



UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE INVESTIGACIONES EN ECOSISTEMAS Y SUSTENTABILIDAD

BIOLOGÍA EVOLUTIVA

**ADAPTACIÓN LOCAL DE *Pinus leiophylla* BAJO MODELOS DE CAMBIO
CLIMÁTICO Y CAMBIO DE USO DE SUELO EN LA FRANJA AGUACATERA DE
MICHOACÁN**

TESIS

(POR ARTÍCULO CIENTÍFICO)

**LOCAL ADAPTATION OF *Pinus leiophylla* UNDER CLIMATE AND LAND USE
CHANGE MODELS IN THE AVOCADO BELT OF MICHOACÁN**

**QUE PARA OPTAR POR EL GRADO DE:
MAESTRA EN CIENCIAS BIOLÓGICAS**

PRESENTA:

VANESSA IZAGUIRRE TORIZ

TUTOR PRINCIPAL DE TESIS: DR. ANTONIO GONZÁLEZ RODRÍGUEZ

INSTITUTO DE INVESTIGACIONES EN ECOSISTEMAS Y SUSTENTABILIDAD, UNAM.

COMITÉ TUTOR: DRA. YESSICA RICO MANCEBO DEL CASTILLO

INSTITUTO DE ECOLOGÍA, A.C., CENTRO REGIONAL DEL BAJÍO

DR. MAURICIO RICARDO QUESADA AVENDAÑO

ESCUELA NACIONAL DE ESTUDIOS SUPERIORES UNIDAD MORELIA, UNAM.

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Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **04 de marzo de 2024**, se aprobó el siguiente jurado para el examen de grado de **MAESTRA EN CIENCIAS BIOLÓGICAS**, en el campo de conocimiento de **BIOLOGÍA EVOLUTIVA**, de la estudiante, **IZAGUIRRE TORIZ VANESSA** con número de cuenta **418125984** por la modalidad de graduación de **tesis por artículo científico** titulado: **LOCAL ADAPTATION OF *Pinus leiophylla* UNDER CLIMATE AND LAND USE CHANGE MODELS IN THE AVOCADO BELT OF MICHOACÁN**, que es producto del proyecto realizado en la maestría que lleva por título: **“ADAPTACIÓN LOCAL DE *PINUS LEIOPHYLLA* BAJO MODELOS DE CAMBIO CLIMÁTICO Y CAMBIO DE USO DE SUELO EN LA FRANJA AGUACATERA DE MICHOACÁN**”, ambos realizados bajo la dirección del **DR. ANTONIO GONZÁLEZ RODRÍGUEZ**, quedando integrado de la siguiente manera:

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Sin otro particular, me es grato enviarle un cordial saludo.

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“POR MI RAZA HABLARÁ EL ESPÍRITU”
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DR. ARTURO CARLOS II BECERRA BRACHO

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Resumen en español

El cambio climático y el cambio de uso de suelo son dos de las principales causas de la pérdida de biodiversidad global, provocando reducciones en el área de distribución de las especies y disminuyendo la diversidad genética que éstas albergan. La disminución de la variación intraespecífica representa una amenaza significativa para la persistencia de las especies, dado que una mayor diversidad genética podría impulsar la adaptación a futuros cambios ambientales.

El rápido cambio climático puede alterar los patrones de adaptación local al modificar las asociaciones genotipo-ambiente que las poblaciones han desarrollado a lo largo de su historia evolutiva. Las poblaciones pueden responder a estas perturbaciones ajustándose a las nuevas condiciones mediante plasticidad fenotípica, evolución adaptativa o migrando a sitios adecuados para su persistencia.

Los métodos en genómica del paisaje nos permiten medir el efecto de las presiones antropogénicas sobre los procesos adaptativos. Recientemente se utilizan métodos como *Gradient Forest (GF)* para medir el *genomic offset* el cual estima la vulnerabilidad de las poblaciones ante el cambio climático. A valores más altos de *genomic offset* una población necesitaría una mayor respuesta a la selección generada por el cambio ambiental para mantenerse adaptada localmente y por lo tanto la población sería más vulnerable. Sin embargo, estos estudios no suelen considerar otros procesos que también ocasiona la pérdida de diversidad genética, como son los cambios de uso del suelo.

En el estado de Michoacán, los bosques de pino-encino abarcaban aproximadamente el 28% del área del estado. Sin embargo, en las últimas décadas estos bosques han experimentado una dramática transformación hacia otros tipos de uso de suelo, entre los que destaca el cultivo de aguacate, con aproximadamente 244,705 hectáreas para el año 2019. Además, se prevé que el cultivo de aguacate seguirá aumentando en el futuro en aproximadamente 178,534 hectáreas adicionales para el año 2050. En este trabajo, usamos a *Pinus leiophylla*, una especie arbórea común en los bosques templados de Michoacán para considerar simultáneamente escenarios de cambio de uso de suelo y cambio climático en estimaciones

de *genomic offset*. Los objetivos fueron: 1) evaluar las asociaciones actuales genotipo-ambiente en poblaciones de *P. leiophylla* en el estado de Michoacán, 2) evaluar la susceptibilidad de las poblaciones considerando simultáneamente la vulnerabilidad de las poblaciones ante escenarios de cambio climático y la creciente expansión de cultivos de aguacate y 3) diseñar estrategias de flujo genético asistido para el manejo de la especie.

Se secuenció mediante ddRADseq (double digest restriction-site associated DNA sequencing), el ADN de 77 individuos pertenecientes a 19 localidades de *P. leiophylla* distribuidas en la franja aguacatera en el estado de Michoacán. Se estimaron los estadísticos de estructura y diversidad genética (heterocigosidad esperada [H_0], diversidad genética [H_s], coeficiente de endogamia [F_{IS}], número de alelos privados por población y el índice de diferenciación [F_{ST}]). Se identificaron 130 SNPs (single nucleotide polymorphisms) atípicos de los 3660 SNPs utilizados para este estudio (3.5% del total) y se determinaron métricas de conectividad entre fragmentos de bosque por dispersión tanto de polen como por semillas. Se modeló el cambio en las frecuencias alélicas a través del paisaje ante escenarios de cambio climático y cambio de uso de suelo, además de evaluar estrategias de flujo génico asistido. Por último, se identificaron las áreas prioritarias para la conservación menos afectadas ante ambos tipos de disturbio.

Se encontró que la diversidad genética adaptativa de las poblaciones presenta patrones heterogéneos a través del paisaje, donde existen poblaciones que serán más vulnerables ante el cambio climático y el cambio de uso de suelo. Sin embargo, hay poblaciones que pueden funcionar como “fuentes de diversidad” para disminuir el riesgo de extinción de las primeras. Se observó que los patrones de conectividad por dispersión mediada por semillas y polen se verán modificados por la futura pérdida de hábitat. Finalmente, las áreas con mayor prioridad para la conservación son aquellas ubicadas en los parches de bosque situados en las zonas este y centro-este del área de estudio, lo cual incluye la Reserva de la Biósfera de la Mariposa Monarca. Esta área es particularmente relevante porque mantendrá poblaciones que podrían adaptarse a los cambios climáticos futuros y serán menos susceptibles a la pérdida de hábitat por cambio de uso de suelo en la región, manteniendo la conectividad entre los parches.

Resumen en inglés (Abstract)

Climate change and land use change are two of the main drivers of global biodiversity decline, causing reductions in the distribution range of species and decreasing the amount of genetic diversity they harbor. The decrease of intraspecific variation represents a significant threat for species persistence, since standing genetic diversity could fuel the adaptation to future environmental change.

