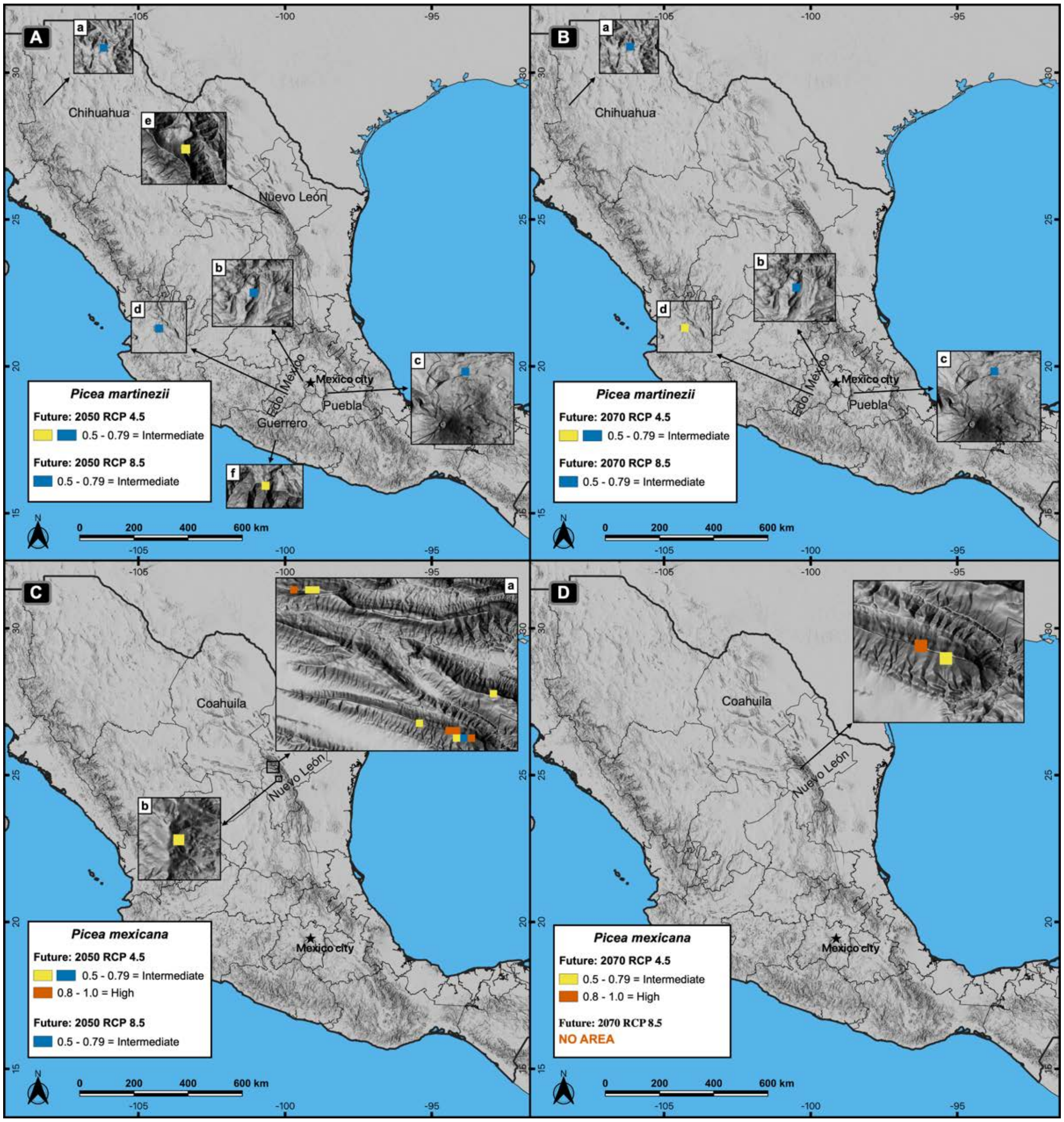

Figure 2.5. Potential distribution of the habitat (modeling based on climate, topography, soil and geological variables) for *Picea martinezii* (A-B) and *P. mexicana* (C-D) for 2050 and 2070, in both the moderate (RCP 4.5) and pessimistic (RCP 8.5) scenarios. Blue pixels shared by RCP 4.5 and 8.5, mean that the same pixel is projected as suitable in both scenarios. The 2050 (A) and 2070 (B) projections for *P. martinezii* show the spread distribution of the less suitable habitat in the Mexican territory, with more area in the moderate (A-a to A-f in 2050; B-a to B-d in 2070) than in the pessimistic (A-a to A-d in 2050; B-a to B-c in 2070) scenarios, and only three pixels of the less suitable habitat remaining by 2070 in the pessimistic scenario at the west of Chihuahua (B-a), center of Estado de México (B-b) and Estado de México-Puebla border (B-c); these three sites are present in all projections for *P. martinezii* from current to future conditions. The 2050 (C) and 2070 (D) projections for *P. mexicana* show the highly suitable habitat only in the moderate scenario, on and near the current populations of La Marta and El Coahuilón in the Coahuila-Nuevo León border (C-a and D) and center of Nuevo León (C-b); in the pessimistic scenario, only a pixel with the less suitable habitat in 2050 (C-b) and no area at all (highly nor less suitable habitat) in 2070 (D).

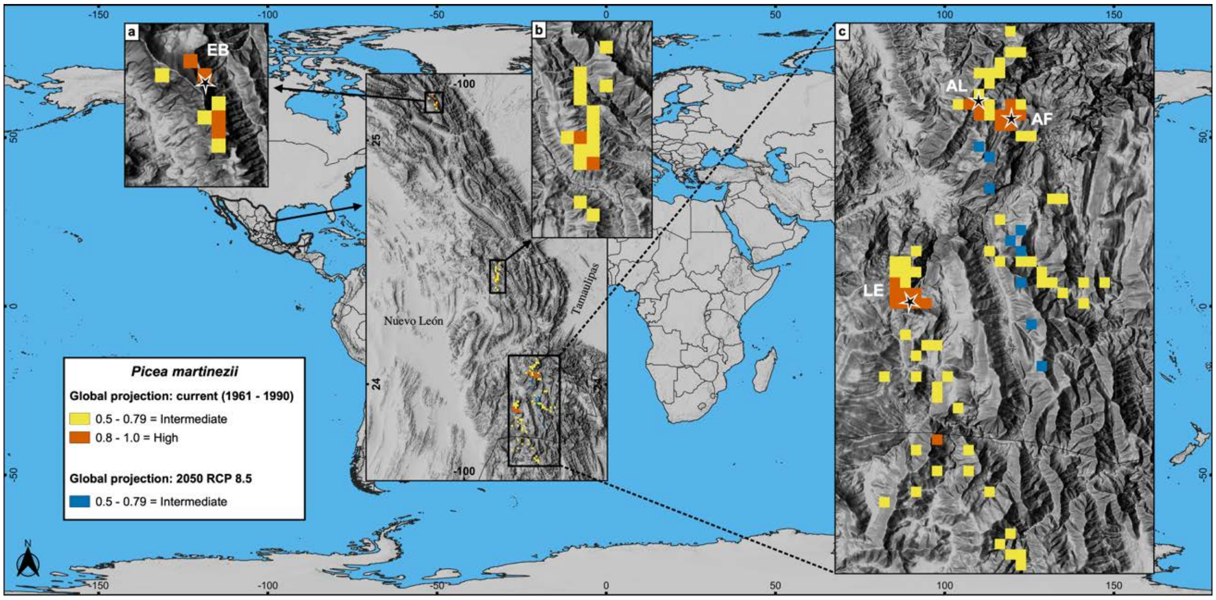

Figure 2.6. Global projections of the potential distribution of the climatically suitable habitat (based only on climatic variables) for *Picea martinezii* in current conditions (1961-1990 reference period) and for 2050 (pessimistic scenario). All sites for the contemporary and future projections are located in Mexico, on and around the current natural populations of El Butano (EB) at the Coahuila-Nuevo León border (a), the center of Nuevo León state (b), and at Agua de Alardín (AL), Agua Fría (AF) and La Encantada (LE) which are at southern Nuevo León state, where a reduced area holding the less suitable habitat will remain by 2050 (c, blue pixels).

P. mexicana in México (when modeling considered climate, soil, geology and topography; Figs. 2.5C and 2.5D), worldwide projections (considering only climate) indicate that there is and there will be suitable climatic habitat ($p \geq 0.5$) in: Laurentian mountains in Canada, Appalachian Mountains in USA, the Andes in south Chile, the Pyrenees in the Spain-France border, the Alps in the France-Switzerland border, the Caucasus mountains at Georgia-Russia border, Khingan mountains in China, Sayan mountains in the Mongolia-Russia border, The Himalayans in India, Sobaek and Taebaek mountains in South Korea, Taebaek mountains in North Korea, the Japanese Alps in Japan, Chungyang mountains in Taiwan, and the Southern Alps in New Zealand (Fig. 2.7). For the projected year 2050 RCP 8.5, a large proportion of those suitable areas under contemporary climate will remain for *P. mexicana* in all regions, except in México, the Appalachian Mountains in USA and the Pyrenees in the Spain-France border (Figs. 2.2 and 2.7B). Outside México, the highly suitable habitat ($p \geq 0.8$) for *P. mexicana* is currently available only in The Himalayans in India (Fig. 2.7A, j) and the

Chungyang mountains in Taiwan (Fig. 2.7A, I), where 10 pixels (about 6.4 km^2; Fig. 2.7B, g) and 25 pixels (about 18.0 km^2; Fig. 2.7B, i), respectively, will remain by 2050.

2.4. Discussion

2.4.1. Demographic census, area extent and exploration of new populations

The results of the first complete population size assessments, as well as better knowledge of population areas and delimitations, provide the basis for the monitoring and management of the two studied species, and therefore, are important for conservationists, local communities, stakeholders and governmental institutions. The seven new natural stands located by field surveys, showed that these species thrive only on very specific habitats at similar elevations than the previously known populations, both in the Sierra Madre Oriental and Sierra Madre Occidental. These new stands may be portions of the nearest known populations, as suggested by their proximity of 0.5 to 5.0 km to each other. Therefore, the total number of populations can be considered as previously reported for these spruce species: four populations of *P. martinezii* and three populations of *P. mexicana* (Ledig et al., 2000a). However, the new stand located at 5.0 km from La Marta, and holding ~6,300 individuals (La Marta 2; Table 2.1), could be considered a fourth sub-population of P. mexicana, although this hypothesis remains to be confirmed by genetic analysis.

Population size assessment of *P. martinezii* (e.g., populations of La Encantada and Agua de Alardín) and *P. mexicana* (e.g., populations of La Marta 3 and La Marta) (Table 2.1) support the findings of Murray & Lepschi (2004), who reported that rare species could be sparse or abundant in different locations. These dissimilar population sizes and their area extents, allowed us to identify the stands which are more prone to local extinction, given their reduced number of individuals (Table 2.1). Overall, the total number of individuals of *P. martinezii* and *P. mexicana*, the new reported stands and the total area occupied by both species confirm their status as rare species (Table 2.1). According to McCune (2016), both species could be classified as extremely rare (i.e. with less than five known populations); according to Rabinowitz (1981) both correspond to rare species that are locally abundant in specific habitats but restricted geographically, or sparse and geographically restricted in specific habitats.

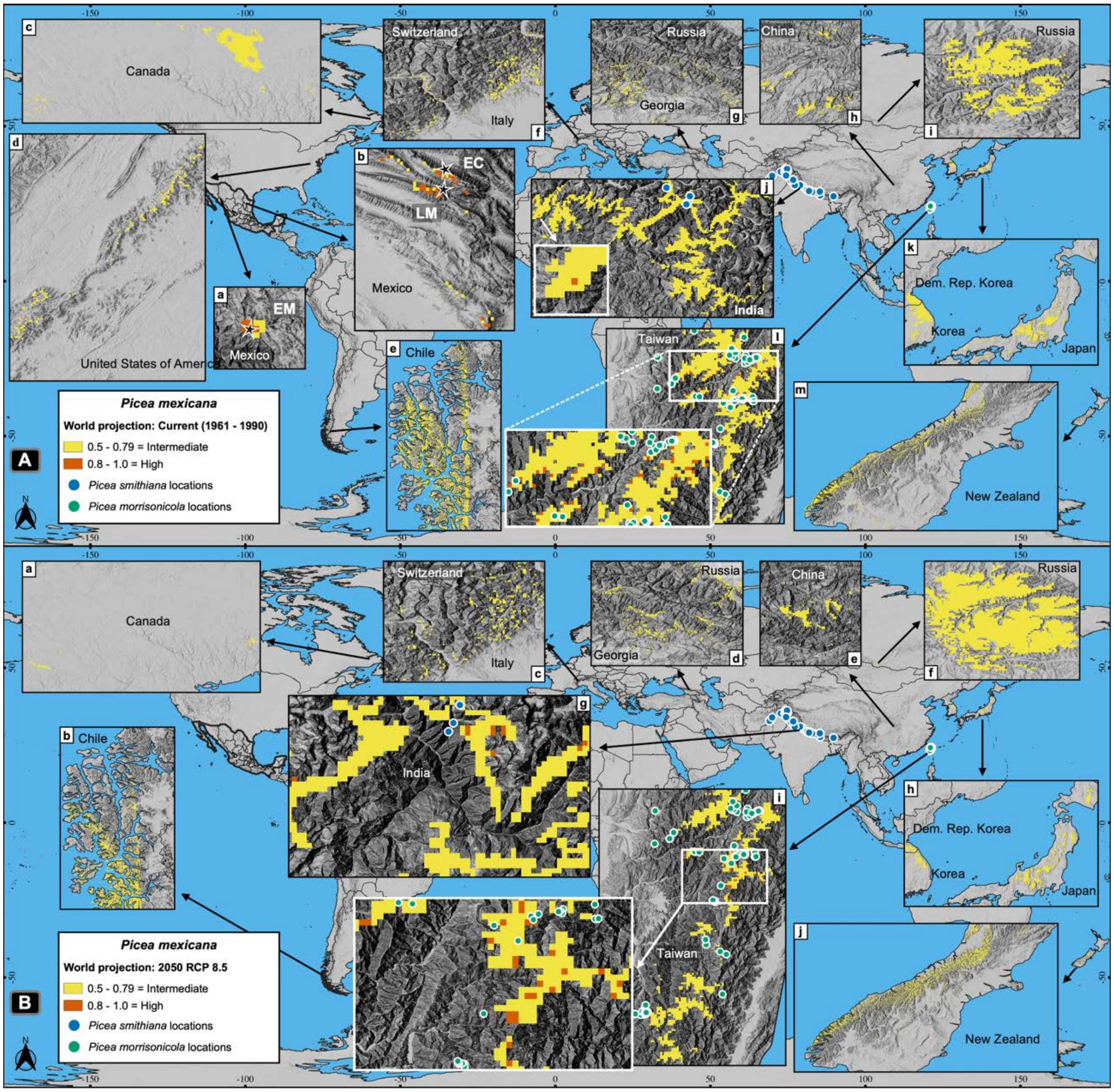

Figure 2.7. Global projections of the potential distribution of the climatically suitable habitat for *Picea mexicana* in contemporary conditions (A) and 2050 (B). All sites for the contemporary projection are located in many mountainous regions of the world, such as: Sierra Madre Occidental (A-a), Sierra Madre Oriental (A-b), Laurentian mountains (A-c), Appalachian Mountains (A-d), southern Andes (A-e), The Pyrenees (not shown), The Alps (A-f), the Caucasus mountains (A-g), Khingan mountains (A-h), Sayan mountains (A-i), The Himalayans (A-j), Sobaek-Taebaek mountains (A-k), the Japanese Alps (A-k), Chungyang mountains (A-l) and the Southern Alps (A-m), where the habitat will remain by 2050, except for Mexico, the Appalachian Mountains and the Pyrenees. The highly suitable habitat by 2050 outside Mexico coincide with the geographical distribution of other endemic spruce species: *Picea smithiana* (blue circles) on The Himalayans in India (A-j and B-g) and *P. morrisonicola* (yellow circles) on Chungyan mountains in Taiwan (A-l and B-i). Contemporary projection (A) shows the natural populations of El Mohinora (EM), La Marta (LM) and El Coahuilón (EC). Occurrences of *P. smithiana* and *P. morrisonicola* were obtained from the Global Biodiversity Information Facility (GBIF, 2021).

2.4.2. Species niche requirements, distribution modelling and model assessment

The results of predictors selection by the VIM function highlight the importance of not only taking into account climatic variables to construct the distribution models for *P. martinezii* and *P. mexicana* (Table 2.2), thus adding to the previous knowledge of the main factors underlying the distribution of the same species (Ledig et al., 2010). According to Sexton et al. (2009), species distributions depend on many environmental factors, and it is known that while some environmental variables represent large scale processes (macro- environment), others define the micro-environmental conditions (Franklin, 2009). In the present study, models constructed for México showed that both micro- and macro-environmental variables may be the factors with the greatest influence on species distributions. For *P. martinezii* the most important predictor was the profile curvature (Table 2.2), which is a proxy of the microenvironment related to groundwater availability. For *P. mexicana* the main factor was minimum temperature of coldest month (Table 2.2), a macro-environmental variable indicating winter severity, and probably related to its adaptation to cold subalpine zones. Similar results to those observed for *P. martinezii* were reported for the rare species *Hesperocyparis forbesii* (Jeps.) Bartel (Tecate cypress), where two proxies of the micro-environment (a topographic and a soil variable) were the most important predictors of the species distribution (Regan et al., 2012). On the other hand, the results obtained for *P. mexicana* were similar to those reported for other widespread forest species, for which similar sets of environmental variables were used and the main drivers of species distributions were found to be climatic variables (Penteriani et al., 2019). However, there were soil and topographic variables among the most important predictors for *P. mexicana*.

Regarding the model metrics, some authors have prevented that the AUC tends to increase when the calibration areas are larger and further from presence records (Lobo, Jiménez-Valverde & Real, 2008; Hijmans, 2012), making preferable to consider additional model evaluators like sensitivity, specificity (Lobo, Jiménez-Valverde & Real, 2008), or TSS which accounts for both omission and commission errors and is not influenced by the sample size of each class (Allouche, Tsoar & Kadmon, 2006; Wehenkel et al., 2020).

The high values of the seven metrics used to evaluate our regional and global models (including AUC, sensitivity, specificity and TSS; Table S2.3), suggested acceptable model performances. Lower model performances have been reported for different widespread species and similar sets of environmental variables and model metrics (Penteriani et al., 2019; López-Sánchez et al., 2021), or only climate variables and AUC (Dyderski et al., 2018). Nevertheless, similar results (only for AUC) have been obtained for *Picea chihuahuana* Martínez (Pinedo-Alvarez et al., 2019), another rare Mexican spruce. The overall better model performance for rare than for widespread species, observed in the comparable metrics, can be explained in part by the reliance on the spatially restricted environmental conditions of the former (McCune, 2016).

Regarding the worldwide projections, to our knowledge, this is the first time that a global study of the suitable climate habitat for a plant species has been carried out; but, similar AUC values have been reported by Taucare-Ríos, Bizama & Bustamante (2016) for an animal species.

Additionally to their high metrics, the regional models (developed with climate, soil and topographic variables) matched with observations in the field (Fig. 2.4), while global models (developed with climate variables) almost matched with observations on terrain (Figs. 2.6 and 2.7A) and identified highly suitable patches where other endemic spruces and similar tree communities thrive in different biogeographic regions (Lin et al., 2012; Bodare et al., 2013; Panthi et al., 2017) (Fig. 2.7).

Ledig et al. (2010) made habitat projections for the same two spruce species by using only climatic variables and a threshold of presence ≥ 0.5, and marked several sites near the natural populations with high potential to hold *P. martinezii* (e.g. a large area at the northwest of El Butano population, at the Coahuila-Nuevo León states border, México) and *P. mexicana* (e.g. mountains Cerro San Rafael, Cerro Potrero de Ábrego and Cerro El Potosí, all above 3,000 m of elevation, in Coahuila and Nuevo León states, México), but where this species does not occur at present. Our models showed that no habitat was available in any site near the current populations of *P. martinezii* using a $\mathrm{PoP_{threshold}} \geq 0.5$ (Fig. 2.4A), as confirmed by field observations. For P. mexicana, our models coincide with the projections of Ledig et al. (2010), for the sites mentioned before, at a $\text{PoP}_{\text{threshold}} \geq 0.5$; however, the absences of *P. mexicana* on those mountains confirmed by field surveys were correctly predicted by our models at a $\text{PoP}_{\text{threshold}} \geq 0.8$, which indicate that the more accurate probability of presence of *P. mexicana* is above this threshold.

2.4.3. Mapping suitable habitat: past and contemporary distributions

The reduced and scattered distributions of the highly suitable habitat for both species during the Last Glacial Maximum, the Middle Holocene and at present (Figs. 2.3 and 2.4), indicate that these species have had restricted habitat for a long period of time, and have survived in climatic refugia. These refugia appear to have been more widespread in the past for *P. mexicana* than for *P. martinezii*. Apparently, the main refugia in the past for *P. mexicana* was at south and central México (Fig. 2.3C). From that high elevations and rugged regions, likely *P. mexicana* migrated to its current locations. That is consistent with pollen records which suggest that members of the *Picea* genus reached the north of Chiapas and the south of Veracruz in the Miocene in southern México (Graham, 1976; Rzedowski, 2006) and the Texcoco lake in central México (bordering with what is today México city) in the Last Glacial Maximum (Lozano-García et al., 1993); although it would be discarded that such *Picea* pollen might belong to *P. martinezii* because of the very scattered marginal areas in the LGM and MH (Fig. 2.3A and 2.3B), it cannot be discarded for *P. chihuahuana* (Pinedo-Alvarez et al., 2019).

2.4.4. Potential for future assisted migration: projections for Mexico and worldwide

Future projections of suitable habitat show an extremely grim situation for both species inside México; which could imply the loss of all natural populations (including recruitment, saplings and trees). For *P. martinezii*, there will be available only a few square kilometers of marginal habitat, and none of them near the current populations when considering climate, soil, geologic and topographic variables for modeling (Fig. 2.5B); however, more areas holding the suitable climatic habitat (considering only climate) will be available near the populations of Agua de Alardín, Agua Fría and La Encantada (Fig. 2.6, c). The reason why more areas appear available inside México, all close to the contemporary populations, is because the global modeling is more inclusive, by considering only climatic variables and excluding the proxies of micro-environment.

As for *P. mexicana*, since it is anticipated a complete loss of the highly suitable habitat in México by 2050 (both considering climate, soil, geology and topography, or only climate), it is needed to take into account the possibility of assisted migration outside the Mexican borders, either to the zones holding the marginal (p = 0.5–0.79) or the highly suitable habitat (p ≥ 0.8) (Fig. 2.7). However, the two zones holding the highly suitable habitat, i.e., Chungyang mountains in Taiwan and the Indian Himalayan region, are inhabited by other two endemic spruce species: *Picea morrisonicola* (Bodare et al., 2013) and *Picea smithiana* (Panthi et al., 2017), respectively (Fig. 2.7B, g, i).

Based on this, assisted migration would be an option for *P. martinezii* only on marginal sites in México (p = 0.5–0.79; Fig. 2.5B, a, b, c; and Fig. 2.6, c). For *P. mexicana*, a possibility would be continental and transcontinental translocations to sites holding the marginal or the highly suitable habitat (Fig. 2.7B). This rises two conservation issues: the first is related to whether or not perform assisted migration to marginal sites which do not cover the main habitat requirements for the species; the second is related to which species (the local or the foreign) should be prioritized for conservation when suitable habitat is found elsewhere but is inhabited by similar endemic species.

We argue that priority for assisted migration should be given to areas with the least potential negative impact on other local spruce species or their close relatives. Such negative implications could be: (i) the unintended introduction of pests and diseases (Simler et al., 2019) or, (ii) introgression through unintended pollen dispersal (Gómez et al., 2015). Therefore, two essential issues should be considered in potential assisted migration projects: (i) a detailed examination of the targeted suitable areas, as well as analyzing the potential impact of physiography and local-scale edaphic variables, as suggested by Wang et al. (2019) for Spiranthes parksii Correl and by Wilson, Roberts & Reid (2011) for Margaritifera margaritifera (L.); and (ii) the costs and benefits balance of this kind of projects, such as the potential negative effects that introduced species could have over local species.

This results give insights of a future biodiversity scenario, where REPS will rely on translocations beyond their native ranges (including across country borders) to subsist in nature, considering the global amount of rare species and their vulnerabilities (Işik, 2011; Enquist et al., 2019). Therefore, new mechanisms of international cooperation need to be discussed to deal with this expected crisis triggered by climatic change (Román-Palacios & Wiens, 2020; Brodie et al., 2021; McDonald & McCormack, 2021).

In this sense, international bodies such as the International Union for Conservation of Nature, the Convention on Biological Diversity, the Ramsar Convention on Wetlands, the Man and Biosphere Programme, or the United Nations Convention to Combat Desertification, would be valuable institutions in providing recommendations, guidelines, and mechanisms to reduce the loss of biodiversity, weighting the risks involved in potential assisted migration projects across country borders (Schwartz et al., 2012; Brodie et al., 2021). In general, such instruments of conservation should be based on the ecological, ethical and social implications and the cost-benefit balance that species translocations beyond nations' borders would imply (IUCN/SSC, 2013), considering the scientific, government and stakeholders support as well as local people acceptance (Pérez et al., 2012).

Finally, a parallel strategy of *in situ* conservation should not be discarded as recommended by the Global Strategy for Plant Conservation (available at URL: https://www.cbd.int/gspc/), considering the possibilities for permanence in local microrefugia (Dawson et al., 2011), as reported for other rare plant species (Patsiou et al., 2014). Such *in situ* conservation activities could include the following: (i) protection of natural recruitment against livestock, plagues, illegal logging, and wildfire, (ii) establishment of artificial recruitment with autochthonous genotypes in well-selected sites near (but not within) the respective population, (iii) removal of competing vegetation (including other tree species) in the vicinity of the natural stands, (iv) assisting biotic dispersal vectors, and (v) monitoring the existing in situ populations (Wehenkel & Sáenz- Romero, 2012).

2.4.5 Potential limitations of the study

Our distribution models included only a set of climate, soil, geologic and topographic environmental factors, with more factors in models for México (42 variables) than for worldwide models (19 climate variables) (Table S2.4). By sampling only abiotic factors, we were not able to search for future suitable areas based on biotic factors like the amount of genetic differentiation among populations, that result in differential degrees of local adaptation (Benito-Garzón, Robson & Hampe, 2019; Zhao et al., 2020) or biotic interactions (Godsoe et al., 2017; Flores-Tolentino et al., 2020), due to the lack of such data for these species.

On the other hand, some new stands of the studied spruces occupy areas as small as 0.1 ha (Table 2.1), similar to microrefugia. As detection of such very small suitable areas depends on grid resolution (Franklin et al., 2013; Patsiou et al., 2014), our models could not identify these microhabitats outside the species ranges, with a resolution of 30 arc-seconds (≈0.7 km^2 or 70 ha). Moreover, SDMs are highly susceptible to produce different results in their geographic projections, and future suitable areas for the species will depend on the used method (Pearson et al., 2006; Qiao, Soberón & Peterson, 2015). Hence, we cannot exclude that there are or will be even better models. However, the findings of potential areas for *P. mexicana* in zones with very similar spruce forest communities in Asia is a good indicator of the quality of these models.

2.4.6. Conclusion

Our findings confirm that *Picea martinezii* and *Picea mexicana* are narrow endemics with varying populations sizes, but viable total populations (Table 2.1), and suggest that the habitats of both tree species were limited since the last glacial age (Figs. 2.2 and 2.3). Considering the surface areas holding the highly suitable habitats ($p \geq 0.8$), contemporary conditions appear to be more suitable than conditions during the Last Glacial Maximum and Middle Holocene for *P. martinezii*; and vice versa for *P. mexicana* (Fig. 2.2). Current highly suitable areas will mostly disappear in the near future (Figs. 2.2 and 2.5). For *Picea martinezii*, the possibility for future assisted migration in northern and central México is only on marginal sites ($p = 0.5$–0.79), all far from its current distribution. Regarding *Picea mexicana*, a complete disappearance of the suitable habitat within México is anticipated; hence, it is needed to discuss the possibility of species translocations beyond the national borders of México, to sites holding the intermediated (p = 0.5–0.79) or the highly ($p \geq 0.8$) suitable climatic habitat, even considering sites so far away as The Himalayans or Taiwan. In the expected stage where species translocations will become necessary to avoid the high extinction rates because of climate change, new mechanisms of international cooperation need to be discussed. In this sense, institutions similar to the International Union for Conservation of Nature, the Convention on Biological Diversity, the Ramsar Convention on Wetlands, the Man and Biosphere Programme, or the United Nations Convention to Combat Desertification would promote this international collaboration and set guidelines and recommendations in assisted migration projects. Meanwhile, in situ conservation should not be discarded, considering marginal microhabitat sites. Future decisions for *ex situ* conservation would be reinforced with data from common garden assays displaying the species' resilience to environmental gradients.

2.5. Additional information and declarations

Funding

The study reported in this paper is an undertaking of the Forest Genetic Resources Working Group, of the North American Forestry Commission. Funding was provided by the Mexican Council of Science and Technology and the Mexican National Forest Commission Joint Fund (CONACyT-CONAFOR-2017-4-292615) to Christian Wehenkel. CONACyT granted graduate fellowships to Eduardo Mendoza-Maya (349164) and Erika Gómez-Pineda (339216). There was no additional external funding received for this study. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Grant Disclosures

The following grant information was disclosed by the authors:
Forest Genetic Resources Working Group.
Mexican Council of Science and Technology and the Mexican National Forest Commission Joint Fund: CONACyT-CONAFOR-2017-4-292615.

Eduardo Mendoza-Maya: 349164.
Erika Gómez-Pineda: 339216.

Competing interest

Christian Wehenkel is an Academic Editor for PeerJ.

Author contributions

- Eduardo Mendoza-Maya conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Erika Gómez-Pineda analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- Cuauhtémoc Sáenz-Romero analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- José Ciro Hernández-Díaz analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Carlos A. López-Sánchez performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.
- J. Jesús Vargas-Hernández analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- José Ángel Prieto-Ruíz analyzed the data, authored or reviewed drafts of the article, and approved the final draft.
- Christian Wehenkel conceived and designed the experiments, performed the
- experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the article, and approved the final draft.

Data availability

The following information was supplied regarding data availability: The raw data to construct the distribution models are available in the Tables S2.1 and S2.2 (presences records); the repository of the absences records are available in the Materials & Methods.

Supplemental Information

Supplemental information for this article can be found online at http://dx.doi.org/10.7717/ peerj.13812#supplemental-information.

2.6. References

Allouche O, Tsoar A, Kadmon R. 2006. Assessing the accuracy of species distribution models: prevalence, kappa and the true skill statistic (TSS). *Journal of Applied Ecology* 43(6):1223–1232 DOI 10.1111/j.1365-2664.2006.01214.x.

Barrio-Anta M, Castedo-Dorado F, Cámara-Obregón A, López-Sánchez CA. 2020. Predicting current and future suitable habitat and productivity for Atlantic populations of maritime pine (*Pinus pinaster* Aiton) in Spain. *Annals of Forest Science* 77(2):41 DOI 10.1007/s13595-020-00941-5.

Benito-Garzón M, Robson TM, Hampe A. 2019. ΔTraitSDMs: species distribution models that account for local adaptation and phenotypic plasticity. *New Phytologist* 222(4):1757–1765 DOI 10.1111/nph.15716.

Bodare S, Stocks M, Yang JC, Lascoux M. 2013. Origin and demographic history of the endemic Taiwan spruce (Picea morrisonicola). *Ecology and Evolution* 3:3320–3333 DOI 10.1002/ece3.698.

Bosso L, Luchi N, Maresi G, Cristinzio G, Smeraldo S, Russo D. 2017. Predicting current and future disease outbreaks of *Diplodia sapinea* shoot blight in Italy: species distribution models as a tool for forest management planning. *Forest Ecology and Management* 400(5):655–664 DOI 10.1016/j.foreco.2017.06.044.

Breiman L. 2001. Random Forests. *Machine Learning* 45(1):5–32 DOI 10.1023/A:1010933404324.

Brodie JF, Lieberman S, Moehrenschlager A, Redford KH, Rodríguez JP, Schwartz M, Seddon PJ, Watson JEM. 2021. Global policy for assisted colonization of species. *Science* 372(6541):456–458 DOI 10.1126/science.abg0532.

Butt N, Chauvenet ALM, Adams VM, Beger M, Gallagher RV, Shanahan DF, Ward M, Watson JEM, Possingham HP. 2021. Importance of species translocations under rapid climate change. *Conservation Biology* 35(3):775–783 DOI 10.1111/cobi.13643.

Castaño-Santamaría J, López-Sánchez CA, Ramón-Obeso J, Barrio-Anta M. 2019. Modelling and mapping beech forest distribution and site productivity under different climate change scenarios in the Cantabrian Range (North-western Spain). *Forest Ecology and Management* 450(4):117488 DOI 10.1016/j.foreco.2019.117488.

Cogoni D, Sulis E, Bacchetta G, Fenu G. 2019. The unpredictable fate of the single population of a threatened narrow endemic Mediterranean plant. *Biodiversity and Conservation* 28(7):1799–1813 DOI 10.1007/s10531-019-01757-0.

CONAFOR. 2009. Inventario Nacional Forestal y de Suelos. *Available at https://www.cnf.gob.mx: 8443/snif/portal/infys/temas/resultados-2004-2009* (accessed 14 November 2019).

Crain BJ, Sánchez-Cuervo AM, White JW, Steinberg SJ. 2015. Conservation ecology of rare plants within complex local habitat networks. *Oryx* 49(4):696–703 DOI 10.1017/S0030605313001245.

Dawson TP, Jackson ST, House JI, Prentice IC, Mace GM. 2011. Beyond predictions: biodiversity conservation in a changing climate. *Science* 332:53–58 DOI 10.1126/science.1200303.

Deschamps B, McNairn H, Shang J, Jiao X. 2012. Towards operational radar-only crop type classification: comparison of a traditional decision tree with a random forest classifier. *Canadian Journal of Remote Sensing* 38(1):60–68 DOI 10.5589/m12-012.

Dyderski MK, Paź S, Frelich LE, Jagodziński AM. 2018. How much does climate change threaten European forest tree species distributions? *Global Change Biology* 24(3):1150–1163 DOI 10.1111/gcb.13925.

Enquist BJ, Feng X, Boyle B, Maitner B, Newman EA, Jørgensen PM, Roehrdanz PR, Thiers BM, Burger JR, Corlett RT, Couvreur TLP, Dauby G, Donoghue JC, Foden W, Lovett JC, Marquet PA, Merow C, Midgley G, Morueta-Holme N, Neves DM, Oliveira-Filho AT, Kraft NJB, Park DS, Peet RK, Pillet M, Serra-Diaz JM, Sandel B, Schildhauer M, Šímová I, Violle C, Wieringa JJ, Wiser SK, Hannah L, Svenning JC, McGill BJ. 2019. The commonness of rarity: global and future distribution of rarity across land plants. *Science Advances* 5(11):1–13 DOI 10.1126/sciadv.aaz0414.

ESRI. 2018. Topographic basemap. Scale Not Given. World Topographic Map. *Available at http:// www.arcgis.com/home/item.html?id=30e5fe3149c34df1ba*

922e6f5bbf808f (accessed 21 October 2021).

Feng L, Sun J, El-Kassaby YA, Yang X, Tian X, Wang T. 2022. Predicting potential habitat of a plant species with small populations under climate change: *Ostrya rehderiana*. *Forests* 13(1):129 DOI 10.3390/f13010129.

Flores-López C, Geada-López CG, López-Upton CJ, López-Ramírez E. 2012. Reproductive indicators in natural populations of *Picea martinezii* T. F. Patterson. *Revista Forestal Baracoa* 31:49–58.

Flores-López C, López-Upton J, Vargas-Hernández JJ. 2005. Reproductive indicators in natural populations of *Picea mexicana* Martínez. *Agrociencia* 39:117–126.

Flores-Tolentino M, García-Valdés R, Saénz-Romero C, Ávila-Díaz I, Paz H, Lopez-Toledo L. 2020. Distribution and conservation of species is misestimated if biotic interactions are ignored: the case of the orchid *Laelia speciosa*. *Scientific Reports* 10(1):1–14 DOI 10.1038/s41598-020-63638-9.

Franklin J. 2009. *Mapping species distributions: spatial inference and prediction*. Cambridge: Cambridge University Press.

Franklin J, Davis FW, Ikegami M, Syphard AD, Flint LE, Flint AL, Hannah L. 2013. Modeling plant species distributions under future climates: how fine scale do climate projections need to be? *Global Change Biology* 19(2):473–483 DOI 10.1111/gcb.12051.

Freeman EA, Moisen GG. 2008. A comparison of the performance of threshold criteria for binary classification in terms of predicted prevalence and kappa. *Ecological Modelling* 217(1–2):48–58 DOI 10.1016/j.ecolmodel.2008.05.015.

GBIF. 2021. What is GBIF? *Available at https://api.gbif.org/v1/* (accessed 10 August 2021).

Godsoe W, Holland NJ, Cosner C, Kendall BE, Brett A, Jankowski J, Holt RD. 2017. Interspecific interactions and range limits: contrasts among interaction types. *Theoretical Ecology* 10(2):167–179 DOI 10.1007/s12080-016-0319-7.

Gómez JM, González-Megías A, Lorite J, Abdelaziz M, Perfectti F. 2015. The silent extinction: climate change and the potential hybridization-mediated extinction of endemic high-mountain plants. *Biodiversity and Conservation* 24(8):1843–1857 DOI 10.1007/s10531-015-0909-5.

Graham A. 1976. Studies in neotropical paleobotany. II. The Miocene communities of Veracruz, México. *Annals of the Missouri Botanical Garden* 63(4):787–842 DOI 10.2307/2395250.

Hall M. 1999. Correlation-based feature selection for machine learning. Thesis, University of Waikato.

Hall M, Frank E, Holmes G, Pfahringer B, Reutemann P, Witten IH. 2009. The WEKA data mining software: an update. *SIGKDD Explorations Newsletter* 11(1):10–18 DOI 10.1145/1656274.1656278.

Hällfors MH, Aikio S, Fronzek S, Hellmann JJ, Ryttäri T, Heikkinen RK. 2016. Assessing the need and potential of assisted migration using species distribution models. *Biological Conservation* 196(7):60–68 DOI 10.1016/j.biocon.2016.01.031.

Hammond WM, Williams AP, Abatzoglou JT, Adams HD, Klein T, López R, Sáenz-Romero C, Hartmann H, Breshears DD, Allen CD. 2022. Global field observations of tree die-off reveal hotter-drought fingerprint for Earth's forests. *Nature Communications* 13(1):1–11 DOI 10.1038/s41467-022-29289-2.

Hengl T, Mendes de JJ, Heuvelink GBM, Ruiperez GM, Kilibarda M, Blagotić A, Shangguan W, Wright MN, Geng X, Bauer-Marschallinger B, Guevara MA, Vargas R, MacMillan RA, Batjes NH, Leenaars JGB, Ribeiro E, Wheeler I, Mantel S, Kempen B. 2017. SoilGrids250m: global gridded soil information based on machine learning. *PLOS ONE* 12(2):1–40 DOI 10.1371/journal.pone.0169748.

Hijmans RJ. 2012. Cross-validation of species distribution models: removing spatial sorting bias and calibration with a null model. *Ecology* 93(3):679–688 DOI 10.1890/11-0826.1.

Hijmans RJ, Cameron SE, Parra JL, Jones PG, Jarvis A. 2005. Very high resolution interpolated climate surfaces for global land areas. *International Journal of Climatology* 25(15):1965–1978 DOI 10.1002/joc.1276.

IPCC. 2013. Climate change 2013: the physical science basis. *Available at http://www. climatechange2013.org/* (accessed 17 November 2019).

Işik K. 2011. Rare and endemic species: why are they prone to extinction? *Turkish Journal of Botany* 35:411–417 DOI 10.3906/bot-1012-90.

IUCN. 2021. The IUCN red list of threatened species. Version 2020-3. *Available at https://www. iucnredlist.org* (accessed 21 January 2021).

IUCN/SSC. 2013. Guidelines for reintroductions and other conservation translocations. Version 1.0. Gland, Switzerland: IUCN Species Survival Commission, viiii + 57.

Jiménez-Valverde A, Lobo JM. 2007. Threshold criteria for conversion of probability of species presence to either-or presence-absence. *Acta Oecologica* 31(3):361–369 DOI 10.1016/j.actao.2007.02.001.

Ledig FT, Bermejo-Velázquez B, Hodgskiss PD, Johnson DR, Flores-López C, Jacob-Cervantes V. 2000b. The mating system and genic diversity in Martínez spruce, an extremely rare endemic of México's Sierra Madre Oriental: an example of facultative selfing and survival in interglacial refugia. *Canadian Journal of Forest Research* 30(7):1156–1164 DOI 10.1139/x00-052.

Ledig FT, Hodgskiss PD, Jacob-Cervantes V. 2002. Genetic diversity, mating system, and conservation of a Mexican subalpine relict, *Picea mexicana* Martínez. *Conservation Genetics* 3(2):113–122 DOI 10.1023/A:1015297621884.

Ledig FT, Mápula-Larreta M, Bermejo-Velázquez B, Reyes-Hernández V, Flores-López C, Capó-Arteaga MA. 2000a. Locations of endangered spruce populations in México and the demography of *Picea chihuahuana*. *Madroño* 47:71–88.

Ledig FT, Rehfeldt GE, Sáenz-Romero C, Flores-López C. 2010. Projections of suitable habitat for rare species under global warming scenarios. *American Journal of Botany* 97(6):970–987 DOI 10.3732/ajb.0900329.