Rapid climate change is altering the patterns of local adaptation by creating novel climates and disrupting genotype-environment associations that populations have developed through their evolutionary history. In consequence, populations may respond to these disturbances either by adjusting to the new conditions via phenotypic plasticity, adaptive evolution or through migration to new places adequate for their survival.

Advances in landscape genomics have increased our ability to measure the effect of pressures caused by anthropogenic activities on the adaptative processes. Methods as *Gradient Forest (GF)* can be used to estimate the *genomic* offset a measure of the vulnerability of populations to climate change. In that sense, higher values of genetic offset indicate that a population would need to respond more to the selection generated by environmental change and therefore that the population would be more vulnerable. However, studies that have analyzed genetic offset usually do not consider other processes that could also increase the loss of genetic diversity, such as changes in land use and habitat fragmentation.

In the state of Michoacán, pine-oak forests potentially covered approximately 28% of the state's total area. Nevertheless, in the last decades Michoacán's pine-oak forests have experienced a dramatic transformation to other land uses, most notably avocado cultivation, which covered approximately 244,705 hectares by the year 2019. Moreover, it is projected that cultivation of avocado will continue to increase in the future by approximately 178,534 additional ha by the year 2050. In this work, we selected *Pinus leiophylla*, a common tree species in Michoacán's temperate forest to simultaneously consider land use and climate change in genomic offset scenarios. The aims were: 1) to

assess current genotype-environment associations in *P. leiophylla* populations in Michoacán, 2) to evaluate the vulnerability of populations simultaneously considering genomic offset on the face of climate change scenarios and the growing expansion of avocado crops, 3) to lay out assisted gene flow strategies for the species' management.

The DNA of 77 individuals belonging to 19 localities of *P. leiophylla* distributed in the Avocado Belt in the state of Michoacán was sequenced using ddRADseq (double digest restriction-site associated DNA sequencing). We estimated the genetic structure and diversity statistics (observed heterozygosity [H_o], gene diversity [H_s], the inbreeding coefficient [F_{is}], private alleles per population and the inbreeding coefficient [F_{ST}]). We identified 130 outlier SNPs (single nucleotide polymorphisms) of the 3660 SNPs used for this study (3.5% of the total) and calculated connectivity metrics among forest fragments as mediated by both pollen and seed dispersal. We modeled the turnover of allele frequencies across the landscape. We modeled how the turnover in allele frequencies across the landscape could change under climate change and land use change scenarios, in addition to evaluating assisted gene flow strategies. Finally, priority conservation areas were identified that could be less affected by both disturbances.

We found that the adaptive genetic diversity of populations shows heterogeneous patterns across the landscape, where there are populations that will be more vulnerable to climate change and land use change. However, there are populations that could serve as "sources of diversity" to reduce the risk of extinction of the more vulnerable ones. We also observed that connectivity patterns under seed and pollen dispersal scenarios will be modified by future habitat loss.

Finally, we found that the areas with higher priority for conservation are those in the forest patches located in the east and center-east part of the study area, which encompasses the Monarch Butterfly Biosphere Reserve. This area is particularly relevant because it maintains populations that could be well adapted to projected future environmental changes and will be less susceptible to loss by land use change, maintaining the connectivity between patches.

Introducción general

La pérdida global de biodiversidad representa una de las mayores amenazas para la salud y el funcionamiento de los ecosistemas. En la actualidad las tasas modernas de extinción de especies son cien veces mayores en comparación con épocas preindustriales (Ceballos et al., 2015), debido principalmente a las actividades antropogénicas como la introducción de especies, la sobreexplotación de recursos, el cambio de uso de suelo y el cambio climático global.

El cambio climático global es reconocido como uno de los principales factores que impulsan este declive de biodiversidad (Urban, 2015), provocando un aumento repentino sin precedentes de la temperatura media del planeta debido a un incremento en la concentración de gases de efecto invernadero en la atmósfera. Esto ocasiona secuelas como reducción de los hábitats, acidificación del océano, aumento en el nivel de los mares, además de ocasionar eventos climáticos extremos cada vez más frecuentes alrededor del mundo. Para el año 2017 el aumento en la temperatura inducido por el hombre alcanzó 1°C promedio por encima de los niveles preindustriales, no obstante, se prevé que para el año 2100 las temperaturas alcancen un aumento promedio de 2.8°C (IPCC) lo que implicaría un impacto negativo para los ecosistemas y en consecuencia una mayor disminución a la biodiversidad que en ellos habita (Habibullah et al., 2022).

La pérdida de biodiversidad puede verse reflejada en todos los componentes de diversidad biológica como son la diversidad ecosistémica, la diversidad de especies y la diversidad genética. Esta última es relevante dado que permite a los organismos adaptarse a los cambios repentinos que ocurran en el ambiente. En este sentido, aquellas poblaciones que alberguen una mayor diversidad genética tendrán una mayor probabilidad de supervivencia dado que contarán con un reservorio genético más amplio que les permita enfrentar nuevas condiciones ambientales (Pauls et al., 2013).

Así mismo, los organismos pueden responder a las alteraciones ambientales, ya sea por plasticidad fenotípica, generando diferentes fenotipos en respuesta a diferentes condiciones ambientales, por mecanismos de adaptación evolutiva, haciendo uso de la

diversidad genética para responder a las presiones selectivas en su ambiente, y/o por migración, la cual puede ser de manera natural o mediada por la acción humana. Esta última conocida como migración asistida. La migración asistida consiste en llevar genotipos que estén adaptados localmente a ciertas condiciones para garantizar su éxito de supervivencia (McLachlan et al., 2007).

En los últimos años la genómica del paisaje, junto con los métodos de secuenciación de nueva generación, nos han permitido evaluar el impacto que el cambio climático ejerce sobre los procesos adaptativos de las poblaciones, así como diseñar estrategias de conservación que permitan incorporar diferentes fuentes de información para tener planes de manejo más adecuados. Para esto se han realizado modelaciones incorporando no solo la distribución geográfica de las especies, lo cual asume que todas las poblaciones responderán de manera homogénea, sino también considerando la diversidad genética y la capacidad adaptativa de las poblaciones (Fitzpatrick & Keller, 2015; Martins *et al.*, 2018; Capblancq *et al.*, 2020; Aguirre-Liguori *et al.*, 2021). Métodos como *gradient forest* (GF, Ellis *et al.*, 2012) buscan integrar ambos componentes estimando el *genomic offset* (GO) de las poblaciones, el cual indica la cantidad de cambio necesaria para que una población tenga la misma asociación genotipo-ambiente actual bajo las condiciones ambientales futuras. De esta forma, a un mayor valor de GO, se necesitará una mayor cantidad de cambio genético y por lo tanto será más difícil para las poblaciones adaptarse a las nuevas condiciones ambientales (Fitzpatrick & Keller, 2015).

No obstante, para intentar pronosticar con mayor certidumbre la respuesta de las poblaciones naturales ante los disturbios, es importante incorporar otros procesos que también ejercen una presión sobre los organismos, como es el cambio de uso de suelo. Actualmente, se estima que tres cuartas partes del medio ambiente terrestre están significativamente alteradas por la acción humana (IPBES, 2019). Estas provocan la pérdida y fragmentación del hábitat, ocasionando que las poblaciones disminuyan su tamaño y conectividad y por consecuencia un decremento en la cantidad de diversidad genética que albergan, repercutiendo en su potencial adaptativo y en su capacidad para lidiar con los cambios ambientales; esto también afecta la manera en cómo las poblaciones responderán

ante los efectos provocados por otros disturbios como el cambio climático, originando un efecto negativo en cascada (Cheptou et al., 2017).

Otro componente importante a considerar es la conectividad entre las poblaciones. En paisajes fragmentados, el surgimiento de barreras a la dispersión, como pueden ser las carreteras, zonas urbanas y parches agrícolas, aumentan el grado de aislamiento de las poblaciones, impidiendo el intercambio de flujo génico entre ellas, y como consecuencia aumentando los efectos negativos de la endogamia y disminuyendo sus niveles de diversidad genética, por lo que se deben diseñar estrategias que permitan el movimiento y adaptación de las poblaciones, evitando el riesgo de extinción de las mismas (Aguilar et al., 2008; Gómez-Fernández et al., 2016)

En México, los bosques templados se encuentran distribuidos entre los 2,000 y 3,400 metros de altitud, compuestos por una gran diversidad de flora y fauna, siendo su vegetación predominante los bosques de pino-encino. Estos bosques proveen de una gran cantidad de servicios ecosistémicos, como son la regulación del ciclo hidrológico y biogeoquímico, captura de carbono, purificación del aire, además de proveer de recursos económicos para numerosas comunidades al servir para el aprovechamiento de recursos maderables. Desafortunadamente, a pesar de su importancia ecológica y social, en la actualidad su distribución se ha reducido de 43.96 millones de ha a 32.41 millones de ha o el 16% del territorio nacional aproximadamente. Además, se estima que el 38.31% de esta superficie se encuentra deteriorada, debido principalmente a la deforestación y el cambio de uso de suelo por agricultura o expansión de zonas urbanas (CONABIO, 2008). Uno de los casos más notorios ha sido en el estado de Michoacán, México, donde el cultivo de aguacate ha incrementado su producción de 90,000 toneladas por año en el año 2003 a aproximadamente 1.3 millones de toneladas para el año 2019, con una extensión de aproximadamente 244,705 ha, debido principalmente al permiso de exportación a Estados Unidos firmado en el año 1997 (Arima et al., 2022; Barsimantov & Navia Antezana, 2012; Latorre-Cárdenas et al., 2023). Este cultivo requiere de condiciones de temperatura y precipitación muy similares a los bosques templados, por lo que este ecosistema es el más amenazado a causa de la expansión de estos huertos (Denvir, 2023). No obstante, este cultivo afecta

también la disponibilidad de agua, al requerir entre dos a siete veces más consumo que un m² de bosque nativo, creando conflictos tanto ecológicos como sociales, los cuales podrían agravarse con los cambios en los regímenes de precipitación proyectados para años con condiciones aún más secas (Gómez-Tagle *et al.*, 2022; Quiroz-Rivera, 2019). Aunado a esto, se proyecta que la expansión de huertos de aguacates seguirá en aumento, con aproximadamente 85,940 hectáreas adicionales para el año 2050 representando una gran amenaza para la persistencia de los bosques templados en el estado (Denvir, 2023).

El género *Pinus* incluye alrededor de 121 especies en todo el mundo (American Conifer Society, 2024). Los pinos son especies pioneras gracias a su resistencia a climas extremos que van desde fuertes condiciones de sequías hasta condiciones de climas extremos, además de que pueden establecerse en suelos pobres en nutrientes, reacondicionando el suelo y facilitando los procesos de sucesión ecológica al proporcionar las condiciones adecuadas que permitan el establecimiento de otras especies en la comunidad (Richardson & Rundel, 2000). Aunado a esto, los pinos son considerados un género arbóreo de gran importancia económica globalmente, al servir como fuente de madera, celulosa y resina (Le Maitre, 1998), lo que desafortunadamente ha provocado la sobreexplotación para numerosas especies de este género. México es considerado el mayor centro de diversidad mundial de pinos, albergando 49 de las especies conocidas, con aproximadamente un 42% de éstas endémicas para nuestro territorio (Gernandt & Pérez-De La Rosa, 2014; Ricker & Hernández, 2010; Perry *et al.*, 1998), destacando la conservación de este género como acción prioritaria para mantener la gran diversidad genética y los servicios ecosistémicos que los pinos ofrecen.

Pinus leiophylla es una especie representativa de los bosques templados de Michoacán, su amplia distribución geográfica abarca desde el suroeste de los Estados Unidos, atravesando la Sierra Madre Occidental, el Cinturón Volcánico Transmexicano y la Sierra Madre del Sur en México (Perry, 1991). *P. leiophylla* es dispersado a través de las semillas y presenta una polinización mediada por viento. Estas características, como a muchas otras especies de árboles, le confieren grandes tamaños efectivos poblacionales, altos valores de diversidad genética y una baja diferenciación genética entre las poblaciones

(Rodríguez-Banderas et al., 2009; Sork et al., 2013). En conjunto, estas características resultan en patrones claros de adaptación local a través de los diferentes gradientes ambientales presentes dentro del rango de distribución de la especie (González-Martínez et al., 2006). No obstante, con la creciente demanda del cultivo de aguacate las poblaciones de *P.leiophylla* se han visto amenazadas por las altas tasas de deforestación que se han visto en el estado Michoacán, poniendo en riesgo su persistencia, disminuyendo su niveles poblacionales, así como sus valores de diversidad genética, repercutiendo en la capacidad adaptativa de las poblaciones ante los efectos del cambio climático, por lo que estudiar cómo responderán las poblaciones de *P. leiophylla* ante los disturbios tanto de cambio climático como de cambio de uso de suelo será clave para garantizar su futura persistencia además de mantener un componente clave de los bosques templados dentro de la franja aguacatera en el estado de Michoacán, los cuáles se han visto gravemente amenazados en los últimos años.

En este trabajo se utilizó a *Pinus leiophylla*, una especie pionera representativa de los bosques templados en Michoacán para evaluar la forma en que la diversidad genética adaptativa de sus poblaciones podría verse afectada bajo escenarios de cambio climático y cambio de uso de suelo, integrando estrategias de flujo génico asistido, además de incorporar métricas de conectividad que permitan identificar áreas de conservación prioritarias en los bosques templados en el estado de Michoacán.

Texto del artículo científico

Title: Local adaptation of *Pinus leiophylla* under climate and land use change models in the Avocado Belt of Michoacán

Authors: Vanessa Izaguirre-Toriz^{1,2}, Jonás A. Aguirre-Liguori³, María Camila Latorre-Cárdenas¹, Eugenio Y. Arima⁴ and Antonio González-Rodríguez^{5*}

¹Instituto de Investigaciones en Ecosistemas y Sustentabilidad, Universidad Nacional Autónoma de México, Morelia 58190, México

²Posgrado en Ciencias Biológicas, Universidad Nacional Autónoma de México (Posgrado en Ciencias Biológicas, Unidad de Posgrado, Edificio D, 1° Piso, Circuito de Posgrados, Ciudad Universitaria, Coyoacán, C.P. 04510, CDMX, México).