Lin CT, Li CF, Zelený D, Chytrý M, Nakamura Y, Chen MY, Chen TY, Hsia YJ, Hsieh CF, Liu HY, Wang JC, Yang SZ, Yeh CL, Chiou CR. 2012. Classification of the high-mountain coniferous forests in Taiwan. *Folia Geobotanica* 47(4):373–401 DOI 10.1007/s12224-012-9128-y.

Lobo JM, Jiménez-Valverde A, Real R. 2008. AUC: a misleading measure of the performance of predictive distribution models. *Global Ecology and Biogeography* 17(2):145–151 DOI 10.1111/j.1466-8238.2007.00358.x.

López-Sánchez CA, Castedo-Dorado F, Cámara-Obregón A, Barrio-Anta M. 2021. Distribution of *Eucalyptus globulus* Labill. in northern Spain: Contemporary cover, suitable habitat and potential expansion under climate change. *Forest Ecology and Management* 481(8):118723 DOI 10.1016/j.foreco.2020.118723.

López-Tirado J, Hidalgo PJ. 2016. Predictive modelling of climax oak trees in southern Spain: insights in a scenario of global change. *Plant Ecology* 217(4):451–463 DOI 10.1007/s11258-016-0589-6.

Lozano-García MS, Ortega-Guerrero B, Caballero-Miranda M, Urrutia-Fucugauchi J. 1993. Late pleistocene and holocene paleoenvironments of Chalco lake, central México. *Quaternary Research* 40(3):332–342 DOI 10.1006/qres.1993.1086.

Martínez M. 1961. Una nueva especie de Picea en México. *Anales del Instituto de Biología UNAM* 32:137–142.

McCune JL. 2016. Species distribution models predict rare species occurrences despite significant effects of landscape context. *Journal of Applied Ecology* 53(6):1871–1879 DOI 10.1111/1365-2664.12702.

McDonald J, McCormack PC. 2021. Rethinking the role of law in adapting to climate change. *Wiley Interdisciplinary Reviews: Climate Change* 12(5):e726 DOI 10.1002/wcc.726.

McLane SC, Aitken SN. 2012. Whitebark pine (*Pinus albicaulis*) assisted migration potential: testing establishment north of the species range. *Ecological Applications* 22(1):142–153 DOI 10.1890/11-0329.1.

Mi C, Huettmann F, Guo Y, Han X, Wen L. 2017. Why choose random forest to predict rare species distribution with few samples in large undersampled areas? Three Asian crane species models provide supporting evidence. *PeerJ* 5(6):e2849 DOI 10.7717/peerj.2849.

Morrone JJ, Escalante T, Rodríguez-Tapia G. 2017. Mexican biogeographic provinces: map and shapefiles. *Zootaxa* 4277(2):277–279 DOI 10.11646/zootaxa.4277.2.8.

Mouillot D, Bellwood DR, Baraloto C, Chave J, Galzin R, Harmelin-Vivien M, Kulbicki M, Lavergne S, Lavorel S, Mouquet N, Paine CET, Renaud J, Thuiller W. 2013. Rare species support vulnerable functions in high-diversity ecosystems. *PLoS Biology* 11(5):e1001569 DOI 10.1371/journal.pbio.1001569.

Müller-Using B, Alanis G. 1984. New records of Chihuahua spruce (*Picea chihuahuana Martínez*) in Nuevo León proposed for the legal protection of two areas of special ecological interest. In: Secretaría de Desarrollo Urbano y Ecología, ed. *Reunión Regional de Ecología Norte, 25, 26 y 27 de abril 1984, Monterrey, N.L. SEDUE - Secretaría de Desarrollo Urbano y Ecología, Subsecretaría de Ecología*. Monterrey: SEDUE, 130–132.

Murray BR, Lepschi BJ. 2004. Are locally rare species abundant elsewhere in their geographical range? *Austral Ecology* 29(3):287–293 DOI 10.1111/j.1442-9993.2004.01365.x.

Ongaro S, Martellos S, Bacaro G, De Agostini A, Cogoni A, Cortis P. 2018. Distributional pattern of Sardinian orchids under a climate change scenario. *Community Ecology* 19(3):223–232 DOI 10.1556/168.2018.19.3.3.

Panthi S, Bräuning A, Zhou ZK, Fan ZX. 2017. Tree rings reveal recent intensified spring drought in the central Himalaya. *Nepal Global and Planetary Change* 157(3):26–34 DOI 10.1016/j.gloplacha.2017.08.012.

Patsiou TS, Conti E, Zimmermann NE, Theodoridis S, Randin CF. 2014. Topo-climatic microrefugia explain the persistence of a rare endemic plant in the Alps during the last 21 millennia. *Global Change Biology* 20(7):2286–2300 DOI 10.1111/gcb.12515.

Patterson TF. 1988. A new species of *Picea* (Pinaceae) from Nuevo León, México. *SIDA, Contributions to Botany* 13:131–135.

Pearson RG, Raxworthy CJ, Nakamura M, Townsend Peterson A. 2007. Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *Journal of Biogeography* 34(1):102–117 DOI 10.1111/j.1365-2699.2006.01594.x.

Pearson RG, Thuiller W, Araújo MB, Martinez-Meyer E, Brotons L, McClean C, Miles L, Segurado P, Dawson TP, Lees DC. 2006. Model-based uncertainty in species range prediction. *Journal of Biogeography* 33(10):1704–1711 DOI 10.1111/j.1365-2699.2006.01460.x.

Pedlar JH, McKenney DW, Aubin I, Beardmore T, Beaulieu J, Iverson L, O'Neill GA, Winder RS, Ste-Marie C. 2012. Placing forestry in the assisted migration debate. *BioScience* 62(9):835–842 DOI 10.1525/bio.2012.62.9.10.

Penteriani V, Zarzo-Arias A, Novo-Fernández A, Bombieri G, López-Sánchez CA. 2019. Responses of an endangered brown bear population to climate change based on predictable food resource and shelter alterations. *Global Change Biology* 25(3):1133–1151 DOI 10.1111/gcb.14564.

Pérez I, Anadón JD, Díaz M, Nicola GG, Tella JL, Giménez A. 2012. What is wrong with current translocations? A review and a decision-making proposal. *Frontiers in Ecology and the Evironment* 10(9):494–501 DOI 10.1890/110175.

Peters RL, Darling JDS. 1985. The greenhouse effect and nature reserves. *BioScience* 35(11):707–717 DOI 10.2307/1310052.

Pinedo-Alvarez C, Renteria-Villalobos M, Aguilar-Soto V, Vega-Mares JH, Melgoza-Castillo A. 2019. Distribution dynamics of Picea chihuahuana Martínez populations under different climate change scenarios in México. *Global Ecology and Conservation* 17(1):e00559 DOI 10.1016/j.gecco.2019.e00559.

QGIS Development Team. 2020. QGIS geographic information system. Open source geospatial foundation project. *Available at http://qgis.osgeo.org*.

Qiao H, Soberón J, Peterson AT. 2015. No silver bullets in correlative ecological niche modelling: insights from testing among many potential algorithms for niche estimation. *Methods in Ecology and Evolution* 6(10):1126–1136 DOI 10.1111/2041-210X.12397.

Rabinowitz D. 1981. Seven forms of rarity. In: Singe H, ed. *The Biological Aspects of Rare Plant Conservation*. Chichester: John Wiley, 205–217.

Regan HM, Syphard AD, Franklin J, Swab RM, Markovchick L, Flint AL, Flint LE, Zedler PH. 2012. Evaluation of assisted colonization strategies under global change for a rare, fire-dependent plant. *Global Change Biology* 18(3):936–947 DOI 10.1111/j.1365-2486.2011.02586.x.

Román-Palacios C, Wiens JJ. 2020. Recent responses to climate change reveal the drivers of species extinction and survival. *Proceedings of The National Academy of Sciences of The United States of America* 117(8):4211–4217 DOI 10.1073/pnas.1913007117.

Rzedowski J. 2006. *Vegetación de México*. México: Comisión Nacional para el Conocimiento y Uso de la Biodiversidad.

Sáenz-Romero C, Rehfeldt GE, Crookston NL, Duval P, St-Amant R, Beaulieu J, Richardson BA. 2010. Spline models of contemporary, 2030, 2060 and 2090 climates for México and their use in understanding climate-change impacts on the vegetation. *Climatic Change* 102(3–4):595–623 DOI 10.1007/s10584-009-9753-5.

Schwartz MW, Hellmann JJ, McLachlan JM, Sax DF, Borevitz JO, Brennan J, Camacho AE, Ceballos G, Clark JR, Doremus H. 2012. Managed relocation: integrating the scientific, regulatory, and ethical challenges. *BioScience* 62(8):732–743 DOI 10.1525/bio.2012.62.8.6.

Sexton JP, McIntyre PJ, Angert AL, Rice KJ. 2009. Evolution and ecology of species range limits. *Annual Review of Ecology, Evolution, and Systematics* 40(1):415–436 DOI 10.1146/annurev.ecolsys.110308.120317.

Simler AB, Williamson MA, Schwartz MW, Rizzo DM. 2019. Amplifying plant disease risk through assisted migration. *Conservation Letters* 12(2):1–9 DOI 10.1111/conl.12605.

Sofaer HR, Jarnevich CS, Pearse IS, Smyth RL, Auer S, Cook GL, Edwards TC, Guala GF, Howard TG, Morisette JT, Hamilton H. 2019. Development and delivery of species distribution models to inform decision-making. *BioScience* 69(7):544–557 DOI 10.1093/biosci/biz045.

Taucare-Ríos A, Bizama G, Bustamante RO. 2016. Using global and regional Species Distribution Models (SDM) to infer the invasive stage of *Latrodectus geometricus* (Araneae: Theridiidae) in the Americas. *Environmental Entomology* 45(6):1379–1385 DOI 10.1093/ee/nvw118.

Wang H-H, Wonkka CL, Treglia ML, Grant WE, Smeins FE, Rogers WE. 2019. Incorporating local-scale variables into distribution models enhances predictability for rare plant species with biological dependencies. *Biodiversity and Conservation* 28(1):171–182 DOI 10.1007/s10531-018-1645-4.

Wehenkel C, del Rocío Mariscal-Lucero S, González-Elizondo MS, Aguirre-Galindo VA, Fladung M, López-Sánchez CA. 2020. Tall *Pinus luzmariae* trees with genes from *P. herrerae*. *PeerJ* 8(6):e8648 DOI 10.7717/peerj.8648.

Wehenkel C, Sáenz-Romero C. 2012. Estimating genetic erosion using the example of *Picea chihuahuana* Martínez. *Tree Genetics and Genomes* 8(5):1085–1094 DOI 10.1007/s11295-012-0488-5.

Williams JN, Seo C, Thorne J, Nelson JK, Erwin S, O'Brien JM, Schwartz MW. 2009. Using species distribution models to predict new occurrences for rare plants. *Diversity and Distributions* 15(4):565–576 DOI 10.1111/j.1472-4642.2009.00567.x.

Wilson CD, Roberts D, Reid N. 2011. Applying species distribution modelling to identify areas of high conservation value for endangered species: a case study using *Margaritifera margaritifera* (L.). *Biological Conservation* 144(2):821–829 DOI 10.1016/j.biocon.2010.11.014.

Zhao W, Sun Y, Pan J, Sullivan AR, Arnold ML, Mao J, Wang X. 2020. Effects of landscapes and range expansion on population structure and local adaptation. *New Phytologist* 228(1):330–343 DOI 10.1111/nph.16619.

Zhiwei X, Xinghua W. 2010. Research for information extraction based on wrapper model algorithm. In: *2010 Second International Conference on Computer Research and Development*. Kuala Lumpur: IEEE, 652–655 DOI 10.1109/ICCRD.2010.141.

2.7. Supplemental materials

Table S2.1. Distribution records for *Picea martinezii* obtained from field surveys.

No. of record	X	Y
1	-99.727227	24.046937
2	-99.729473	24.045693
3	-99.71575	24.037917
4	-99.713161	24.032339
5	-99.703583	24.039861
6	-99.701667	24.04225
7	-99.694056	24.041611
8	-99.699278	24.044306
9	-99.697306	24.041306
10	-99.782972	23.895333
11	-99.780583	23.898556
12	-99.791535	23.890743
13	-99.791618	23.890372
14	-99.79195	23.890803
15	-99.792018	23.890236
16	-99.792202	23.889685
17	-99.791416	23.891232
18	-99.790506	23.891951
19	-99.79097	23.891999
20	-99.7788	23.890500
21	-99.7734	23.890700
22	-99.773472	23.8899444
23	-99.734083	24.0448056
24	-99.734361	24.0427500
25	-99.733278	24.0421111
26	-99.731444	24.0423333
27	-99.731444	24.0419722
28	-99.730972	24.0420278
29	-99.731083	24.0417222
30	-99.730944	24.0402778
31	-99.705472	24.0396667
32	-99.708778	24.0406389
33	-99.711	24.0399167
34	-99.711361	24.0385556
35	-99.711889	24.0386111
36	-99.711917	24.0382500

Table S2.1. Continua…

No. of record	X	Y
37	-99.712472	24.0379722
38	-99.712472	24.0360833
39	-100.12294	25.1723790
40	-100.12291	25.1728800
41	-100.12306	25.1732720
42	-100.12341	25.1734330
43	-100.12505	25.1755410
44	-100.12802	25.1801120
45	-100.12828	25.181882
46	-100.12248	25.171408

Table S2.2. Distribution records for *Picea mexicana* obtained from field surveys.

No. of record	X	Y
1	-100.36423	25.1995528
2	-100.36244	25.1988611
3	-100.36692	25.1983333
4	-100.36189	25.2019722
5	-100.36383	25.2016389
6	-100.36286	25.2042778
7	-100.41316	25.2130556
8	-100.41681	25.2134444
9	-100.40115	25.2091722
10	-100.38627	25.2053694
11	-100.40831	25.2110000
12	-100.40867	25.2097222
13	-100.41189	25.2114667
14	-100.36925	25.2087778
15	-100.37064	25.2078056
16	-100.37017	25.2083056
17	-100.36967	25.2079167
18	-100.36906	25.2032222
19	-100.35464	25.2469722
20	-100.35569	25.2475278
21	-100.35628	25.2475556
22	-100.35953	25.2488056
23	-100.34706	25.2452778
24	-100.34517	25.2496944
25	-100.35989	25.2498056

Table S2.2. Continua…

No. of record	X	Y
26	-100.365	25.2508333
27	-100.36361	25.2513889
28	-100.36306	25.2513889
29	-100.35778	25.2502778
30	-100.35333	25.2480556
31	-100.354	25.2471111
32	-100.35464	25.2469722
33	-100.35628	25.2475556
34	-100.35953	25.2488056
35	-100.36183	25.2496944
36	-100.34539	25.2446667
37	-100.36875	25.2548333
38	-100.36972	25.2557778
39	-100.32722	25.2424444
40	-100.33148	25.2443917
41	-100.33319	25.2435
42	-107.03897	25.9619444
43	-107.04608	25.9620833
44	-107.04654	25.9605194
45	-107.04094	25.9599306
46	-107.0395	25.9595556
47	-107.04761	25.959694
48	-107.03274	25.9644333
49	-107.02602	25.9684
50	-107.04178	25.967203

Table S2.3. Model fit metrics for species distributions as indicated by Random Forest analysis applied to the occurrence data for *Picea martinezii* and *Picea mexicana*, with a cross validation n = 10. Model fit metrics included the area under the receiver operator curve (AUC), the overall accuracy (OA), Matthews correlation coefficient (MCC), true skill statistic (TSS), Cohen's kappa, sensitivity, specificity and probability of presence (PoP).

Species	**Presences**	**Absences**	**Total**	**Prevalence**	**AUC**	**OA**	**MCC**	**TSS**	**Kappa**	**Sensitivity**	**Specificity**	**$\text{PoP}_{\text{threshold}}$**
Model for Mexico (30-arc second resolution)												
Picea martinezii	35	32,293	32,328	0.00108	0.986	0.999	0.971	0.971	0.971	0.971	1.000	0.73
Picea mexicana	38	32,571	32,609	0.00117	1.000	0.999	0.919	0.895	0.918	0.895	1.000	0.83
World model (30-arc second resolution)												
Picea martinezii	46	22,004	22,050	0.00209	1.000	0.999	0.956	0.957	0.956	0.957	1.000	0.73
Picea mexicana	50	32,571	32,568	0.00154	1.000	0.999	0.904	0.922	0.904	0.922	1.000	0.83

Table S2.4. Descriptive statistics of the 42 environmental variables used to characterize the abiotic niches and to construct the distribution models of *Picea martinezii* and *Picea mexicana*, from the presence locations. Min = minimum, SD = standard deviation, Max = maximum.

Class	**Variable**	**Description**	**Source**	***Picea martinezii***				***Picea mexicana***			
				Mean	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
Climate	Bio_1	Annual mean temperature (ºC)	WorldClim	13.5	1.9	10.6	15.5	9.3	0.7	7.9	10.3
	Bio_2	Mean diurnal range (mean of monthly (max temp - min temp)) (ºC)	WorldClim	14.6	0.6	13.6	15.2	14.0	1.0	13.1	15.8
	Bio_3	Isothermality (BIO2/BIO7) (* 100) (ºC)	WorldClim	6.6	0.0	6.6	6.7	6.4	0.2	6.1	6.6
	Bio_4	Temperature seasonality (standard deviation *100) (ºC)	WorldClim	22.9	2.1	20.2	25.1	24.7	5.2	20.8	33.6
	Bio_5	Max temperature of warmest month (ºC)	WorldClim	24.1	2.1	21.1	26.4	20.0	1.4	17.9	22.7
	Bio_6	Min temperature of coldest month (ºC)	WorldClim	2.3	1.2	0.3	3.5	-1.6	1.3	-4.0	-0.2
	Bio_7	Temperature annual range (BIO5-BIO6) (ºC)	WorldClim	21.9	1.0	20.5	22.9	21.7	2.3	19.9	25.6
	Bio_8	Mean temperature of wettest quarter (ºC)	WorldClim	14.9	1.7	12.0	16.6	11.5	1.3	9.5	13.9
	Bio_9	Mean temperature of driest quarter (ºC)	WorldClim	10.9	1.0	9.1	11.9	8.3	0.9	6.7	10.0
	Bio_10	Mean temperature of warmest quarter (ºC)	WorldClim	16.1	2.1	12.9	18.3	12.1	1.1	10.2	14.3
	Bio_11	Mean temperature of coldest quarter (ºC)	WorldClim	10.2	1.6	7.6	11.9	5.8	0.8	4.7	6.9
	Bio_12	Annual precipitation (mm)	WorldClim	787.9	67.9	654.0	855.0	858.4	209.0	686.0	1211.0
	Bio_13	Precipitation of wettest month (mm)	WorldClim	164.5	28.9	123.0	199.0	161.7	66.0	112.0	273.0
	Bio_14	Precipitation of driest month (mm)	WorldClim	19.1	2.1	17.0	22.0	23.8	1.6	22.0	27.0
	Bio_15	Precipitation seasonality (coefficient of variation)	WorldClim	68.5	7.5	59.0	77.0	62.4	13.2	54.0	85.0
	Bio_16	Precipitation of wettest quarter (mm)	WorldClim	359.9	41.0	292.0	409.0	426.0	173.2	301.0	717.0

Table S2.4. Continua…

Class	Variable	Description	Source	*Picea martinezii*				*Picea mexicana*			
				Mean	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
	Bio_17	Precipitation of driest quarter (mm)	WorldClim	70.3	6.6	64.0	82.0	81.2	4.5	74.0	90.0
	Bio_18	Precipitation of warmest quarter (mm)	WorldClim	281.6	27.2	224.0	303.0	354.6	165.6	240.0	632.0
	Bio_19	Precipitation of coldest quarter (mm)	WorldClim	72.0	8.4	64.0	87.0	112.2	38.7	83.0	186.0
Soil	DB	Absolute deep to bed rock (cm)	SoilGrids250m	316.5	97.2	194.0	427.0	513.4	275.1	105.0	1128.0
	DB200	Depth to bedrock (R horizon) up to 200 cm	SoilGrids250m	156.3	31.5	107.0	191.0	143.9	16.8	108.0	165.0
	R	Probability occurrence of R horizon (%)	SoilGrids250m	42.1	6.7	32.0	58.0	46.8	7.0	36.0	61.0
	SC	Soil organic carbon content (g kg^{-1})	SoilGrids250m	102.3	20.0	61.0	130.0	32.9	5.5	24.0	53.0
	BD	Bulk density of the fine earth fraction (< 2mm) (kg m^{-3})	SoilGrids250m	1092.7	59.9	1036.0	1218.0	1171.9	31.8	1039.0	1222.0
	CLAY	Percentage of clay in soil (Weight %)	SoilGrids250m	38.4	1.0	37.0	40.0	37.7	4.6	34.0	47.0
	CF	Coarse fragments (Volumetric %)	SoilGrids250m	24.0	1.6	21.0	25.0	21.3	0.8	20.0	24.0
	SAND	Percentage of sand in soil (Weight %)	SoilGrids250m	32.9	0.7	32.0	34.0	31.6	3.4	25.0	35.0
	SILT	Percentage of silt in soil (Weight %)	SoilGrids250m	27.4	1.0	26.0	29.0	30.8	1.3	28.0	33.0
	CEC	Cation-exchange capacity (cmol(c) kg^{-1})	SoilGrids250m	29.0	2.4	27.0	36.0	28.7	1.2	27.0	32.0
	SS	Soil organic carbon stock (ton ha^{-1})	SoilGrids250m	39.5	5.1	32.0	50.0	54.2	13.1	39.0	108.0
	pH_H_2O	Soil pH in H_2O solution	SoilGrids250m	6.5	0.2	6.3.0	7.0	6.5	0.5	5.6.0	7.0
	pH_KCl	Soil pH in KCl solution	SoilGrids250m	5.6	0.1	5.5.0	5.9.0	5.6	0.6	4.4.0	6.2
	FSC	Full soil code	INEGI	12.4	4.7	6.0	18.0	18.3	3.1	14.0	24.0
Geology	Geo	Geological units	INEGI	8.0	0.0	8.0	8.0	12.9	8.6	5.0	27.0
	LIT	Lithostratigraphy	INEGI	14.0	0.0	14.0	14.0	12.9	1.8	10.0	14.0
Topo-graphy	ASP	Aspect (º)	INEGI	88.2	104.1	11.3	286.9	150.6	135.8	1.7	349.4
	CU	Curvature	INEGI	0.0	0.2	-0.3	0.4	0.2	0.3	-0.2	0.9
	PLC	Plan curvature	INEGI	0.0	0.1	-0.2	0.2	0.0	0.1	-0.1	0.2
	PRC	Profile curvature	INEGI	0.0	0.2	-0.3	0.2	-0.2	0.3	-0.8	0.1
	SLP	Slope (%)	INEGI	16.8	10.0	9.1	33.8	24.7	10.7	6.5	48.2
	TSI	Terrain shape index	INEGI	0.0	0.2	-0.3	0.2	-0.1	0.2	-0.6	0.2
	WI	Wetness index	INEGI	11.8	1.3	10.2	14.2	10.7	0.7	9.8	13.2

Table S2.5. Model projections for the contemporary conditions in Mexico (using climate, soil, geological and topographic variables). The categories of probability of presences (*p*), the pixel count for each category of presence, and the real presences/absences of *Picea martinezii* and *Picea mexicana* on the corresponding predicted areas by the models are shown.

Species	*p*	Pixel count	Real presences (RP)	%RP	Real absences (RA)	%RA
Picea martinezii	< 0.5	-	0	0.0	All pixels	100.0
	0.5-0.6	12	0	0.0	12	100.0
	0.6-0.7	3	1	33.3	2	66.7
	0.7-0.8	0	-	-	-	-
	0.8-0.9	1	1	100.0	0	0.0
	0.9-1.0	6	6	100.0	0	0.0
Picea mexicana	< 0.5	-	0	0.0	All pixels	100.0
	0.5-0.6	16	0	0.0	16	100.0
	0.6-0.7	15	2	13.3	13	86.7
	0.7-0.8	14	2	14.3	12	85.7
	0.8-0.9	9	5	55.6	4	44.4
	0.9-1.0	10	9	90.0	1	10.0

CAPÍTULO 3. INDICADORES REPRODUCTIVOS DE *Picea martinezii* T.F. PATT. Y *Picea mexicana* MARTÍNEZ

Capítulo de libro LAS PICEAS (*Picea*, PINACEAE) DE MÉXICO
ISBN – Versión digital: 978-607-8383-36-8

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3.1. Introducción

El género *Picea* comprende tres especies en México: *Picea chihuahuana* Martínez, *Picea martinezii* T.F. Patt. y *Picea mexicana* Martínez; estas especies tienen diferente distribución y se ubican en poblaciones aisladas (Ledig *et al.,* 2000a). *Picea chihuahuana* se distribuye en la Sierra Madre Occidental en 40 poblaciones localizadas en los estados de Chihuahua y Durango; *P. martinezii* se distribuye en la Sierra Madre Oriental en cuatro poblaciones conocidas en el estado de Nuevo León; y *P. mexicana* tanto en la Sierra Madre Occidental como en la Oriental, en tres poblaciones conocidas en los estados de Chihuahua y Coahuila (Ledig *et al.,* 2000a).

Picea martinezii se caracteriza por su afinidad ecológica con elementos de bosque mesófilo de montaña; se desarrolla en elevaciones entre los 2,250 y 2,650 m, asociada con especies de coníferas como *Pinus, Abies* y *Taxus*, así como con *Quercus, Tilia, Ostrya, Cornus, Ilex, Juglans* y *Crataegus* (Patterson, 1988). *Picea mexicana* se encuentra en elevaciones de 3,000 a 3,600 m en hábitat de tipo boreal, en asociación con especies de los géneros *Pinus* (*P. rudis, P. arizonica, P. ayacahuite* y *P. cooperi*), *Abies* (*A. vejarii* y *A. durangensis*), *Pseudotsuga mensiezii*, *Populus tremuloides, Salix paradoxa* y *Sambucus* sp. (Flores-López, 2014).

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En general, las poblaciones fragmentadas tienden a sufrir mayores niveles de endogamia, lo cual conlleva a una menor capacidad reproductiva y mayor riesgo de extinción (Frankham, 1996). Uno de los principales problemas de producción de semillas en poblaciones naturales es la gran cantidad de semillas vanas y abortivas causado por falta de disponibilidad de polen, así como por las condiciones ambientales adversas (Flores-López, 2004; Flores-López *et al.,* 2005).

De estos factores, la limitación de polen ha sido el más estudiado (Vaughton y Ramsey, 1995). En ausencia de polinización cruzada adecuada, tal como sucede en poblaciones pequeñas o árboles aislados, se producen grandes cantidades de semillas vanas y las semillas desarrolladas pueden producir plántulas anormales como resultado de la depresión por endogamia (Sorensen y Miles, 1974; Frankham, 1996; Ledig *et al.,* 1997; Mosseler, 1998 y Ledig *et al.,* 2000b). Es decir, las poblaciones pequeñas y aisladas pueden experimentar autopolinización dando como resultado reducciones de vigor en el número de semillas por cono y de planta de semillero; esto como resultado de alelos deletéreos existentes en el pool genético de la especie, los cuales aparecen en condición homocigota en la progenie. Por lo tanto, el conocimiento de las características biológicas de la maduración de conos y semillas es importante para el manejo de las poblaciones y la programación de colectas.

Uno de los métodos para evaluar la producción de semillas y la pérdida de estas ha sido el análisis de conos y semillas (Bramlett, 1974). Este tipo de análisis proporciona la información requerida para evaluar la productividad, la cual puede expresarse en términos de potencial (PS) y eficiencia (ES) de semilla. El PS representa el número máximo de semillas que los estróbilos son capaces de producir (definido como dos veces el número de escamas fértiles de un cono); la ES es la cantidad de semillas llenas con relación al potencial de semillas. Por lo tanto, la eficiencia de las semillas mide la productividad de un cono en relación con su capacidad biológica (Bramlett *et al.,* 1977).

Los análisis de conos y semillas evalúan además características designadas como indicadores reproductivos, tales como largo del cono, peso seco del cono, escamas fértiles, proporción de óvulos abortados y óvulos rudimentarios, proporción de semillas vanas y semillas llenas, índice de endogamia y eficiencia reproductiva (Bramlett *et al.,* 1977; Mosseler *et al.,* 2000; Flores-López *et al.,* 2005). Estos análisis son considerados como una herramienta básica en el monitoreo de la viabilidad reproductiva y la endogamia de las poblaciones, especialmente las pequeñas y aisladas (Bramlett *et al.,* 1977; Flores-López *et al.,* 2005; Flores-López *et al.,* 2012). Además, estos análisis permiten relacionar los parámetros reproductivos con parámetros de diversidad y estructura genética de las poblaciones.

Los estudios enfocados en el análisis de conos y semillas en coníferas son cuantiosos. Entre las especies más estudiadas se encuentran *Pinus greggii* Engelm. (Ramírez-García *et al.,* 2007), *Pinus oaxacana* Mirov. (Alba-Landa y Márquez Ramírez 2006), *Pinus cembroides* Zucc. (González-Ávalos *et al.,* 2006), *Pinus pseudostrobus* Lindl. (Hernández-Carmona *et al*., 2003) y *Pinus hartwegii* Lindl. (Alba-Landa *et al*., 2003) que son especies de amplia distribución con poblaciones grandes. Sin embargo, son pocos los trabajos que se concentran en especies en riesgo o pequeñas poblaciones, tal es el caso de *Pinus maximartinezii* Rzedowski, *Pinus catarinae* M.F. Robert-Passini., *Pinus johannis* M.-F. Robert., *Pinus nelsonii* Shaw. y *Pinus pinceana* Gordon; siendo sus poblaciones las que ocupan mayor importancia para la conservación, ya que su fuente de semilla está adaptada a condiciones particulares, por lo que estas semillas pueden ser de gran utilidad en la adaptación de dichas especies ante el cambio climático (Rajora y Mosseler, 2001).

Respecto al género *Picea* en México, Flores-López *et al.,* (2005) encontraron una variación significativa de las características reproductivas tanto entre poblaciones como entre árboles en *Picea mexicana* en dos años de recolecta. De la misma manera estimaron altos índices de endogamia (73%), baja eficiencia de semillas (13%) y una alta proporción de óvulos abortados en sus poblaciones. En cuanto a *Picea martinezii*, Flores-López *et al.,* (2012) encontraron un potencial de semillas alto (266 por cono) pero una baja eficiencia de semilla en las poblaciones (7%). Así mismo se estimó gran pérdida de semilla por óvulos abortados y rudimentarios y semillas vanas, confirmando la presencia de endogamia asociada a autofecundaciones y cruzamientos entre individuos relacionados genéticamente.

Estudios de diversidad genética en *P. mexicana* y *P. martinezii* demostraron que ambas especies presentan diferentes grados de endogamia, cuyo monitoreo (y manejo) a través de los indicadores reproductivos es crítico para su conservación, ya que esta afecta la regeneración (Ledig *et al.,* 2000b; Ledig *et al.,* 2002).

El presente estudio tuvo como objetivo estimar los siguientes indicadores reproductivos en las poblaciones conocidas de *P. martinezii* y *P. mexicana*, para el año de 2018: a) potencial de semillas, b) eficiencia de semillas, c) peso seco del cono, d) número de semillas llenas por cono, e) proporción de semillas llenas, f) proporción de semillas vanas, g) proporción de óvulos abortados, h) proporción de óvulos rudimentarios, i) proporción de semillas dañadas por insectos, hongos o bacterias, j) eficiencia reproductiva, k) índice de endogamia, l) germinación de las semillas, m) longitud del cono, y n) diámetro del cono; esto como una acción de monitoreo de la viabilidad reproductiva de estas especies en peligro de extinción, tomando como referencia los estudios previos para *P. martinezii* (año de colecta 2006; Flores-López *et al.,* 2012) y *P. mexicana* (años de colecta 1999 y 2001; Flores-López *et al.,* 2005).

3.2. Materiales y métodos

Las especies se distribuyen en la Sierra Madre Occidental (SMOc) y la Sierra Madre Oriental (SMOr): las poblaciones de El Butano, Agua de Alardín, Agua Fría y La Encantada de *P. martinezii* se localizan en el estado de Nuevo León (SMOr); las poblaciones de El Mohinora, La Marta y El Coahuilón de *P. mexicana* se localizan en los estados de Chihuahua (SMOc) y Coahuila (SMOr) (Fig. 3.1, Cuadro 3.1) (Ledig *et al.,* 2000a; Flores-López *et al.,* 2012; Mendoza-Maya *et al.,* 2015).

Durante los meses de octubre a noviembre de 2018 se recolectaron conos de 13 a 30 árboles en cada población (184 muestras en total; Cuadro 3.1). El muestreo de los árboles se realizó de manera selectiva a lo largo y ancho de las poblaciones, con una distancia mínima de 50 m entre árboles, para reducir la probabilidad de parentesco. Sin embargo, algunos árboles tuvieron distancias < 50 m, pero ≥ 10 m entre sí, por la falta de conos en árboles ubicados a 50 m. Los árboles seleccionados cumplieron con las siguientes características fenotípicas: árboles totalmente rectos, dominantes y/o co-dominantes, con diámetros normales mayores de 10 cm (en algunos casos de 7 cm de DAP) y con presencia de conos. La recolecta de los conos se realizó utilizando equipo de escalado y gancho corta conos en diferentes partes de la copa del árbol (alta, media y baja); seleccionando 30 a 70 conos maduros, cerrados y en buen estado. Los árboles seleccionados se georreferenciaron y se registró su altura y DAP.

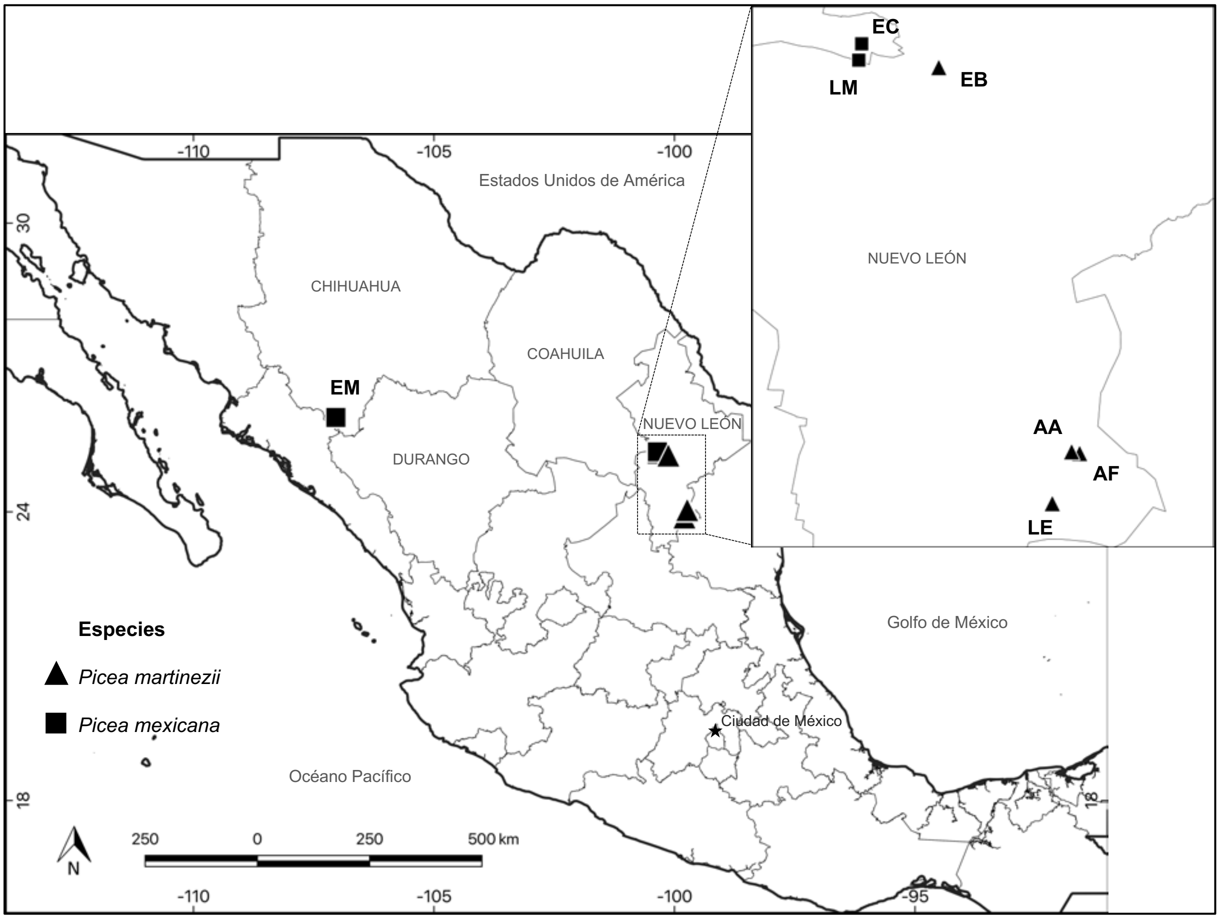

Figura 3.1 Mapa de las poblaciones conocidas de *Picea mexicana* (cuadros) y *Picea martinezii* (triángulos) en México. Nota: EM = El Mohinora, EC = El Coahuilón, LM = La Marta, EB = El Butano, AA = Agua de Alardín, AF = Agua Fría, LE = La Encantada.

En campo, los conos recolectados se colocaron en bolsas recipientes que permitieran una aireación adecuada y se identificaron con la fecha de recolecta, la procedencia y el número de árbol. En laboratorio, muestras de 5 a 10 conos (del total de conos recolectados) por árbol se separaron individualmente en bolsas de papel, y se identificaron con la procedencia, el número de árbol y el número de cono.

Cuadro 3.1 Localización de las poblaciones de *Picea mexicana, Picea martinezii* y árboles recolectados por población.

Población	Propiedad	Municipio	Coordenadas geográficas	Elevación (msnm)*	No. árboles recolectados
			Picea mexicana		
La Marta	Propiedad privada Felipe de la Peña	Arteaga, Coah.	25° 11’ 55” N 100° 21’ 52” O	3 494	30
El Coahuilón	Ejido Nuncio	Arteaga, Coah.	25° 14’ 51” N 100° 21’ 17” O	3 528	30
El Mohinora	Ejido El Tule y Portugal	Guadalupe y Calvo, Chih.	25° 57’ 41” N 107° 02’ 32” O	3 113	30
			Picea martinezii		
Agua Fría	Propiedad Privada: Agua Fría	Aramberri, NL	24° 02’ 17” N 99° 42’ 39” O	1 820	21
Agua de Alardín	Propiedad Privada: Agua de Alardín	Aramberri, NL	24° 02’ 34” N 99° 44’ 04” O	2 120	30
El Butano	Ejido la Trinidad	Montemorelos, NL	25° 10 ’41” N 100° 07’ 37” O	2 180	30
La Encantada	Ejido La Encantada	Zaragoza, NL	23° 53’ 24” N 99° 47’ 30” O	2 515	13
Total					184

* Metros sobre el nivel del mar.

3.2.1. Procedimiento de análisis de los conos

Los indicadores reproductivos se estimaron a través del procedimiento de análisis de conos y semillas de acuerdo con la metodología de Bramlett *et al.* (1977) y Flores-López *et al.* (2005).

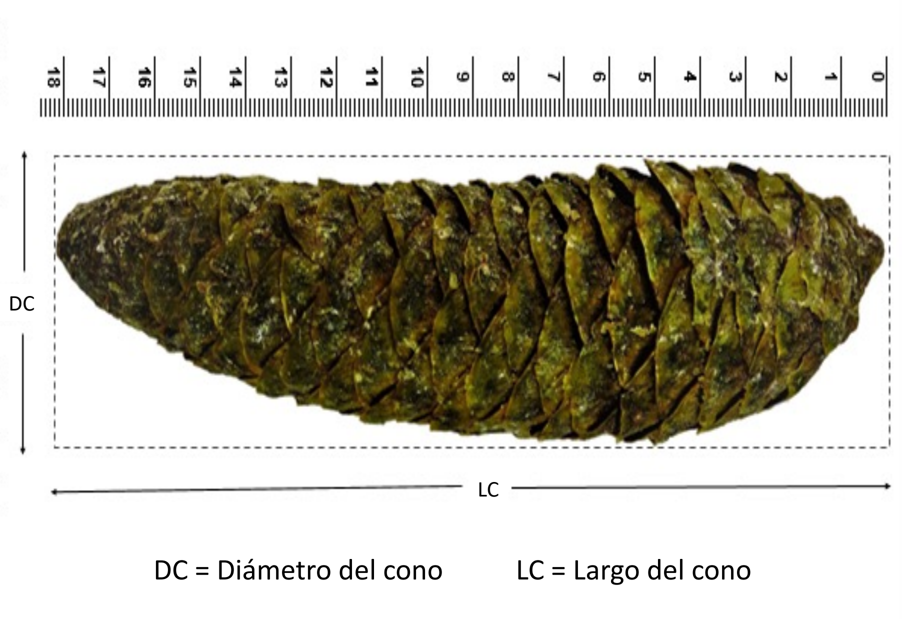

Figura 3.2 Variables evaluadas de los conos de *Picea martinezii* T.F. Patt. y *Picea mexicana* Martínez.