³Departamento de Ecología Tropical, Campus de Ciencias Biológicas y Agropecuarias, Universidad Autónoma de Yucatán, Mérida, México

⁴Department of Geography and the Environment, University of Texas at Austin, Austin, TX 78712, USA

⁵Laboratorio Nacional de Innovación Ecotecnológica para la Sustentabilidad (LANIES), Instituto de Investigaciones en Ecosistemas y Sustentabilidad, UNAM campus Morelia

Author for correspondence: Antonio González-Rodríguez Email: agrodrig@iies.unam.mx

Summary

Climate change and land use change are two main drivers of global biodiversity decline, decreasing the amount of genetic diversity that populations harbor and altering the patterns of local adaptation. Methods in landscape genomics allow measuring the effect of these anthropogenic disturbances on the adaptation of populations. However, both factors have rarely been considered simultaneously. We modeled the spatial turnover in allele frequencies of 19 localities of *Pinus leiophylla* across the Avocado Belt in Michoacán state, Mexico which could change under climate change and land use change scenarios, in addition to evaluating assisted gene flow strategies and connectivity metrics across the landscape to identify priority conservation areas. We found that localities at the center-east regions

would be more vulnerable to climate change, while localities in the west area will be more threatening by actions of land use change. However, assisted gene flow actions could reduce their risk of extinction for both scenarios. Connectivity patterns will also be modified by future habitat loss, with the central and eastern parts having the highest connectivity values. These results show that the areas with the highest priority for conservation are in the eastern zones, which include the Monarch Butterfly Biosphere Reserve. This work is useful as a framework that incorporates distinct layers of information to provide a robust representation of the response of populations to future anthropogenic disturbances.

Keywords: Genomic offset, local adaptation, conservation genomics, land use change, climate change, habitat loss.

Introduction

Climate change and habitat loss due to land use change are two of the main drivers of global biodiversity decline (Hansen *et al.*, 2001; Oliver & Morecroft, 2014), causing reductions in the distribution range of species and decreasing the amount of genetic diversity they harbor. A recent study estimated that on average, 10 to 16% of global genetic diversity has been lost due to decreasing habitat (Exposito-Alonso *et al.*, 2022). Moreover, projections for the year 2070 estimate that between 6.2 and 10.7% of habitat will be lost per decade for amphibians, mammals and bird species causing the upcoming loss of 2 to 5% of genetic diversity in these groups (Powers & Jetz, 2019; Exposito-Alonso *et al.*, 2022). The decrease of intraspecific variation may have considerable effects on ecological communities and ecosystem processes (Des Roches *et al.*, 2018) as it represents a significant threat to species persistence, since standing genetic diversity could fuel the adaptation to future environmental change (Frankham *et al.*, 1999; Barrett & Schluter, 2008; Laikre *et al.*, 2020).

Rapid climate change is altering the patterns of local adaptation by creating novel climates and disrupting genotype-environment associations that populations have developed through their evolutionary history (Gougherty *et al.*, 2021). In consequence, populations may respond to these disturbances either by adjusting to the new conditions via phenotypic plasticity or through adaptive evolution. There is increasing evidence that

local adaptation to variation in environmental conditions across species' distribution ranges is common (Hoffmann & Sgró, 2011; Gugger *et al.*, 2021), suggesting that studying the evolutionary potential at the population level could allow understanding of how species respond to changing environments. Nevertheless, species with long generation times may not have enough time to adapt to rapidly changing environmental conditions, causing an *adaptation lag*, and future maladaptation (Kremer *et al.*, 2012; Browne *et al.*, 2019). Alternatively, populations could migrate to sites where they could become locally adapted; or they could receive, either naturally or mediated by humans, adaptive genetic variation through gene flow, creating novel genotype-environment relationships (Aitken & Whitlock, 2013; Rhoné *et al.*, 2020).

The recent development of next generation sequencing and the advances in landscape genomics have increased our ability to measure the effect of the pressures on adaptive processes caused by anthropogenic activities and climate change. Methods such as gradient forest (Ellis *et al.*, 2012) have been recently used to estimate the genomic offset of populations to climate change (Fitzpatrick & Keller, 2015; Martins *et al.*, 2018; Capblancq *et al.*, 2020; Aguirre-Liguori *et al.*, 2021). The genomic offset is obtained in three steps. First, a model that describes the association between the genetic composition of populations and their current climatic conditions is constructed. Second, this model is used to infer the expected genetic composition of the populations projected in future conditions so that populations can stay locally adapted. Finally, the genetic offset is measured as the Euclidian distance between the present and future genetic compositions and quantifies the amount of genetic change that a population would need to maintain the existing gene-environment relationships (Fitzpatrick & Keller, 2015). Higher values of genetic offset indicate that a population would need to respond more to the selection pressure generated by climate change and, therefore, that the population would be more vulnerable. By estimating the genetic offset of populations, it is possible to identify areas that should be prioritized for conservation and design robust management plans (Yu *et al.*, 2022).

Genetic offsets are powerful tools that could indicate how populations will respond to climate change. However, studies that have analyzed genetic offset usually do not

consider other processes that could also affect or limit the response of populations by increasing the loss of genetic diversity, such as changes in land use and fragmentation of populations (Selwood *et al.*, 2015). Therefore, it is important to estimate the joint impact of anthropogenic disturbances on the vulnerability of the populations to better predict how these will respond in the future. Yet, it is still not easy to predict how land use and habitat fragmentation will occur in the future, since this requires accurate models of different scenarios of habitat change, considering both environmental and socioeconomic variables that could determine the speed and magnitude of these land transitions.

Mexico is considered a center of diversification for the *Pinus* and *Quercus* genera, harboring around 47 of 121 pine species, and 161 of 450 oak species (38 and 35% of the species genus, respectively; Perry *et al.*, 1998; Valencia-A., 2004). In the state of Michoacán, pine-oak forests potentially covered approximately 1,623,100 ha or 28% of the state's total area (Denvir *et al.*, 2022), hosting a great diversity of flora and fauna and approximately 16 pine and 30 oak species (Leal Nares & Villaseñor Gómez, 2016). These forests provide fundamental ecosystem services, such as carbon sequestration and storage, water and air purification, regulation of the hydrological cycle, and a large number of cultural and aesthetic values (Leal Nares & Villaseñor Gómez, 2016; Gómez-Tagle Chavez *et al.*, 2019; Denvir, 2023). Nevertheless, despite their high biological and social importance, in the last decades Michoacán's pine-oak forests have experienced a dramatic transformation to other land uses most notably avocado cultivation. Michoacán is the principal producer of avocado in Mexico, with 73% of the total production in the country, covered approximately 244,705 ha by the year 2019 which represent 45% of the international market, followed by Jalisco state (Khan *et al.*, 2021; Latorre-Cárdenas *et al.*, 2023). This expansion has caused an enormous deforestation in the last years, putting the persistence of temperate forest in the region at great risk. Moreover, it is projected that cultivation of avocado in Michoacán will continue to increase in the future by approximately 178,534 additional ha by the year 2050, making the pine-oak forest the most vulnerable ecosystem in the region due to the combination of climate change and avocado expansion (Denvir, 2023). Prioritizing the conservation of pine-oak forest is of vital importance not only for their invaluable biological

worth but also because they could maintain the ecological and ecosystem services in the region.

Pinus leiophylla (Pinaceae), is a representative species of the pine-oak forest with high ecological and economic value since it is an important source of wood and resin. Likewise, is frequently used in restoration projects due to its ability to tolerate stress conditions, as water scarcity and frost (Martínez Trinidad *et al.*, 2002). In this work, we selected *P. leiophylla* as a study system to simultaneously consider land use and climate change in genomic offset scenarios and adaptative management proposals for this species.

The specific aims of this work were: 1) to assess current genotype-environment associations in *P. leiophylla* localities in Michoacán, 2) to evaluate the vulnerability of localities simultaneously considering genomic offset on the face of climate change scenarios and the growing expansion of avocado crops, 3) to lay out assisted gene flow strategies for the species' management.

Materials and methods

Study system and sample collection

Pinus leiophylla is present across highly heterogeneous environments from the Southwest of the United States through the Sierra Madre Occidental, the Trans Mexican Volcanic Belt, and the Sierra Madre del Sur in Mexico (Perry, 1991). As all pines, *P. leiophylla* is wind pollinated, a trait that is often associated with high genetic variation, high effective population sizes, and low genetic differentiation between populations (Rodríguez-Banderas *et al.*, 2009; Sork *et al.*, 2013). In turn, these characteristics result in a high adaptative capacity and clear patterns of local adaptation of populations (González-Martínez *et al.*, 2006).

Our sampling was conducted in the “Avocado Belt”, which is a region of avocado production covering 62 municipalities in the state of Michoacán, with 244,705 ha of avocado crops (Latorre-Cárdenas *et al.*, 2023). The area is located within the Trans-Mexicana Volcanic Belt and is topographically complex with mountains, plateaus, valleys and hills, which result in a high heterogeneity of climatic gradients and a variety of soils including luvisols, acrisols

and andosols. Elevation ranges from 1,300 to 3,600 m, and the mean temperature and annual precipitation range from 10 to 24°C and from 800 to 1600 mm, respectively (Ramírez-Mejía *et al.*, 2022).

We sampled 77 individuals from 19 localities encompassing the temperature and precipitation gradients (fig 1, Supporting Information Table S1). At each site, we randomly chose 2-5 adult trees with a diameter at breast height (DBH) of at least 20 cm and separated by a minimum distance of 30 m from each other to avoid sampling related individuals. We decided to maximize the number of populations to sample more environmental variation across our species' range, as it has been shown that the more populations sampled, the fewer individuals are needed to obtain accurate estimates of genetic diversity (F_{ST} , H_o , H_s ; Nazareno *et al.*, 2017; Aguirre-Liguori *et al.*, 2020). Fresh leaf tissue was stored at -80°C until DNA extraction.

DNA extraction, library construction, sequencing, and variant detection

Genomic DNA was extracted from approximately 30 mg of frozen tissue using the cetyltrimethylammonium bromide (CTAB) protocol described by Doyle & Doyle, 1987. We assessed the purity and concentration of the extracted DNA using absorbance ratios at 260/280 and 260/230 nm using a NanoDrop 2000 spectrophotometer (Thermo Scientific) and fluorometric quantification (Qubit 2.0 BR assay; Invitrogen). For each individual that had an adequate quality and concentration of DNA, we collected >1 µg DNA that was sent to Floragenex (www.floragenex.com) for Rad tag sequencing.

Genomic libraries were created using the ddRAD-Seq protocol described by Truong *et al.* (2012). Briefly, genomic DNA of each sample was digested using the restriction enzymes *Pst*I and *Mse*I, and an adapter was ligated to the DNA fragments. The adapter-ligated fragments were sequenced using Illumina NovaSeq 6000. The sequenced samples were demultiplexed and trimmed for further analysis.

A *de novo* RAD reference genome was constructed using the individual that had the highest number of unique RAD sequences. Next, we used custom scripts to cluster identical

sequences. The assembly for the reference individual was realigned against itself using BWA (Li & Durbin, 2009). BOWTIE (Langmead *et al.*, 2009) was then used to align the reads of each individual to the RAD reference genome. SAMTOOLS (Li *et al.*, 2009) and custom scripts were used to detect and filter SNPs that had a minimum sequencing depth per sample of 15x, individual per locus genotype quality scores of at least 20 and a minimum of 10x sequence coverage (ID 1076599 - BioProject – NCBI).

Subsequently, we used PLINK v1.9 (Purcell *et al.*, 2007) and vcftools v0.1.16 (Danecek *et al.*, 2011) to retain diallelic SNPs that had minor allele frequencies (MAF) \geq 0.025 and had less than 20% of missing data. We also removed SNPs that were in linkage disequilibrium ($LD < 0.5$) within a window size of 50 bp and a window shift of 5 (Purcell *et al.*, 2007). We used HDplot (McKinney *et al.*, 2017) to remove possible paralogs. We removed all regions that had observed heterozygosities (H_o) greater than 0.5 and a D value outside the range of -15 and 15, which could indicate potentially duplicate loci due to deviation of allelic ratios expectation. After these filtering procedures we obtained 3660 SNPs.

Spatial modelling and environmental variables

We obtained from Latorre-Cárdenas *et al.* (2023) the land use and land cover classification (LULC) map of the Avocado Belt for 2019 with a resolution of 30 m, consisting of six classes: (1) temperate forest, (2) avocado orchards, (3) water bodies (4) agriculture and pasture areas (which included all types of croplands except the avocado orchards), (5) settlements and (6) dry forest and shrublands of low and medium deciduous forests (fig. 1). In total our study area included 786,812 ha of temperate forest. Based on the LULC layer for 2019, we followed the procedure of Arima *et al.* (2022) to project the expansion of new avocado orchards by the year 2050 and created a temperate forest layer for 2050 subtracting from the 2019 forest layer the areas where avocado is projected to be established by 2050. For downstream analysis we only considered the temperate forest patches that had an area equal to or greater than 10 ha to avoid including patches that are too small for prioritizing conservation areas. These maps allowed us to evaluate the effects of fragmentation, habitat

loss, and the avocado expansion on the patterns of genetic diversity distribution and connectivity of *P. leiophylla* in temperate forest patches.

We downloaded 19 bioclimatic variables from WorldClim (<http://www.worldclim.com/version2>) representing the mean observations between 1970-2000 with a resolution of 30 arc/sec (Fick & Hijmans, 2017). For the future climate we downloaded the 2041-2060 layers using the Miroc6 model under the 370 shared socioeconomic pathway (SSP) scenario, which is in an intermediate range of global climate projections, with a resolution of 30 arc/sec. (<http://worldclim.com/version2>). To remove the correlation among the climatic variables, we kept those that had Pearson correlation coefficients <0.8 : isothermality (BIO3), minimum temperature of coldest month (BIO6), temperature annual range (BIO7), mean temperature of driest quarter (BIO9), precipitation seasonality (BIO15), precipitation of wettest quarter (BIO16), precipitation of driest quarter (BIO17), precipitation of warmest quarter (BIO18) and precipitation of coldest quarter (BIO19).

Genetic diversity and structure

We used the *basic.stats* function in the *hierfstat* package (Goudet, 2005) in R 3.4.1 (R CoreTeam, 2015) to obtain the per SNP site and mean observed heterozygosity (H_o), gene diversity (H_s), and the inbreeding coefficient (F_{IS}) per locality. The number of private alleles was estimated with the package *poppr* version 2.9.3 (Kamvar *et al.*, 2014). We also obtained the genetic differentiation among localities using the Weir and Cockerham F_{ST} (Weir & Cockerham, 1984). Finally, we evaluated if there is a correlation between the connectivity values of the forest patches (for seed and pollen dispersal scenarios, see below) and the values of gene diversity (H_s).

To determine the genetic structure, we first used the software Admixture v1.3 (Alexander *et al.*, 2009) with K values ranging from 1 to 19. From this analysis, we selected the K value with the lowest cross-validation error. Second, we performed a PCA with the *adeigenet* package in R using individual genotypes, with missing data imputed by the mean

allele frequency (Jombart, 2008). We also analyzed genetic variation within and among localities with a nonparametric analysis of molecular variance (AMOVA) (Stewart & Excoffier, 1996) using Arlequin software v3.0 (Excoffier *et al.*, 2005).