El procedimiento de análisis de los conos fue el siguiente:

1. Con un vernier digital (aproximación de 0.1 mm) se midió el largo (desde la base hasta el ápice) y diámetro (parte más amplia) del cono cerrado (Fig. 3.2).
2. Se procedió al secado de los conos en estufa con flujo de aire durante 24 a 72 h a 45 oC, hasta obtener una apertura completa de las escamas.
3. Se extrajeron las semillas del cono de manera manual.
4. Se separaron las escamas del cono de forma sistemática, iniciando desde las escamas basales hacia las terminales. Se determinó el número de escamas fértiles e infértiles, de acuerdo con la metodología de Bramlett *et al.,* (1977). Las escamas fértiles se clasificaron en seis categorías con base en el tipo de semillas contenidas (semillas desarrolladas = SD, óvulos abortados = OA, y óvulos rudimentarios = OR) en combinaciones de dos: escamas fértiles con 2SD, 2OA, 2OR, 1SD-1OA, 1SD-1OR, 1OA-1OR (Figura 4.3).
5. Las escamas se identificaron y guardaron, junto con todas las estructuras remanentes del cono, para su posterior secado en estufa a 105 ºC durante 24 h y la lectura del peso seco del cono en balanza electrónica (precisión de 0.01 g).

6. Las semillas extraídas de cada cono, en el paso tres, se separaron en desarrolladas y óvulos abortados o rudimentarios. Las semillas desarrolladas fueron beneficiadas y evaluadas para identificar y registrar las dañadas con ayuda de un estereomicroscopio. Posteriormente, se separaron las semillas desarrolladas en vanas y llenas mediante la técnica de flotado en alcohol 70% (v/v). Las semillas desarrolladas llenas se pesaron y almacenaron a 4 ºC previa identificación.

Con base en los análisis de conos y semillas se evaluaron las siguientes variables: longitud del cono (LC), diámetro del cono (DC), peso seco del cono (PSC), escamas fértiles (EF), escamas infértiles (EI), clasificación de escamas fértiles (paso No. 4 del procedimiento de análisis y Fig. 3.2), total de escamas (TE), óvulos abortados (OA), óvulos rudimentarios (OR), semillas desarrolladas (SD), semillas vanas (SV), semillas llenas (SLL), semillas dañadas por insectos, hongos o bacterias (SDIHB) y peso de las semillas llenas (PesoSLL). A partir de estas variables, se calcularon los valores de los indicadores reproductivos.

3.2.2. Indicadores reproductivos

Con las variables derivadas del análisis de conos y semillas, se estimaron los parámetros reproductivos mediante las siguientes fórmulas:

1. Potencial de semilla (PS) = 2 x total de escamas fértiles. Donde el total de escamas fértiles = 2SD + 2OA + 2OR + 1SD1OA + 1SD1OR + 1OA1OR (paso No. 4 del procedimiento de análisis de los conos y Fig. 3.3).
2. Eficiencia de semilla (ES) = total de semillas llenas / potencial de semillas.
3. Peso seco del cono.
4. Número de semillas llenas.
5. Proporción de semillas llenas = semillas llenas (SLL) / semillas desarrolladas (SD) x 100.
6. Proporción de semillas vanas = total de semillas vanas (SV) / semillas desarrolladas (SD) x 100.

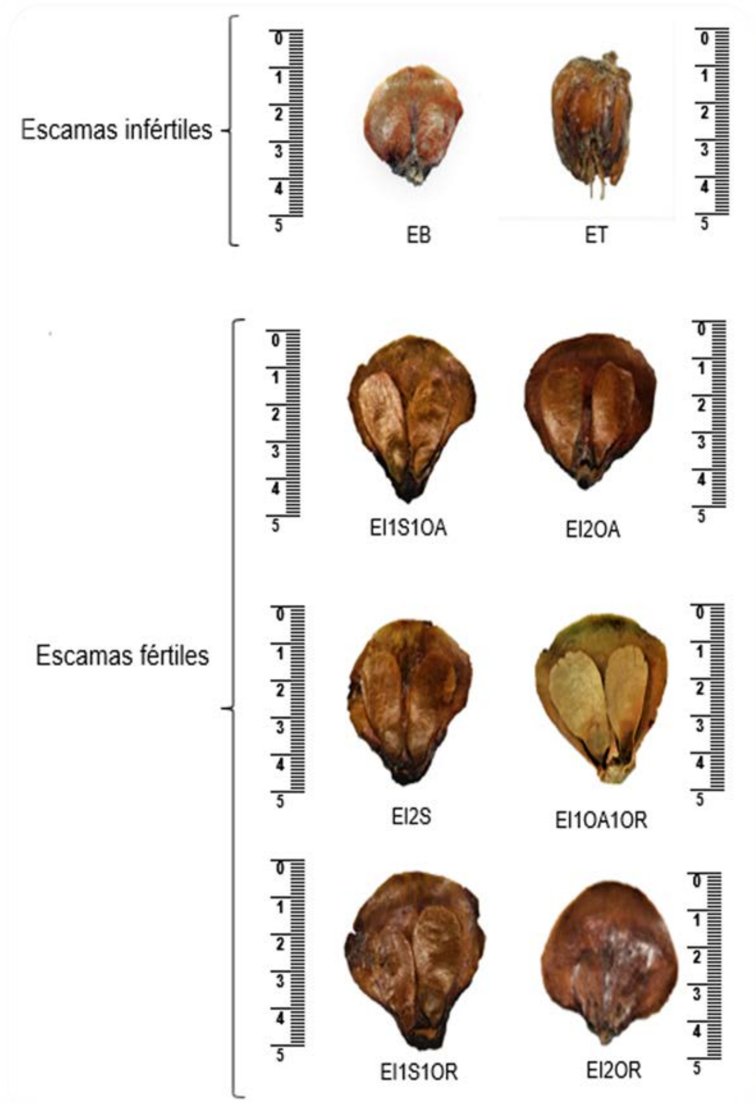

Figura 3.3. Características morfológicas de las escamas evaluadas para la estimación de indicadores reproductivos en *Picea martinezii* y *Picea mexicana*. (EB = Escama basal con óvulos rudimentarios, ET = Escama terminal con óvulos rudimentarios, 1SD-1OA = Escama con una semilla desarrollada y un óvulo abortado, E2OA = Escama con dos óvulos abortados, E2S = Escama con dos semillas desarrolladas, E1OA-1OR = Escama con un óvulo abortado y un óvulo rudimentario, E1S-1OR = Escama con una semilla desarrollada y un óvulo rudimentario, E2OR = Escama con dos óvulos rudimentarios).

7. Proporción de óvulos abortados = óvulos abortados (OA) / potencial de semillas (PS) x 100.

   Donde OA = EI1S1OA + EIOA1OR + [EI2OA x 2]. (Paso No. 4 del procedimiento de análisis de los conos y Fig. 3.3).

8. Proporción de óvulos rudimentarios = óvulos rudimentarios (OR) / potencial de semillas (PS) x 100. Donde OR = EI1S1OR + EI1OA1OR + [EI2OR x 2]. (Paso No. 4 del procedimiento de análisis de los conos y Fig. 3.3).
9. Proporción de semillas dañadas = [total de semillas dañada por insectos, hongos o bacterias (SDIHB) / Semillas desarrolladas (SD)] x 100.
10. Eficiencia reproductiva (ER) = peso de las semillas llenas / peso seco del cono (PSC).
11. Índice de endogamia (IE) = semillas vanas (SV) / total de semillas desarrolladas (TSD). Donde TSD = semillas llenas (SLL) + semillas vanas (SV) + semillas dañadas por insectos, hongos o bacterias (SDIHB).
12. Porcentaje de germinación calculado a partir de 31 semillas por árbol por población.

3.2.3. Análisis estadísticos

Se realizaron pruebas de normalidad para los datos de los diferentes indicadores reproductivos. Con base en estos análisis, se realizaron pruebas de Kruskal-Wallis (para datos no paramétricos) y pruebas de Dunn (utilizando la corrección de Bonferroni, partiendo de $\alpha = 0.05$) para detectar diferencias en la germinación de semillas entre poblaciones y especies. Finalmente, se estimó el coeficiente de correlación de Spearman (*rs*) (Hauke y Kossowski, 2011) entre variables reproductivas, como una aproximación para reducir el grupo de variables en futuras evaluaciones. Todos los análisis se realizaron con el software libre R (R Core Team, 2015).

3.3. Resultados

3.3.1. Picea martinezii

El potencial de semillas promedio para *P. martinezii* es de 280 semillas por cono, con un rango de 259 a 292 (Figura 3.4), donde el primer valor corresponde a la población Agua Fría que se diferencia del resto. Sin embargo, la especie presenta altas pérdidas de semillas (Figura 3.4), por lo tanto, baja eficiencia de semillas (ES = 16.0 %) y eficiencia reproductiva (ER = 0.31), así como un elevado índice de endogamia (IE = 0.68) (Cuadro 3.2).

Entre las causas o factores que explican la pérdida de semilla sobresalen el porcentaje de óvulos abortados y de semillas vanas, mientras que los óvulos rudimentarios y las semillas dañadas representan una causa casi nula en la pérdida de semillas de esta especie (Cuadro 3.2).

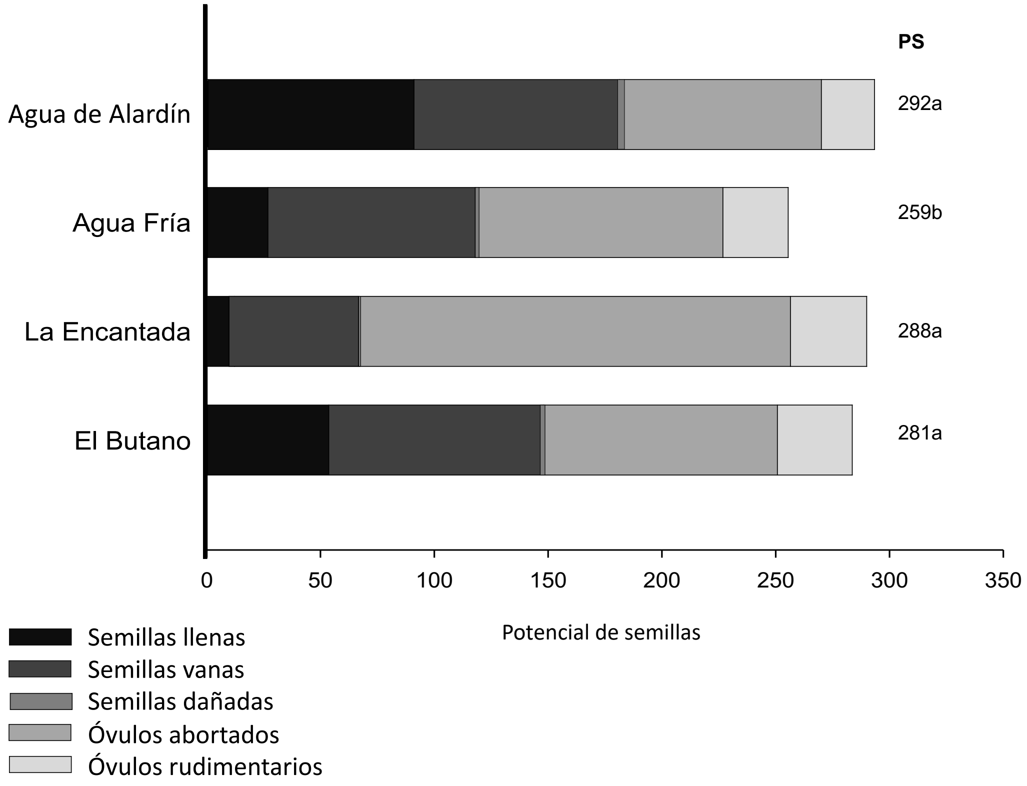

Figura 3.4 Potencial de semillas (PS) de cuatro poblaciones de *Picea martinezii* en México. Las medias seguidas de letras iguales no son estadísticamente diferentes (α = 0.05).

Con respecto al análisis de correlación entre los principales indicadores reproductivos de *P. martinezii*, se detectaron correlaciones significativas entre la eficiencia de semillas (ES) y la proporción de semillas llenas (PSLL) con el índice de endogamia (IE) y con la eficiencia reproductiva (ER) (Cuadro 3.3 y Fig. 3.5).

Finalmente, se determinó un porcentaje de germinación de semillas promedio de 72.9 % para *P. martinezii*, con diferencias significativas de germinación entre poblaciones; El Butano y Agua de Alardín con el mayor porcentaje de germinación, y Agua Fría con el menor (Cuadro 4.4).

Cuadro 3.2 Indicadores reproductivos de conos y semillas de *Picea martinezii* en el año de colecta 2018.

Indicadores reproductivos	Promedio
Peso seco del cono (gr)	32.56
Número de semillas llenas por cono	45.71
Proporción de semillas llenas	31.14
Proporción de semillas vanas	67.95
Proporción de óvulos abortados	44.79
Proporción de óvulos rudimentarios	8.39
Proporción de semillas dañadas por insectos, hongos o bacterias	0.91
Eficiencia reproductiva [2] (mg/g)	31.0
Índice de endogamia [3]	0.68
Longitud del cono (cm)	13.64
Diámetro del cono (cm)	3.44

Nota: [1] Peso de semilla llena en mg / Peso seco del cono en g. [2] Semillas vanas / Semillas desarrolladas.

Cuadro 3.3 Coeficientes de correlación de Spearman entre los principales indicadores reproductivos en las poblaciones de *Picea martinezii* T.F. Patt. y *Picea mexicana* Martínez, en el año de colecta 2018.

	Picea martinezii					***Picea mexicana***				
	LC	PSC	PSLL	IE	ER	LC	PSC	PSLL	IE	ER
ES	0.37**	0.42**	0.86**	-0.86**	0.97**	0.24**	0.04	0.89**	-0.86**	0.90**
LC		0.83**	0.25**	-0.25**	0.29**		0.47**	0.18**	-0.17**	0.34**
PSC			0.32**	-0.32**	0.31**			-0.02**	0.03	-0.01
PSLL				-0.99**	0.85**				-0.98**	0.81**
IE					-0.85**					-0.78**

Nota: ES= Eficiencia de semillas, LC = Longitud del cono, PSC = Peso seco del cono, PSLL = Proporción de semillas llenas, IE = Índice de endogamia y ER = Eficiencia reproductiva.
** Valores con significancia ($p<0.0001$).

Cuadro 3.4 Porcentaje de germinación de semillas de *Picea martinezii* y *Picea mexicana*. Las medias en una misma fila seguidas de letras iguales no son estadísticamente diferentes (α = 0.05).

Germinación [1]	***Picea martinezii***				***Picea mexicana***		
	EB	**AA**	**AF**	**LE**	**EM**	**LM**	**EC**
Promedio población	78.49a	79.46a	61.29b	72.46ab	80.14a	74.41a	80.86a
Promedio especie	72.93a				78.47a		

Nota: [1] Promedio de germinación estimado a partir de 31 semillas por árbol. EB = El Butano, AA = Agua de Alardín, AF = Agua Fría, LE = La Encantada, EM = El Mohinora, LM = La Marta, EC = El Coahuilón.

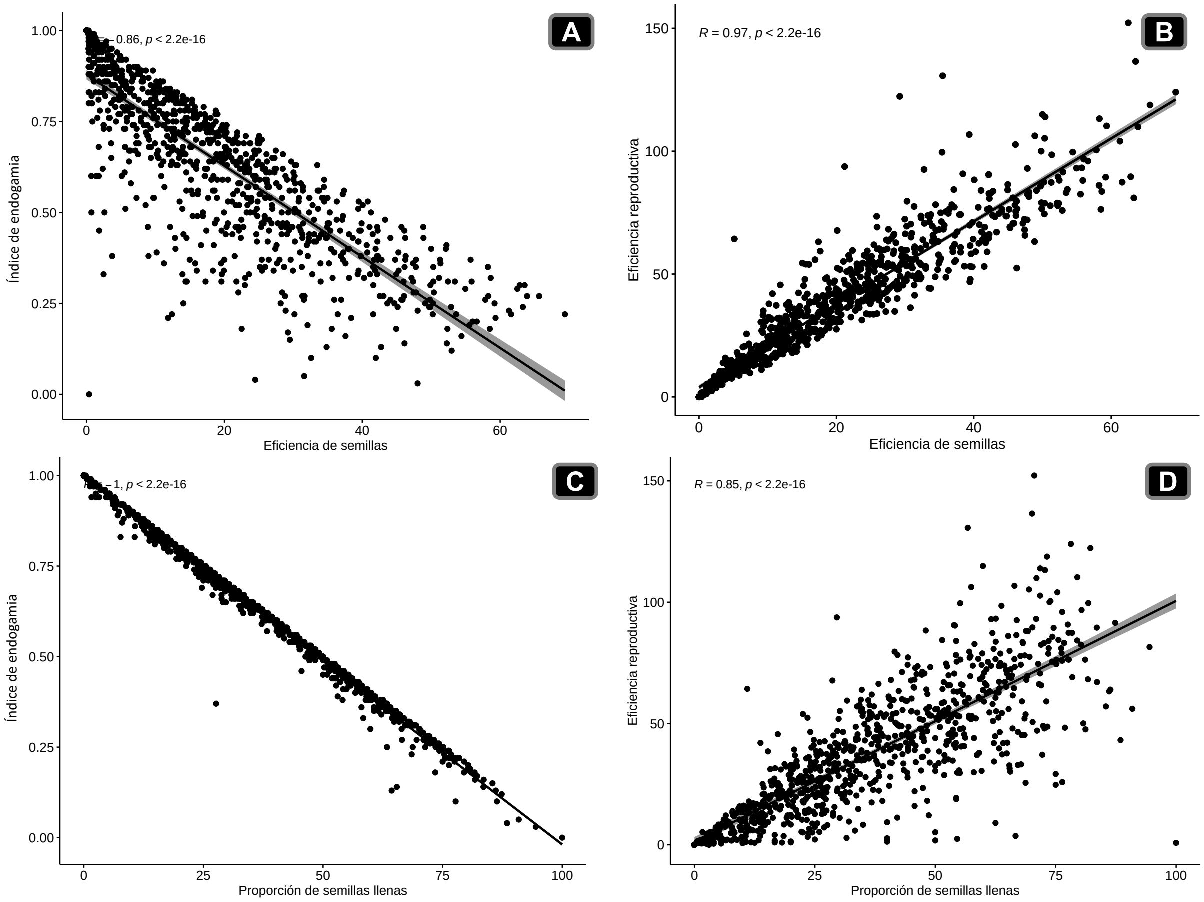

Figura 3.5 Coeficiente de correlación de Spearman entre la eficiencia de semillas con el índice de endogamia (A) y la eficiencia reproductiva (B); y entre la proporción de semillas llenas con el índice de endogamia (C) y la eficiencia reproductiva (D) en *Picea martinezii*.

3.3.2. *Picea mexicana*

El potencial de semillas promedio para *P. mexicana* es de 147 semillas por cono, con un rango de 142 a 150, sin encontrarse diferencias significativas entre poblaciones (Fig. 3.6). Esta especie también presenta altas proporciones de óvulos abortados, óvulos rudimentarios y semillas vanas (Fig. 3.6), baja eficiencia de semillas (ES = 28%) y eficiencia reproductiva (ER = 24.0), así como un elevado índice de endogamia (IE = 0.55) (Cuadro 3.5).

Al igual que en *P. martinezii*, entre las causas o factores que explican la pérdida de semilla en *P. mexicana* sobresalen los porcentajes de semillas vanas y de óvulos abortados, y en menor medida por los óvulos rudimentarios, mientras que las semillas dañadas representan una causa muy reducida en la pérdida de semillas en las poblaciones (Fig.3.6).

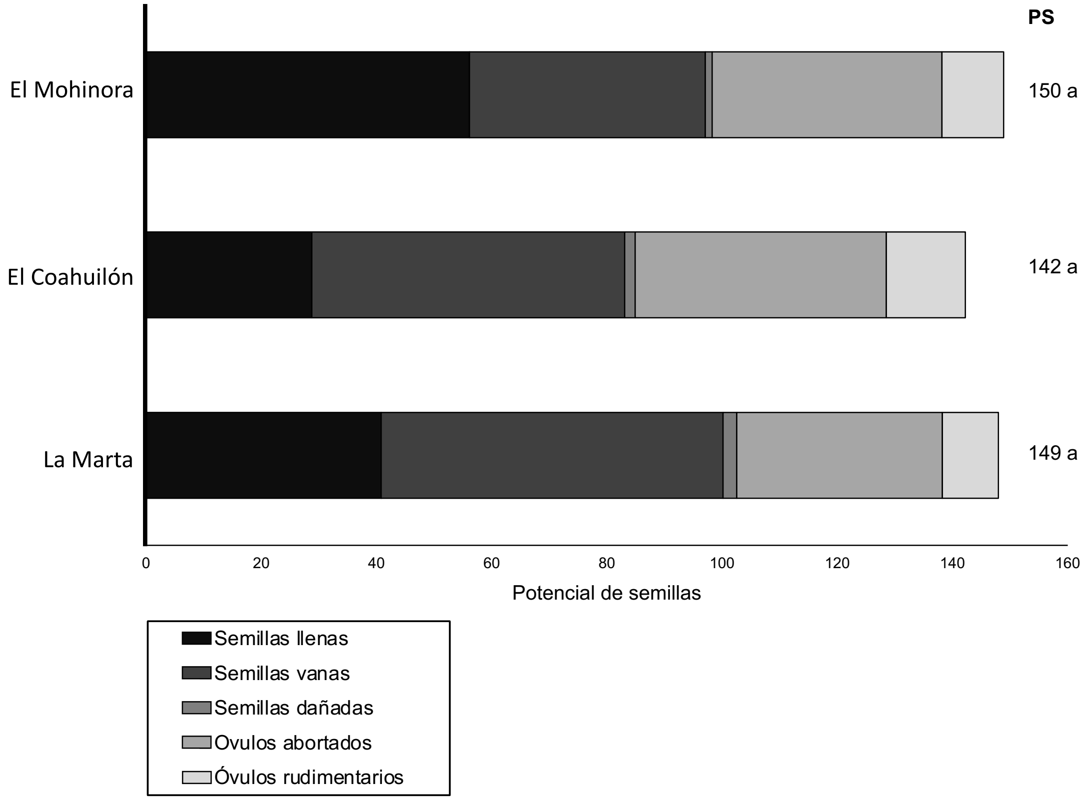

Figura 3.6. Potencial de semillas (PS) de las tres poblaciones de *Picea mexicana* en México. Las medias seguidas de letras iguales no son estadísticamente diferentes (α = 0.05).

Cuadro 3.5 Indicadores reproductivos de conos y semillas de *Picea mexicana* en el año de colecta 2018.

Indicadores reproductivos	Promedio
Peso seco del cono (gr)	9.25
Número de semillas llenas por cono	41.89
Proporción de semillas llenas	43.07
Proporción de semillas vanas	54.81
Proporción de óvulos abortados	27.19
Proporción de óvulos rudimentarios	8.27
Proporción de semillas dañadas por insectos, hongos o bacterias	2.11
Eficiencia reproductiva (mg/g) [2]	24.0
Índice de endogamia [3]	0.55
Longitud del cono (cm)	5.35
Diámetro del cono (cm)	2.38

Con respecto al análisis de asociación entre los principales indicadores reproductivos de *P. mexicana*, se detectaron correlaciones significativas entre la eficiencia de semillas (ES) y la proporción de semillas llenas (PSLL) con el índice de endogamia (IE) y con la eficiencia reproductiva (ER) (Cuadro 3.3 y Fig. 3.7).

En *P. mexicana* se estimó un valor promedio de germinación de semillas de 78.5 %, sin diferencias significativas de germinación entre poblaciones (Cuadro 3.4). No se detectaron diferencias significativas en los porcentajes de germinación entre especies (Cuadro 3.4).

3.4. Discusión

Los resultados muestran que el potencial de semillas promedio para *Picea martinezii* (PS = 280) es de los más altos en comparación con otras coníferas como *Pinus patula* (PS = 82, Alba-Landa, 1999), *P. tecunumanii* (PS = 125, Isaza *et al.,* 2002), *P. oocarpa* (PS = 137, Isaza *et al.,* 2002), *P. maximinoi* (PS = 140, Isaza *et al.,* 2002), *Pinus greggii* (161, Alba-Landa *et al.,* 2005), *P. oaxacana* (PS = 186, Vázquez *et al.,* 2004), *P. hartwegii* (PS = 199, Alba-Landa *et al.,* 2005). El potencial de semilla

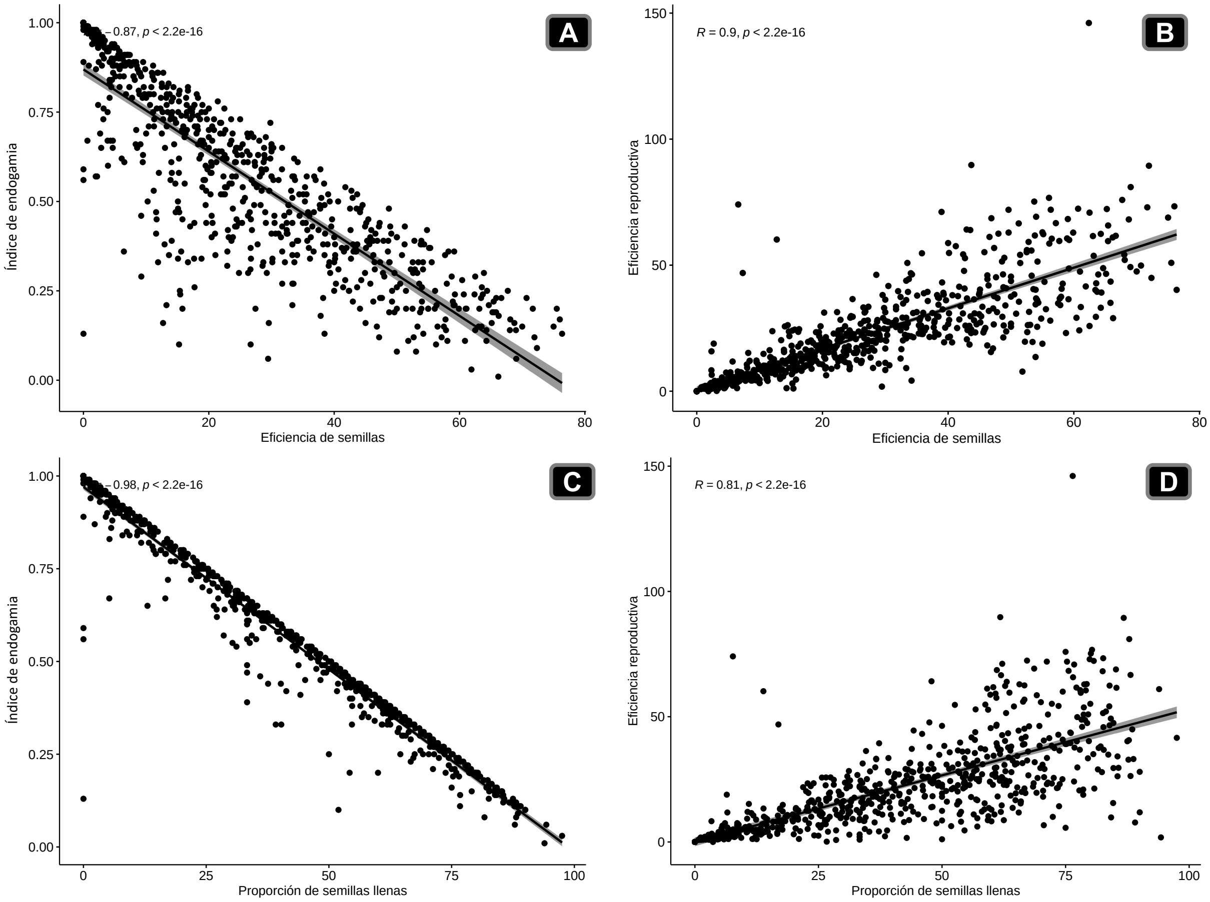

Figura 3.7 Coeficientes de correlación de Pearson entre la eficiencia de semillas y el índice de endogamia (A) y la eficiencia reproductiva (B); y entre la proporción de semillas llenas y el índice de endogamia (C) y la eficiencia reproductiva (D) en *Picea mexicana* Martínez.

que se reporta aquí para *Picea martinezii* es mayor incluso al encontrado por Flores-López *et al.,* (2012) (PS = 266). Una variación similar en diferentes temporadas de producción se reporta para *Pinus cooperi* (PS = 103 y 115, Prieto y Martínez, 1993).

En cuanto a la eficiencia de semillas, *P. martinezii* (ES = 19) presenta los porcentajes más bajos en comparación con otras coníferas como *Pinus gregii* (ES = 78.9, Alba-Landa *et al.,* 2005), *P. hartwegii* (ES = 71.5, Alba-Landa *et al.,* 2005), *Pinus cooperi* (ES = 68.8, Prieto y Martínez, 1993), *P. patula* (ES = 64.6, Alba-Landa, 1999), aunque similar al encontrado para especies como *Pseudotsuga mensiezii* (ES = 24.5, Zavala y Méndez, 1996), *Pinus tecunumanii* (ES = 8.0, Isaza *et al.,* 2002) o *P. maximinoi* (ES = 8.0, Isaza *et al.,* 2002).

El índice de endogamia promedio de *Picea martinezii* calculado en este estudio (IE = 0.68) fue menor al reportado por Flores-López *et al.,* (2012) para esta misma especie (IE = 0.75). No se cuenta con un valor de referencia para la eficiencia reproductiva (ER) para *P. martinezii*, sin embargo, el valor calculado es mayor que para *Picea rubens* que tiene una distribución más amplia, pero con poblaciones aisladas (Mosseler *et al.,* 2000).

Por otra parte, el potencial de semillas de *P. mexicana* (PS = 147) se ubica dentro del rango encontrado para otras pináceas como *P. oocarpa* (Isaza *et al.,* 2002) o *P. maximinoi* (Isaza *et al.,* 2002). La eficiencia de semillas (ES = 28) es menor que para especies de pinos como *Pinus gregii* (Alba-Landa *et al.,* 2005), *P. hartwegii* (Alba-Landa *et al.,* 2005), *Pinus cooperi* (Prieto y Martínez, 1993) y *P. patula* (Alba-Landa, 1999), pero similar a especies como *Pseudotsuga mensiezii* (Zavala y Méndez, 1996), *Pinus tecunumanii* (Isaza *et al.,* 2002) o *P. maximinoi* (Isaza *et al.,* 2002). El índice de endogamia (IE = 0.55) es menor al reportado por Flores-López *et al.,* (2005) (IE = 0.79). La eficiencia reproductiva de *P. mexicana* se ha mantenido estable en un lapso de 20 años (ER1999-2001 = 0.24 *vs* ER2018 = 0.24), valor que es mayor al encontrado para *Picea rubens* (Mosseler *et al.,* 2000).

En general, las diferencias entre los diferentes indicadores reproductivos reportados aquí con los evaluados previamente para ambas especies pueden deberse al recambio de los individuos participantes en cada evento reproductivo (población efectiva). Sin embargo, el incremento en la eficiencia de semillas y la reducción de los índices de endogamia en ambas especies indican un posible incremento en el tamaño efectivo de población en ellas, mediante la integración de árboles jóvenes a la edad reproductiva; lo cual es posible en el lapso de 12 y 20 años transcurridos desde la última evaluación en *P. martinezii* y en *P. mexicana*, respectivamente. Estas hipótesis podrían evaluarse con estudios fenológicos y demográficos más extensos.

Por otra parte, ambas especies presentaron alto porcentaje de germinación de semillas (Cuadro 4.4), similar al de otras coníferas de amplia distribución, como *Pinus patula* Sch. et Cham. (75-84 %) y *Pinus pseudostrobus* Lindl. (70-81%) (Aparicio-Rentería *et al.*, 1999). Aunque la pérdida de semillas en forma de semillas vanas, óvulos abortados y óvulos rudimentarios es muy alta en ambas especies, las pocas semillas desarrolladas llenas tienen elevada viabilidad. Por lo tanto, un factor crítico es determinar las causas intrínsecas o extrínsecas implicadas en esta elevada pérdida de semillas.

Las causas que impiden que una semilla no culmine su proceso de crecimiento y desarrollo pueden ser factores endógenos adversos (Varnell, 1976; Prieto y Martínez, 1993). De acuerdo con Karrfalt y Belcher (1977), la baja eficiencia de semilla se debe generalmente a tres causas: escasa polinización, autopolinización y daños por insectos. Esto se observa en los resultados registrados en varias especies de *Picea* (Caron y Powell, 1989). Adicionalmente, Frankham (1996) incluye el reducido tamaño poblacional entre los factores que pueden tener efectos directos en la viabilidad reproductiva, al reducirse la diversidad genética e incrementarse la frecuencia de alelos deletéreos mediante endogamia o deriva génica. La comparación a nivel de especie entre los indicadores de *P. martinezii* y *P. mexicana* con otras pináceas sugiere que ambas especies aún se encuentran dentro del rango normal en cuanto a capacidad reproductiva. Sin embargo, a nivel poblacional el estatus podría ser diferente, por lo que es necesario evaluar el efecto del tamaño poblacional y la diversidad genética sobre este importante indicador reproductivo.

Los métodos de manejo para contrarrestar la pérdida de semillas podrían ser el incremento de la diversidad genética dentro de las poblaciones mediante la polinización controlada (Mendoza-Maya *et al.,* 2015), el incremento del tamaño de población mediante reforestaciones con individuos seleccionados que posean la mayor eficiencia reproductiva y el menor índice de endogamia determinados mediante análisis de conos, en diferentes eventos reproductivos. La selección de los árboles semilleros podría apoyarse también con marcadores moleculares que permitan identificar alelos deletéreos y su efecto en la viabilidad reproductiva, y de esta manera seleccionar a los individuos con la menor cárga de alelos deletéreos y más adecuación. Acciones de manejo adicionales son la identificación y saneamiento de plagas y enfermedades, lo cual se puede realizar mediante sitios permanentes de monitoreo. Todo esto puede permitir, en el mediano y largo plazo, la conservación de estas especies *in situ*. Sin embargo, es importante considerar los escenarios climáticos futuros (Ledig *et al.,* 2010), que indican que la conservación *ex situ* será necesaria.

La evaluación de los indicadores reproductivos es una herramienta esencial para el monitoreo de la viabilidad de las poblaciones (Bramlett *et al.,* 1977, Mosseler *et al.,* 2000), al evaluar caracteres implicados directamente en la adecuación de las especies a los sitios que habita. Sin embargo, es una metodología laboriosa que implica una inversión considerable de tiempo, el cual muchas veces es limitado. De acuerdo con los resultados de las asociaciones entre los principales indicadores reproductivos (Figs. 4.5 y 4.7), es razonable suponer que la evaluación de la proporción de semillas llenas (PSLL) o la eficiencia de semillas (ES), dentro del grupo completo de indicadores reproductivos, permite una estimación confiable del índice de endogamia y de la eficiencia reproductiva. Esto permitiría reducir en gran medida la carga de trabajo y el tiempo de evaluación.

3.4. Referencias

Alba-Landa, J. 1999. Potencial de producción de semilla de la progenie de un huerto semillero de segunda generación de selección de *Pinus patula* Schl. et Cham. en el estado de Veracruz. ISIMA, Durango, México, pp. 133-134.

Alba-Landa, J., J. Márquez-Ramírez y H. S. Bárcenas. 2005. Potencial de producción de semillas de *Pinus greggi* Engelm. en tres cosechas de una población ubicada en Carrizal Chico, Zacualpan Veracruz, México. Foresta Veracruzana 7(2): 37-40.

Alba-Landa, J., A. Aparicio-Rentería y J. Márquez-Ramírez. 2003. Potencial y eficiencia de producción de semillas de *Pinus hartwegii* Lindl. de dos poblaciones de México. Foresta Veracruzana, 5(1): 25-28.

Alba-Landa., J. y J. Márquez-Ramírez. 2006. Potencial y eficiencia de producción de semillas de *Pinus oaxacana* Mirov. de Los Molinos, Perote, Veracruz. Foresta Ve- racruzana 8(1): 31-36.

Aparicio-Rentería, A., H. Cruz-Jiménez y J. Alba-Landa. 1999. Efecto de seis sustratos sobre la germinación de *Pinus patula* Sch. et Cham., *Pinus montezumae* Lamb. y *Pi- nus pseudostrobus* Lindl. en condiciones de vivero. Foresta Veracruzana 1(2): 31-34.

Bramlett, D. L. 1974. Seed potential and seed efficiency. *In*: John Kraus (ed.). Seed yield from southern pine seed orchards colloquium proceedings. Ga. For. Res. Counc., Macon, Ga. U. S. A. pp. 1-7.

Bramlett, D. L., E. W. Belcher Jr., G. L. DeBarr, J. L Hertel, R. P. Karrfalt, C. W. Lantz, T. Miller, K. D. Ware y H. O. III Yates. 1977. Cone analysis of Southern pines: a guidebook. Gen. Tech. Rep. SE-13. Asheville, N.C. USDA, Forest Service, Southeastern Forest Experiment Station, Asheville, N.C. U. S. A. 28 pp.

Caron, G. E. y G. R. Powell. 1989. Cone size and seed yield in young *Picea mariana* trees. Canadian Journal Forest Resources 19: 351-358.

Dunn, O. J. 1964. Multiple comparisons using rank sums. Technometrics 6(3): 241-252.

Flores-López, C. 2004. Indicadores reproductivos en tres poblaciones de *Picea mexicana* Martínez de México. Tesis de Maestria en Ciencias. Colegio de Postgraduados. Montecillo, Texcoco, Edo. de México. 46 pp.

Flores-López, C. 2014. Líneas para la conservación de los recursos genéticos de *Picea mexicana* Martínez y *Picea martinezii* Patterson. Tesis Doctor en Ciencias Forestales. Facultad de Forestal y Agronomía, Centro de Estudios Forestales, Universidad de Pinar Del Río. República de Cuba. 90 pp.

Flores-López C., J. López-Upton y J. J. Vargas-Hernández. 2005. Indicadores reproductivos en poblaciones naturales de *Picea mexicana* Martínez, 1-10 pp.

Flores-López, C., C. G. Geada-López, J. López-Upton y E. López-Ramírez. 2012. Reproductive indicators in natural populations of *Picea martinezii* T. F. Patterson. Revista Forestal Baracoa 31(2): 49–58.

Frankham, R. 1996. Relationship of genetic variation to population size in wildlife. Conservation Biology 10 (6): 1500-1508.

González-Ávalos, J., E García-Moya, J. J. Vargas-Hernández, A. Trinidad-Santos, A. Romero-Manzanares y V. M. Cetina-Alcalá. 2006. Evaluación de la producción y análisis de conos y semillas de *Pinus cembroides* Zucc. Revista Chapingo. Serie Ciencias Forestales y del Ambiente 12(2): 133-138.

Hauke, J., and Kossowski, T. 2011. Comparison of values of Pearson's and Spearman's correlation coefficients on the same sets of data. Quaest. Geogr., 30: 87–93.

Hernández-Carmona., O., E. O. Ramírez-García y L. Mendizábal-Hernández. 2003. Variación en semillas de cinco procedencias de *Pinus pseudostrobus* Lindl. Foresta Veracruzana 5(2): 23-28.

Isaza, N., W. S. Dvorak y J. López-Upton. 2002. Producción de semillas del género *Pinus* en huertos y rodales semilleros de Smurfit Cartón de Colombia, Informe de Investigación no. 187, Smurfit Cartón de Colombia, Cali, Colombia, 9 pp.

Karrafalt, R. P. y E. W. Belcher. 1977. Evaluation of seed production by cone analysis. Northeastern Forest Tree Improvement Conference. University of Maryland, Center for Environmental and Estaurine Studies. College Park, Maryland. Georgia. pp. 84-89.

Ledig, F. T., V. Jacob C., P. D. Hodgskiss y T. Eguiluz-Piedra. 1997. Recent evolution and divergence among populations of rare Mexican endemic, Chihuahua spruce, following Holocene climatic warming. Evolution 51 (6): 1815-1827.

Ledig, F. T., M. Mápula-Larreta, B. Bermejo-Velázquez, V. Reyes-Hernández, C. Flores-López y M. A. Capó-Arteaga. 2000a. Locations of endangered spruce populations in Mexico and the demography of *Picea chihuahuana*. Madroño 47:71-78.

Ledig, F. T., B. Bermejo-Velázquez, P. D. Hodgskis, D. R. Johnson, C. Flores-López y V. Jacob-Cervantes. 2000b. The mating system and genetic diversity in Martínez spruce, an extremely rare endemic of Mexico's Sierra Madre Oriental: an example of facultative selfing and survival in interglacial refugia. Canadian Journal of Forest Research 30: 1156-1164.

Ledig, F. T., G. E. Rehfeldt, C. Sáenz-Romero y C. Flóres-López. 2010. Projections of suitable habitat for rare species under global warming scenarios. American Journal of Botany 97: 970-987.

Ledig, F. T., P. D. Hodgskiss y V. Jacob-Cervantes. 2002. Genetic diversity, mating system, and conservation of a Mexican subalpine relict, *Picea mexicana* Martínez. Conservation Genetics 3:113-122.

Mendoza-Maya, E., J. Espino-Espino, C. Z. Quiñones-Pérez, C. Flores-López, C. Wehenkel, J. J. Vargas-Hernández y C. Sáenz-Romero. 2015. Propuesta de conservación de tres especies mexicanas de picea en peligro de extinción. Revista Fitotecnia Mexicana 38(3): 235-247.

Mosseler, A. 1998. Miniumm viable population size and the conservation of forest genetic resources. *In*: S. Puri (Ed.). Tree improvement: applied research and technology transfer. Science Publishers, Inc. U.S.A. pp. 191-205.

Mosseler, A., J. E. Major, J. D. Simpson, B. Daigle, K. Lange, Y.-S. Park, K.H. Johnsen, y O.P. Rajora. 2000. Indicators of populations viability in red spruce, *Picea rubens*. I. Reproductive traits and fecundity. Canadian Journal of Botany 78:928-940.

Patterson, T. E. 1988. A new species of *Picea* (Pinaceae) from Nuevo Leon, Mexico. SIDA 13: 131-135.