Identification of outlier SNPs

We used a genome-environment association test (GEA) to identify potentially non neutral SNPs, employing latent factors mixed models (LFMM; Frichot *et al.*, 2013) implemented in the package *LEA* in R (Frichot & François, 2015). LFMM tests for correlations between allelic frequencies and environmental variables after controlling for genetic structure using latent factors. To select the optimal number of latent factors we used $K=1$ as the number of inferred clusters (K) based on the admixture analysis (see Results). We ran LFMM analysis using the allelic frequencies of all SNPs and the nine bioclimatic variables described above. Five independent runs were made using 10,000 iterations with a burn-in of 5000. Adjusted p -values (q) were calculated using the genomic inflation factor (λ) procedure (Devlin & Roeder, 1999). For each run we analyzed the histograms of p values to ensure that the confounding effects of population structure was well controlled. Finally, to correct for multiple testing the resulting p -values were adjusted using the false-discovery rate method with a significance threshold of $Q < 0.01$. The outlier SNPs detected by LFMM were defined as the outlier set.

Annotation and gene ontology

We used the Blast2Go v6.0.3 program to identify the putative gene functions and Gene Ontology (GO) terms using the flanking sequences of each SNP of the outlier set. We first performed a homology search using BLASTN and BLASTX on the NCBI non-redundant (nr) public database with an e-value threshold set to 1×10^{-5} . Second, the putative function of the sequences was assigned according to the highest BLAST hits and GO terms associated with the BLAST hits.

Gradient forest

To model the current patterns of genetic variation of *P. leiophylla* through the Avocado Belt we used Gradient Forest (GF), a machine-learning approach that models the turnover in

genetic composition across the landscape (Fitzpatrick & Keller, 2015). GF identifies nonlinear associations between allele frequencies and environmental variables and determines how allele frequencies change along the gradient. We used the *gradient forest* package in R (Ellis *et al.*, 2012) using the allele frequencies of each locality for the full set and for the outlier set of SNPs. We decided to incorporate both sets to seek for differences in genomic turnover patterns across the landscape between putatively adaptative SNPs and reference SNPs, as has been done in previous studies (Martins *et al.*, 2018; Nielsen *et al.*, 2021). To obtain the GF models we used the same nine bioclimatic variables for the current climate (1970-2000). We ran gradient forest with 1000 regression trees per SNP and a variable correlation threshold of 0.5 (Fitzpatrick and Keller, 2015).

Once we had the GF models, we performed a principal components analysis (PCA) on the predictions and used the first three axes to obtain a red-green-blue color palette. The color scale was used to map the turnover in genotype-environment associations across the landscape, where similar colors indicate a similarity in the expected patterns of locality genetic composition (Fitzpatrick & Keller, 2015). We mapped the resulting prediction only considering actual forest fragments according to our LULC map. To estimate the difference in genetic composition patterns between the models obtained with the two datasets (full and outlier), we performed a Procrustes analysis with the *vegan* package in R (Oksanen *et al.*, 2022).

Local, forward, and reverse offsets

To assess the genomic offset of localities under future climate change we used the same nine bioclimatic variables for the years 2041-2060. First, we used the *predict* function in R to infer the expected genetic composition across the present and future landscapes. Next, we estimated the genomic offsets as the Euclidean distance between the genomic composition under the present and future scenarios (local offset). The genetic offsets across the landscape were mapped to identify the regions of temperate forests that are expected to become vulnerable because of climate change. Finally, we used ArcMap program v10.1 to subtract the projected areas of expansion of avocado crops by 2050 to evaluate the effects occasioned by both, climate change and habitat loss.

Genomic offsets predict the risk of maladaptation of the populations *in situ* (local offset). However, populations can migrate to more suitable areas within their current range for their survival (forward offset), or in turn, receive genotypes of any population in the current range that is more pre-adapted to future climatic conditions (reverse offset). To provide a more realistic approach of the vulnerability of the localities to climate change, we quantified the forward and reverse offset following the procedure of Gougherty *et al.* (2021). Briefly, the forward offset was calculated between current to future climate. We obtained the predicted minimum offset between each grid under current climatic conditions within the current forest range and all the grids within the temperate forest layer for 2050 in future climate. Reverse offset was calculated between future to current climate, by predicting the minimum offset between each grid under future climatic conditions within the current forest extent, and all grids within the temperate forest layer for 2050 in current climate conditions. Finally, we mapped the local, forward and reverse offsets using a red-green-blue image with a histogram equalization performed on each band to ensure the visualization of the full range of colors.

Isolation by distance and environment

To estimate the contribution of geographic distance and environmental variation in the patterns of genetic differentiation, we performed redundancy analyses (RDA) and partial redundancy analyses (pRDA) using a variance partitioning approach implemented in the *varpart* and *rda* functions of the package *vegan* in R (Oksanen *et al.*, 2022). These analyses were performed using as dependent matrix the allelic frequencies of each locality, with the climate and geographic variables of each locality as explanatory matrices. To obtain the matrix of climatic variables we performed a PCA with the same nine bioclimatic variables to avoid the redundancy between variables and used the first three axes, which explained 72% of the accumulated variation. The geographic distance matrix consisted of the geographic coordinates of locality (latitude and longitude). We performed this analysis with: (1) the full set of SNPs; (2) the outlier set; and (3) the SNPs with a positive R^2 obtained from the gradient forest analyses (see below). Significance was tested performing 999 permutations.

Connectivity and future land use change

Land use change associated with avocado crops has caused an enormous habitat reduction of pine-oak forest through the Avocado Belt in recent years (Mas *et al.*, 2017) altering the patterns of connectivity between natural populations restricted to forest patches. The degree of connectivity is given by attributes of the landscape (structural connectivity) and the organism (functional connectivity). High connectivity values mean that the organisms could move easily between the patches thereby maintaining genetic diversity, avoiding inbreeding, and contributing to the recolonization process. To estimate how the expansion of avocado crops has affected the connectivity patterns between the patches of temperate forest within the Avocado Belt, we obtained the Composite Connectivity Index (CCI) values from Latorre-Cárdenas *et al.* (2023) using two different scenarios based on the seed and pollen dispersal capacity of pine and oak species. CCI considers the contribution of every focal patch (f) to the connectivity in adjacent patches and their contribution to connectivity in the total landscape (Latorre-Cárdenas *et al.*, 2023). To forecast how connectivity patterns will be modified in future scenarios of habitat loss, we estimated CCI values on the projected remnants patches of forest for the year 2050 according to our model of avocado expansion following the same procedure described in Latorre-Cárdenas *et al.* (2023).

Next, we performed a weighted overlay technique in ArcMap program v10.1 to identify the priority areas for conservation that probably will be less affected by avocado expansion and that will have lower values of local offset and high connectivity. This technique uses overlays of different raster layers by giving a weight to each layer according to its importance. We reclassified each raster on a scale of four categories with the Jenks natural breaks classification method (Jenks, 1963), assigning the value of 1 to the areas with lowest local genomic offset and 4 to areas with higher values. For the connectivity layers, 1 corresponded to areas with higher connectivity and 4 to areas with the lowest connectivity. The mean overall weights were set to 50% for the values of local offset and 25% to each of the two dispersal (seed and pollen) scenarios. Next, we weighted the overlay of the three layers by summing the product of each category by its weight. Finally, the values obtained

were converted into a map with four priority classes: 1 = high priority, 2 = intermediate priority, 3 = low priority and 4 = not priority.