Prieto R., J. A y J. Martínez A. 1993. Análisis de conos y semillas en dos áreas semilleras de *Pinus cooperi*. Folleto científico No 1. SARH, INIFAP, Centro de Investigación Regional del Norte Centro. Campo Experimental "Valle del Guadiana". Durango, Dgo. México. 18 pp.

Rajora, O. P. y A. Mosseler. 2001. Challenges and opportunities for conservation of forest genetic resources. Euphytica 118(2): 197-212.

Ramírez-García., E. O., J. Márquez-Ramírez y O. Hernández-Carmona. 2007. Estudio de conos de *Pinus greggii* Engelm. de una plantación en el municipio de Naolinco, Veracruz, México. Foresta Veracruzana 9(2): 39-44.

R Core Team. 2015. R: A Language and Environment for Statistical Computing; R Foundation for Statistical Computing: Vienna, Austria. Available online: http://www.R-project.org/

Sorensen, F. C. y R. S. Miles. 1974. Self-pollination on Douglas-fir and ponderosa pine seeds and seedlings. Silvae Genetica 23 (5): 135-165.

Varnell, R. J. 1976. Cone and seed production in Flash Pine: effects of tree dimensions and climatic factors. Departament of Agriculture Southeastern Forest Experiment Station Asheville, Nort Carolina, U. S. A. 145: 1-10.

Vaughton, G. y M. Ramsey. 1995. Pollinators and seed production. *In*: seed development and germination. Edited by J. Kigel, G. Galili. New York, USA. pp. 475-489.

Vázquez, O. G., E. O. Ramírez-García y L. Alba-Landa. 2004. Variación de conos y potencial de producción de semillas de *Pinus oaxacana* Mirov. en una población del estado de Tlaxcala, México. Foresta Veracruzana 6(2): 31-36.

Zavala, C. F. y J. T. Méndez M. 1996. Factores que afectan la producción de semillas en *Pseudotsuga macrolepis* Flous en el estado de Hidalgo, México. Acta Botanica Mexicana 36: 1-13.

CAPÍTULO 4. GENOMIC AND EVOLUTIONARY DRIVERS OF REPRODUCTIVE FITNESS IN TWO ENDANGERED FOREST TREES

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Abstract

Reproductive success has a capital role on individual fitness, population viability, conservation and breeding. However, the genetic basis and evolutionary dynamics of this trait is still poorly understood. We disentangle various components of reproductive success and demographic data for two endangered forest trees, *Picea martinezii* and *Picea mexicana*, to search for putative genomic drivers of such components and to infer strategies to face extinction vortices. Results showed: *i*) a positive association between population size and reproductive traits related to seed development, but no association with seed germination and genetic diversity; *ii*) the genetic basis of traits related to seed development, fitted a polygenic model of adaptation with more genomic drivers for *P. martinezii* and not shared ones between species; *iii*) functional annotations of candidate fitness-loci revealed proteins associated to embryo development; *iv*) fitness-loci contained derived alleles with putative deleterious or advantageous effects, respectively distributed in small or large populations, which agree with the reproductive fitness and demographic structure of populations; *v*) a genetic purge is occurring at seed development for both spruces, and it is possibly more advanced for *P. mexicana*. The risk of species and populations of being in extinction vortices is discussed as well.

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Key words: conservation genomics, effective population size, Genome-Wide Association Studies (GWAS), genomic architecture of complex traits, Heterozygosity-Fitness Correlations (HFCs), *Picea martinezii*, *Picea mexicana*, extinction vortex.

4.1. Introduction

Reproductive success is one of the most important factors for defining individual fitness (Caignard *et al.*, 2019). This trait can be expressed as age at sexual maturity, fertility (the production of viable gametes or zygotes), fecundity (the total seasonal or lifetime reproductive output) or the amount of progeny that survive and reproduce (Frankel & Soulé, 1981; Wiens *et al.*, 1987). Ecologically, reproductive success is directly related to within-population individual recruitment, which at its turn determines the potential of a population to persist, expand and adapt over time (Brys *et al.*, 2003). However, despite its capital role for breeding and conservation, the genomic architecture and evolutionary dynamics of reproductive success is still poorly understood (Frankham, 2009; Caignard *et al.*, 2019). Most particularly, additional research is needed to understand whether or not there are genes underlying reproductive success, whether they are shared between related species or are distinctive to each taxa (Satake & Kelly, 2021).

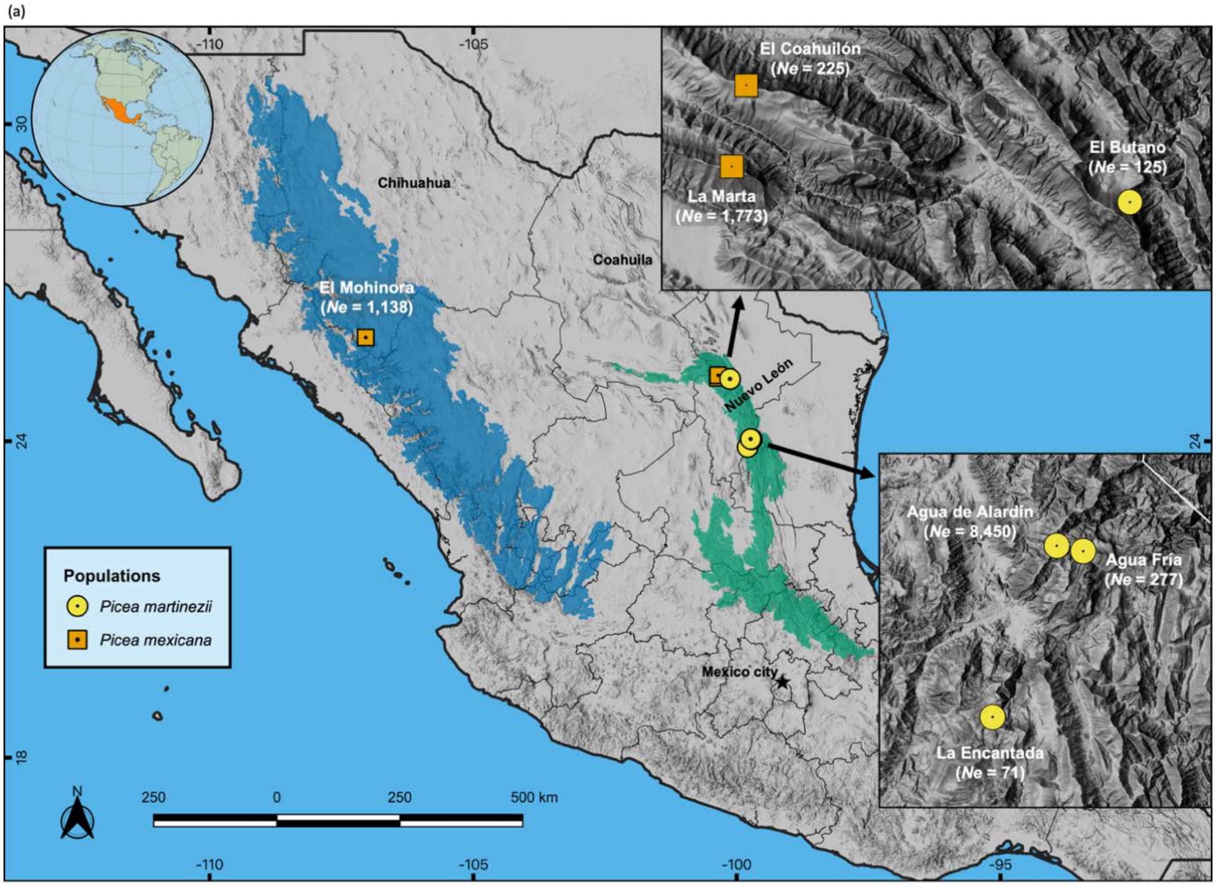
From an evolutionary point of view, reproductive success may have a direct impact on the effective population size (*Ne*), a basic parameter for many conservation and management plans (Hoban *et al.*, 2020). Defined as the sample size of an ideal population that maintains the same levels of genetic drift and heterozygosity (*He*) than a natural population, *Ne* usually encompasses adult individuals that successfully reproduce, and whose number is generally much smaller than the complete census of a population (*Nc*; Ellegren and Galtier, 2016). Population genetics theory predicts a decrease of *He* in finite populations at a rate of $1 / 2Ne$ per generation, which implies a more rapid decline in smaller than in larger populations (Charlesworth, 2009; Ellegren & Galtier, 2016; Kardos *et al.*, 2023). In conservation biology, the link between *Ne*, *He* and individual fitness has led to the model of extinction vortices, where small, isolated populations may become extinct through a down-ward spinning cycle that increases genetic drift and inbreeding, which at their turn diminishes genetic diversity, and then translates in the long-term decrease of individual fitness and population viability (Gilpin & Soulé, 1986). However, a high reproductive output or fecundity, such as the one observed in long-lived organisms that dominate entire ecosystems (e.g., forest trees), may help delay or even avoid extinction vortices (Petit & Hampe, 2006). Thus, it should be of interest to explore how the putative genes underlying reproductive success (if any) respond to selective and stochastic evolutionary forces in threatened forest tree taxa, to complement their conservation plans (Sanjayan *et al.*, 2020; Nabutanyi & Wittmann, 2021).

The link between *Ne*, *He* and individual fitness and the genetic basis of inbreeding depression are usually explored through heterozygosity–fitness correlations (HFCs; David, 1998; Slate *et al.*, 2004; Grueber *et al.*, 2008; Szulkin *et al.*, 2010), although such studies have been so far more frequent in animals than in plants (e.g., Grueber *et al.*, 2008; Brommer *et al.*, 2015; Bateson *et al.*, 2016; Yates *et al.*, 2019; Botero-Delgadillo *et al.*, 2020a,b). Several hypotheses have been postulated to account for possible genomic drivers of heterozygosity–fitness correlations. These include: the additive effect of deleterious alleles distributed across the genome (i.e., the general effect hypothesis), which may become exposed in inbred (and thus less heterozygous) individuals (Szulkin *et al.*, 2010; Rodríguez-Quilón *et al.*, 2015); and the action of specific loci that directly (direct effect hypothesis) or indirectly (local effect hypothesis) affect individual fitness, and for which heterozygous individuals have an advantage (Szulkin *et al.*, 2010). The general effect hypothesis is often tested through HFCs with multilocus or genome-wide heterozygosity, while local and direct effects require genotype-phenotype correlations (GWAS), which imply intrinsic challenges to pinpoint fitness-loci (Kardos *et al.*, 2016; Hohenlohe *et al.*, 2021); particularly for species with overly large genomes and/or underdeveloped genomic resources. To date, studies supporting the general– (e.g., Reed & Frankham, 2003; Leimu *et al.*, 2006; Botero-Delgadillo *et al.*, 2020a), local– (e.g., Brommer *et al.*, 2015; Rodríguez-Quilón *et al.*, 2015) and direct–effect hypotheses (Bateson *et al.*, 2016) are available for species with different life spans, mating systems and geographical distributions. Although examples also exist for null (Lammi *et al.*, 1999; Yates *et al.*, 2019) or negative associations

(Altukhov *et al.*, 1986; Strauss & Libby, 1987; Botero-Delgadillo *et al.*, 2020b) between heterozygosity and fitness.

The capacity for detecting different kinds of HFCs often depends on an increased among-population variance for fitness-related traits, which results from contrasting historical *Ne* changes (Szulkin *et al.*, 2010). This variance is expected to increase with time and bottleneck severity, and diminish with increasing levels of gene flow (Charlesworth & Willis, 2009; Kyriazis *et al.*, 2021; Waller, 2021). Thus, recently collapsed, expanding or connected populations should not be able to completely purge their genetic load and will exhibit a direct relationship between inbreeding, heterozygosity and individual fitness, to allow the detection of general-effect loci. On the other hand, populations that have collapsed and remained small and isolated for long periods of time should have purged most of their genetic load and exhibit low levels of genome-wide heterozygosity (because of genetic drift), except for fitness-related regions, at which heterozygosity should be higher, either because the heterozygous has an advantage or because one of the homozygous is extremely deleterious (Charlesworth & Willis, 2009; Waller, 2021); such populations should allow detecting direct- and local-effect loci.

Martínez (*Picea martinezii* Patterson) and Mexican (*P. mexicana* Martínez) spruces are two relict and rare endemic conifers confined to very narrow and fragmented habitats in the north-facing slopes of specific mountains in northern Mexico (Ledig *et al.*, 2000a; Mendoza-Maya *et al.*, 2022) (Fig. 4.1). They are both endangered, and bear reduced genetic diversity (Ledig *et al.*, 2000b, 2002; Jaramillo-Correa *et al.*, 2006; Mendoza-Maya *et al.*, 2015; IUCN, 2023). Martínez spruce is restricted to four known stands clustered in two groups in the montane cloud and conifer-deciduous mixed forests (at elevations between 1,800 and 2,600 m) of the northern Sierra Madre Oriental (SMOr; Fig. 4.1); while Mexican spruce has a disjunct distribution in montane to subalpine zones (at elevations from 3,000 to 3,600 m), with two known populations in the SMOr and one stand in the Sierra Madre Occidental (SMOc; Mendoza-Maya *et al.*, 2022) (Fig. 4.1). While formal phylogeographic studies are still pending (i.e., using Approximate Bayesian Computation modelling and parameter estimation), evidence suggest that these spruces were subjected to contrasting demographic dynamics since



(b)

Picea martinezii				*Picea mexicana*			
Population pair	**Distance (km)**	**F_{ST}**	**Nm**	**Population pair**	**Distance (km)**	**F_{ST}**	**Nm**
Agua de Alardín – Agua Fría	2.5	0.072	3.2	El Coahuilón – La Marta	5.5	0.040	6.0
Agua de Alardín – La Encantada	17.8	0.182	1.1	El Mohinora – La Marta	676.5	0.110	2.0
Agua Fría – La Encantada	18.3	0.178	1.2	El Mohinora – El Coahuilón	676.7	0.099	2.3
Agua de Alardín – El Butano	132.0	0.136	1.6				
Agua Fría – El Butano	133.2	0.128	1.7				
El Butano – La Encantada	146.6	0.225	0.9				
Mean		**0.154**		**Mean**		**0.083**	

Figure 4.1. Locations and pairwise geographical and genetic distances of the studied *Picea mexicana* (squares) and *P. martinezii* populations (circles). (a) Map of locations including estimates of effective population sizes (*Ne*, determined as 10% of the total census; Hoban *et al.*, 2021), as well as mountain ranges referred to in text: Sierra Madre Occidental (SMOc, dark blue) and Sierra Madre Oriental (SMOr, bluish green). (b) Pairwise geographic distance, F_{ST} and migrants per generation (*Nm*) between populations. Note that the populations of Agua de Alardín and La Encantada are also known in some refences as Cañada Puerto (e.g., Ledig *et al.*, 2000a) and La Tinaja (Ledig *et al.*, 2000b), respectively. All F_{ST} pairwise comparisons were significant at *p*-values < 0.001.

the Last Glacial Maximum (~22,000 years before); while Martínez spruce probably collapsed and remained at favorable microsites within its glacial distribution (Ledig *et al.*, 2000b), Mexican spruce likely represents the remnants of an ancient migration from central Mexico (Mendoza-Maya *et al.*, 2022). These characteristics make them interesting models to study the genomic components of reproductive success and individual fitness, and their putative resilience to extinction vortices. More specifically, Martínez spruce should show more marked general HFCs than Mexican spruce, while this last taxon should exhibit more local- and direct-effect loci than the former. The large phylogenetic distance and complete lack of natural hybridization between these two spruces (Lockwood *et al.*, 2013) should further allow detecting shared and species-specific direct effects fitness-loci.

In this study, we aimed to disentangle various components of reproductive success for these two endangered spruces and associate them with genomic and demographic data. First, we evaluated various HFCs models and identified putative genomic components associated to reproductive success. We then compare such components between species to infer common strategies to face extinction vortices. This study represents one of the first attempts for identifying the genomic basis of reproductive fitness in trees and exemplifies how to develop evolutionary-informed conservation strategies from genomic information for threatened species.

4.2. Materials and Methods

4.2.1. Sampling and reproductive fitness analysis

Taking profit that fertility in conifers can be traced down from the production of reproductive structures (strobili) and gametes to mature embryos and latent-viable seeds (Williams, 2009), we collected cones from 13 to 30 trees (depending on bearing-cone trees availability) from each known populations of both species (Mendoza-Maya *et al.*, 2022; Fig. 4.1), in October-November 2018. This resulted in 94 and 90 individuals sampled for *P. martinezii* and *P. mexicana*, respectively (Supporting Information Table S1). Collected individuals were ideally separated by up to 50 m from each other; however, for the smaller populations (i.e., La Encantada, Agua Fría, El Butano and El Coahuilón), we had to reduce such distance to as little as 10 m. All sampled trees had a straight stem with a diameter at breast heigh (DBH) larger than 7.0 cm and were dominant or co-dominant in height. Individual coordinates and elevation are available in Supporting Information Table S1. Then, from each sampled individual we selected between five and ten cones with no apparent physical or insect damage and dried them inside individual paper bags at 45 ºC for 24 to 72 h in an air-flow drier, until their scales were totally open.

We analyzed the cones following Mosseler *et al.* (2000) and O'Connell *et al.* (2006). Briefly, we classified scales of dismantled cones as non-fertile (basal- and terminal-cone scales containing no seed) and fertile (middle-cone scales containing seeds); seeds were classified as vestigial (undeveloped non-pollinated ovules) or developed (pollinated ovules). Besides, we sorted out developed seeds into empty and filled; the former are fertilized ovules that reached normal seed coats in shape and size, but lacked developed megagametophytes and embryos, and the later those that contained megagametophytes and embryos. Then, we calculated eight reproductive indicators per individual tree, which, excepting the production of strobili, cover all stages of fertility in conifers (see Table 1 for definition and detailed formulas): 1) SP = seed potential; 2) UO = percentage of vestigial seeds, which can be used as a proxy for pollen unavailability (O'Connell et al., 2006); 3) ES = percentage of empty seeds; 4) FS = percentage of filled seeds; 5) SE = seed efficiency; 6) RE = reproductive efficiency, which is a proxy for the resources (biomass and energy) allocated to seed production (O'Connell *et al.*, 2006); 7) EI = endogamy index, an estimator of tree consanguinity (O'Connell *et al.*, 2006); and 8) SG = seed germination. We evaluated this last trait as a percentage from 31 filled seeds per tree, as described by Wehenkel *et al.* (2023). Briefly, seeds were soaked in hydrogen peroxide (2%, v/v) for 24 hours and then sowed in 170-cm^3 plastic tubes containing a 3 : 1 : 1 mixture of peat moss : agrolite : vermiculite, bonified with 3 kg m^{-3} of a controlled-release fertilizer (Multicote®). Germination was performed in glasshouse at ~22 ºC; germination percentage was recorded after 28 days of sowing.

We correlated all reproductive indicators (excepting SP and SG) to reduce redundancy. To do so, we arcsine-transformed percentage data, and removed variables with $r_s \geq 0.9$. Then, we performed a Principal Component Analysis (PCA) separately for each species (R package FactoMineR v2.4; Lê *et al.*, 2008; R Core Team, 2020) (details of PCAs in Supporting Information Methods S4.1). To determine the number of principal components to retain, we used the standard eigenvalue threshold ≥1.0 (Abdi & Williams, 2010). Then, we obtained individual loads for each retained ordination axis (PCs; Supporting Information Methods S1 and Table S1). Seed germination was excluded from PCAs as it represents a separated fitness trait, being a further step in the process of producing new seedlings (i.e., after seed development). Reproductive fitness-traits PCs (Table 4.1) and seed germination were used in all subsequent analyses. Differences in reproductive fitness were estimated for species (*t*-tests) and populations (Tukey-Kramer tests).

Table 1. Reproductive indicators evaluated in *Picea martinezii* and *Picea mexicana*.

	Reproductive indicator	**ID**	**Formula or description**	**PCA**[1]
1	Seed potential	SP	Fertile scales * 2[2]	Not included
2	Undeveloped ovules (%)	UO	(undeveloped ovules / seed potential) * 100	Retained
3	Filled seeds (%)	FS	(filled seeds / developed seeds) * 100	Discarded
4	Empty seeds (%)	ES	(empty seeds / developed seeds) * 100	Discarded
5	Seed efficiency (%)	SE	(filled seeds / seed potential)	Discarded
6	Reproductive efficiency (mg/g)	RE	[weight of filled seeds (mg) / weight of dry cones (g)]	Retained
7	Endogamy index	EI	(empty seeds / developed seeds)	Retained
8	Seed germination (%)	SG	From a sample of 31 seeds per tree	Not included

[1] Reproductive traits retained or discarded after the Principal Components Analysis, according with thresholds for the correlation among variables (r_s≥0.9) and eigenvalues ≥1 for Principal Components retention (Supporting Information Methods 4.1).

[2] Cone scales containing undeveloped ovules or developed seeds; empty or filled.

4.2.2. Genomic variants and genetic parameters

We collected young needles for the same individuals above (Section 4.2.1), dried them on paper bags at 45 ºC for 72 h, and stored them at -20 ºC until use. We isolated DNA from 15.0 mg of tissue using the CTAB protocol (Hodgetts *et al.*, 2001) in volumes corresponding to minipreps (i.e., small-scale isolations of DNA performed on test tubes of 1.5 ml) with some modifications to the lysis buffer: we replaced polyethylene glycol (PEG) by polyvinylpyrrolidone (PVP_{40}; 1.0 %) and LiCl by NaCl 5 M; we further added 5.0 µl of 2-mercaptoethanol and 50 µl of Proteinase-K.

We measured DNA concentrations with a NanoDrop 2000 (ThermoFisher Scientific) and prepared libraries for next-generation sequencing and GBS (genotyping by sequencing) following Abed *et al.* (2019). Briefly, 200 ng of DNA per individual was double-digested with *Pst*I (R0106L) and *Msp*I (R3140L; both from New England Biolabs) and barcodes were ligated with T4 DNA ligase (New England Biolabs) in the same plate. Samples were pooled, purified, and amplified at the Plate-forme d'analyses génomiques, Institut de biologie intégrative et des systems-IBIS (Université Laval). Paired-end sequencing (2 x 140-160 bp) was finally performed on an Illumina Nova Seq 6000 System (Centre d'expertise et de services, Génome Québec).

Raw sequence reads were cleaned with Trim Galore software (available at URL: https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/) by removing low quality reads and adapters. Data was processed for each separate species following the ref-map Stacks pipeline, with standard parameters for assembly and single nucleotide polymorphism (SNP) calling (Catchen *et al.*, 2013), using the reference genome of *Picea glauca* (Birol *et al.*, 2013). SNP filtering was performed with vcftools (v0.1.17; Danecek *et al.*, 2011), setting the following options: --mac 2, --max-missing 0.8, --minDP 10, --maxDP 120. Observed heterozygosity (*Ho*) and inbreeding coefficient (*F*) were calculated per individual with the R packages ‘adegenet’ (v2.1.4; Jombart, 2008) and ‘popkin’ (v1.3.13; Ochoa & Storey, 2021), respectively. Genetic differentiation among populations was evaluated by computing F_{ST} in Arlequin (v3.5.2; Excoffier & Lischer, 2010) and the number of migrants exchanged per generation (*Nm)* following the equation:

$$Nm = \frac{1 - F_{ST}}{4F_{ST}}$$

4.2.3. Evaluating the risks of being in an extinction vortex

The conceptual framework to search for the expected effects when a species is located at different parts of a potential extinction vortex are illustrated in Fig. 4.2.

4.2.3.1. Demographic structure and effective population size (Ne)

Initially, an extinction vortex is expected to reduce both *Ne* and population census size because of a reduced reproductive fitness of the breeding individuals (Fig. 4.2a). We evaluated this by determining the demographic structure of each studied population by making a special emphasis in recruitment. In the 2018-2019 period, we

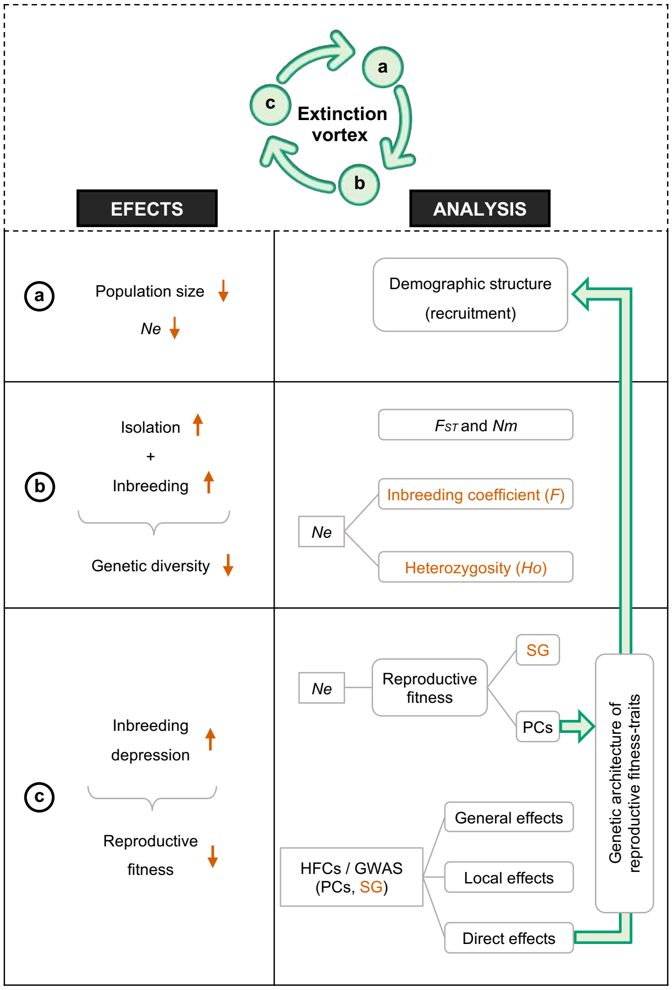


Figure 4.2. Flow of analyses to test for the expected effects when a species is located at different parts of a potential extinction vortex. (a) Demographic structure. (b) Isolation, inbreeding and genetic diversity. (c) Reproductive fitness. Right panels: green arrow direction denotes synergic effects and continuity of the vortex cycle; red-text indicate the genetic factors or phenotypic-traits yielding null associations. Left panels: red arrow indicates whether a parameter is expected to increase (upward) or decrease (downward). *Ne* = effective population size; F_{ST} = population differentiation; *Nm* = number of migrants per generation; SG = Seed germination; PCs = Principal Components summarizing seed production (mostly loaded by reproductive efficiency and endogamy index); HFCs = Heterozygosity-Fitness Correlations; GWAS = Genome Wide Association Analyses.

recorded and categorized all trees higher than 0.3 m in different developmental stages based on individuals' diametric categories (measured at breast heigh, 1.3 m: Cat0 to Cat$_n$), following Kneeshaw & Burton (1997). Individuals smaller than 1.3 m were placed in Cat0. Then, living stems < 2.0 m tall (encompassing Cat0 and Cat1 individuals) were considered seedlings or new regenerants; individuals ≥ 2.0 m tall but < Cat7 (i.e., Cat2 to Cat6) as saplings or juveniles; and Cat7 and higher were composed of diametric categories for adult reproductive trees. We assumed that seedlings and saplings (Cat0 to Cat6) constituted the population recruitment (i.e., new progeny and juveniles), while individuals with Cat≥7 could be used as a proxy for *Ne* (hereafter $Ne_{Cat\geq 7}$), as all trees at this stage develop both male and female strobili (Williams, 2009). While this mainly occurs at age 30 for other spruces (but with records at 4-year-old trees, Nienstaedt & Zasada, 1990), regression models constructed for *P. martinezii* and *P. mexicana*, from data retrieved from Hernández-Díaz *et al.* (2022), indicate that these Cat≥7 trees have minimum ages of ~31 and ~54, respectively (Supporting Information Fig. S4.1). However, because estimating contemporary *Ne* in forest trees is still a largely contentious subject (e.g., Fady & Bozzano, 2021; Hoban *et al.*, 2021; Santos-del-Blanco *et al.*, 2022), we used an additional estimator based on the more widely accepted *Ne*/*Nc* = 0.1 threshold, which assumes *Ne* is *c*. 10% of the total number of breeding individuals (Charlesworth, 2009; Hoban *et al.*, 2021), hereafter $Ne_{10\%Nc}$. We based this calculation on total population censuses, as mature female-strobili were observed in all diametric categories (surprisingly even in some Cat0 individuals; Supporting Information Fig. S4.2)

4.2.3.2. Association Ne-genetic diversity

In a second stage, a species within an extinction vortex would reduce its genetic diversity, because of the combined effects of isolation, inbreeding and genetic drift (Fig. 4.2b). We tested this at the population level by using linear regression models between our two *Ne* proxies and both the mean observed heterozygosity (*Ho*) and inbreeding (*F*) per population (Fig. 4.2b). Because this correlation should not be species-specific (Hamrick *et al.*, 1991), we combined populations from both species to increase statistical power. To account for differences in sample size, we multiplied mean values of phenotypic and genetic parameters by the correction factor $n / (n\text{-}1)$ (Quiñones-Pérez *et al.*, 2017), where *n* is the number of sampled trees per population (Supporting Information Table S4.2).

4.2.3.3. Inbreeding depression and reproductive fitness traits

In a third stage, an extinction vortex is expected to increase inbreeding and genetic load, which further reduces *Ne* (Fig. 4.2c). We explored this through linear regressions at population level between *Ne* proxies and fitness-traits (i.e., reproductive

PCs and seed germination) as above (Section 4.2.3.2). Second, we tested for a correlation between individual heterozygosity and these same fitness traits to search for possible general HFCs; we further looked for putative direct-effects loci through Genome Wide Association Analyses (GWAS) between SNP presence and individual fitness.

Following Rodríguez-Quilón *et al.* (2015), we assessed general HFCs through the correlation of genome-wide observed heterozygosity (*Ho*) and the retained fitness-traits per individual using the R package 'inbreedR' (v0.3.3; Stoffel *et al.,* 2016). However, given that the general-effect hypothesis assumes that there is no influence of population structure (Slate *et al.*, 2004), we first performed a PC using all SNPs and removed putatively adaptive loci that could be loading population differentiation using the R package 'pcadapt' (v4.3.3; Luu *et al.*, 2017), after correcting for Bonferroni (alpha = 0.05). A second assumption is that inbreeding increases the co-variation of un-linked loci from different regions of the genome (i.e., identity disequilibrium, ID; Szulkin *et al.*, 2010). To test for ID, we measured genome-wide loci covariation with g_2 (the covariance in heterozygosity standardized by average heterozygosity; David *et al.*, 2007) and HHC (heterozygosity-heterozygosity correlations; Szulkin *et al.*, 2010). High values of this last parameter further indicates if we have enough power to detect HFCs (Miller *et al.*, 2014).

Because the number of loci controlling complex traits such as those related with reproductive fitness is assumed to be high and mostly adjust to a dominance model (Frankham, 2009; Lobell *et al.*, 2017; Caignard *et al.*, 2019; Wu & Nilsson, 2023), direct-effect genes related to such traits (i.e., the fitness-loci) should be detected through GWAS (Uffelmann *et al.,* 2021). To avoid spurious associations caused by population structure (Neale & Savolainen, 2004; Uffelmann *et al.*, 2021), we continued using the same SNPs data sets as for the genome-wide general HFCs effects (i.e., without loci related to population structure). To differentiate between putatively local and direct-effect loci, we first partitioned each species SNP dataset into linked and unlinked loci through linkage pruning with PLINK (v.1.9, available at URL: https://www.cog-genomics.org/plink/1.9/; Purcell *et al.*, 2007). In conifers, linkage disequilibrium (LD) is expected to be negligible for loci separated by more than 100 kb (Moritsuka *et al.*, 2012). Therefore, we constructed windows of 100 kb in the assembled genomes and sorted all loci showing LD values higher or lower than $r^2 = 0.1$ using window steep sizes of one SNP. Linkage pruning generated sets of 5,438 and 18,469 linked SNPs and 12,492 and 11,691 unlinked SNPs for *P. martinezii* and *P. mexicana*, respectively. We then used multiple regressions (Uffelmann *et al.*, 2021) to test for associations between SNPs of each data set and reproductive fitness-traits, using PLINK (v.1.9). We retained candidates after false discovery rate correction (FDR_BH) for local effects and Bonferroni correction for direct effects. To discard spurious genotype-phenotype associations, we performed additional tests using pseudo-phenotypic variables constructed with random numbers within ranges of the original ones. Finally, to test for variations in the general HFCs when using different sets of loci (Miller *et al.*, 2014), we re-run HFCs by using *Ho* computed only with the candidate SNPs detected for local and direct effect tests.

4.2.4. Genomic architecture of reproductive-fitness traits

In a fourth stage, smaller populations within an extinction vortex should accumulate and express more deleterious alleles than the larger ones (Frankham *et al.*, 2002). This implies that candidate loci associated with fertility should be enriched for non-synonymous mutations and mostly affect elements of metabolic routes driving seed and embryo development. Furthermore, the derived alleles (identified by comparison with the reference genome of *P. glauca*) should be more frequent in the stands with low individual fertility than in populations with high tree fertility. To test this, we selected sequence reads containing direct-effect candidate SNPs (candidate fitness-loci) and performed blast searches on the *Picea glauca* Transcriptome Assembly (TSA; nucleotide to nucleotide blast), and on green plant gene-protein databases (BLASTx; nucleotide to protein; Sayers *et al.*, 2021). Following Wegrzyn *et al.* (2014), we used a threshold of 1E-5 to determine sequence homology and annotated both gene function (GO-term) and SNP effects (deduced from the slope parameter BETA of the individual-loci regressions computed in GWAS). We then looked for eventual heterozygote advantages and dominance by comparing individual fertility for each genotype at each locus through Wilcoxon tests, based on alternative genetic effect models described by Nicolini *et al.* (2018). We finally determined the derived allele frequency distribution for the successfully annotated SNPs.

4.3. Results

4.3.1. Reproductive fitness analysis

After independent PCA for each species, only the first principal component summarizing fitness was significant, and thus retained, for both taxa; these explained 72.8% and 66.6% of the total fitness variance for *P. martinezii* and *P. mexicana*, respectively. Interestingly, this first PC was loaded by the same variables for both species: reproductive efficiency (RE; contribution = 44.0% and *r* = -0.98 in *P. martinezii*; contribution = 46.7% and *r* = -0.97 in *P. mexicana*) and endogamy index (EI; contribution = 34.5% and *r* = 0.87 in *P. martinezii*; contribution = 38.3% and *r* = 0.87 in *P. mexicana*) (Supporting Information Methods S4.1). Observed correlations between PC1 and their loading variables (i.e., negative with RE and positive with EI for both species) implied lower reproductive fitness at higher PC1 values, for both species. However, for a more intuitive interpretation of results below, we decided to invert the signs of individual PC1 values by multiplying individual load by -1 (Supporting Information Table S4.1 and Methods S4.1).

Overall, mean PC1 loads were statistically similar between species according to *t*-test (-0.472 *vs* 0.002, *p* = 0.66; Fig. 4.3), although differences among populations within species were large and significant, showing diminishing fitness at stands with smaller *Ne* (Fig. 4,3). Such a relationship was significant independently of the *Ne* proxy used (see below), and after correcting for the potential bias generated by an unbalanced sample size among populations, particularly for *P. martinezii* (*n* = 11-30). This was verified by repeating analyses using five random subsamples of eleven individuals for each one of the most vastly sampled stands (Supporting Information Fig. S4.3).

Along with PC1s, seed germination (SG) was the other main reproductive fitness-trait related to fertility in both spruces (Supporting Information Methods S4.1). As for PC1s, SG also fitted a normal distribution of individual values (Supporting Information Fig. S4.4) and showed no significant differences between species

(Supporting Information Fig. S4.5a). However, one population of *P. martinezii* (Agua Fría) had significantly lower SG values than the rest (Supporting Information Fig. S4.5b,c).

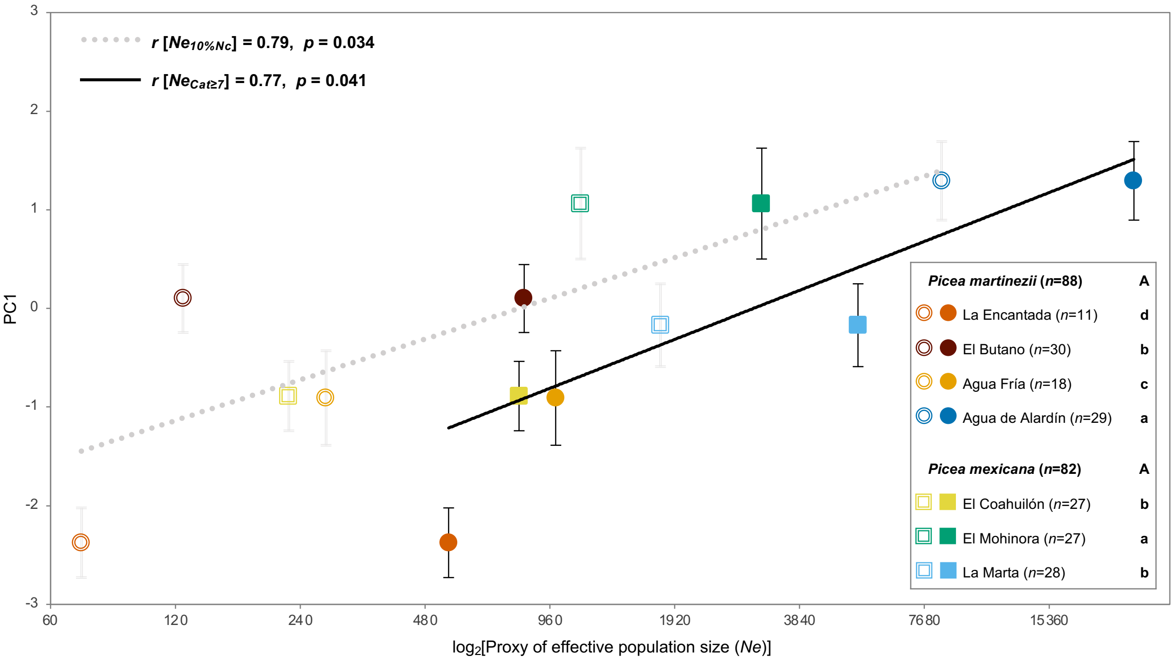


Figure 4.3. Association between two proxies of effective population size (*Ne*) and PC1 loads (summarizing reproductive fitness) for *Picea martinezii* (circles) and *Picea mexicana* (squares). Empty symbols and dotted line represent *Ne* values and regression calculated as 10% of complete population censuses ($Ne_{10\%Nc}$); and filled symbols and continuous line, *Ne* values and regression for the number of adult individuals per stand (i.e., Cat≥7 trees; $Ne_{Cat\geq7}$). Populations color-codes, names and number of successfully genotyped and phenotyped (*n*) trees are noted in the inset at the bottom right. Results for comparisons of PC1 values between species (*t*-test; upper-case letters) and populations within species (Tukey-Kramer tests; lower-case letters) are also shown in this inset; where different letters denote significantly different PC1s. Whiskers within graph indicate 95% confidence intervals.

4.3.2. Genomic variants and genetic parameters

In total, ~170-172 billion bases and 1.2 billion reads were sequenced for each spruce; reads aligned to 624,164,343 and 602,656,682 scaffolds of the *P. glauca* reference genome for *P. martinezii* and *P. mexicana*, respectively; this resulted in 151,554 and 222,115 loci per species. The mean sample coverage for built loci was 96.0x (*sd* = 32.5x) for *P. martinezii* and 94.1x (*sd* = 29.3x) for *P. mexicana*. Total raw sets of 218,778 SNPs were then called for the former species and 297,503 SNPs for the later. After filtering, 18,072 and 30,230 SNPs were respectively retained for *P.*

martinezii and *P. mexicana*, which were successfully genotyped for 88 *P. martinezii* individuals and 82 *P. mexicana* trees (Fig. 4.3). Despite having less and more broadly separated populations, *P. mexicana* was more genetically diverse than *P. martinezii* (*H*o = 0.218 *vs.* *H*o = 0.154), less inbred (*F* = 0.220 *vs.* *F* = 0.236) (Supporting Information Table S4.2), and more genetically homogeneous (F_{ST} = 0.083 *vs.* F_{ST} = 0.154; Fig. 4.1). While this might be the result of more phylogenetic proximity of *P. mexicana* and the reference species (*P. glauca*), it may also indicate contrasting demographies between taxa.

4.3.3. Evaluating the risks of being in an extinction vortex

4.3.3.1. Demographic structure and effective population sizes (Ne)

Ne proxies were significantly different from each other, with $Ne_{10\%Nc}$ (10% of total census) being at least three times lower than $Ne_{Cat\geq7}$ (adult Cat≥7 trees) for a same population (Fig. 4.3). Independently of these differences, both indices showed the same demographic trend, with populations La Encantada and Agua de Alardín, and stands El Coahuilón and La Marta having the smallest and largest *Ne* for *P. martinezii* and *P. mexicana*, respectively. Both species further showed higher recruitment with increasing *Ne*; however, for *P. martinezii*, the two smallest and more isolated populations (La Encantada and El Butano) had a higher proportion of mature trees than of saplings and seedlings, while its two largest ones showed the opposite pattern (Fig. 4.4a). For *P. mexicana*, recruitment always contributed more to total population census than adult trees (Fig. 4.4b).

4.3.3.2. Genetic diversity and population genetic differentiation

Although non statistically significant, genetic diversity (*Ho*) tended to increase and inbreeding to decrease (*F*) with effective population size, particularly for *P. martinezii*. Populations of *P. mexicana* showed more homogenous values of both *Ho* and *F* (Supporting Information Table S4.2). F_{ST} was higher and *Nm* lower between the two smallest and more isolated populations of *P. martinezii* (La Encantada and El Butano, distance ≈ 147 km, F_{ST} = 0.225, *Nm* = 0.9) than between largest and more closely located ones (Agua de Alardín and Agua Fría, dist. ≈ 3 km, F_{ST} = 0.072, *Nm* = 3.2; Fig. 4.1). Surprisingly, these two populations were more genetically close to El

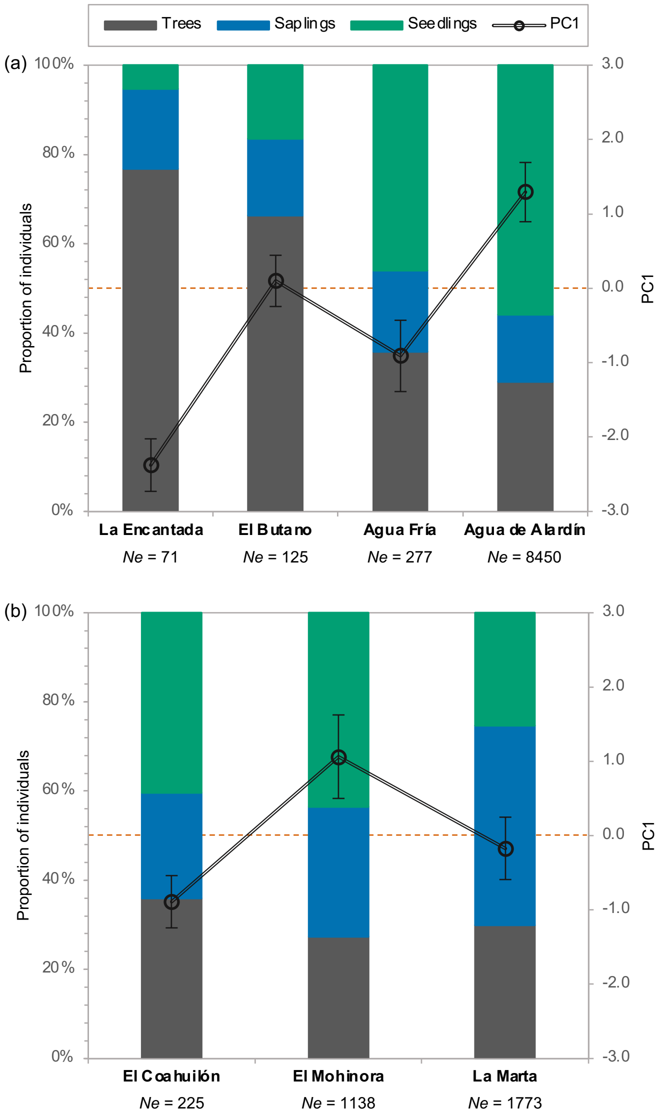


Figure 4.4. Demographic structure of known populations of *Picea martinezii* (a) and *Picea mexicana* (b). Left *y*-axis shows the proportions of individuals separated as adult trees (Cat≥7 individuals; grey bars), saplings and seedlings (blue and green bars, respectively). Right *y*-axis displays reproductive PC1 loads (grey lines and empty circles). Horizontal dashed red lines inside graphs indicate the 50% threshold for demographic inversions; that is, frequencies above which recruitment (saplings and seedlings) is lower compared to adult trees (which indicates demographic constraints; see Fig. 2a). *Ne* = Effective population size estimated as 10% of total census ($Ne_{10\%Nc}$).

Butano (~133 km away, F_{STmean} = 0.132, Nm_{mean} = 1.6) than to La Encantada (~18 km distant, F_{STmean} = 0.180, Nm_{mean} = 1.1). For *P. mexicana*, genetic differentiation matched the geographic distribution of populations, with the two populations from the Sierra Madre Oriental showing the lowest F_{ST} and largest Nm (F_{ST} = 0.040, Nm = 6.0; ~6km away from each other), compared with the highest F_{ST}, which was observed between these stands and the lone population from Sierra Madre Occidental (F_{STmean} = 0.105, Nm_{mean} = 2.1; ~677 km away from other stands; Fig. 4.1).

4.3.3.3. Inbreeding depression and reproductive fitness-traits

The expected positive relationship between *Ne* and reproductive fitness (Fig. 4.2c), symptomatic of inbreeding depression, was only observed when using PC1 as a proxy of reproductive fitness (r = 0.79, p = 0.034 for $Ne_{10\%Nc}$; r = 0.77, p = 0.041 for $Ne_{Cat\geq 7}$; Fig. 4.3); such a relationship was non-significant when employing seed germination (SG) (r = 0.29, p = 0.52 for $Ne_{10\%Nc}$; r = 0.34, p = 0.46 for $Ne_{Cat\geq 7}$). Inbreeding depression was further inferred by testing for a positive correlation between individual fitness and mean genomic heterozygosity (general HFC). However, before testing for such an association, we first removed 142 and 70 SNPs significantly contributing to population structure in *P. martinezii* and *P. mexicana*, respectively (Supporting Information Methods S4.2). Retained genome-wide SNPs showed strong heterozygosity-heterozygosity correlations (HHCs = 0.91-0.92; Supporting Information Table S4.3) and a weak but significant correlation with PC1 in *P. martinezii* (r = 0.29, p = 0.006), but no association in *P. mexicana* (r = -0.06, p = 0.615; Fig. 4.5a,b). As for the *Ne*-fitness correlations, SG had no significant general-effect HFCs in either *P. martinezii* (r = 0.12, p = 0.249) or *P. mexicana* (r = 0.10, p = 0.357), which indicates no inbreeding depression related to seed viability.

HFCs were also evaluated after identifying local- and direct-effect candidate loci. A total of 627 and 472 local-effect candidates were associated with PC1 for *P. martinezii* and *P. mexicana*, respectively. On the other hand, 49 and 11 were retained as candidates for direct effects for *P. martinezii* and *P. mexicana*, respectively (Supporting Information Fig. S4.6 and Fig. S4.7). Spurious associations were discarded as no genotype-phenotype correlations were observed when using pseudo-PC1s (Supporting Information Fig. S4.8). Furthermore, no local nor direct effects were found for SG in both spruces (Supporting Information Fig. S4.9). Mean individual heterozygosity determined with the retained candidates showed opposite correlations with individual fitness (PC1) between species, with more heterozygous individuals of *P. martinezii* having lower individual reproductive fitness than the less heterozygous ones (Fig. 4.5c,e), and more heterozygous trees of *P. mexicana* showing higher reproductive fitness than the less heterozygous ones (Fig. 4.5d,f).

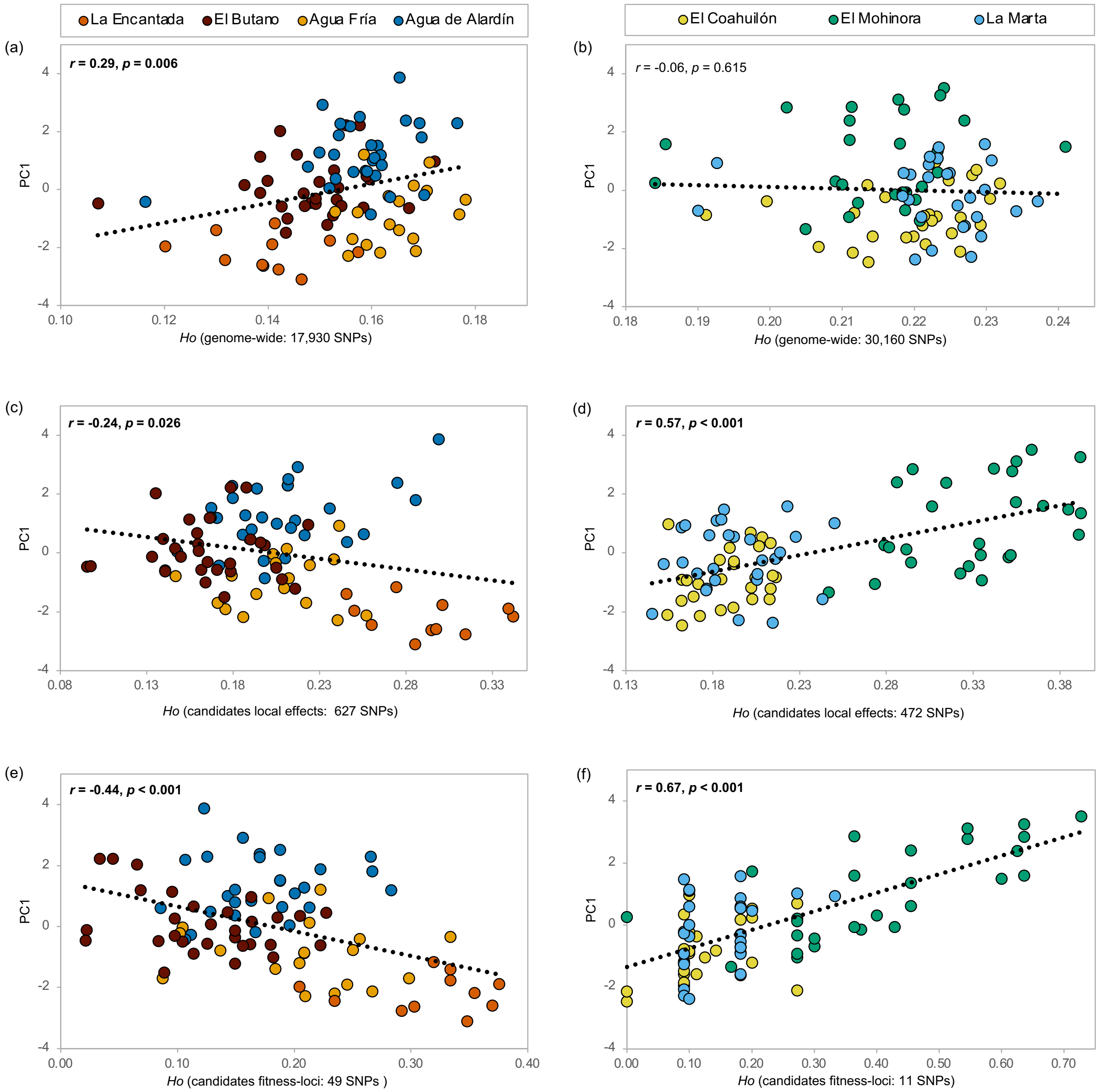


Figure 4.5. Heterozygosity-Fitness Correlations (HFCs) for PC1 loads (summarizing reproductive fitness) of *Picea martinezii* (a,c,e) and *Picea mexicana* (b,d,f). Observed heterozygosity (*Ho*) was computed independently for each species for complete SNP sets (genome-wide heterozygosity, after excluding outliers for population structure) and for candidate SNPs for local and direct effects detected through GWAS. Spurious HFCs when using *Ho* of candidate SNPs (c-f) were discarded by running tests with similar sets of SNPs than for local and direct effects, but randomly chosen for each species (Supporting Information Table S4).

4.3.4. Genomic architecture of reproductive traits

Only 21 out of the 49 candidates for *P. martinezii* (Fig. 4.6a-u) and seven out of the 11 ones for *P. mexicana* (Fig. 4.6v-ab) were successfully annotated by comparison with the *P. glauca* transcriptome. There were no shared candidate fitness-loci between species. Annotated regions involved proteins related to cell division, maintenance, and homeostasis, as well as regulation of gene expression, cell differentiation and development. Interestingly, some of these proteins have been previously associated to seed and ovule development and embryo-lethal phenotypes in different plant species (Supporting Information Table S4.5).

The genetic effects observed for the annotated candidates, surprisingly disclosed only few cases of heterozygote advantage (overdominance); two loci for *P. martinezii* and three for *P. mexicana*. However, all these cases were undistinguishable from dominance in both spruces, due to the lack of one genotype (e.g., Fig. 4.6r,v). Dominance was the most common genetic effect in both species (e.g., Fig. 4.6b-d), although it was unclearly defined in some cases because an almost absence of one homozygote genotype (e.g., Fig. 4.6h,x). Furthermore, dosage effects were observed at four loci for *P. martinezii* and one locus for *P. mexicana* (e.g., Fig. 4.6a,ab).

Regardless the fitted model by genotype for each candidate SNP, the slope parameter (BETA) of individual loci regressions showed overall contrasting effects of reproductive fitness at local and direct effects for both spruces (see Supporting Information Table S4.6 for the whole sets of candidate SNPs). For the successfully annotated SNPs, nine candidates (43%) carried putatively beneficial derived alleles, and twelve of them (57%) putatively deleterious derived alleles in *P. martinezii* (Fig. 4.6a-u). For *P. mexicana*, six out of seven (86%) were presumptive beneficial and one (14%) deleterious (Fig. 4.6v-ab). Finally, while the putatively beneficial derived alleles had higher frequencies at the largest population of *P. martinezii* (Agua de Alardín) and lower at smaller stands, the supposed deleterious derived alleles were more frequent at the smallest population of this species (La Encantada) and almost absent from the largest one (Agua de Alardín; Fig. 4.7a-b). For *P. mexicana*, the putatively beneficial derived alleles were more frequent in the population exhibiting the highest fertility (El

Mohinora) instead of the largest one (La Marta), which only had the putatively harmful derived allele in high frequency (Figs. 4.6y and 4.7c).

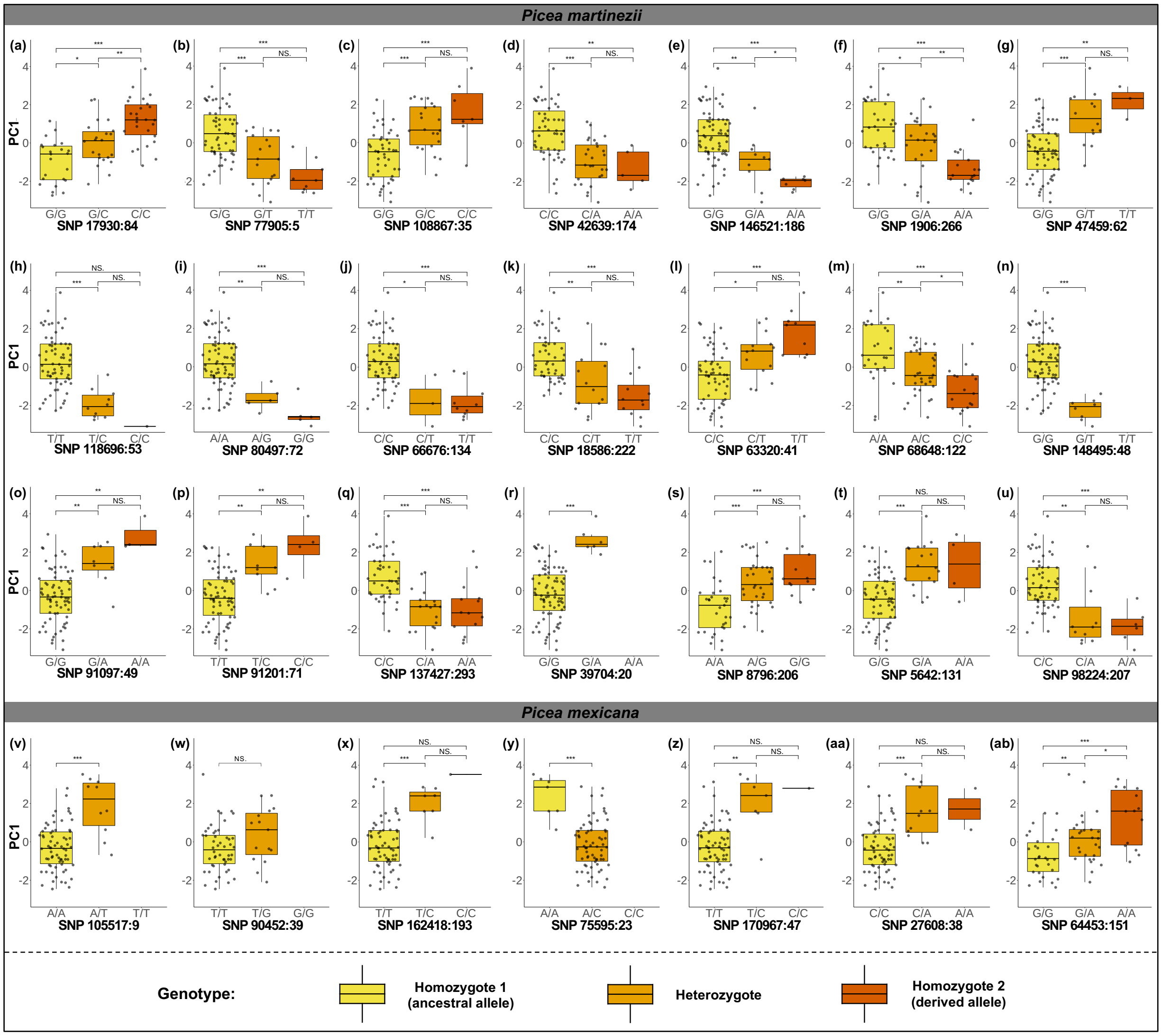


Figure 4.6. Reproductive fitness (PC1 loads) for individual genotypes of successfully annotated candidate SNPs for *Picea martinezii* (top; a-u) and *P. mexicana* (bottom; v-ab). Derived allele was inferred through comparisons with the reference genome of *P. glauca*. Upper side of sub-figures show differences among genotype effects according to Wilcoxon tests (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant). Functional annotations are shown in Supporting Information Table S5. Frequency distributions of derived alleles for each SNP are shown in Fig. 7.

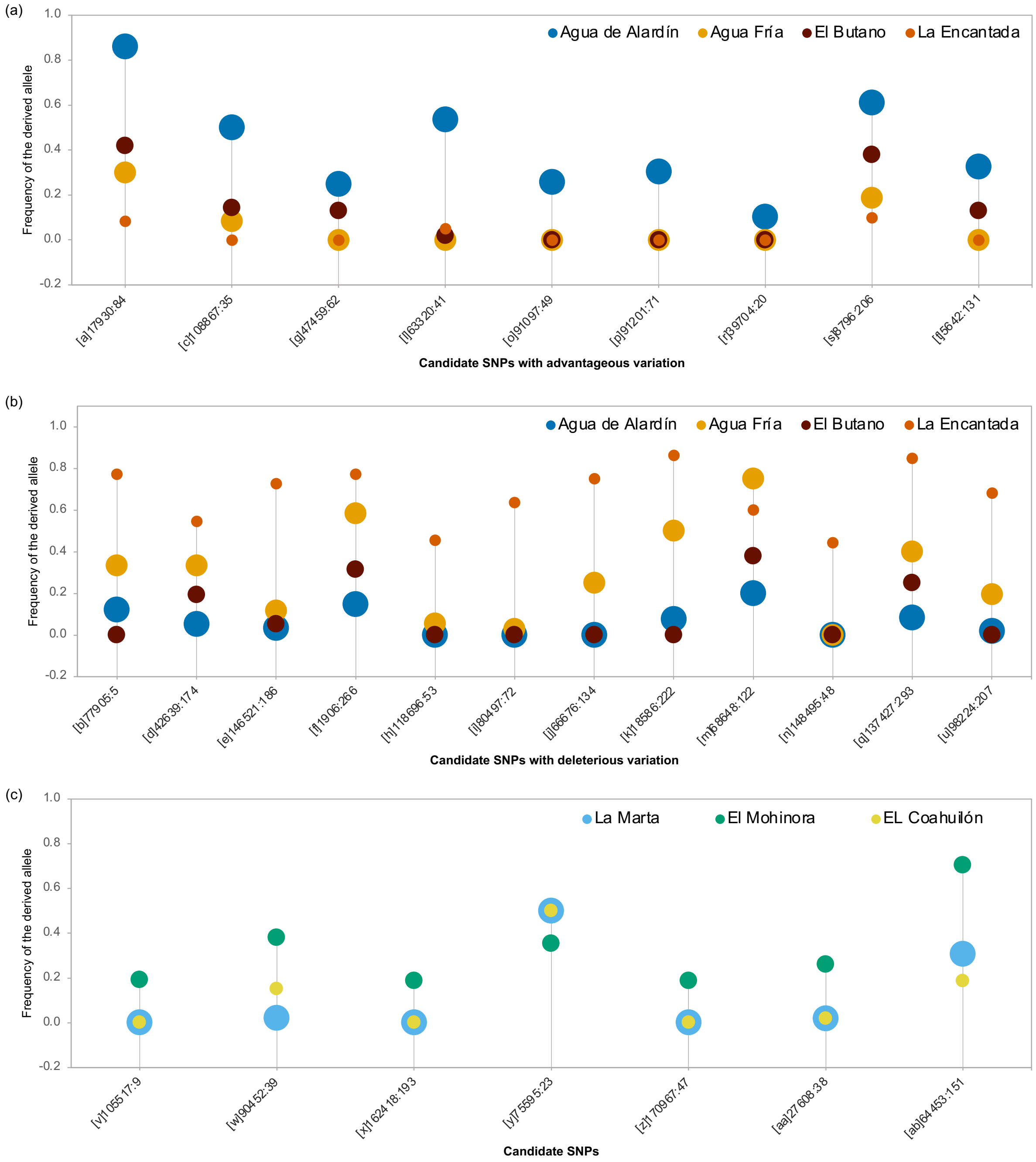


Figure 4.7. Frequency distribution of the derived allele for the successfully annotated candidate SNPs related to reproductive fitness in *Picea martinezii* (a-b) and *P. mexicana* (c). Candidates for *Picea martinezii* species were sub-divided in (a) putatively advantageous (derived allele related to increased fitness) or (b) possibly deleterious (derived allele associated with diminished fitness) following results shown in Fig. 6. Different sized points inside graphs symbolize $Ne_{10\%Nc}$ for each population.

4.4. Discussion

In this study, we explored the genomic basis of reproductive fitness in two unrelated and endangered spruces from northern Mexico (*Picea martinezii* and *P. mexicana*). Contrary to most studies in forest taxa, that explore individual fitness on growth and other economically-relevant traits (e.g., Du *et al.*, 2018; Baison *et al.*, 2019; Chen *et al.*, 2021; but see also McKown *et al.*, 2014), we directly addressed fitness in seed-related traits, which is one of the main stages where most of the selective purging is expected to occur (Husband & Schemske, 1996; Williams & Savolainen, 1996; Ledig *et al.*, 2000b; O'Connell *et al.*, 2006). We combined such measures with demographic and genomic data, which allowed us to uncover species-specific evolutionary trends. While we observed the expected general pattern of diminishing reproductive fitness with decreasing *Ne* (although with relatively low significance), we only detected the anticipated relationship between genome-wide heterozygosity and individual fitness in *P. martinezii*. For *P. mexicana*, we only found such a correlation when using candidate SNPs associated to reproductive fitness. The number of candidates and their putative effects on fitness further differed between spruces, which suggests that species-specific evolutionary factors can decouple the expected correlation between *Ne*, genetic diversity, and reproductive fitness. Differences in population demographic structure between *P. martinezii* and *P. mexicana* further point to a more predominant role for purifying selection in the first than in the second taxa, despite that the last has less populations and fewer adult trees than *P. martinezii*. The singularities observed for *P. mexicana* indicate that equating fitness to either *Ne* or *He*, as traditionally proposed in Conservation Biology (Leimu *et al.*, 2006), might not always be adequate, and that the specificities of adaptive fitness should be explored for more effective conservation programs (e.g, Booy *et al.*, 2000; Yates *et al.*, 2019), particularly for long-lived organisms with high reproductive outputs, like forest trees.

Other limitations illustrated here include those of commonly used estimators of *Ne* and the conventional *Ne* > 500 threshold for evaluating populations and species at risk (Frankham *et al.*, 2002), especially for forest trees (see also Santos-del-Blanco *et al.*, 2022). For instance, if we consider that all adult trees are equally contributing to *Ne* and simply use individual counts as a proxy ($Ne_{\mathrm{Cat}\geq 7}$), genetic erosion or inbreeding depression should be disregarded in all populations of both spruces. However, when using the most conservative 10% of the total population census as an estimator ($Ne_{10\%Nc}$), all populations but one of *P. martinezii* (Agua de Alardín) and two of *P. mexicana* (El Mohinora and La Marta) should be genetically eroded or experiencing inbreeding depression. Even so, the largest population of this last species (La Marta) showed both demographic and individual reproductive signs of inbreeding depression, as the number of seedlings was reduced when compared to that of saplings and adults, and adult trees had lower mean individual reproductive fitness (PC1) than mature trees from smaller and more isolated populations (e.g., El Mohinora; Figs. 4.1, 4.3, 4.4). Such factors highlight that both historical, stochastic, and adaptive evolutionary processes must be accounted for when evaluating evolutionary potential for endangered species. For instance, simulations have shown that populations that have collapsed and remained at small sizes for long periods of time should have purged most of their genetic load and may now exhibit high reproductive outputs, while recently collapsed stands should be still purging their genetic load and see their current reproductive fitness diminished (Kyriazis *et al.*, 2021).

While formal phylogeographic studies are still pending for both *P. martinezii* and *P. mexicana*, our results coincide with previous observations and hypothesized premises. Indeed, *P. martinezii* apparently collapsed between the late Pleistocene and the Holocene and has survived at small pockets of favorable microsites within its glacial distribution (Ledig *et al.*, 2000b). This led us to hypothesize that this species should adjust to a general correlation pattern, with larger populations exhibiting higher genome-wide diversity and higher mean reproductive fitness than smaller stands. On the other hand, *P. mexicana*, likely underwent a more ancient collapse and a northward migration from central Mexico (Jaramillo-Correa *et al.*, 2006; Mendoza-Maya *et al.*, 2022) and currently shows signs of strong selection against inbreeds (Ledig *et al.*, 2002), which led to decoupled *Ne*, *He*, fitness correlations.

Genome-wide variation at *P. martinezii* fitted the general HFC model (Supporting Information Table S4.3 and Fig. 4.5a), under which fitness is assumed to be driven by the additive effect of deleterious alleles distributed across the genome (Rodríguez-Quilón *et al.*, 2015). Such alleles become exposed in inbred, and thus less heterozygous, individuals, which have lower fitness than the offspring of genetically unrelated individuals, which should be more heterozygous (Szulkin *et al.*, 2010). Such a model mostly implies dominance (i.e., only the recessive homozygotes have diminished fitness), but it does not necessarily exclude overdominance (i.e., heterozygote advantage; White *et al.*, 2007). For the particular case of *P. martinezii*, most of the fitness-related candidates fitted the dominance model (71 %; e.g., Fig 4.6), and only one could be fitting the overdominance model (Fig. 4.6r; although one of the homozygous was not observed and overdominance cannot be fully confirmed). For the remaining loci (19%), heterozygote individuals had intermediate fitness relative to both homozygotes (e.g., Fig. 4.6a,f). Interestingly, for most of the 21 retained and annotated candidates for this species, individuals bearing derived alleles had lower fitness than those carrying the ancestral variants. This indicates an incipient purging of genetic load and the accumulation of harmful species-specific variation that is consistent with the hypothesized recent collapse proposed for this species (Ledig *et al.*, 2000b). Detailed studies surveying the overall distribution of fitness effects (e.g., Eyre-Walker & Keightley, 2007) are thus necessary for this taxon.

Genome-wide variation was, on the other hand, not related to individual fitness in *P. mexicana*. HFC for this species was only observed when surveying heterozygosity for the retained candidates. The number of such candidates was lower than the one obtained for *P. martinezii*, and many of them fitted dominance expectations, as for *P. martinezii* (86%; Fig. 4.6v-aa). However, contrary to this last species, individuals carrying derived alleles at candidate loci in *P. mexicana* had on average higher fitness than those bearing ancestral variants. This suggests that genetic load purging is more advanced in this species than in *P. martinezii*, and is even promoting the fixation of advantageous alleles. This coincides with previous inferences of inbreeding tolerance and a more ancient population collapse (Ledig *et al.*, 2002; Jaramillo-Correa *et al.*, 2015), which are known to decouple correlations between *Ne*, *He* and fitness, and favor alleles that increase reproductive output (Kyriazis *et al.*, 2021). This is illustrated at the lone population of *P. mexicana* in the Sierra Madre Occidental (El Mohinora; Fig. 4.7c). This stand has the highest tree fertility in the species, despite being smaller and more isolated than the largest *P. mexicana* stand (Figs 4.1, 4.3 and 4.4). This population further has the highest frequencies for all putatively advantageous derived alleles in the species.

Although all candidates reported here still need confirmation and further testing in controlled conditions to unravel the putative selective processes (e.g., antagonist selection, conditional neutrality, etc.; Alonso-Blanco & Méndez-Vigo, 2014), their numbers indicate that reproductive-fitness fits a polygenic model of adaptation, as proposed for other complex fitness-traits in plants (Alonso-Blanco & Méndez-Vigo, 2014; Caignard *et al.*, 2019). While these candidates were not shared between spruces, they contained genes associated to functions that were common to both species, including gene expression, cell differentiation and embryo development (Supplementary Information Table S4.5). Genes within these pathways are good candidates to understanding the mechanisms that allow forest trees to circumvent extinction vortices, and should be explored in other genetically impoverished and thriving species, such as *Pinus resinosa* or *Juglans cinerea* in North America, and *P. pinea* in Europe (Walter & Epperson, 2005; Boraks & Broders, 2016; Jaramillo-Correa *et al.*, 2020).

In the studied spruces, and in forest trees in general, most of the selection on reproductive fitness seem to operate early, when embryos develop into viable seeds, instead of during and after germination, including the seedling stages (Husband & Schemske, 1996; Williams & Savolainen, 1996; but see Capblancq *et al.*, 2021). This is supported by the correlation between *Ne* and fitness-seed traits (PC1) and the lack of such correlation when using seed germination as a proxy of fitness (SG; Fig. 4.2c; section 4.3.3.3). It must be noted that the observed SG was particularly high for such endangered taxa (Supporting Information Fig. S4.5a); it was indeed similar to that reported for large populations of widespread and non-threatened boreal spruces, like *P. rubens* (SG >60%; Capblancq *et al.*, 2021) or *P. glauca* (SG = 94.1%; O'Connell *et al.,* 2006). Moreover, similar to our study, smaller stand sizes of transcontinental *P. glauca* showed decreased reproductive success (filled seeds) when compared to larger populations, and there were no differences among stands of contrasting sizes at further developmental stages (i.e., seed germination and seedlings survival; O'Connell *et al.*, 2006; see also Wehenkel *et al.*, 2023, who reported no survival issues for *P. martinezii* and *P. mexicana* seedlings under nursery conditions). Such observations further point that growth and other economically-relevant traits might not be the most adequate proxies for individual fitness, and could explain the difficulty of previous surveys in finding HFCs in forest trees (e.g., Rodríguez-Quilón *et al.*, 2015) and in endangered species (e.g., Grueber *et al.*, 2011).

Other inconsistencies of empirical studies looking for HFCs that could be explained by our results include contra-intuitive reports of negative HCF (e.g., Altukhov *et al.*, 1986; Strauss & Libby, 1987). Such studies were performed with isozymes, whose variation could reflect the accumulation of subtle and partially deleterious variants that are still to be purged (Pyhäjärvi *et al.*, 2011), as suspected herein when surveying HFC in *P. martinezii* with candidate SNPs (Fig. 4.5c). Indeed, our results suggest that the contrasting HFC patterns observed in empirical studies are the likely result of contrasting demographic processes between species (e.g., population decline, collapse severity and time spanned since population decline; Kyriazis *et al.*, 2021), and individual loci effects. Revisiting classic HFCs studies using information derived from NGS technologies and putting results into the adequate demographic context should thus help resolving all these apparent contradictions.

According to our results, and as previously concluded by Ledig *et al.* (2000b, 2002), the spruces under study, as a species, have not yet entered into extinction vortices. However, our results point to severe risks of extinction for the two smallest *P. martinezii* populations (La Encantada and El Butano) and suggest that close monitoring is needed for the medium-sized Agua Fría stand. These populations only have but a few hundred adult trees (a proxy for *Ne*), which might be insufficient to avoid inbreeding depression in the short term (Fig. 4.4a). Indeed, limited seed output have already been recorded in La Encantada and El Butano for over 12 years (Flores-López *et al.*, 2012), and adult trees at Agua Fría showed very low fertility in the present study (Fig. 4.4a). Such risks are highlighted by the fact that these stands bear putatively harmful alleles at high frequencies and have lost most of the possibly advantageous variation (Fig. 4.7). However, the location of Agua Fría, next to the largest population of the species (Fig. 4.1), might facilitate incoming gene flow, and dilute the effects of any existing deleterious variation. The other stands are, on the other hand, more isolated and should be the focus of special conservation programs, probably including assisted migration and reforestation.

The smallest population of *P. mexicana* (El Coahuilón) should be also monitored. This stand has apparently enough regeneration to avoid demographic constraints (Figs. 4.1 and 4.3), but it has low individual fertility (Fig. 4.4) and carried virtually no putatively advantageous alleles (Fig. 4.7). However, as for the last case above (Agua Fría), this stand is closely located to largest population of the species, which might facilitate incoming gene flow and reduce chances of inbreeding depression. On the other hand, the case of most isolated population of the species (El Mohinora, Fig. 4.1) is particularly noteworthy. Despite being the only stand in the Sierra Madre Occidental (more than 670 km apart from the other populations), it has the highest individual fertility, and the most abundant regeneration of the species; it also has putatively favorable alleles at higher frequencies than the other *P. mexicana* populations. This stand represents a very interesting model for surveying mechanisms that help delay or even avoid extinction vortices in forest trees, like gene load purging and local adaptation (Petit & Hampe, 2006). It further reinforces the necessity of integrating historical demography and phylogeography in conservation programs (López-Delgado & Meirmans, 2022; Evans *et al.*, 2023). However, for this and all other populations of both species, important external issues must be tackled to assure their long-term survival; particularly climate change and anthropic disturbances (Ledig *et al.*, 2000b, 2010; Mendoza-Maya *et al.*, 2022).

4.6. References

Abdi H, Williams LJ. 2010. Principal component analysis. *WIREs Computational Statistics* 2: 433–459.

Abed A, Légaré G, Pomerleau S, St-Cyr J, Boyle B, Belzile F. 2019. Genotyping-by-sequencing on the Ion Torrent platform in barley. In: Harwood WA, ed. Barley: Methods and Protocols, Methods in Molecular Biology. New York: Springer Science+Business Media, 233–252.

Allendorf FW, Luikart G. 2007. *Conservation and the Genetics of Populations*. Padstow: Blackwell Publishing.

Alonso-Blanco C, Méndez-Vigo B. 2014. Genetic architecture of naturally occurring quantitative traits in plants: an updated synthesis. *Current Opinion in Plant Biology* 18: 37–43.

Altukhov YP, Gafarov NI, Krutovskii K V, Dukharev VA. 1986. Allozyme polymorphism in a natural population of Norway spruce *Picea abies* (L.) Karst. III. Correlation between levels of individual heterozygosity and relative number of inviable seeds. *Soviet genetics (USA)* 22: 1580–1585.

Baison J, Vidalis A, Zhou L, Chen Z-Q, Li Z, Sillanpää MJ, Bernhardsson C, Scofield D, Forsberg N, Grahn T, *et al.* 2019. Genome-wide association study identified novel candidate loci affecting wood formation in Norway spruce. *The Plant Journal* 100: 83–100.

Bateson ZW, Hammerly SC, Johnson JA, Morrow ME, Whittingham LA, Dunn PO. 2016. Specific alleles at immune genes, rather than genome-wide heterozygosity, are related to immunity and survival in the critically endangered Attwater's prairie-chicken. *Molecular Ecology* 25: 4730–4744.

Birol I, Raymond A, Jackman SD, Pleasance S, Coope R, Taylor GA, Yuen MM Saint, Keeling CI, Brand D, Vandervalk BP. 2013. Assembling the 20 Gb white spruce (*Picea glauca*) genome from whole-genome shotgun sequencing data. *Bioinformatics* 29: 1492–1497.

Booy G, Hendriks RJJ, Smulders MJM, Van Groenendael JM, Vosman B. 2000. Genetic diversity and the survival of populations. *Plant biology* 2: 379–395.

Boraks A, Broders KD. 2016. Population genetic diversity of the rare hardwood butternut (*Juglans cinerea*) in the northeastern USA. *Tree Genetics & Genomes* 12: 43.

Botero-Delgadillo E, Gilsenan C, Mueller JC, Kempenaers B. 2020a. Negative effects of individual heterozygosity on reproductive success in a wild bird population. *Molecular Ecology* 29: 3196–3216.

Botero-Delgadillo E, Quirici V, Vásquez RA, Kempenaers B. 2020b. Heterozygosity-fitness correlations in a continental island population of Thorn-tailed Rayadito. *Journal of Heredity* 111: 628–639.

Brommer JE, Kekkonen J, Wikström M. 2015. Using heterozygosity–fitness correlations to study inbreeding depression in an isolated population of white-tailed deer founded by few individuals. *Ecology and evolution* 5: 357–367.

Brys R, Jacquemyn H, Endels P, Hermy M, De Blust G. 2003. The relationship between reproductive success and demographic structure in remnant populations of *Primula veris*. *Acta Oecologica* 24: 247–253.

Caignard T, Delzon S, Bodénès C, Dencausse B, Kremer A. 2019. Heritability and genetic architecture of reproduction-related traits in a temperate oak species. *Tree genetics & genomes* 15: 1–12.

Capblancq T, Munson H, Butnor JR, Keller SR. 2021. Genomic drivers of early-life fitness in *Picea rubens*. *Conservation Genetics* 22: 963–976.

Catchen J, Hohenlohe PA, Bassham S, Amores A, Cresko WA. 2013. Stacks: an analysis tool set for population genomics. *Molecular ecology* 22: 3124–3140.

Charlesworth B. 2009. Fundamental concepts in genetics: effective population size and patterns of molecular evolution and variation. *Nature Reviews Genetics* 10: 195–205.

Charlesworth D, Willis JH. 2009. The genetics of inbreeding depression. *Nature Reviews Genetics* 10: 783–796.

Chen Z-Q, Zan Y, Milesi P, Zhou L, Chen J, Li L, Cui B, Niu S, Westin J, Karlsson B, *et al.* 2021. Leveraging breeding programs and genomic data in Norway spruce (*Picea abies* L. Karst) for GWAS analysis. *Genome Biology* 22: 179.

Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, Handsaker RE, Lunter G, Marth GT, Sherry ST. 2011. The variant call format and VCFtools. *Bioinformatics* 27: 2156–2158.

David P. 1998. Heterozygosity–fitness correlations: new perspectives on old problems. *Heredity* 80: 531–537.

David P, Pujol B, Viard F, Castella V, Goudet J. 2007. Reliable selfing rate estimates from imperfect population genetic data. *Molecular Ecology* 16: 2474–2487.

Du Q, Lu W, Quan M, Xiao L, Song F, Li P, Zhou D, Xie J, Wang L, Zhang D. 2018. Genome-Wide Association Studies to improve wood properties: challenges and prospects. *Frontiers in Plant Science* 9.

Ellegren H, Galtier N. 2016. Determinants of genetic diversity. *Nature Reviews Genetics* 17: 422–433.

Evans A, de Kort H, Brys R, Duffy KJ, Jersáková J, Kull T, Selosse M-A, Tsiftsis S, Minasiewicz J, Jacquemyn H. 2023. Historical biogeography and local adaptation explain population genetic structure in a widespread terrestrial orchid. *Annals of Botany* 131: 623–634.

Excoffier L, Lischer HEL. 2010. Arlequin suite ver. 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources* 10: 564–567.

Eyre-Walker A, Keightley PD. 2007. The distribution of fitness effects of new mutations. *Nature Reviews Genetics* 8: 610–618.

Fady B, Bozzano M. 2021. Effective population size does not make a practical indicator of genetic diversity in forest trees. *Biological Conservation* 253: 108904.

Flores-López C, Geada-López CG, López-Upton J, López-Ramírez E. 2012. Reproductive indicators in natural populations of *Picea martinezii* T. F. Patterson. *Revista Forestal Baracoa* 31: 49–58.

Frankel OH, Soulé ME. 1981. *Conservation and evolution*. Cambridge: Cambridge University Press.

Frankham R. 2009. Genetic architecture of reproductive fitness and its consequences. In: van der Werf J, Graser H-U, Frankham R, Gondro C, eds. Adaptation and Fitness in Animal Populations: Evolutionary and Breeding Perspectives on Genetic Resource Management. Dordrecht: Springer Netherlands, 15–39.

Frankham R, Ballou JD, Briscoe DA. 2002. *Introduction to Conservation Genetics*. Cambridge: Cambridge University Press.

Gilpin ME, Soulé ME. 1986. Minimum viable populations: processes of species extinction. In: Soulé ME, ed. Conservation Biology: The Science of Scarcity and Diversity. Massachusetts: Sinauer Associates, 19–34.

Grueber CE, Wallis GP, Jamieson IG. 2008. On inbreeding depression in threatened species. *Molecular Ecology* 17: 3978–3984.

Grueber CE, Waters JM, Jamieson IG. 2011. The imprecision of heterozygosity-fitness correlations hinders the detection of inbreeding and inbreeding depression in a threatened species. *Molecular Ecology* 20: 67–79.

Hamrick JL, Godt MJW, Murawski DA, Loveless MD. 1991. Correlations between species traits and allozyme diversity: implications for conservation biology. In: Falk DA, Holsinger KE, eds. Genetics and Conservation of Rare Plants. New York: Oxford University Press New York, 75–86.

Hernández-Díaz JC, Díaz-Carrillo OA, Villanueva-Díaz J, Wehenkel C. 2022. Análisis del incremento corriente anual y del incremento medio anual en siete poblaciones de *Picea*. In: González-Elizondo MS, Wehenkel C, eds. Las piceas (*Picea*, Pinaceae) de México. 8: Comisión Nacional Forestal, 132–152.