Results

Genetic diversity and structure

Across localities the mean gene diversity was $H_s = 0.153$, ranging from 0.142 to 0.163, the mean observed heterozygosity was $H_o = 0.144$, ranging from 0.127 to 0.157 and the mean inbreeding coefficient was $F_{IS} = 0.06$, ranging from -0.08 to 0.065 (Supporting Information Table S2). The number of private alleles per locality ranged from zero to six. We did not detect a significant correlation between current connectivity indexes of the forest patches and the values of gene diversity (H_s) for either the seed or pollen dispersal scenarios (Supporting Information Fig. S1), suggesting that the current generation of adult *P. leiophylla* trees does not show an effect of fragmentation and habitat loss on genetic diversity.

Genetic differentiation among localities was low, with a global F_{ST} value of 0.026. Accordingly, the AMOVA showed that most of the variation was found within localities (97.05%) and only a small but significant proportion of variation was found among them (2.95%, $p < 0.0001$) (Supporting Information Table S3). In turn, ADMIXTURE showed $K = 1$ as the optimal value for K . The PCA explained only 6.02% of the variance among localities based on the first two PCs, and also supported the presence of a single genetic group in the area (Supporting Information Fig. S2).

Identification of outlier SNPs

In total, we identified 130 SNPs (3.5% of the total) that were significantly associated with climate variables. The majority of these SNPs were associated with the mean temperature of driest quarter (BIO9), followed by the minimum temperature of coldest month (BIO6) and the precipitation of warmest quarter (BIO18) (Supporting Information Table S4).

Annotation and gene ontology

Ten of the 130 outliers SNPs had a match in the BLAST search. The RAD_kmer_0003444 was associated with six climatic variables and was found to be homologous to a protein coding sequence previously identified in other pines as *P. taeda*, *P. sylvestris* and *P. pinaster*, and shown to be involved in the response to water deficit stress (protein LP3-2) (Padmanabhan *et al.*, 1997; Eveno *et al.*, 2008). The other nine SNPs fell within proteins of unknown function. In addition, we obtained 19 GO terms associated with a broad range of biological processes such as biosynthesis, transport, metabolic processes and localization.

Gradient forest

According to the GF models, the full data set explained a slightly higher proportion of variation (mean $R^2 = 11.71\%$) than the outlier set (mean $R^2 = 10.74\%$) (table 2). The minimum temperature of coldest month (BIO6), and the precipitation of wettest quarter (BIO16) were consistently within the three most important environmental predictors in the two GF models, with similar R^2 -weighted importance for both data sets (Supporting Information Fig. S3).

The allele turnover showed different genotype-environment associations across the landscape for the two models. The turnover is stronger for the model constructed with the outlier set than for the full set. However, the Procrustes analysis revealed that the difference in predicted patterns of allele distribution was restricted to some patches in the east and west areas, showing a deviation between the full and potentially non-neutral genetic composition (fig. 2).

Local, forward, and reverse offsets

First, we estimated the local genetic offset of localities, using the full and outlier data sets. We found that the estimates of local genomic offset for both data sets revealed a similar pattern between models, with greater values found in the outlier set model. However, we tested the correlation between the offsets estimated by both datasets and found that they

were highly correlated ($R^2 = 0.79$, $p < 0.05$, Supporting Information Fig. S4). Therefore, henceforth we will focus only on the outlier set model.

Local genomic offset predictions presented the highest values in the center-east regions, indicating that localities in these areas would be at higher risk due to future disruption of present genotype–environment associations. In contrast, we found that areas in the easternmost and some patches at the west and center of the distribution range had lower local genomic offsets (fig. 3, Supporting Information Fig. S5).

Forward genetic offsets showed higher values at the eastern and northern part of the distribution, indicating that these localities are maladapted to all future climates within the future temperate forest range and that the impacts of climate change cannot be mitigated by moving to more suitable areas. In contrast, for the most part of the distribution we found low values of reverse genetic offset, with moderate to higher values in some small patches at the center-east and west. This means that these localities may not have similar genotype–environment relationship across the current forest range that would be preadapted to their future conditions (fig. 3, Supporting Information Fig. S5).

Isolation by distance and environment

The RDA analysis revealed that geography did not explain any percentage of the genetic variation for the full set or the outlier set, while for the set of SNPs with positive R^2 values in the gradient forest analysis, geography explained 2.9% of the variation. However, none of these components were significant ($p > 0.05$, table 1). Environmental variation neither explained a large portion of the genetic variation for the full (1.4%) and the outlier SNP sets (3.5%) with none of these being significant. In contrast, for the SNPs with positive R^2 , environmental variables explained 18.2% of the genetic variation ($p < 0.01$, table 1). Geography alone, when controlling for environmental variation, explained a small proportion of the genetic variation for the full (1.4%), outlier (0.9%) and positive R^2 sets (5.3%), but none of these were significant ($p > 0.05$, table 1). Environmental variation alone, when controlling for geography, accounted for a nonsignificant 3.4% of the variation for the full set ($p > 0.05$), while for the outlier and positive R^2 sets it explained 4.8% and 20.7%,

respectively ($p < 0.05$, table 1). This means that the pure effects of climate are greater on shaping the spatial pattern of genetic variation in outlier sets, while geography is negligible.

Connectivity and future land use change

Using our model of avocado expansion by the year 2050, we estimated an expected distribution extent of temperate forest of 674,906 ha, with a loss of approximately 58,589 additional ha, compared to the present. These transformations will affect mainly the south-west area, where are some of the *P. leiophylla* localities that will be potentially less affected by climate change (lower values of local offset, fig. 4) and where Zurumucapio is located, the locality with the higher number of private alleles (Supporting Information Table S2). According to these results, one locality will be completely lost, while three other localities are in forest patches that will be reduced to less than 60% of their current area. The rest of the localities are in forest patches that will probably conserve over 90% of their current extension (Supporting Information Table S5), assuming avocado expansion as the only driver of deforestation.

Next, we analyzed how the future expansion of avocado crops could impact the connectivity of the localities. We found that the connectivity patterns for the seed and pollen dispersal scenarios will be modified by future habitat loss (see Latorre-Cárdenas *et al.*, 2023). According to our models, the central and eastern patches will present higher CCI values for both dispersal scenarios (fig. 5). Contrary to the present, the patches in the west area where the avocado expansion is projected to be more intense will show the lowest CCI values, meaning that they will be less connected and will have the lowest contribution to the total connectivity across the area.

Finally, we identified that the areas with the highest priority for conservation considering the future avocado extension, low local offset, and high connectivity are those located in the eastern patches, which encompass the Monarch Butterfly Biosphere Reserve, while the localities with the lower priorities are those located at the center-west patches (fig. 4).

Discussion

Climate change and land use change are two of the main drivers of local extinction, contributing to the ongoing loss of global biodiversity. Genomic data have improved our understanding of how organisms respond to such disturbances, but most studies do not consider both factors simultaneously. In this study, we analyzed how climate change and land use change associated to the expansion of avocado cultivation will impact localities of *P. leiophylla* in the Avocado Belt in Michoacán. To our knowledge, this is the first time that the effects of climate change and land use change have been modelled together, providing more realistic information to evaluate the vulnerability of tree localities and prioritizing conservation of those localities that will be less affected by 2050.