Hoban S, Bruford M, D'Urban Jackson J, Lopes-Fernandes M, Heuertz M, Hohenlohe PA, Paz-Vinas I, Sjögren-Gulve P, Segelbacher G, Vernesi C, *et al.* 2020. Genetic diversity targets and indicators in the CBD post-2020 Global Biodiversity Framework must be improved. *Biological Conservation* 248: 108654.

Hoban S, Paz-Vinas I, Aitken S, Bertola L, Breed MF, Bruford M, Funk C, Grueber C, Heuertz M, Hohenlohe P. 2021. Effective population size remains a suitable, pragmatic indicator of genetic diversity for all species, including forest trees. *Biological Conservation* 253: 108906.

Hodgetts RB, Aleksiuk MA, Brown A, Clarke C, Macdonald E, Nadeem S, Khasa D. 2001. Development of microsatellite markers for white spruce (*Picea glauca*) and related species. *Theoretical and Applied Genetics* 102: 1252–1258.

Hohenlohe PA, Funk WC, Rajora OP. 2021. Population genomics for wildlife conservation and management. *Molecular Ecology* 30: 62–82.

Husband BC, Schemske DW. 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution* 50: 54–70.

IUCN (International Union for Conservation of Nature). 2023. The IUCN Red List of Threatened Species. Version 2022-2.

Jaramillo-Correa JP, Bagnoli F, Grivet D, Fady B, Aravanopoulos FA, Vendramin GG, González-Martínez SC. 2020. Evolutionary rate and genetic load in an emblematic Mediterranean tree following an ancient and prolonged population collapse. *Molecular Ecology* 29: 4797–4811.

Jaramillo-Correa JP, Beaulieu J, Ledig FT, Bousquet 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.

Jaramillo-Correa J-P, Rodríguez-Quilón I, Grivet D, Lepoittevin C, Sebastiani F, Heuertz M, Garnier-Géré PH, Alía R, Plomion C, Vendramin GG, *et al.* 2015. Molecular proxies for climate maladaptation in a long-lived tree (*Pinus pinaster* Aiton, Pinaceae). *Genetics* 199: 793–807.

Jombart T. 2008. adegenet: a R package for the multivariate analysis of genetic markers. *Bioinformatics* 24: 1403–1405.

Kardos M, Husby A, McFarlane SE, Qvarnström A, Ellegren H. 2016. Whole-genome resequencing of extreme phenotypes in collared flycatchers highlights the difficulty of detecting quantitative trait loci in natural populations. *Molecular Ecology Resources* 16: 727–741.

Kardos M, Zhang Y, Parsons KM, Kang H, Xu X, Liu X, Matkin CO, Zhang P, Ward EJ, Hanson MB. 2023. Inbreeding depression explains killer whale population dynamics. *Nature Ecology & Evolution*: 1–12.

Kneeshaw DD, Burton PJ. 1997. Canopy and age structures of some old sub-boreal *Picea* stands in British Columbia. *Journal of Vegetation Science* 8: 615–625.

Kyriazis CC, Wayne RK, Lohmueller KE. 2021. Strongly deleterious mutations are a primary determinant of extinction risk due to inbreeding depression. *Evolution letters* 5: 33–47.

Lammi A, Siikamäki P, Mustajärvi K. 1999. Genetic diversity, population size, and fitness in central and peripheral populations of a rare plant *Lychnis viscaria*. *Conservation Biology* 13: 1069–1078.

Lê S, Josse J, Husson F. 2008. FactoMineR: an R package for multivariate analysis. *Journal of statistical software* 25: 1–18.

Ledig FT, Bermejo-Velázquez B, Hodgskiss PD, Johnson DR, Flores-López C, Jacob-Cervantes V. 2000a. The mating system and genic diversity in Martínez spruce, an extremely rare endemic of México's Sierra Madre Oriental: an example of facultative selfing and survival in interglacial refugia. *Canadian Journal of Forest Research* 30: 1156–1164.

Ledig FT, Hodgskiss PD, Jacob-Cervantes V. 2002. Genetic diversity, mating system, and conservation of a Mexican subalpine relict, *Picea mexicana* Martínez. *Conservation Genetics* 3: 113–122.

Ledig FT, Mápula-Larreta M, Bermejo-Velázquez B, Reyes-Hernández V, Flores-López C, Capó-Arteaga MA. 2000b. Locations of endangered spruce populations in Mexico and the demography of *Picea chihuahuana*. *Madroño* 47: 71–88.

Ledig FT, Rehfeldt GE, Sáenz-Romero C, Flores-López C. 2010. Projections of suitable habitat for rare species under global warming scenarios. *American Journal of Botany* 97: 970–987.

Leimu R, Mutikainen PIA, Koricheva J, Fischer M. 2006. How general are positive relationships between plant population size, fitness and genetic variation? *Journal of Ecology* 94: 942–952.

Lobell AS, Kaspari RR, Serrano Negron YL, Harbison ST. 2017. The genetic architecture of ovariole number in *Drosophila melanogaster*: genes with major, quantitative, and pleiotropic effects. *G3 Genes|Genomes|Genetics* 7: 2391–2403.

Lockwood JD, Aleksić JM, Zou J, Wang J, Liu J, Renner SS. 2013. A new phylogeny for the genus *Picea* from plastid, mitochondrial, and nuclear sequences. *Molecular Phylogenetics and Evolution* 69: 717–727.

López-Delgado J, Meirmans PG. 2022. History or demography? Determining the drivers of genetic variation in North American plants. *Molecular Ecology* 31: 1951–1962.

Luu K, Bazin E, Blum MGB. 2017. pcadapt: an R package to perform genome scans for selection based on principal component analysis. *Molecular ecology resources* 17: 67–77.

McKown AD, Klápště J, Guy RD, Geraldes A, Porth I, Hannemann J, Friedmann M, Muchero W, Tuskan GA, Ehlting J, *et al.* 2014. Genome-wide association implicates numerous genes underlying ecological trait variation in natural populations of *Populus trichocarpa*. *New Phytologist* 203: 535–553.

Mendoza-Maya E, Espino-Espino J, Quiñones-Pérez CZ, Flores-López C, Wehenkel C, Vargas-Hernández JJ, Sáenz-Romero C. 2015. Proposal for conservation of three endangered species of Mexican spruce. *Revista Fitotecnia Mexicana* 38: 235–247.

Mendoza-Maya E, Gómez-Pineda E, Sáenz-Romero C, Hernández-Díaz JC, López-Sánchez CA, Vargas-Hernández JJ, Prieto-Ruíz JÁ, Wehenkel C. 2022. Assisted migration and the rare endemic plant species: the case of two endangered Mexican spruces. *PeerJ* 10: e13812.

Miller JM, Malenfant RM, David P, Davis CS, Poissant J, Hogg JT, Festa-Bianchet M, Coltman DW. 2014. Estimating genome-wide heterozygosity: effects of demographic history and marker type. *Heredity* 112: 240–247.

Moritsuka E, Hisataka Y, Tamura M, Uchiyama K, Watanabe A, Tsumura Y, Tachida H. 2012. Extended linkage disequilibrium in noncoding regions in a conifer, *Cryptomeria japonica*. *Genetics* 190: 1145–1148.

Mosseler A, Major JE, Simpson JD, Daigle B, Lange K, Park YS, Johnsen KH, Rajora OP. 2000. Indicators of population viability in red spruce, *Picea rubens*. I. Reproductive traits and fecundity. *Canadian Journal of Botany* 78: 928–940.

Nabutanyi P, Wittmann MJ. 2021. Models for eco-evolutionary extinction vortices under balancing selection. *The American Naturalist* 197: 336–350.

Neale DB, Savolainen O. 2004. Association genetics of complex traits in conifers. *Trends in Plant Science* 9: 325–330.

Nicolini P, Amorín R, Han Y, Peñagaricano F. 2018. Whole-genome scan reveals significant non-additive effects for sire conception rate in Holstein cattle. *BMC Genetics* 19: 14.

Nienstaedt H, Zasada JC. 1990. *Picea glauca* (Moench) Voss. In: Burns RM, Honkala BH, eds. Agricultural Handbook 654. Silvics of North America. Washington, D.C.: United States Department of Agriculture and Forest Service, 204–226.

O'Connell LM, Mosseler A, Rajora OP. 2006. Impacts of forest fragmentation on the reproductive success of white spruce (*Picea glauca*). *Botany* 84: 956–965.

Ochoa A, Storey JD. 2021. Estimating F_{ST} and kinship for arbitrary population structures. *PLOS Genetics* 17: e1009241.

Petit RJ, Hampe A. 2006. Some evolutionary consequences of being a tree. *Annu. Rev. Ecol. Evol. Syst.* 37: 187–214.

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, De Bakker PIW, Daly MJ. 2007. PLINK: a tool set for whole-genome association and population-based linkage analyses. *The American Journal of Human Genetics* 81: 559–575.

Pyhäjärvi T, Kujala ST, Savolainen O. 2011. Revisiting protein heterozygosity in plants—nucleotide diversity in allozyme coding genes of conifer *Pinus sylvestris*. *Tree Genetics & Genomes* 7: 385–397.

Quiñones-Pérez CZ, González-Elizondo M del S, Wehenkel C. 2017. Ruling out genetic erosion in *Picea chihuahuana* Martínez. *New Forests* 48: 201–215.

R Core Team. 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. URL https://www.R-project.org/

Reed DH, Frankham R. 2003. Correlation between fitness and genetic diversity. *Conservation biology* 17: 230–237.

Rodríguez-Quilón I, Santos-del-Blanco L, Grivet D, Jaramillo-Correa JP, Majada J, Vendramin GG, Alia R, González-Martínez SC. 2015. Local effects drive heterozygosity–fitness correlations in an outcrossing long-lived tree. *Proceedings of the Royal Society B: Biological Sciences* 282: 20152230.

Sanjayan M, Crooks KR, Mills LS. 2020. Michael E. Soulé (1936–2020). *Nature Ecology & Evolution* 4: 1296–1297.

Santos-del-Blanco L, Olsson S, Budde KB, Grivet D, González-Martínez SC, Alía R, Robledo-Arnuncio JJ. 2022. On the feasibility of estimating contemporary effective population size (*Ne*) for genetic conservation and monitoring of forest trees. *Biological Conservation* 273: 109704.

Satake A, Kelly D. 2021. Studying the genetic basis of masting. *Philosophical Transactions of the Royal Society B* 376: 20210116.

Sayers EW, Beck J, Bolton EE, Bourexis D, Brister JR, Canese K, Comeau DC, Funk K, Kim S, Klimke W, *et al.* 2021. Database resources of the National Center for Biotechnology Information. *Nucleic Acids Research* 49: D10–D17.

Slate J, David P, Dodds KG, Veenvliet BA, Glass BC, Broad TE, McEwan JC. 2004. Understanding the relationship between the inbreeding coefficient and multilocus heterozygosity: theoretical expectations and empirical data. *Heredity* 93: 255–265.

Stoffel MA, Esser M, Kardos M, Humble E, Nichols H, David P, Hoffman JI. 2016. inbreedR: an R package for the analysis of inbreeding based on genetic markers. *Methods in Ecology and Evolution* 7: 1331–1339.

Strauss SH, Libby WJ. 1987. Allozyme heterosis in radiata pine is poorly explained by overdominance. *The American Naturalist* 130: 879–890.

Szulkin M, Bierne N, David P. 2010. Heterozygosity-fitness correlations: a time for reappraisal. *Evolution* 64: 1202–1217.

Uffelmann E, Huang QQ, Munung NS, de Vries J, Okada Y, Martin AR, Martin HC, Lappalainen T, Posthuma D. 2021. Genome-wide association studies. *Nature Reviews Methods Primers* 1: 59.

Waller DM. 2021. Addressing Darwin's dilemma: can pseudo-overdominance explain persistent inbreeding depression and load? *Evolution* 75: 779–793.

Walter R, Epperson BK. 2005. Geographic pattern of genetic diversity in *Pinus resinosa*: contact zone between descendants of glacial refugia. *American Journal of Botany* 92: 92–100.

Wegrzyn JL, Liechty JD, Stevens KA, Wu L-S, Loopstra CA, Vasquez-Gross HA, Dougherty WM, Lin BY, Zieve JJ, Martínez-García PJ, *et al.* 2014. Unique features of the Loblolly pine (*Pinus taeda* L.) megagenome revealed through sequence annotation. *Genetics* 196: 891–909.

Wehenkel C, Torres-Valverde JM, Hernández-Díaz JC, Mendoza-Maya E, Carrillo-Parra A, Solis-González S, López-Upton J. 2023. Adaptive trait variation in seedlings of rare endemic Mexican spruce provenances under nursery conditions. *Forests* 14: 790.

White TL, Adams WT, Neale DB. 2007. *Forest Genetics*. London: CABI Publishing.

Wiens D, Calvin CL, Wilson CA, Davern CI, Frank D, Seavey SR. 1987. Reproductive success, spontaneous embryo abortion, and genetic load in flowering plants. *Oecologia* 71: 501–509.

Williams CG. 2009. *Conifer Reproductive Biology*. Dordrecht: Springer Science+Business Media.

Williams CG, Savolainen O. 1996. Inbreeding depression in conifers: implications for breeding strategy. *Forest Science* 42: 102–117.

Wu H, Nilsson O. 2023. Threatened forests: as the Northern forests suffer from the effects of climate change, genomics has great potential to help them adapt. *EMBO reports* 24: e57106.

Yates MC, Bowles E, Fraser DJ. 2019. Small population size and low genomic diversity have no effect on fitness in experimental translocations of a wild fish. *Proceedings of the Royal Society B* 286: 20191989.

4.7. Supporting Information

The following Supporting Information is available for this article:

Figures S4.1-S4.9

Tables S4.1-S4.7

Methods S4.1-S4.2

FIGURES

Fig. S4.1

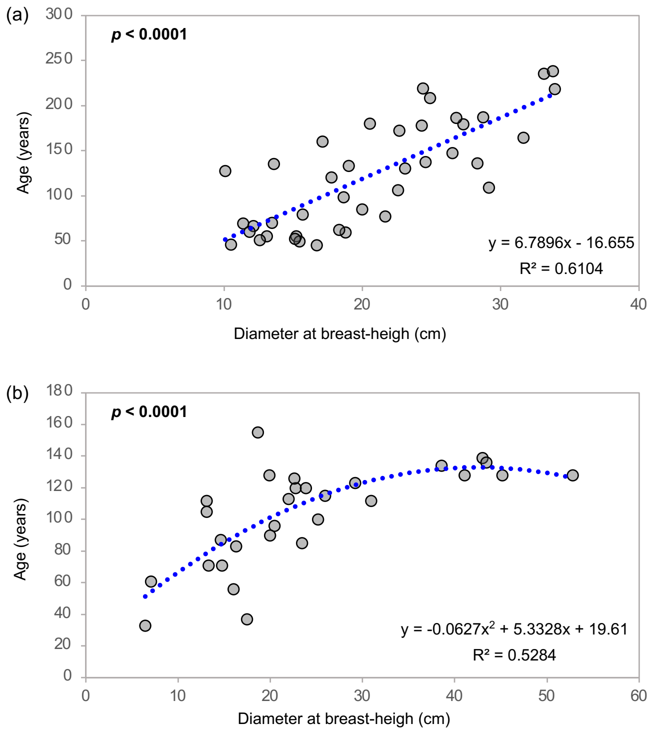


Figure S4.1. Tree age estimation for *Picea martinezii* (a) and *Picea mexicana* (b). Regression models are based on total diameters at breast heigh (DBH; 1.3 m height) and ages estimated through growth-rings analyses, for 29-30 sampled trees per species.

Fig. S4.2

Figure S4.2. Seedlings and saplings of *P. mexicana* bearing mature female strobili. Cat0 (a) and Cat2/Cat4 (b) individuals at El Mohinora population are shown.

Fig. S4.3.

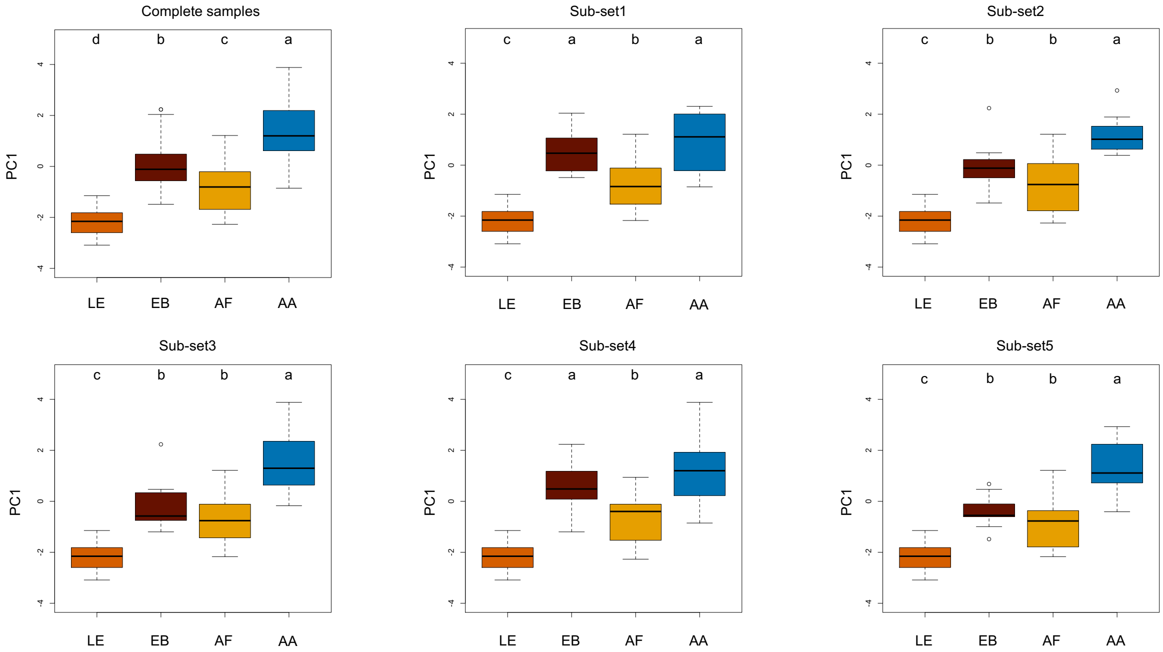


Figure S4.3. Variation of reproductive PC1 among populations of *Picea martinezii* with complete (as in Fig. 3 of main text; n = 11-30) and uniform (sub-sets 1-5: n = 11) sample sizes. Populations are sorted according to increasing *Ne*: LE = La Encantada, EB = El Butano, AF = Agua Fría, AA = Agua de Alardín. Same upper letters indicate non-significant differences, with alpha = 0.05.

Fig. S4.4

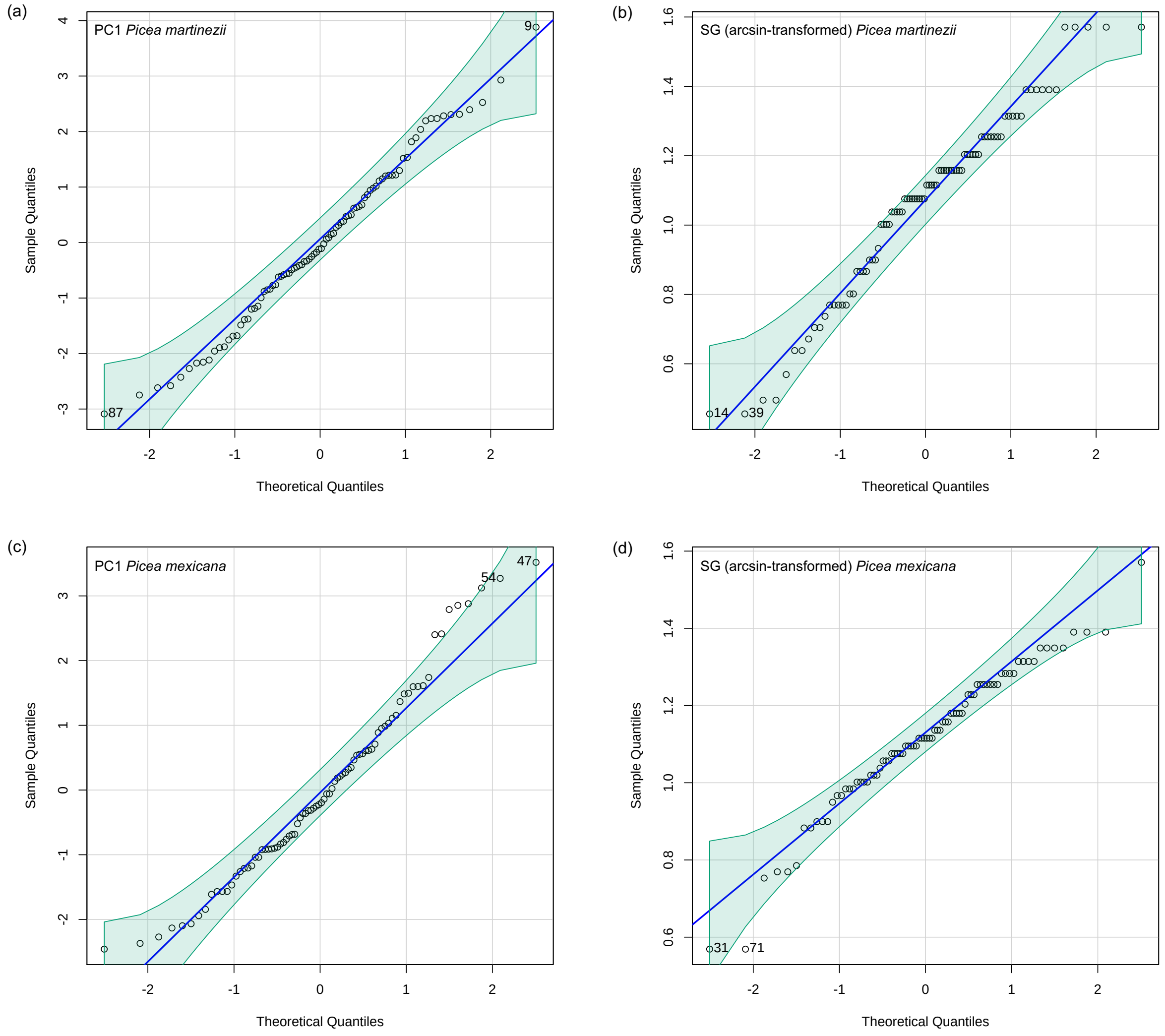


Figure S4.4. Normality tests for the reproductive traits (PC1 and seed germination) of *Picea martinezii* (a-b) and *Picea mexicana* (c-d). Continuous-blue-lines indicate perfect distribution fits; green-shaded areas indicate 95% confidence intervals.

Fig. S4.5

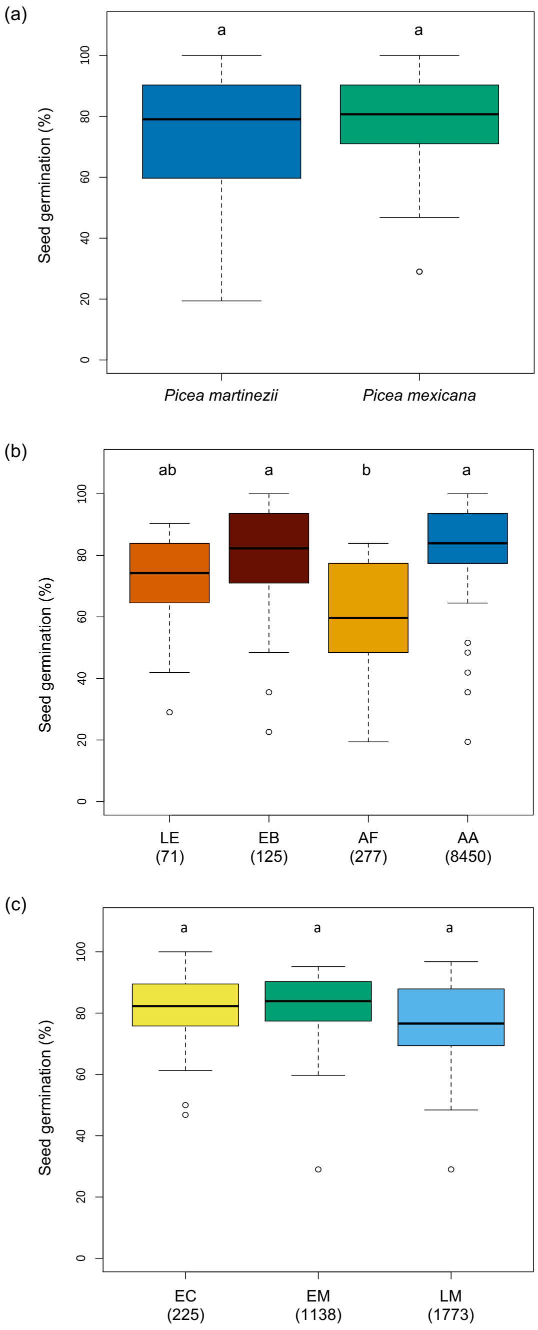


Figure S4.5. Seed germination among species (a) and populations of *Picea martinezii* (b) and *Picea mexicana* (c). Sub-figures (b) and (c) show the proxies of the effective population size ($Ne_{10\%Nc}$) inside parenthesis after population IDs. LE = La Encantada, EB = El Butano, AF = Agua Fría, AA = Agua de Alardín, EC = El Coahuilón, EM = El Mohinora, LM = La Marta. Same upper letters indicate non-significant differences for the trait, with alpha = 0.05.

Fig. S4.6

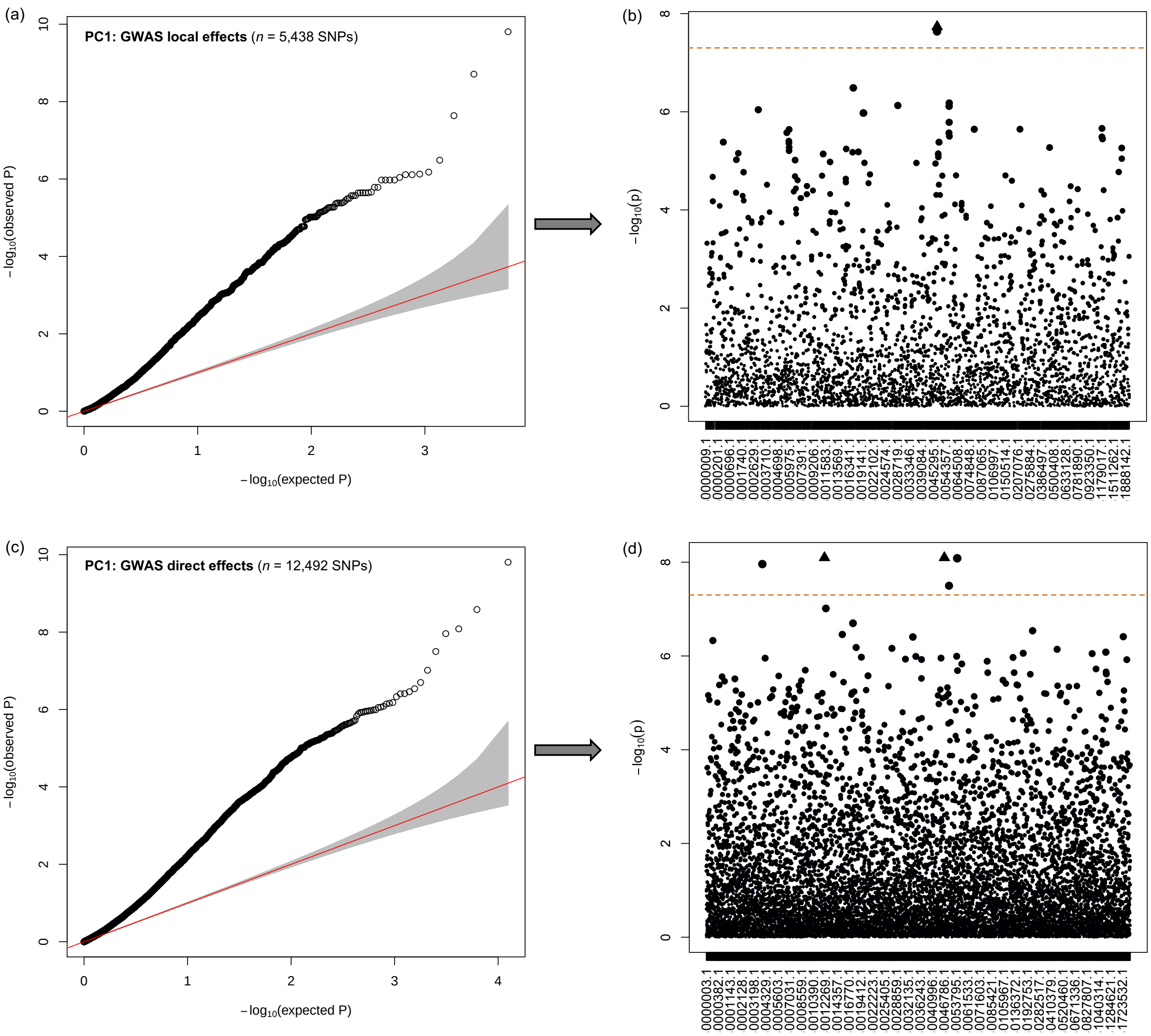


Figure S4.6. Plot visualizations of GWAS implemented for PC1 of *Picea martinezii*. (a-b) Local effects (linked-loci). (c-d) Direct effects (unlinked-loci). In Q-Q plots (a,c), red-continuous-lines represent the null hypotheses for associations between SNPs (black-open-circles) and fitness-traits, with 95% confidence intervals marked as grey-shaded areas. In Manhattan plots (b,d), *x*-axis show scaffolds of assembled genome (starting with codes ALWZ05S, not shown); top red-dotted-lines indicate threshold of significances for unadjusted *p*-values (5e-8); SNPs overpassing *y*-axis-limit are plotted as black-filled-triangles instead of black-filled-circles.

Fig. S4.7

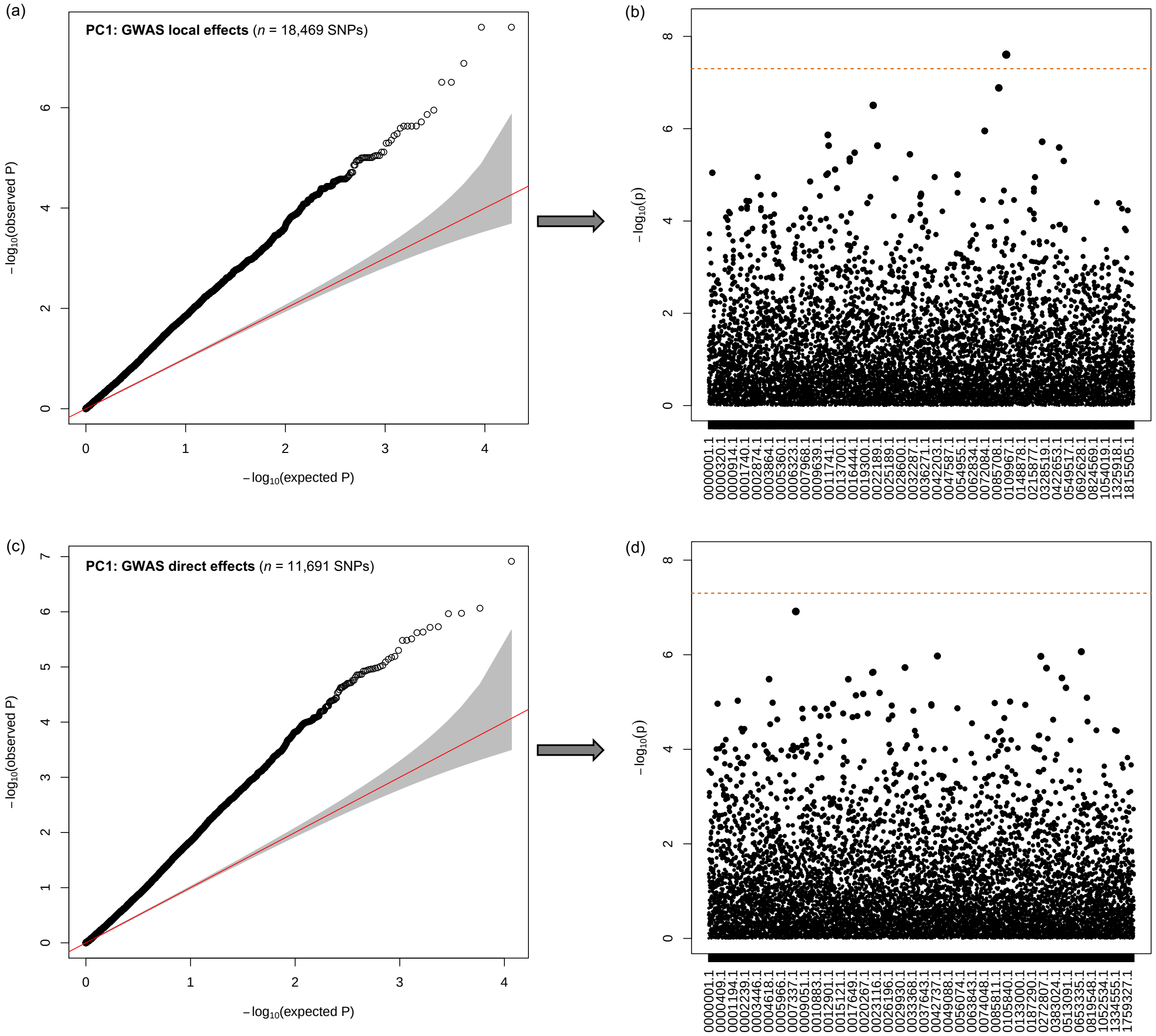


Figure S4.7. Plot visualizations of GWAS implemented for PC1 of *Picea mexicana*. (a-b) Local effects (linked-loci). (c-d) Direct effects (unlinked-loci). In Q-Q plots (a,c), red-continuous-lines represent the null hypotheses for associations between SNPs (black-open-circles) and fitness-traits, with 95% confidence intervals marked as grey-shaded areas. In Manhattan plots (b,d), *x*-axis show scaffolds of assembled genome (starting with codes ALWZ05S, not shown); top red-dotted-lines indicate threshold of significances for unadjusted *p*-values (5e-8); SNPs are plotted as black-filled-circles.

Fig. S4.8

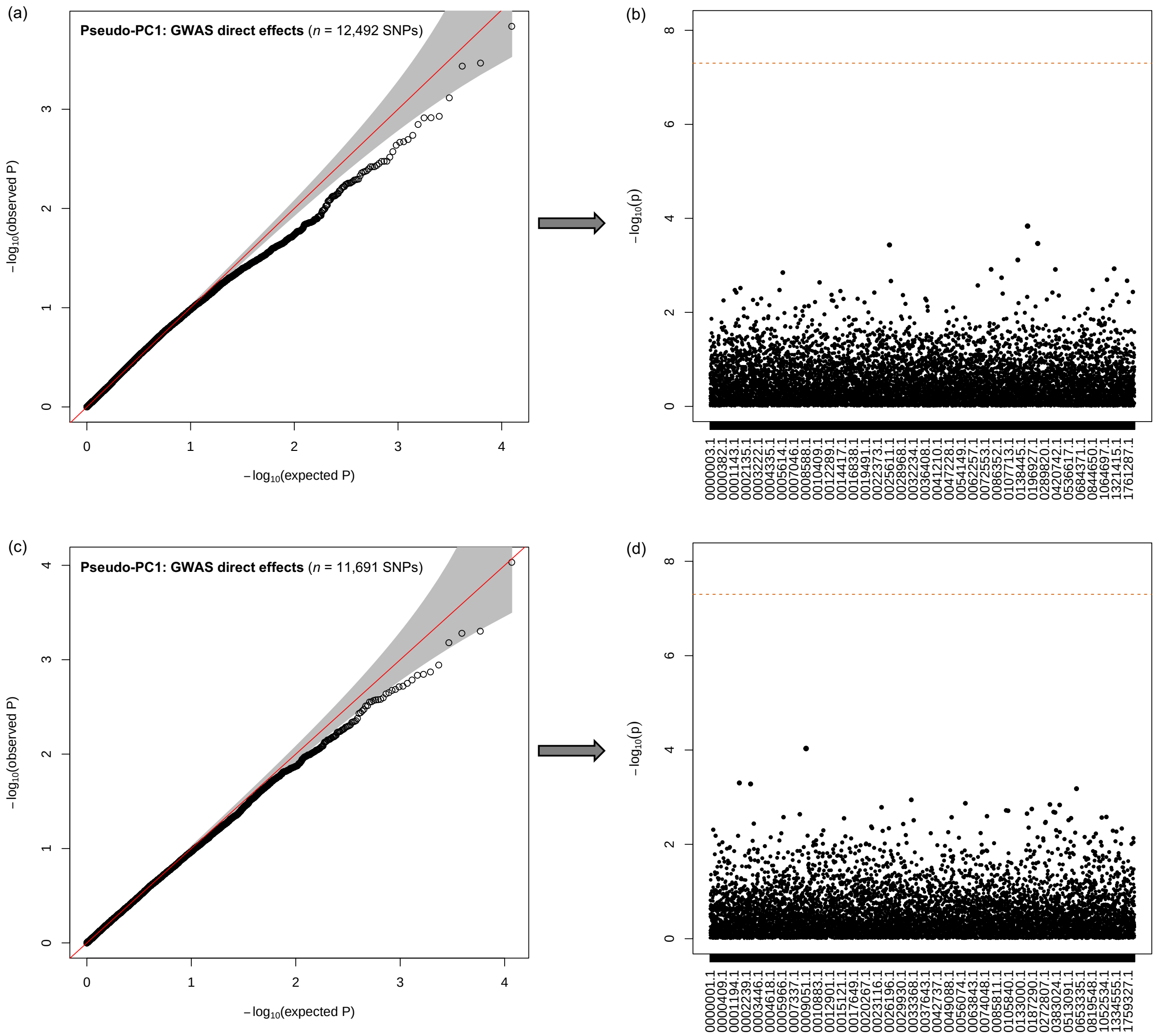


Figure S4.8. Plot visualizations for the no-significant GWAS (after Bonferroni) using SNP-sets for direct effects (unlinked loci) and pseudo-PC1s for *Picea martinezii* (a-b) and *Picea mexicana* (c-d). In Q-Q plots (a,c), red-continuous-lines represent the null hypotheses for associations between SNPs (black-open-circles) and pseudo-PC1s, with 95% confidence intervals marked as grey-shaded areas. In Manhattan plots (b,d), *x*-axis show scaffolds of assembled genome (starting with codes ALWZ05S, not shown); top red-dotted-lines indicate threshold of significances for unadjusted *p*-values (5e-8); SNPs are plotted as black-filled-circles. Note: similar results were found using SNP-sets for local effects (linked loci) in both species, after FDR.

Fig. S4.9

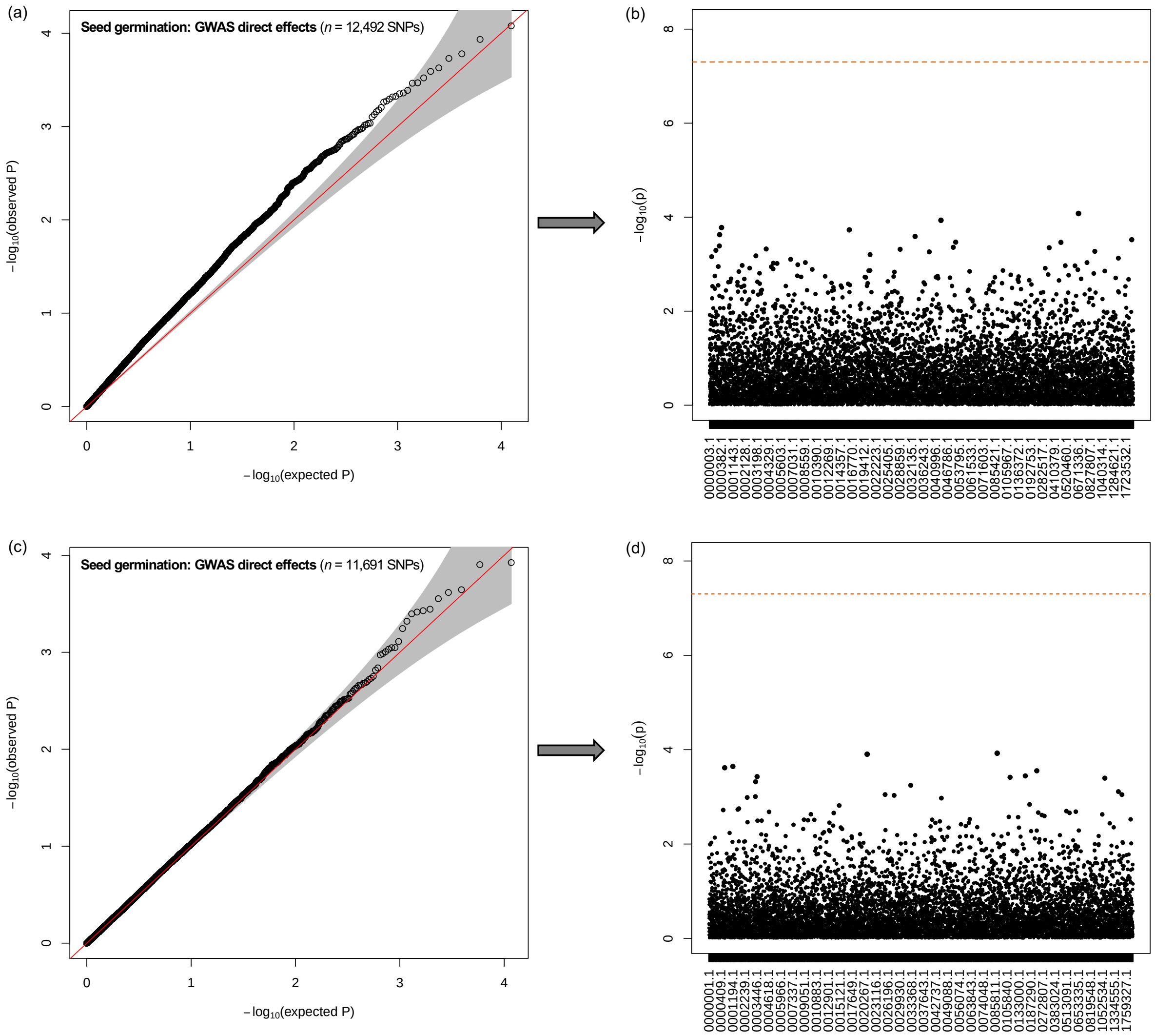


Figure S4.9. Plot visualizations for the no-significant GWAS (after Bonferroni) using SNP-sets for direct effects (unlinked loci) and seed germination (SG) for *Picea martinezii* (a-b) and *Picea mexicana* (c-d). In Q-Q plots (a,c), red-continuous-lines represent the null hypotheses for associations between SNPs (black-open-circles) and SG, with 95% confidence intervals marked as grey-shaded areas. In Manhattan plots (b,d), *x*-axis show scaffolds of assembled genome (starting with codes ALWZ05S, not shown); top red-dotted-lines indicate threshold of significances for unadjusted *p*-values (5e-8); SNPs are plotted as black-filled-circles. Note: similar results were found using SNP-sets for local effects (linked loci) in both species, after FDR.

TABLES

Table S4.1. Dasometric data, location, genetic parameters and summarized reproductive fitness of the sampled trees of *Picea martinezii* and *Picea mexicana*, in October-November 2018.

Tree- ID[1]	**DBH**[2]	**Heigh**	**Decimal coordinates**		**Elevation**	***Ho***[3]	***F***[4]	**PC1*(-1)**[5]
	(cm)	**(m)**	**Longitude**	**Latitude**	**(m)**			
AA1	53.4	29.1	-99.731083	24.041639	2177	0.180	0.188	-0.254
AA2	80.7	50.0	-99.731556	24.041667	2184	0.162	0.199	1.517
AA3	60.3	38.0	-99.73175	24.041889	2184	0.186	0.124	2.311
AA4	34.2	30.0	-99.731833	24.042111	2138	0.171	0.197	1.199
AA5	46.3	32.0	-99.731861	24.042361	2138	0.165	0.239	0.383
AA6	55.2	23.0	-99.731944	24.0415	2184	0.160	0.246	0.060
AA7	42.1	27.0	-99.730806	24.041333	2225	0.176	0.207	-0.853
AA8	47.5	25.0	-99.73325	24.041806	2164	0.170	0.201	0.499
AA9	37.2	30.0	-99.734417	24.041083	2191	0.177	0.178	3.882
AA10	56.2	30.0	-99.734667	24.040917	2191	0.182	0.159	2.306
AA11	51.0	37.0	-99.734778	24.041028	2191	0.173	0.206	1.535
AA12	22.3	22.0	-99.734833	24.041139	2237	0.178	0.172	2.394
AA13	48.8	32.0	-99.734917	24.041028	2237	0.169	0.156	-0.175
AA14	27.0	26.0	-99.734583	24.0415	2181	0.162	0.256	1.297
AA15	26.7	21.0	-99.734472	24.041528	2181	0.171	0.236	1.888
AA16	49.3	32.0	-99.734417	24.041556	2181	0.176	0.157	1.816
AA17	41.0	27.0	-99.7345	24.0415	2181	0.166	0.211	0.651
AA18	38.6	28.0	-99.734417	24.04175	2181	0.170	0.204	1.016
AA19	63.5	37.0	-99.734639	24.042083	2156	NA[6]	NA	NA
AA20	49.2	32.0	-99.734694	24.04225	2156	0.166	0.203	1.109
AA21	10.9	12.0	-99.73425	24.041889	2144	0.170	0.217	2.524
AA22	44.3	28.0	-99.734361	24.042222	2156	0.171	0.210	0.632
AA23	43.7	29.0	-99.735167	24.042556	2164	0.150	0.267	0.809
AA24	32.2	25.0	-99.734917	24.042583	2164	0.162	0.241	1.218
AA25	50.4	30.0	-99.734833	24.042833	2164	0.160	0.226	2.194
AA26	26.0	28.0	-99.734667	24.0425	2156	0.162	0.252	2.929
AA27	36.2	28.0	-99.734917	24.042583	2164	0.163	0.235	2.283
AA28	53.2	30.0	-99.735056	24.042222	2183	0.162	0.224	0.619
AA29	40.6	22.0	-99.735306	24.042861	2164	0.174	0.195	0.863
AA30	39.8	17.0	-99.733694	24.044306	2108	0.129	0.423	-0.411
AF1	56.0	32.0	-99.715639	24.038833	1855	0.200	0.121	-0.840
AF2	53.0	35.0	-99.712722	24.036139	1943	0.175	0.149	0.940

Tree- ID[1]	DBH[2]	Heigh	Decimal coordinates		Elevation	*Ho*[3]	*F*[4]	PC1*(-1)[5]
	(cm)	(m)	Longitude	Latitude	(m)			
AF3	31.5	23.0	-99.712556	24.036472	1915	0.176	0.164	0.147
AF4	38.7	24.2	-99.711	24.039667	1818	0.173	0.227	-2.271
AF5	46.5	23.0	-99.711167	24.040056	1821	NA	NA	NA
AF6	38.2	21.0	-99.711667	24.03975	1829	0.176	0.165	-1.680
AF7	36.4	23.0	-99.711528	24.039889	1829	0.179	0.179	-1.379
AF8	28.0	17.0	-99.710667	24.040306	1826	0.172	0.152	-0.023
AF9	49.3	35.0	-99.709917	24.040611	1814	0.174	0.179	-0.400
AF10	46.7	33.0	-99.702333	24.041056	1813	0.171	0.190	-0.207
AF11	18.0	17.0	-99.702444	24.043111	1814	0.174	0.210	-1.895
AF12	33.9	23.0	-99.707333	24.039333	1842	0.190	0.113	-0.334
AF13	39.6	25.0	-99.7075	24.039139	1861	0.167	0.222	-1.686
AF14	29.0	25.0	-99.708111	24.0395	1828	NA	NA	NA
AF15	41.7	15.2	-99.711306	24.040083	1821	NA	NA	NA
AF16	44.5	23.0	-99.711361	24.040056	1821	0.180	0.163	-2.117
AF17	46.7	36.0	-99.71075	24.039028	1840	0.176	0.238	-0.760
AF18	38.4	28.0	-99.71075	24.038194	1857	0.178	0.187	-1.187
AF19	19.8	21.0	-99.711194	24.037944	1882	0.176	0.219	-0.773
AF20	21.7	17.5	-99.711333	24.037778	1882	0.170	0.213	1.213
AF21	41.0	31.0	-99.711806	24.036583	1900	0.172	0.197	-2.173
EB1	37.6	21.0	-100.12842	25.181444	2156	0.160	0.274	1.211
EB2	33.2	19.0	-100.12806	25.181361	2174	0.168	0.198	0.483
EB3	40.6	23.1	-100.128	25.180861	2174	0.154	0.287	2.039
EB4	45.2	29.0	-100.12753	25.179944	2182	0.163	0.243	-1.200
EB5	45.7	25.0	-100.12631	25.177917	2182	0.178	0.140	0.979
EB6	51.2	26.0	-100.12611	25.177306	2195	0.164	0.254	0.275
EB7	31.0	23.1	-100.12586	25.177972	2173	0.149	0.300	0.309
EB8	44.0	25.6	-100.12592	25.178167	2172	0.150	0.308	-0.108
EB9	34.0	23.3	-100.12581	25.178139	2172	0.167	0.212	2.236
EB10	34.3	20.3	-100.126	25.177722	2182	0.162	0.306	1.147
EB11	42.3	26.7	-100.12564	25.177528	2173	0.167	0.213	-0.608
EB12	28.2	22.0	-100.12511	25.177306	2176	0.162	0.233	-0.342
EB13	35.4	27.0	-100.12522	25.177139	2176	0.148	0.324	0.165
EB14	47.2	28.8	-100.12481	25.176444	2180	0.180	0.206	0.364
EB15	48.4	31.3	-100.125	25.176306	2195	0.156	0.227	2.234
EB16	45.0	27.3	-100.125	25.176028	2195	0.164	0.267	-0.119

Tree- ID[1]	DBH[2] (cm)	Heigh (m)	Decimal coordinates Longitude	Decimal coordinates Latitude	Elevation (m)	*Ho*[3]	*F*[4]	PC1*(-1)[5]
EB17	52.5	27.5	-100.12478	25.175389	2195	0.124	0.464	-0.463
EB18	75.6	28.0	-100.12256	25.171861	2233	0.163	0.241	-0.881
EB19	46.3	25.3	-100.12247	25.171417	2233	0.161	0.259	-0.442
EB20	54.9	27.8	-100.12242	25.171583	2233	0.163	0.237	0.678
EB21	34.8	21.0	-100.12264	25.17125	2313	0.164	0.264	-0.563
EB22	40.9	25.0	-100.12197	25.171333	2219	0.161	0.237	0.087
EB23	40.9	18.7	-100.12153	25.171306	2219	0.157	0.284	-0.995
EB24	47.0	29.5	-100.12167	25.171361	2219	0.156	0.255	-0.488
EB25	45.5	21.0	-100.12156	25.171278	2219	0.164	0.227	-0.553
EB26	40.3	23.5	-100.12214	25.171389	2254	0.153	0.287	-0.579
EB27	47.2	25.2	-100.12261	25.171	2313	0.151	0.282	-1.486
EB28	34.7	23.8	-100.12303	25.171056	2313	0.162	0.257	-0.299
EB29	37.9	24.7	-100.12317	25.171028	2375	0.174	0.164	-0.621
EB30	32.7	26.0	-100.12331	25.171306	2375	0.167	0.205	0.468
LE1	33.8	24.5	-99.792	23.89025	2578	0.163	0.279	-2.748
LE2	34.7	21.3	-99.791639	23.890556	2563	0.138	0.390	-1.956
LE3	36.2	22.0	-99.791722	23.890528	2563	0.154	0.344	-1.388
LE4	25.1	19.1	-99.792111	23.890194	2576	NA	NA	NA
LE5	36.5	19.5	-99.792083	23.890333	2568	0.147	0.332	-2.427
LE6	39.8	20.5	-99.791889	23.890556	2563	0.163	0.294	-2.617
LE7	38.8	23.0	-99.791611	23.890306	2563	0.160	0.297	-2.580
LE8	50.5	27.0	-99.791056	23.891944	2534	0.166	0.284	-1.147
LE9	45.0	27.0	-99.791194	23.891667	2534	0.164	0.234	-1.755
LE10	35.2	23.5	-99.791333	23.891806	2534	NA	NA	NA
LE11	36.0	24.4	-99.7915	23.891639	2542	0.172	0.205	-2.156
LE12	30.8	20.9	-99.791361	23.892028	2529	0.164	0.259	-3.088
LE13	36.2	20.5	-99.791694	23.890444	2563	0.157	0.286	-1.880
EC1	18.8	8.0	-100.34542	25.244806	3517	NA	NA	NA
EC2	7.0	4.0	-100.3452	25.244853	3513	0.212	0.206	-0.899
EC3	14.2	7.0	-100.34603	25.244889	3517	NA	NA	NA
EC4	23.6	11.0	-100.34703	25.245444	3475	0.221	0.209	-0.811
EC5	39.4	17.1	-100.34689	25.245333	3475	0.191	0.319	-0.834
EC6	8.6	16.0	-100.34683	25.245528	3475	0.219	0.189	0.538
EC7	24.9	17.0	-100.34669	25.245472	3475	0.216	0.207	0.984
EC8	29.2	19.0	-100.34667	25.245278	3520	0.211	0.240	0.184
EC9	48.2	22.0	-100.347	25.245639	3475	0.207	0.264	-1.942

Tree- ID[1]	DBH[2]	Heigh	Decimal coordinates		Elevation	*Ho*[3]	*F*[4]	PC1*(-1)[5]
	(cm)	(m)	Longitude	Latitude	(m)			
EC10	20.3	12.0	-100.34764	25.245611	3505	0.224	0.184	-1.173
EC11	20.3	11.0	-100.35464	25.246917	3556	0.217	0.195	-0.922
EC12	14.0	8.3	-100.3545	25.247167	3553	0.218	0.217	-1.568
EC13	15.7	10.0	-100.35431	25.247111	3553	0.213	0.237	-1.569
EC14	22.8	12.0	-100.35439	25.247278	3553	0.226	0.187	0.709
EC15	45.7	20.0	-100.35464	25.247333	3553	0.217	0.209	-1.039
EC16	36.0	20.7	-100.35992	25.249917	3457	0.220	0.238	-2.459
EC17	35.0	22.0	-100.35969	25.249889	3438	0.210	0.248	-2.132
EC18	38.8	20.0	-100.35986	25.250028	3457	0.217	0.210	-1.844
EC19	23.3	16.0	-100.35992	25.250167	3457	0.196	0.290	-0.362
EC20	27.7	19.0	-100.36003	25.250056	3457	0.218	0.212	-1.205
EC21	38.8	24.0	-100.36039	25.250111	3482	0.212	0.222	-1.612
EC22	27.9	13.0	-100.36019	25.250389	3410	0.212	0.198	-1.468
EC23	30.5	13.0	-100.35964	25.2508367	3390	0.221	0.179	-0.281
EC24	16.5	10.0	-100.35939	25.25	3438	0.217	0.212	-0.882
EC25	24.5	14.0	-100.35908	25.250028	3426	0.218	0.195	-2.098
EC26	17.8	10.0	-100.35892	25.249944	3426	0.226	0.175	0.246
EC27	16.2	7.4	-100.35864	25.249722	3466	NA	NA	NA
EC28	12.9	7.6	-100.35875	25.249611	3481	0.209	0.232	-0.227
EC29	10.2	9.0	-100.35956	25.24925	3493	0.219	0.246	-0.761
EC30	48.7	25.0	-100.35947	25.249528	3493	0.219	0.201	0.346
EM1	73.2	32.1	-107.03889	25.961778	3051	0.186	0.344	0.270
EM2	46.1	20.8	-107.03906	25.961611	3051	0.216	0.222	-0.684
EM3	40.7	19.0	-107.03889	25.961722	3051	NA	NA	NA
EM4	45.2	20.2	-107.03975	25.962	3020	0.212	0.217	-0.319
EM5	49.0	20.0	-107.03975	25.961278	3062	0.206	0.271	-1.331
EM6	28.0	16.0	-107.03903	25.960333	3100	0.210	0.214	-1.039
EM7	49.0	18.0	-107.04031	25.961639	3040	0.206	0.249	2.400
EM8	47.1	19.0	-107.04267	25.961639	3080	0.207	0.248	2.879
EM9	31.1	14.0	-107.03972	25.961028	3062	0.217	0.227	-0.141
EM10	45.5	20.0	-107.03939	25.960833	3093	0.218	0.222	-0.059
EM11	55.1	18.0	-107.03847	25.960861	3081	0.219	0.193	2.413
EM12	31.0	16.0	-107.04136	25.9615	3042	0.209	0.256	0.321
EM13	44.3	19.0	-107.04103	25.9625	3023	0.205	0.253	0.211
EM14	35.3	20.0	-107.04083	25.961056	3062	0.211	0.206	1.367

Tree- ID[1]	**DBH**[2]	**Heigh**	**Decimal coordinates**		**Elevation**	***Ho***[3]	***F***[4]	**PC1*(-1)**[5]
	(cm)	**(m)**	**Longitude**	**Latitude**	**(m)**			
EM15	44.2	18.0	-107.04097	25.960528	3088	0.214	0.222	2.790
EM16	29.3	16.0	-107.04064	25.959639	3147	0.207	0.249	-0.918
EM17	42.4	16.0	-107.04128	25.959917	3122	0.201	0.245	-0.429
EM18	49.1	21.0	-107.04136	25.959139	3184	0.225	0.143	1.496
EM19	39.3	20.0	-107.03919	25.960167	3130	0.217	0.223	-0.059
EM20	62.3	23.0	-107.03997	25.958944	3170	0.212	0.214	0.134
EM21	50.1	21.0	-107.04022	25.959083	3170	0.215	0.203	3.518
EM22	30.0	18.0	-107.04261	25.959472	3195	0.192	0.340	1.600
EM23	54.8	20.0	-107.04256	25.959806	3150	0.206	0.224	1.613
EM24	58.8	24.0	-107.04383	25.960583	3179	0.212	0.206	0.631
EM25	70.7	24.0	-107.04492	25.960222	3231	NA	NA	NA
EM26	33.6	16.0	-107.04614	25.960306	3226	0.205	0.224	3.123
EM27	68.8	23.0	-107.047	25.960444	3226	0.206	0.249	1.740
EM28	65.1	21.0	-107.04678	25.960972	3193	0.200	0.280	2.854
EM29	35.5	13.2	-107.04603	25.962028	3123	NA	NA	NA
EM30	8.5	15.0	-107.04653	25.962	3123	0.211	0.204	3.271
LM1	59.5	23.7	-100.36449	25.199233	3434	0.230	0.152	-0.362
LM2	83.8	23.0	-100.36433	25.198972	3476	0.216	0.219	0.552
LM3	40.9	20.0	-100.36364	25.199028	3480	0.217	0.219	-0.312
LM4	46.2	20.5	-100.36342	25.199083	3480	0.183	0.324	-0.688
LM5	27.1	19.0	-100.36375	25.19925	3434	0.220	0.204	1.108
LM6	68.6	23.0	-100.36361	25.198889	3480	0.224	0.209	-2.067
LM7	73.6	19.3	-100.36308	25.199278	3448	0.221	0.204	1.485
LM8	41.4	20.5	-100.36261	25.199222	3448	0.224	0.190	-1.210
LM9	66.8	20.8	-100.36267	25.1995	3448	0.213	0.223	-0.196
LM10	39.3	19.6	-100.36256	25.1995	3454	0.216	0.193	-1.260
LM11	48.3	19.5	-100.36308	25.199722	3448	0.223	0.188	-0.250
LM12	46.5	19.0	-100.36322	25.199667	3441	0.218	0.210	0.887
LM13	36.8	21.0	-100.36336	25.199778	3403	0.219	0.198	0.607
LM14	33.5	20.0	-100.36353	25.199778	3403	0.225	0.187	-0.910
LM15	22.8	12.0	-100.36369	25.199778	3403	0.227	0.187	-2.271
LM16	41.4	19.0	-100.365	25.200389	3375	0.207	0.215	-2.370
LM17	55.9	19.0	-100.36483	25.200472	3375	NA	NA	NA
LM18	28.9	18.0	-100.36522	25.200361	3375	NA	NA	NA
LM19	18.5	17.0	-100.36533	25.20025	3403	0.219	0.210	0.465

Tree- ID[1]	**DBH**[2]	**Heigh**	**Decimal coordinates**		**Elevation**	***Ho***[3]	***F***[4]	**PC1*(-1)**[5]
	(cm)	**(m)**	**Longitude**	**Latitude**	**(m)**			
LM20	37.0	20.0	-100.36428	25.200194	3397	0.220	0.213	-0.914
LM21	23.3	16.0	-100.36339	25.200111	3403	0.216	0.195	-0.522
LM22	28.2	15.0	-100.36306	25.200417	3372	0.226	0.181	0.022
LM23	23.6	18.0	-100.36289	25.200556	3372	0.210	0.313	0.951
LM24	45.7	24.2	-100.36564	25.199972	3425	0.218	0.221	0.612
LM25	14.5	11.0	-100.36539	25.200583	3399	0.225	0.181	1.597
LM26	30.5	18.0	-100.36553	25.200361	3399	0.223	0.200	0.561
LM27	33.8	19.0	-100.36561	25.19975	3425	0.230	0.166	-0.711
LM28	19.0	12.0	-100.36531	25.199833	3403	0.221	0.208	1.153
LM29	44.2	20.0	-100.36544	25.199583	3452	0.227	0.179	1.031
LM30	35.5	20.2	-100.36544	25.199917	3425	0.226	0.183	-1.568

[1] ID-prefixes indicate populations of *P. martinezii* (AA = Agua de Alardín, AF = Agua Fría, EB = El Butano, LE = La Encantada) and *P. mexicana* (EC = El Coahuilón, EM = El Mohinora, LM = La Marta).

[2] DBH = Diameter at breast heigh (1.3 m).

[3] Observed heterozygosity.

[4] Endogamy coefficient.

[5] Individual loads for retained ordination axis of PC1 (mainly loaded by reproductive efficiency and endogamy index); values were multiplied by -1 to invert signs and perform further analyses (see Methods S1 for details).

[6] NA = Non-available data because of a high percentage of missing genomic data (≥20%) or lack of seeds in some collected cones.

Table S4.2. Genetic parameters and fitness-traits used for the association analyses with *Ne*. *n* = Successfully genotyped and phenotyped trees per population, *n*/(*n*-1) = Correction factor accounting for differences in sample size, *Ho* = Genome-wide observed heterozygosity, *F* = Inbreeding coefficient, SG = Seed germination, PC1 = Principal Component 1 (mainly loaded by reproductive efficiency and endogamy index).

Population	***n***	***n* / (*n*-1)**	**Unadjusted**				**Adjusted**			
			Ho	***F***	**SG**	**PC1**	***Ho***	***F***	**SG**	**PC1**
					Picea martinezii					
La Encantada	11	1.100	0.143	0.291	0.999	-2.158422	0.158	0.320	1.099	-2.374265
El Butano	30	1.034	0.150	0.255	1.129	0.097626	0.155	0.264	1.168	0.100993
Agua Fría	18	1.059	0.164	0.183	0.880	-0.856841	0.174	0.193	0.931	-0.907243
Agua de Alardín	29	1.036	0.158	0.215	1.146	1.249551	0.164	0.222	1.186	1.294178
Species	**88**		**0.154**	**0.236**						
					Picea mexicana					
El Coahuilón	27	1.038	0.218	0.219	1.133	-0.854703	0.227	0.228	1.176	-0.887576
El Mohinora	27	1.038	0.214	0.235	1.136	1.024192	0.222	0.244	1.180	1.063584
La Marta	28	1.037	0.222	0.206	1.073	-0.163436	0.230	0.214	1.113	-0.169490
Species	**82**		**0.218**	**0.220**						

Table S4.3. Associations among loci underpinning the Heterozygosity-Fitness Correlations (HFCs) for *Picea martinezii* and *Picea mexicana*. Observed heterozygosity (*Ho*) was computed with the complete sets of SNPs (genome-wide heterozygosity, excluding outliers giving population structure) as well as candidates for local and direct effects detected through GWAS. g_2 = multilocus identity disequilibrium, *HHCs* = heterozygosity-heterozygosity correlations. Numbers inside parenthesis indicate confidence intervals for each parameter.

Species	Loci used for general effects	g_2	*HHCs*
Picea martinezii	*Ho* (genome-wide: 17,930 SNPs)	0.008	0.92
		(0.005-0.011)	(0.90-0.95)
	Ho (candidates local effects: 627 SNPs)	0.063	0.82
		(0.042-0.083)	(0.74-0.87)
	Ho (candidates fitness-loci: 49 SNPs)	0.120	0.37
		(0.64-0.187)	(0.16-0.52)
Picea mexicana	*Ho* (genome-wide: 30,160 SNPs)	0.004	0.91
		(0.002-0.007)	(0.88-0.93)
	Ho (candidates local effects: 472 SNPs)	0.082	0.87
		(0.059-0.100)	(0.82-0.91)
	Ho (candidates fitness-loci: 11 SNPs)	0.376	0.41
		(0.207-0.546)	(0.18-0.59)

Table S4.4. Heterozygosity-Fitness Correlations (HFCs) performed with randomly sampled loci with similar *n*-sizes than candidates for local and direct effects. Pearson correlation coefficients (*r*) are fallowed by both sample sizes of loci (*n*) and *p*-values inside parenthesis.

Species	HFCs	
	Local effects	Direct effects
Picea martinezii	0.08 (*n* = 627; *p* = 0.43)	0.03 (*n* = 49; *p* = 0.81)
Picea mexicana	0.04 (*n* = 472; *p* = 0.69)	0.02 (*n* = 11; *p* = 0.88)

Table S4.5. Functional annotation of candidate fitness-loci associated to PC1 of *Picea martinezii* and *Picea mexicana*.

	SNP_ID	BETA[1]	R^2	p[2]	Annotation (BLASTx)	NCBI Accession	Function	Reference
					Picea martinezii			
a	17930:84	1.078	0.38	0.0001	VQ-like protein	PWA79563.1	Transcriptional regulation; seed development	Jing and Lin (2015)
b	77905:5	-1.238	0.31	0.0004	LRR receptor-like	XP_021806888.1	Ovule development, early embryogenesis	Hecht *et al*. (2001), Diévart and Clark (2004)
c	108867:35	1.257	0.30	0.0036	Dynamin-related protein 4C-like	XP_009779141.1	Internal membranes-system fusions/divisions; cytokinesis and cell expansion	Bednarek and Backues (2010), Ford and Chappie (2019)
d	42639:174	-1.302	0.28	0.0044	DNA polymerase epsilon catalytic subunit A-like	XP_047319798.1	DNA replication and repair	Pospiech and Syväoja (2003)
e	146521:186	-1.265	0.27	0.0049	Asparagine-tRNA ligase	XP_006850236.2	Attachment of aminoacids to tRNAs	Englert and Beier (2005), Berg *et al*. (2005)
f	1906:266	-1.072	0.30	0.0059	Lec-RLK	XP_002984805.2	Stress response, regulation of growth and development	Sun *et al*. (2020)
g	47459:62	1.510	0.27	0.0083	Subtilisin-like protease	XP_006837470.1	Protein turnover, stress resistance, embryogenesis	Schaller *et al*. (2011), Figueiredo *et al*. (2018)
h	118696:53	-2.03	0.28	0.0091	Peroxidase	CAA2962059.1	Oxidative stress response, homeostasis	Duroux and Welinder (2003)
i	80497:72	-1.468	0.25	0.0128	Spermidine synthase	AFF18800.1	Embryo development, embryo-lethal phenotypes identified	Imai *et al*. (2004)
j	66676:134	-1.141	0.27	0.0129	Neutral ceramidase 1-like	XP_031286335.1	Oxidative stress response, programmed cell death control	Li *et al*. (2015), Zienkiewicz *et al*. (2020)
k	18586:222	-1.047	0.28	0.0140	Ubiquitin regulatory X (UBX) domain	XP_016512643.1	Ubiquitination pathway	Schubertha and Buchberger (2008)
l	63320:41	1.139	0.26	0.0147	F-box protein	ABR16978.1	Ubiquitination pathway	Zhang *et al*. (2019)

	SNP_ID	BETA[1]	R^2	p[2]	Annotation (BLASTx)	NCBI Accession	Function	Reference
m	68648:122	-1.047	0.27	0.0150	RING-H2 finger protein	XP_006828605.1	Seed/embryo development, embryo-lethal phenotypes identified	Xu and Li (2003)
n	148495:48	-2.513	0.25	0.0151	E3 ubiquitin-protein ligase	XP_046849240.1	Phytohormone signaling: regulation of hormone biosynthesis and hormone perception	Kelley (2018)
o	91097:49	1.621	0.25	0.0163	Aspartate-tRNA ligase	XP_042509532.1	Attachment of amino acids to tRNAs	Englert and Beier (2005), Berg *et al*. (2005)
p	91201:71	1.440	0.24	0.0286	Eukariotic translation initiation factor 3 subunit E	XP_006837958.1	Gene expression	Rabee *et al*. (2019)
q	137427:293	-0.966	0.26	0.0303	BAG family molecular chaperone regulator 5	XP_042059738.1	Pos-translational protein processing; cell division/differentiation	Takayama and Reed (2001)
r	39704:20	2.820	0.23	0.0310	Phox/Bem1p domain	OVA20025.1	Protein-protein interactions; control of auxin responses, development	Korasick *et al*. (2015)
s	8796:206	1.014	0.25	0.0387	Protein Kinases	XP_011627426.1	Central processor unit; gene expression, metabolism, cell growth/division regulation	Hardie (1999)
t	5642:131	1.295	0.23	0.0435	DnaJ-class molecular chaperone	ABK24462.1	Protein homeostasis control	Pulido and Leister (2018)
u	98224:207	-1.259	0.25	0.0487	Phosphoinositide phospholipase C 4-like	XP_039066498.1	Stress response	Xia *et al*. (2017)
					Picea mexicana			
v	105517:9	2.178	0.26	0.0125	Adenosine kinase 2-like	XP_017415683.1	Cell functioning; housekeeping activities	Moffat *et al*. (2000)

	SNP_ID	BETA[1]	R^2	p[2]	Annotation (BLASTx)	NCBI Accession	Function	Reference
w	90452:39	1.219	0.28	0.0219	Histone-lysine N-methyltransferase	XP_006851161.1	Gene expression regulation, plant reproduction; germ cells and seeds development	Yao and Shen (2011), Shi *et al*. (2015)
x	162418:193	2.050	0.27	0.0225	Phosphopyruvate hydratase (enolase)	TJX57852.1	Plant development and reproductive success	Eremina *et al*. (2015)
y	75595:23	-2.528	0.27	0.0273	Tubulin beta chain	KAE8797749.1	Microtubules component: morphogenesis, cell growth and division	Jost *et al*. (2004)
z	170967:47	1.999	0.25	0.0363	Pentatricopeptide repeat (PPR)-containing protein	ADE77588.1	Embryo development; embryo-lethal phenotypes identified	Cushing *et al*. (2005)
aa	27608:38	1.530	0.24	0.0385	Pentatricopeptide repeat (PPR)-containing protein	XP_006849215.2	Embryo development; embryo-lethal phenotypes identified	Cushing *et al*. (2005)
ab	64453:151	0.980	0.27	0.0387	Phytochrome-associated serine/threonine protein phosphatase	KAF5749838.1	Pos-translational protein processing (phosphorylation)	Bheri *et al*. (2021)

[1] Regression slope parameter.

[2] Significance after Bonferroni correction (alpha 0.05).

References

Bednarek SY, Backues SK. 2010. Plant dynamin-related protein families DRP1 and DRP2 in plant development. *Biochemical Society Transactions* 38: 797–806.

Berg M, Rogers R, Muralla R, Meinke D. 2005. Requirement of aminoacyl-tRNA synthetases for gametogenesis and embryo development in *Arabidopsis*. *The Plant Journal* 44: 866–878.

Bheri M, Mahiwal S, Sanyal SK, Pandey GK. 2021. Plant protein phosphatases: what do we know about their mechanism of action? *The FEBS Journal* 288: 756–785.

Cushing DA, Forsthoefel NR, Gestaut DR, Vernon DM. 2005. *Arabidopsis* emb175 and other ppr knockout mutants reveal essential roles for pentatricopeptide repeat (PPR) proteins in plant embryogenesis. *Planta* 221: 424–436.

Diévart A, Clark SE. 2004. LRR-containing receptors regulating plant development and defense. *Development* 131: 251–261.

Duroux L, Welinder KG. 2003. The Peroxidase gene family in plants: a phylogenetic overview. *Journal of Molecular Evolution* 57: 397–407.

Englert M, Beier H. 2005. Plant tRNA ligases are multifunctional enzymes that have diverged in sequence and substrate specificity from RNA ligases of other phylogenetic origins. *Nucleic Acids Research* 33: 388–399.

Eremina M, Rozhon W, Yang S, Poppenberger B. 2015. ENO2 activity is required for the development and reproductive success of plants, and is feedback-repressed by AtMBP-1. *The Plant Journal* 81: 895–906.

Figueiredo J, Sousa Silva M, Figueiredo A. 2018. Subtilisin-like proteases in plant defence: the past, the present and beyond. *Molecular Plant Pathology* 19: 1017–1028.

Ford MGJ, Chappie JS. 2019. The structural biology of the dynamin-related proteins: new insights into a diverse, multitalented family. *Traffic* 20: 717–740.

Hardie DG. 1999. Plant protein serine/threonine kinases: classification and functions. *Annual Review of Plant Physiology and Plant Molecular Biology* 50: 97–131.

Hecht V, Vielle-Calzada J-P, Hartog M V, Schmidt EDL, Boutilier K, Grossniklaus U, de Vries SC. 2001. The Arabidopsis somatic embryogenesis receptor kinase 1 gene is expressed in developing ovules and embryos and enhances embryogenic competence in culture. *Plant Physiology* 127: 803–816.

Imai A, Matsuyama T, Hanzawa Y, Akiyama T, Tamaoki M, Saji H, Shirano Y, Kato T, Hayashi H, Shibata D, *et al.* 2004. Spermidine synthase genes are essential for survival of *Arabidopsis*. *Plant Physiology* 135: 1565–1573.

Jing Y, Lin R. 2015. The VQ motif-containing protein family of plant-specific transcriptional regulators. *Plant physiology* 169: 371–378.

Jost W, Baur A, Nick P, Reski R, Gorr G. 2004. A large plant beta-tubulin family with minimal C-terminal variation but differences in expression. *Gene* 340: 151–160.

Kelley DR. 2018. E3 ubiquitin ligases: key regulators of hormone signaling in plants. *Molecular & Cellular Proteomics* 17: 1047–1054.

Korasick DA, Chatterjee S, Tonelli M, Dashti H, Lee SG, Westfall CS, Fulton DB, Andreotti AH, Amarasinghe GK, Strader LC, *et al.* 2015. Defining a two-pronged structural model for PB1 (Phox/Bem1p) domain interaction in plant auxin responses. *Journal of Biological Chemistry* 290: 12868–12878.

Li J, Bi F-C, Yin J, Wu J-X, Rong C, Wu J-L, Yao N. 2015. An *Arabidopsis* neutral ceramidase mutant ncer1 accumulates hydroxyceramides and is sensitive to oxidative stress. *Frontiers in Plant Science* 6: 00460.

Moffatt BA, Wang L, Allen MS, Stevens YY, Qin W, Snider J, von Schwartzenberg K. 2000. Adenosine kinase of *Arabidopsis*. Kinetic properties and gene expression. *Plant Physiology* 124: 1775–1785.

Pospiech H, Syväoja JE. 2003. DNA polymerase e - More than a polymerase. *TheScientificWorldJOURNAL* 3: 858125.

Pulido P, Leister D. 2018. Novel DNAJ-related proteins in *Arabidopsis thaliana*. *New Phytologist* 217: 480–490.

Raabe K, Honys D, Michailidis C. 2019. The role of eukaryotic initiation factor 3 in plant translation regulation. *Plant Physiology and Biochemistry* 145: 75–83.

Schaller A, Stintzi A, Graff L. 2012. Subtilases – versatile tools for protein turnover, plant development, and interactions with the environment. *Physiologia Plantarum* 145: 52–66.

Schuberth C, Buchberger A. 2008. UBX domain proteins: major regulators of the AAA ATPase Cdc48/p97. *Cellular and Molecular Life Sciences* 65: 2360–2371.

Shi J, Dong A, Shen W-H. 2015. Epigenetic regulation of rice flowering and reproduction. *Frontiers in Plant Science* 5: 00803.

Sun Y, Qiao Z, Muchero W, Chen J-G. 2020. Lectin receptor-like kinases: the sensor and mediator at the plant cell surface. *Frontiers in Plant Science* 11: 596301.

Takayama S, Reed JC. 2001. Molecular chaperone targeting and regulation by BAG family proteins. *Nature Cell Biology* 3: E237–E241.

Xia K, Wang B, Zhang J, Li Y, Yang H, Ren D. 2017. *Arabidopsis* phosphoinositide-specific phospholipase C 4 negatively regulates seedling salt tolerance. *Plant, Cell & Environment* 40: 1317–1331.

Xu R, Quinn Li Q. 2003. A RING-H2 zinc-finger protein gene RIE1 is essential for seed development in *Arabidopsis*. *Plant Molecular Biology* 53: 37–50.

Yao X, Shen W. 2011. Crucial function of histone lysine methylation in plant reproduction. *Chinese Science Bulletin* 56: 3493–3499.

Zhang X, Gonzalez-Carranza ZH, Zhang S, Miao Y, Liu C-J, Roberts JA. 2019. F-Box proteins in plants. *Annual Plant Reviews online* 2: 307–328.

Zienkiewicz A, Gömann J, König S, Herrfurth C, Liu Y-T, Meldau D, Feussner I. 2020. Disruption of *Arabidopsis* neutral ceramidases 1 and 2 results in specific sphingolipid imbalances triggering different phytohormone-dependent plant cell death programmes. *New Phytologist* 226: 170–188.

Table S4.6. Advantageous and deleterious candidates associated to reproductive PC1s, for local and direct effects. Overall Heterozygosity Fitness Correlation (HFC) outputs, when using heterozygosity of candidates, are indicated. Numbers inside parenthesis indicate total sets of SNPs analyzed for each type of association and species. Deleterious/beneficious effects were inferred from the slope parameter (BETA) of individual loci regressions (see Table S5 for the successfully annotated SNPs).

Association	**SNPs effects**	**SNPs detected and HFC output**	
		Picea martinezii	***Picea mexicana***
Local effects	Deleterious	399	77
	Beneficious	228	395
	Total	**627**	**472**
		(5,438)	(18,469)
	HFC	Negative	Positive
Direct effects	Deleterious	34	1
	Beneficious	15	10
	Total	**49**	**11**
		(12,492)	(11,691)
	HFC	Negative	Positive

METHODS

Methods S4.1. Principal Components Analyses (PCA) for the reproductive traits of *Picea martinezii* and *Picea mexicana*.

The total set of reproductive indicators (Table 4.1 of main text) was reduced for each species by removing those highly correlated before PCA, as indicated by Spearman correlations tests ($r_s \geq 0.9$). For retention of only one of the highly correlated variables, we choose those capturing more biological information, according to variable descriptions in main text. Hence, filled seeds (FS), empty seeds (ES) and seed efficiency (SE) were removed from the PCA analyses for both species (Table Methods S4.1-1). Seed potential was not included on PCAs; it served to make derived reproductive-traits comparable among species.

Table Methods S4.1-1. Correlations (above diagonals) and respective significances (under diagonals) between the reproductive indicators of *Picea martinezii* and *Picea mexicana*. UO = Undeveloped ovules, FS = Filled seeds, ES = Empty seeds, SE = Seed efficiency, RE = Reproductive efficiency, EI = Endogamy index. Correlations higher than 0.9 are marked in bold.

	UO	FS	ES	SE	RE	EI
			Picea martinezii			
UO	-	-0.24	0.24	-0.62	-0.56	0.24
FS	0.027	-	**-1.00**	0.88	0.88	**-1.00**
ES	0.024	0.000	-	-0.88	-0.88	**1.00**
SE	0.000	0.000	0.000	-	**0.97**	-0.88
RE	2.3E-08	0.000	0.000	0.000	-	-0.88
EI	0.024	3.6E-113	3.2E-163	6.8E-30	1.3E-29	-
			Picea mexicana			
UO	-	-0.18	0.18	-0.50	-0.45	0.19
FS	0.105	-	**-0.99**	0.89	0.85	**-0.99**
ES	0.096	0.000	-	-0.89	-0.84	**1.00**
SE	2.9E-06	1.0E-29	2.7E-28	-	**0.93**	-0.89
RE	2.4E-05	6.8E-24	5.7E-23	0.000	-	-0.84
EI	0.094	8.4E-71	3.9E-157	2.4E-28	6.3E-23	-

Principal Component 1 (PC1) was the most important in both tree species, as indicated by the eigenvalue threshold ≥ 1.0 and the explained variances: 72.8% in *P. martinezii* and 66.6% in *P. mexicana* (Table Methods S4.1-2). Hence, only PC1s were used in downstream analysis, which were mainly loaded by reproductive efficiency (RE) and endogamy index (EI) in both spruces (Table Methods S4.1-3 and Fig. Methods S4.1-1). In both cases, PC1s were negatively correlated with RE and positively correlated with EI (Table Methods S4.1-4); therefore, they indicate higher reproductive fitness at lower PC1 values, and *vice versa*. For a more intuitive interpretation of further analyses (i.e. using a positive PC1-fitness association), we inverted the signs of the individual loads of retained ordination axis of PC1 (Table S4.1).

Table Methods S4.1-2. Eigenvalues of the Principal Components and percentage of variance explained.

Component	Eigenvalues	Percentage of variance	Cumulative percentage of variance
		Picea martinezii	
1	**2.185**	72.83	72.83
2	0.759	25.30	98.13
3	0.056	1.88	100.00
		Picea mexicana	
1	**1.998**	66.59	66.59
2	0.888	29.59	96.19
3	0.114	3.81	100.00

Table Methods S4.1-3. Contributions of variables (%) to the Principal Components.

Variable	PC1	PC2	PC3
	Picea martinezii		
Undeveloped ovules (OU)	21.47	69. 30	9.25
Endogamy index (EI)	34.51	29.76	35.73
Reproductive efficiency (RE)	44.02	0.97	55.02
	Picea mexicana		
Undeveloped ovules (OU)	15.00	78.01	7.00
Endogamy index (EI)	38.27	21.31	40.42
Reproductive efficiency (RE)	46.74	0.68	52.58

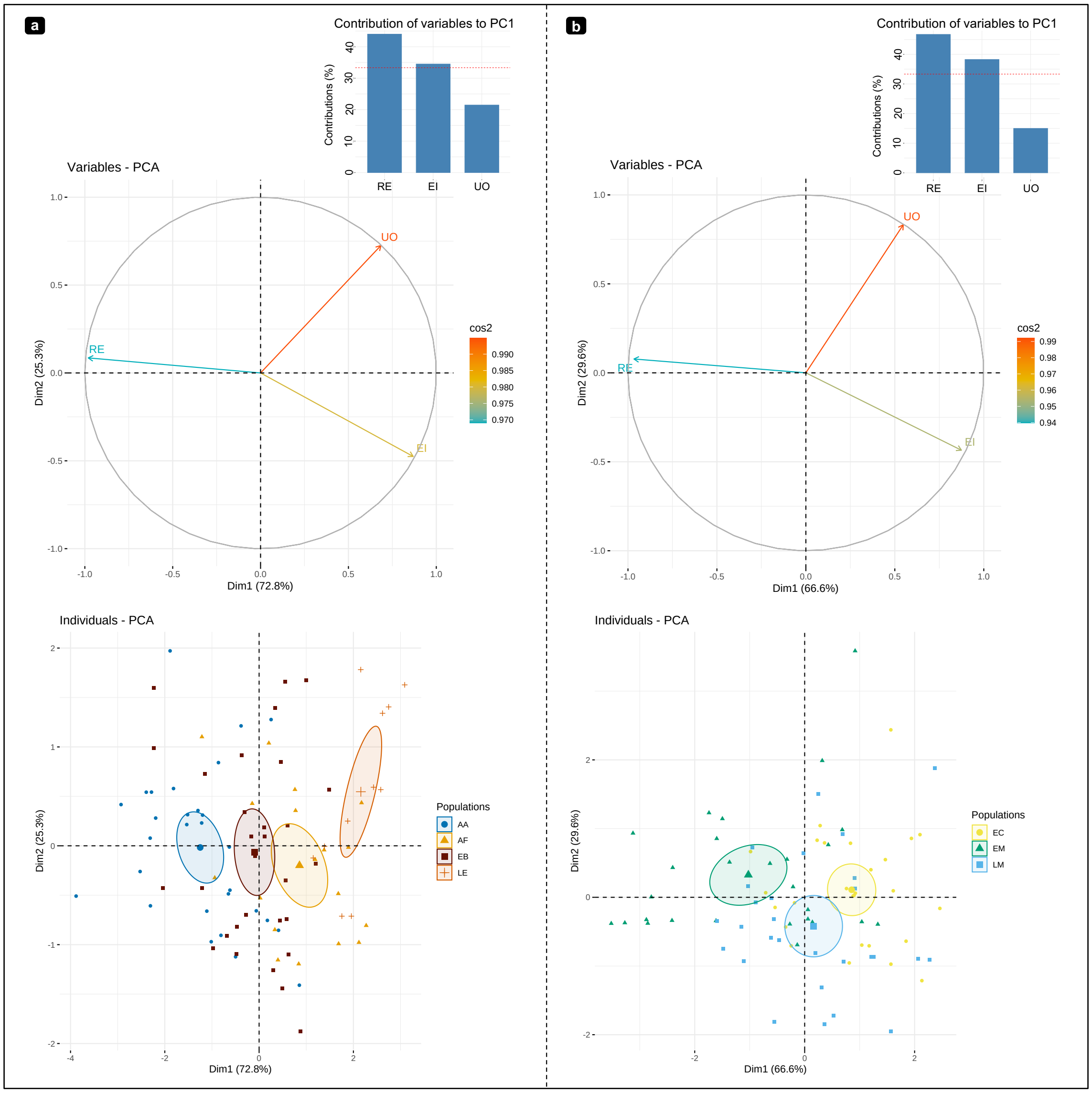


Figure Methods S4.1-1. Grouping of reproductive variables and sampled trees of *Picea martinezii* (a) and *Picea mexicana* (b) according to the Principal Components Analyses. Upper bar-plots show the main variables loading PC1s, which were retained for the further analyses. RE = Reproductive efficiency, EI = Endogamy index, UO = Undeveloped ovules, cos2 = quality of representation of the variables on the factor map. Populations of AA = Agua de Alardín, AF = Agua Fría, EB = El Butano, LE = La Encantada, EC = El Coahuilón, EM = El Mohinora, LM = La Marta.

Table Methods S4.1-4. Correlations between variables and Principal Components (*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; NS, not significant).

Variable	**PC1**	**PC2**
	Picea martinezii	
Undeveloped ovules (OU)	0.69***	0.73***
Endogamy index (EI)	0.87***	-0.48***
Reproductive efficiency (RE)	-0.98***	0.09^{ns}
	Picea mexicana	
Undeveloped ovules (OU)	0.55***	0.83***
Endogamy index (EI)	0.87***	-0.44***
Reproductive efficiency (RE)	-0.97***	0.08^{ns}

Methods S4.2. Population genetic structures of *Picea martinezii* and *Picea mexicana*.

Single nucleotide polymorphisms contributing to population structure were identified separately for each species with the R package “PCAdapt” (v4.3.3; Luu et al., 2017, cited in main text). PCAdapt has reported higher performances than similar algorithms through different demographic models. This software performs genome scans for variant detection with atypical values based on Principal Component Analysis, and assumes that variants highly related to population structure are candidates for local adaptation (Luu *et al.*, 2017). Filtered SNPs after vcftools (VCF files containing 18,072 variants for *P. martinezii* and 30,230 variants for *P. mexicana*; see Section 4.3.2 of main text) were transformed to PLINK format (.bed) and then used to perform PCAs with PCAdapt’s default options: K = 3 or 4 (as indicated by the optimal components for each species, see below); method = c(“mahalanobis”, “componentwise”); min.maf = 0.05; ploidy = 2. The cutoff for outlier detection was Bonferroni (alpha = 0.05).

According to scree-plots (k-groups; Fig. Methods S4.2-1a,b), the optimal components for each species were consistent with the number of evaluated populations: four of *P. martinezii* and three of *P. mexicana*, as showed by individuals grouping in projections onto PC1 and PC2 (Fig. Methods S4.2-1c,d). *P. martinezii* showed a stronger population structure than *P. mexicana*, as indicated by the number of outliers as well as the smaller *p*-values for the PCs-outliers associations in the former (Fig. Methods S4.2-1e,f). After Bonferroni correction, 142 and 70 SNPs were respectively identified as the main contributors for structure in *P. martinezii* and *P. mexicana*. All retained k-groups were associated with outliers (Table Methods S4.2-1). These variants were then removed from the initial data sets (VCFs), remaining 17,930 SNPs for *P. martinezii* and 30,160 for *P. mexicana* for further analyses; i.e. genome wide Heterozygosity-Fitness Correlations (HFCs) and genotype-phenotype associations (GWAS; see Section 4.2.3.3 of main text).

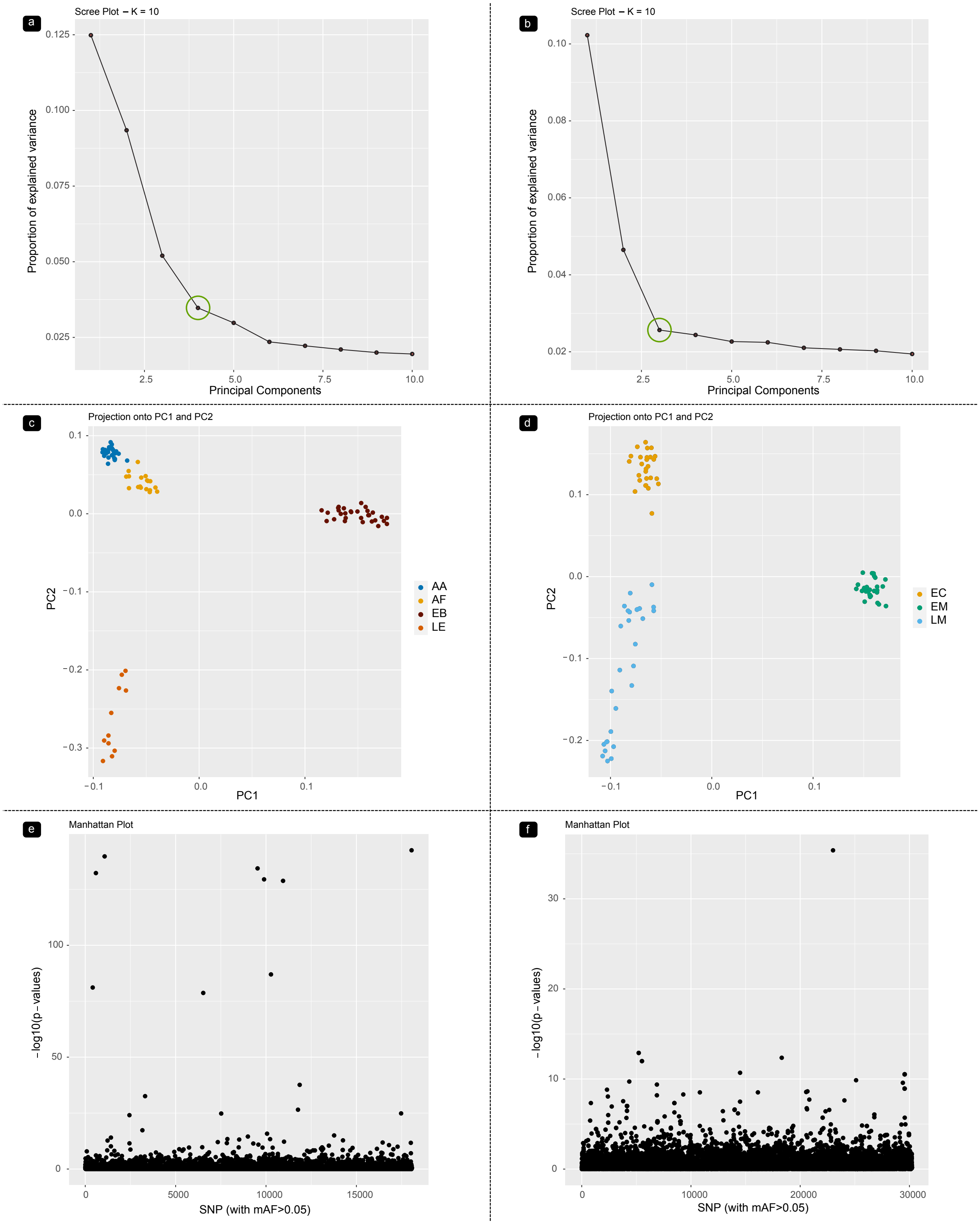


Figure Methods S4.2-1. Identification of candidates for population structure in *Picea martinezii* (a,c,e) and *Picea mexicana* (b-d-f). In (c): AA = Agua de Alardín, AF = Agua Fría, EB = El Butano, LE = La Encantada; in (d) EC = El Coahuilón, EM = El Mohinora, LM = La Marta.

Table Methods S4.2-1. Outliers detected for Principal Components (k-groups).

Principal Component	*Picea martinezii*	*Picea mexicana*
PC1	25	7
PC2	96	28
PC3	1	35
PC4	20	-
Total	**142**	**70**

CAPÍTULO 5. CONSIDERACIONES FINALES Y RECOMENDACIONES

5.1. Estatus demográfico y reproductivo

El presente documento muestra por primera vez el censo completo de cada una de las poblaciones conocidas de *Picea martinezii* y *P. mexicana*, así como el registro de siete nuevos rodales; dos para la primera especie y cinco para la segunda (Cuadro 2.1).

Por lo anterior, podría parecer que el estatus general de conservación de las especies mejora. Sin embargo, cabe remarcar que los nuevos rodales de *P. martinezii* son en realidad muy pequeños y aportan muy pocos individuos totales a la especie (34 en total; Cuadro 2.1). Por otra parte, de los cinco nuevos rodales de *P. mexicana*, cuatro se encuentran en el trayecto del mismo Cerro de La Marta (Coahuila; ~11 km de longitud por encima de los 3,000 m de elevación), mientras que el quinto rodal está en el mismo Cerro El Mohinora (Chihuahua; a ~1.0 km de la población principal); además, solamente dos de ellos aportan un número considerable de nuevos individuos a la especie (7,550 individuos; Cuadro 2.1), lo que aumenta en ~25% el total de individuos remanentes de la especie, con respecto al censo de las poblaciones conocidas. Por otra parte, el censo poblacional y la estructura demográfica mostraron que tres de las siete poblaciones conocidas para ambas especies son poblaciones estables con presencia abundante o densidad relativamente alta de individuos reproductivos; a excepción de La Encantada, El Butano y Agua Fría de *P. martinezii* y El Coahuilón de *P. mexicana*, en donde el tamaño poblacional fue menor de mil individuos adultos.

De acuerdo con McCune (2016), *P. martinezii* continuaría siendo una especie extremadamente rara con menos de cinco poblaciones, mientras que *P. mexicana* podría dejar de serlo si se determina que los nuevos rodales registrados son poblaciones separadas de la población conocida más cercana. Por otra parte, de acuerdo con Rabinowitz (1981), si se determina que todos los nuevos rodales son poblaciones separadas de las poblaciones anteriormente conocidas más cercanas, las especies seguirían siendo raras debido a su restricción geográfica, pero localmente abundantes o escasas. Finalmente, debido a que la aptitud reproductiva de los individuos incrementa con el tamaño de la población (Fig. 4.3), se recomienda reducir la depresión endogámica en las poblaciones más pequeñas de cada especie: La Encantada, El Butano y Agua Fría de *P. martinezii*, así como en El Coahuilón de *P. mexicana*. Para esto, se recomienda que los individuos donadores de polen provengan de Agua de Alardín para *P. martinezii* y de La Marta para *P. mexicana* (ver sección 5.3).

5.2. Consideraciones para la conservación *in situ* y *ex situ*

Los resultados del censo poblacional y las proyecciones de hábitat al futuro mostraron la necesidad de combinar la conservación *in situ* y *ex situ*, para asegurar la permanencia de las especies bajo estudio.

Por una parte, la conservación *in situ* es necesaria para todas las poblaciones conocidas, y de ser posible, para los nuevos rodales (Cuadro 2.1). Si no fuera posible incluir los nuevos rodales registrados en un programa de conservación, sería recomendable tomar muestras de germoplasma para realizar reforestaciones en las poblaciones conocidas más cercanas. Por otra parte, en algunas de las poblaciones antes conocidas se siguen observando perturbaciones de hábitat previamente señaladas (Mendoza-Maya *et al*., 2015); tales como el pastoreo por ganado vacuno y caprino. Dicha problemática fue observada en las poblaciones de El Mohinora y El Coahuilón de *P. mexicana* (por ganado vacuno), así como en Agua Fría de *P. martinezii* (por ganado caprino). Por lo anterior, es clara la necesidad de desarrollar investigación en el campo de las ciencias sociales para: *i*) identificar alternativas productivas y económicas que permitan remover el ganado de las poblaciones de *Picea* sin la pérdida de los beneficios económicos que supone dicha actividad; y *ii*) elaborar planes de conservación que incluyan beneficios sociales y en los que se incentive la participación de los pobladores locales, mediante empleos y otros estímulos.

La participación de las comunidades y los propietarios de los predios en donde se encuentran las poblaciones de *Picea* es indispensable para su conservación. Dicha participación podría realizarse en: *i*) el mantenimiento de los cercos protectores; *ii*) la apertura y mantenimiento de brechas cortafuegos; *iii*) la recolecta de semillas de árboles seleccionados (ver sección 5.3); *iv*) la producción de planta en vivero; *v*) la reforestación para incrementar la densidad y el tamaño de las poblaciones remanentes; *vi*) el monitoreo de la reforestación y de las poblaciones naturales en su conjunto. Otra oportunidad que se puede derivar de planes integrales de manejo y conservación, es la participación de profesionales locales enfocados de manera permanente en actividades de concientización sobre la importancia del bosque, los recursos forestales y las especies amenazadas. Dichas actividades podrían realizarse en lugares de acceso al público en general, pero especialmente en las escuelas locales de todos los niveles.

Respecto a la conservación *ex situ*, las proyecciones a futuro indican que el hábitat natural más adecuado de estas especies desaparecerá por completo y no estará disponible en sitios fuera de la distribución natural, dentro de México (Fig. 2.5). Por lo tanto, se espera que las especies se sometan a un estrés ambiental severo *in situ* por la reducción en la disponibilidad de agua o el incremento de las temperaturas, sin posibilidades de migrar o translocarse hacia otros ambientes propicios. En dicha situación, las especies tienen dos opciones: adaptarse o extinguirse (Aitken *et al*., 2008). En este caso, la falta de hábitat favorable en el futuro también reduciría las posibilidades de reintroducción o repoblación del germoplasma conservado en bancos, el cuál pierde su viabilidad con el paso del tiempo. Tal es el caso de *Pinus echinata*, que registró una reducción de la viabilidad de sus semillas a 25% después de 50 años de almacenamiento (Barnett & Vozzo, 1985). En conjunto, esto elevaría el riesgo de extinción de las dos especies tanto en condiciones naturales (*in situ*) como del germoplasma almacenado *ex situ*.

Para reducir el riesgo de extinción de las especies bajo estudio, se recomienda iniciar proyectos de migración asistida adecuados a las posibilidades de cada especie. Para *P. martinezii*, las posibilidades se restringen a hábitats marginales cerca de las poblaciones del sur de Nuevo León (Fig. 2.6). En el caso de *P. mexicana*, las posibilidades son extensas, pero sólo fuera de México (Fig. 2.7). Sin embargo, en el último caso se deberían elegir las regiones o países en los que los pros superen a los contras; es decir, en donde los posibles riesgos ecológicos en los ecosistemas receptores sean menores (ej., donde *P. mexicana* tenga el menor potencial para hibridar con otras especies locales del mismo género, para la exclusión competitiva y para convertirse en especie invasora) y en donde la cooperación entre países sea más factible, mediante más facilidades para resolver los problemas legales. La recolecta de las semillas para la migración asistida debería ser a granel y de individuos distribuidos en toda la superficie de las poblaciones conocidas y nuevos rodales registrados, para conservar la mayoría del acervo genético de las especies. Otra posibilidad es asegurar la colecta de germoplasma de individuos con un mayor potencial para producir progenie de mayor adaptabilidad (ej. aptitud reproductiva; ver sección 5.3). Lo ideal sería establecer copias de cada población, pero la concentración de muestras de todo el acervo genético de las especies en poblaciones únicas sería viable también.

5.3. Consideraciones para la selección de individuos para reforestación y flujo génico

Los resultados de las asociaciones genotipo-fenotipo permitieron identificar loci con efectos directos sobre la aptitud reproductiva en las dos especies bajo estudio (Fig. 4.6). A su vez, los efectos de los alelos derivados en estos loci pudieron diferenciarse en ventajosos o deletéreos (Figs. 4.6 y 4.7). Con esta información, se estimaron los efectos generales de la carga de dichos alelos sobre la aptitud reproductiva de manera general por especie (Fig. 5.1) y se identificaron los individuos portadores de cada tipo de variante en las poblaciones (Figs. 5.2-5.4). Se recomienda que la selección de los individuos para realizar flujo génico desde las poblaciones donadoras (Agua de Alardín y La Marta; Fig. 5.2) se base en una mayor carga de alelos ventajosos, mientras que la selección de individuos en las poblaciones con elevada depresión endogámica (La Encantada, El Butano y Agua Fría de *P. martinezii*; El Coahuilón de *P. mexicana*) se base en la menor carga de alelos deletéreos (Fig. 5.3-5.4). De esta forma, se reduciría la depresión endogámica de los individuos en las poblaciones receptoras y se promovería una mayor adaptabilidad en la progenie.

De manera similar, la recolecta de semillas para realizar reforestación y/o recuperación de las poblaciones naturales debería basarse en la selección de los individuos que porten mayores frecuencias de alelos derivados con efectos ventajosos. Al igual que la aptitud reproductiva, otras características fenotípicas relevantes para explorar la adaptabilidad al cambio climático son la resistencia a condiciones de sequía o a mayor temperatura. Esto permitiría detectar genotipos e individuos más resistentes a las condiciones adversas proyectadas para las poblaciones y rodales naturales. Estos genotipos e individuos podrían utilizarse como fuentes de germoplasma para reforestaciones futuras.

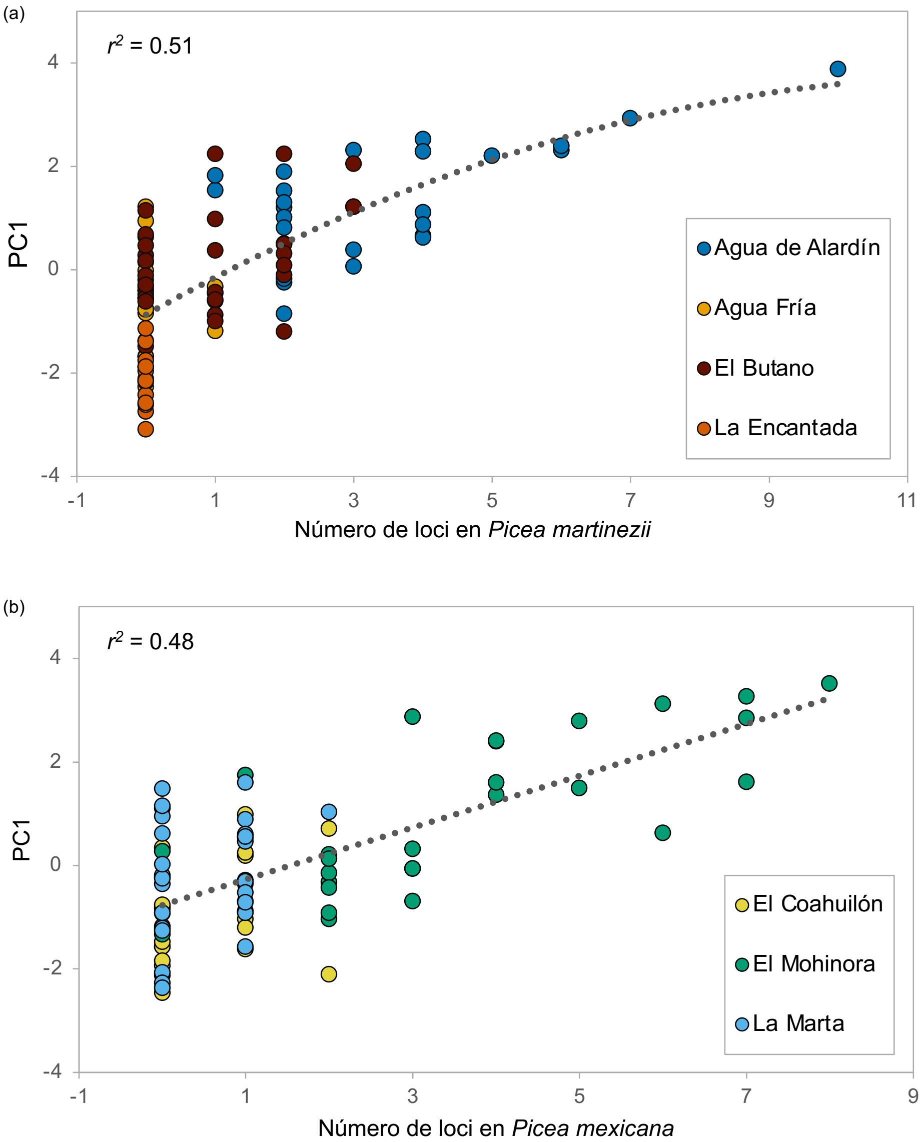


Figura 5.1. Asociación entre el número de loci homocigotos para el alelo derivado con efectos ventajosos y la aptitud reproductiva (PC1) de *Picea martinezii* (a) y *Picea mexicana* (b). PC1 = Componente Principal 1, conformado por la eficiencia reproductiva y el índice de endogamia para ambas especies (ver Supplementary Information Methods S4.1).

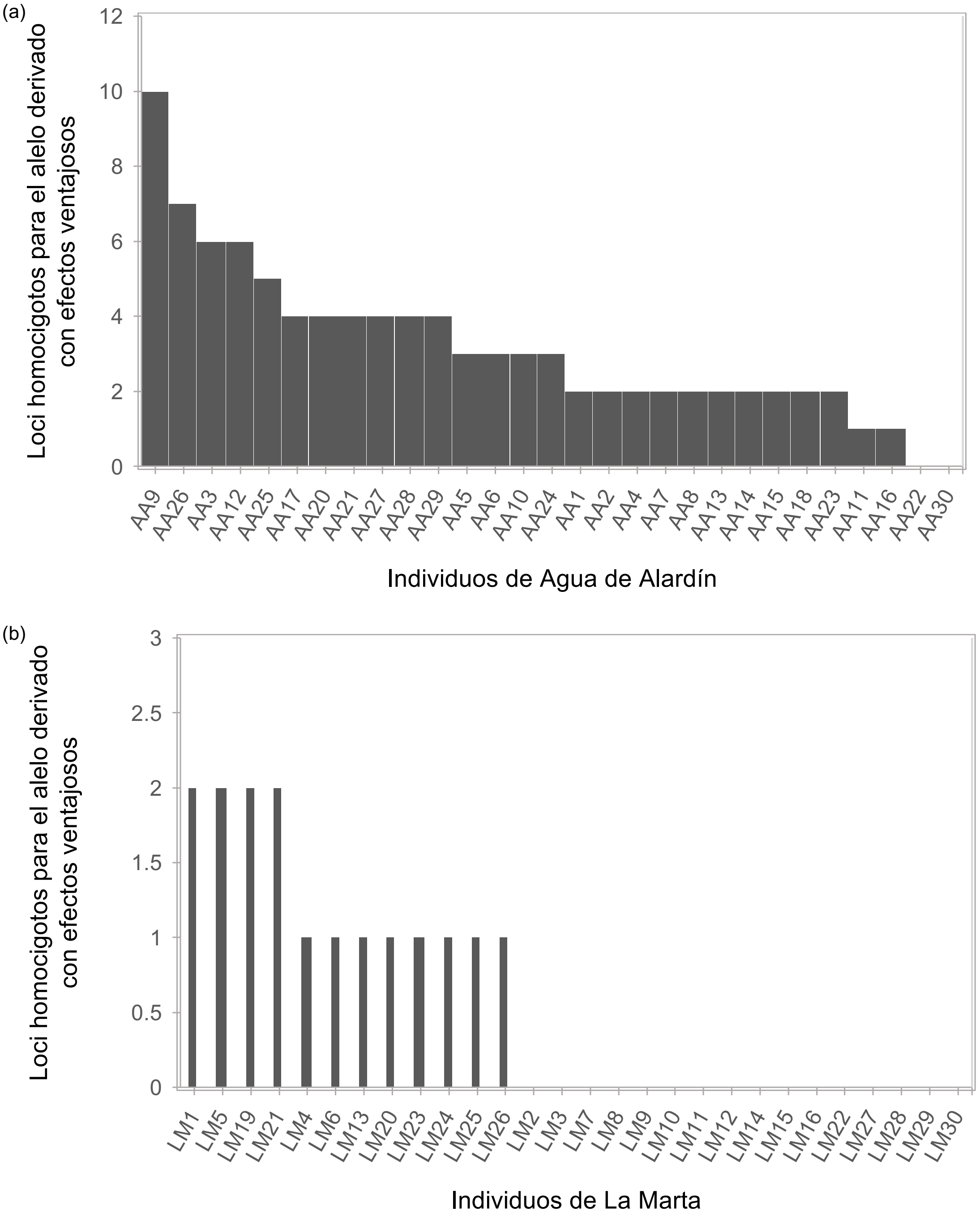


Figura 5.2. Número de loci homocigotos para el alelo derivado con efectos ventajosos por individuo en las poblaciones sugeridas como donadoras de polen para *Picea martinezii* (a) y *Picea mexicana* (b). Los individuos se muestran ordenados de manera descendente de acuerdo con la carga de loci homocigotos.

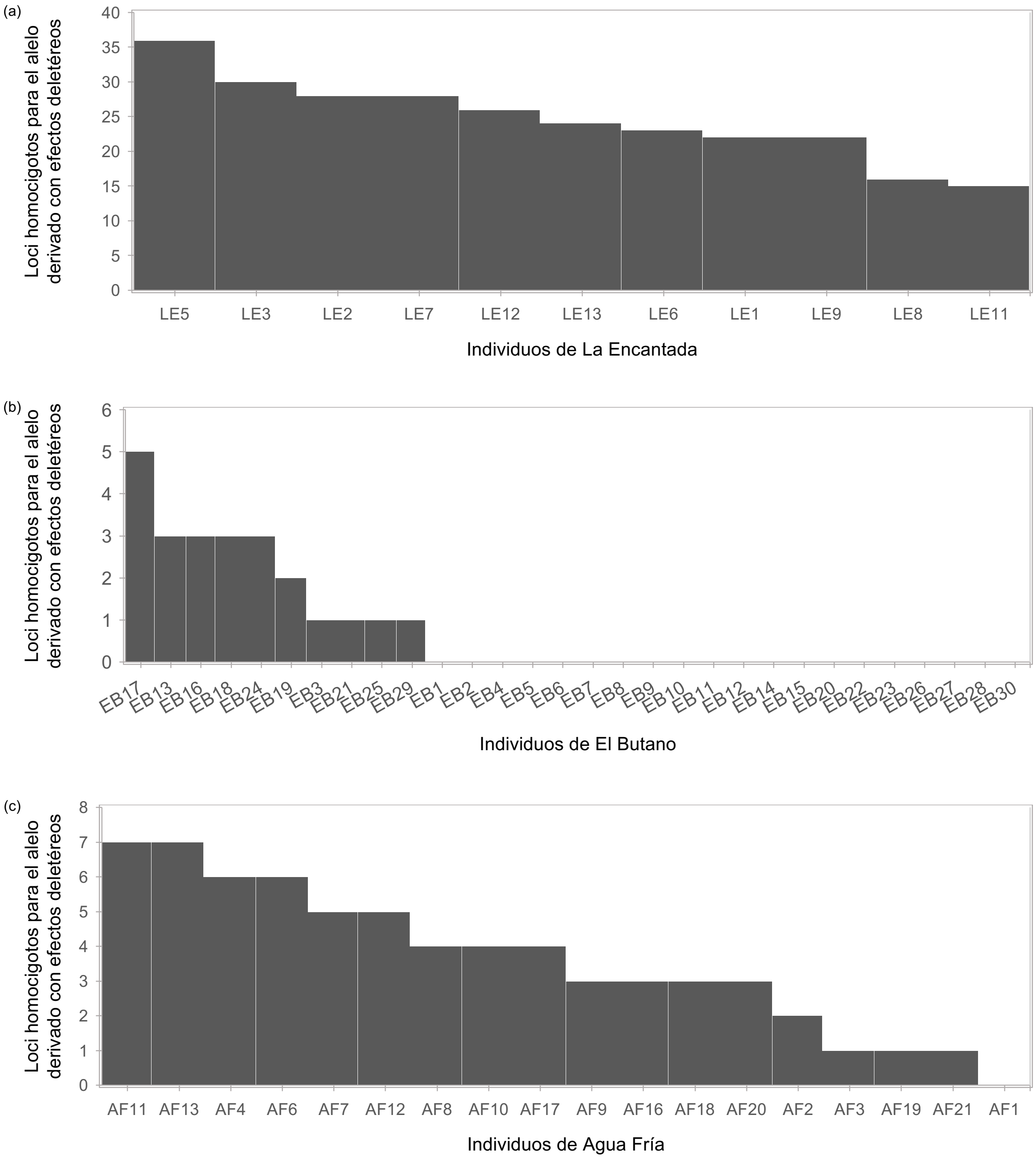


Figura 5.3. Número de loci homocigotos para el alelo derivado con efectos deletéreos por individuo en las poblaciones sugeridas como receptoras de polen para *Picea martinezii*. Los individuos de las poblaciones de (a) La Encantada, (b) El Butano y (c) Agua Fría, se muestran ordenados de manera descendente de acuerdo con la carga de loci homocigotos.

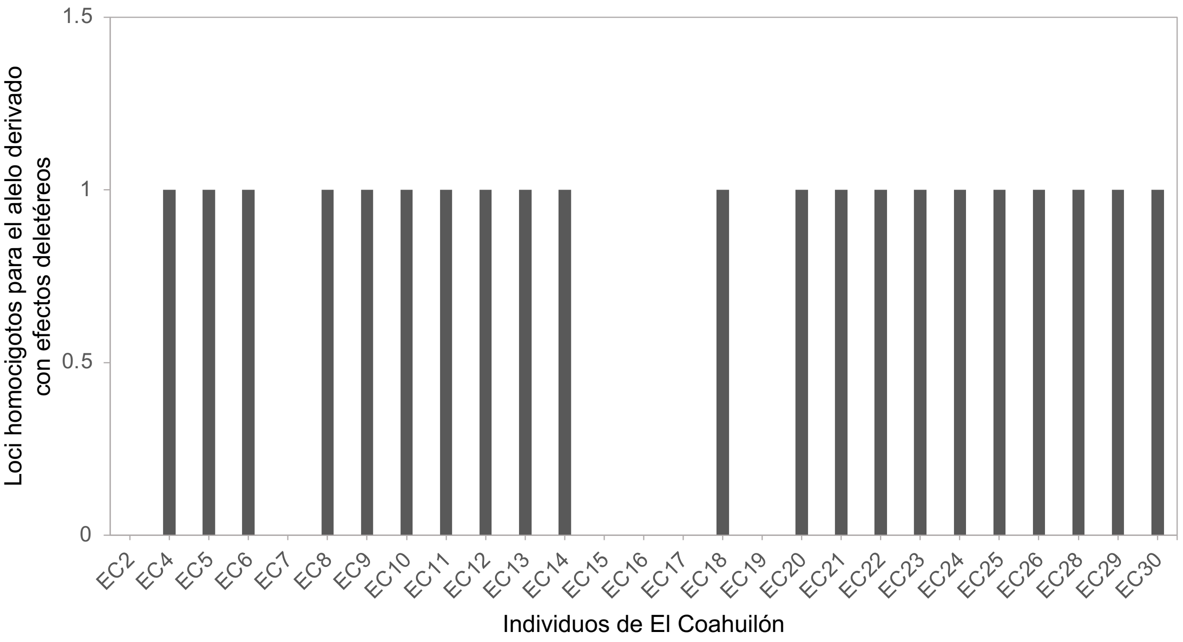


Figura 5.4. Número de loci homocigotos para el alelo derivado con efectos deletéreos por individuo en la población de El Coahuilón, sugerida como receptora de polen para *Picea mexicana*.

5.4. Literatura citada

Aitken, SN; Yeaman, S; Holliday, JA; Wang, T; Curtis-McLane, S. 2008. Adaptation, migration or extirpation: climate change outcomes for tree populations. Evolutionary Applications 1(1):95-111. DOI: https://doi.org/10.1111/j.1752-4571.2007.00013.x.

Barnett, JP; Vozzo, JA. 1985. Viability and vigor of Slash and Shortleaf pine seeds after 50 years of storage. Forest Science 31(2):316-320. DOI: https://doi.org/10.1093/forestscience/31.2.316.

McCune, JL. 2016. Species distribution models predict rare species occurrences despite significant effects of landscape context. Journal of Applied Ecology 53(6):1871-1879. DOI: https://doi.org/10.1111/1365-2664.12702.

Mendoza-Maya, E; Espino-Espino, J; Quiñones-Pérez, CZ; Flores-López, C; Wehenkel, C; Vargas-Hernández, JJ; Sáenz-Romero, C. 2015. Proposal for conservation of three endangered species of Mexican spruce. Revista Fitotecnia Mexicana 38(3):235-247. DOI: https://doi.org/10.35196/rfm.2015.3.235.

Rabinowitz, D. 1981. Seven forms of rarity. In Singe, H (ed.). Chichester, John Wiley. p. 205-217.

CAPÍTULO 6. CONCLUSIONES GENERALES

Los censos poblacionales mostraron que *Picea martinezii* y *Picea mexicana* son especies endémicas, raras, con hábitat reducido y poblaciones muy variables, pero viables en conjunto. Los modelos de distribución sugieren que el hábitat de ambas especies ha sido reducido desde el Ultimo Máximo Glacial (hace ~22 mil años), y que *P. mexicana* probablemente migró desde el centro de México hacia su ubicación actual, mientras que la distribución potencial pasada no es clara para *P. martinezii*, la cual pareciera restringida a micro-refugios en el Holoceno Medio (hace ~6 mil años) principalmente. En ambas especies, el hábitat marginal (p = 0.5-0.79) ha tenido una reducción neta desde el Último Máximo Glacial; por su parte, el hábitat más apropiado (p = 0.8-1.0) se ha reducido para *P. mexicana*, pero es más abundante en las condiciones contemporáneas para *P. martinezii*. Las proyecciones al futuro sugieren que el hábitat más apropiado (p = 0.8-1.0) desaparecerá casi por completo en las poblaciones naturales de ambas especies en un escenario moderado de cambio climático (RCP4.5) y completamente en un escenario pesimista (RCP8.5). De acuerdo con estas proyecciones, las posibilidades de conservación *ex situ* para *P. martinezii* se restringen a México en sitios marginales (p = 0.5-0.79), y para *Picea mexicana* sólo habría posibilidad en extensas áreas fuera de los límites territoriales de México, principalmente con hábitat marginal.

Referente a la aptitud reproductiva de las especies, la eficiencia de semillas de ambas es baja con respecto a la de otras coníferas. El índice de endogamia (IE) de las poblaciones muestreadas fue ligeramente menor a los valores reportados previamente para ambas especies, mientras que la eficiencia reproductiva (ER) se ha mantenido estable. Por otra parte, se detectó una alta pérdida de semillas en forma de óvulos abortados y semillas vanas; sin embargo, las pocas semillas que alcanzan la maduración tienen altos porcentajes de germinación. El porcentaje de semillas llenas (PSLL) es uno de los indicadores reproductivos base que podrían explorarse para estimar otros indicadores más representativos como la eficiencia de semillas, la eficiencia reproductiva o el índice de endogamia en menor tiempo; es decir, sin la necesidad de separar las escamas infértiles y fértiles, para estimar del potencial de semillas (PS). Los indicadores reproductivos más representativos de la aptitud reproductiva fueron la eficiencia reproductiva (ER) y el índice de endogamia (IE).

Los análisis de asociación entre factores demográficos (proxi de tamaño efectivo de población; *Ne*), genéticos (diversidad genética, *Ho*; coeficiente de endogamia, *F*) y reproductivos (eficiencia reproductiva e índice de endogamia integrados en la variable PC1, y la germinación de semillas), indican un desacople entre dichos factores en las piceas estudiadas, en el cual los parámetros genéticos no se asocian con el tamaño poblacional, pero sí con la aptitud reproductiva. Esto indica que el tamaño poblacional tiene influencia sobre factores adaptativos regulados por loci específicos, pero no sobre la diversidad genética general, la cual tiende a ser elevada por el sistema de apareamiento de las coníferas. Sin embargo, la asociación entre el tamaño de población con solo una de las dos variables fenotípicas evaluadas (PC1), indican depresión endogámica diferencial para diferentes fases iniciales del desarrollo de los individuos. El ensamble de genomas con genoma de referencia, usando datos genómicos de los individuos adultos muestreados para el análisis reproductivo en las poblaciones conocidas (*n* = 13-30), permitió estimar una mayor diferenciación (F_{ST}) y aislamiento genético (N_m) en *P. martinezii* que en *P. mexicana*. Dicha diferenciación genética fue más elevada que la estimada previamente con ensambles *de novo*, usando datos genómicos de individuos adultos y regeneración, incluyendo individuos muestreados en los nuevos rodales localizados (*n* = 30-50 por población).

Los análisis de asociación heterocigosidad-fitness (HFCs) y genotipo-fenotipo (GWAS) mostraron que *Picea martinezii* tiene mayor depresión endogámica que *P. mexicana*, debido a la detección de efectos generales (efectos de la heterocigosidad general) y un mayor número de loci con efectos locales y directos principalmente deletéreos. Estos resultados indican colapsos poblacionales recientes para dicha especie, con una purga genética en proceso. En contraste, en *P. mexicana* los nulos efectos generales y la mayor carga de alelos ventajosos en los loci candidatos con efectos directos, sugieren una purga más avanzada de la variación deletérea y colapsos poblacionales más ancestrales. Los resultados de las asociaciones genotipo-fenotipo concuerdan con la hipótesis de herencia poligénica para la aptitud reproductiva, cuya estructura genética mostró: *i*) loci candidatos asociados a proteínas reportadas para el desarrollo embrionario; *ii*) loci con alelos derivados de efectos deletéreos o ventajosos; *iii*) la distribución de cada tipo de diversidad concordó con el tamaño de población en *P. martinezii*, con mayores frecuencias de homocigotos deletéreos en poblaciones pequeñas que en las grandes, y viceversa para los homocigotos ventajosos; *iv*) mientras que en *P. mexicana* las mayores frecuencias de alelos derivados ventajosos se registraron en la población de tamaño mediano y más aislada geográficamente. Aunado a la asociación con el tamaño de población, la detección de efectos generales o loci de efectos directos sólo para PC1 y no para la germinación de semillas (SG), sugiere una purga de la variación deletérea en fases iniciales del desarrollo embrionario.

En general, se pudo estimar una correspondencia entre el tamaño poblacional, la distribución de los alelos deletéreos o ventajosos asociados directamente a la aptitud reproductiva, el nivel de depresión por endogamia para esta característica y la estructura demográfica de las poblaciones. Por lo anterior, se concluye que las especies no están en vórtices de extinción como conjunto. Sin embargo, el aislamiento genético, el reducido tamaño poblacional (*Ne*), la aptitud reproductiva y su estructura genética, así como la estructura demográfica (con menor frecuencia de individuos de reclutamiento que adultos), indican que La Encantada y El Butano de *P. martinezii* están en grave riesgo de extinción. Por lo tanto, la permanencia de estas poblaciones dependerá de las acciones que se implementen para el rescate genético y demográfico de las mismas. Adicionalmente, las poblaciones de Agua Fría (también de *P. martinezii*) y El Coahuilón de *P. mexicana*, requieren atención especial debido a que podrían presentar la misma problemática en el corto o mediano plazo.