Our population genomic analysis of *P. leiophylla* revealed a very low level of differentiation ($F_{ST} = 0.026$) with similar or higher gene diversity values ($H_S = 0.153$) than other pines (Chhatre *et al.*, 2013; Dauphin *et al.*, 2021; Figueroa-Corona *et al.*, 2022), indicating high rates of historical gene flow, as is common in wind-pollinated, outcrossing tree species (González-Martínez *et al.*, 2006; Sork, 2018). This lack of structure reduces a confounding factor in our analysis to detect signals of local adaptation (Dalongeville *et al.*, 2018). Moreover, previous studies have demonstrated that pines, despite having weak genetic differentiation, usually display strong patterns of local adaptation (Eckert *et al.*, 2015; Lind *et al.*, 2017). In this work, we performed a genotype-environment association analysis, which detected 130 putatively adaptive SNPs, whose genetic variance was mainly explained by the environment as opposed to the full set of SNPs (table 1). The genomic context of these candidates revealed one protein related to water deficit stress (protein LP3-2), which has been suggested to be involved in adaptive mechanisms in *P. albicaulis*, *P. pinaster* and *P. halepensis* (Grivet *et al.*, 2011; Lind *et al.*, 2017).

The map resulting from the gradient forest analysis revealed patterns of genotype-environment associations across the distribution range of *P. leiophylla* within the Avocado Belt, with similar trends for the full SNP set and the putatively adaptive set (fig. 2). However, differences between both sets are evident in some forest patches in the eastern and western parts. The discrepancies between the putatively adaptive and full models

could indicate areas where local adaptation might be occurring. Although these areas do not present extreme climatic values compared to the rest of the distribution range of *P. leiophylla* in the Avocado Belt, the differences observed by comparing both models could indicate that localities might be adapting to intermediate climatic values that could be exerting a selection pressure. It is also possible that there could be local adaptation to other factors correlated with the variables we measured, such as soil type or biotic interactions that would be shaping the patterns of allele distribution seen in this study. For both of our GF models the minimum temperature of the coldest month was the most important variable predicting the turnover of allele distribution. This variable is also a major determinant in the distribution of other conifer species (Yun *et al.*, 2018; Akyol & Örucü, 2019), indicating that cold temperatures could play a role as a major factor in shaping allele distribution patterns, with some localities possibly being more resistant to colder temperatures.

Our projections of genomic offsets for the year 2050 were highly correlated between the full set and the adaptive set as has been observed in studies with *Pseudotsuga menziesii* var. *menziesii*, *P. menziesii* var. *glauca*, *Pinus banksiana*, and *Picea rubens* (Lachmuth *et al.*, 2023; Lind *et al.*, 2023) as well as in animals (*Cyclograpsus punctatus*, *Parechinus angulosus*, *Scutellastragranularis*; Nielsen *et al.*, 2021). One reason could be the fact that a vast number of adaptations are due to polygenic effects rather than to single loci (Savolainen *et al.*, 2013; Fagny & Austerlitz, 2021). This could explain why the neutral and non-neutral data provide a similar pattern, but more studies are necessary to test this.

The genomic offset models show that localities of *P. leiophylla* located in the center-east of the Avocado Belt are at greater risk of maladaptation because of climate change (fig. 3). However, the reverse offset analyses suggest that the local offset of most localities could be mitigated by actions of assisted gene flow. Notwithstanding, these measures are context-specific and should be analyzed with caution depending on each locality. For example, some localities with low local offset might not need assisted gene flow actions to persist, while for others it will be necessary to carry out strategies of assisted gene flow to avoid the risk of extinction. Additionally, *P. leiophylla* populations outside the study distribution area could serve as sources of diversity through migration and diminishing the local offset for those

localities that are predicted to be maladapted to future climate, reducing their risk of extinction.

Land use change is another pressure expected to greatly affect forest tree species within the Avocado Belt (Charre-Medellín *et al.*, 2021; Arima *et al.*, 2022; Denvir, 2023). The projection of avocado cultivation expansion by the year 2050, indicates a more intensive transformation at the west and center of the study region, probably causing the total loss of the forest patch where the Zurumucapio locality (Id. 18, supporting Information Table S1) is located and reducing the original patch extension to less than 60% for three other localities. To maintain the genetic diversity of these localities, it will be required to carry out strategies of assisted migration to new locations where they would have a low forward genetic offset. Another conservation strategy could be to promote with avocado producers the maintenance of forest strips that include individuals of *P. leiophylla* from the localities that are more threatened by the expansion of avocado orchards. In fact, the Zurumucapio locality is interesting because it grows at the upper limit of variables related to temperature and at the lowest limit of variables related to precipitation (Supporting Information Table S1). It is likely that this locality is locally adapted to hotter and drier climates and might be an interesting donor of adaptive genetic diversity. Also, this locality has the highest number of private alleles, and could contain significant genetic diversity that could serve as a fuel to future adaptation to climate change.

Avocado expansion will also modify the current patterns of connectivity on the remaining patches. For both, the seed and pollen dispersal scenarios, we found that the central and western localities will be the least connected, as a result of the projected establishment of avocado crops. These alterations will leave those areas as isolated patches causing risk of inbreeding and experiencing the negative effect of genetic drift, also these patches cannot contribute to the regeneration process of the temperate forest, suggesting that conservation actions through human intervention might be necessary.

Finally, our results indicate that the localities in the eastern part of the Avocado Belt will have low local genomic offset, a probable low reduction of their original patch extension and will maintain higher values of connectivity (fig. 4). This area is particularly relevant

because it includes localities that could be well adapted to projected future environmental changes and will be less susceptible to loss by land use change, maintaining the connectivity between patches. Also, this area encompasses the Monarch Butterfly Biosphere Reserve (MBBR), an area that provides important ecosystem services (Galicia & Zarco-Arista, 2014) so it must be a conservation target to preserve viable localities of *P. leiophylla* that contribute to the persistence of temperate forests in the region and their ecological interactions. Although this area is supposed to be already protected, illegal deforestation to extract wood sources and land use change due to avocado plantations are still a strong threat to the persistence of the MBBR, so more strategies must be implemented that guarantee their future protection. (REFXXX)

Although genomic offset has proven to be a powerful tool to incorporate genomic information to understand how species will respond on the face of climate change, this approach must be validated to estimate how related is the genomic offset with the real performance of the populations (Rellstab *et al.*, 2021) taking into account the demographic history and the genomic architecture of the populations (Láruson *et al.*, 2022). However, recent studies have used common garden experiments to test if genetic offsets are adequate to measure reductions in fitness. The results have shown that genomic offsets are better predictors of future maladaptation than climatic or geographic differences alone, showing a correlation between higher genomic offset values with lower values of population performance (Capblancq & Forester, 2021; Fitzpatrick *et al.*, 2021; Lachmuth *et al.*, 2023; Lind *et al.*, 2023). Also, it is important to note that genetic offset only models the quantity of genomic change necessary to survive given the actual genotype-environment association that populations present. However, other factors could also be playing an important role in the fate of the populations, such as genetic drift or biotic interactions and should be considered in future studies (Aguirre-Liguori *et al.*, 2021). Nevertheless, since trees are long-lived species, genetic offsets offer an opportunity to estimate the effects of climate change (which are already causing a damage in global biodiversity) in taxa for which phenotypic information or common garden experiments are lacking and whose attainment will cost a lot of time and resources. We believe that this work is useful as a framework to

incorporate distinct layers of information to provide a more robust representation of the response of the populations to future anthropogenic disturbances and serves as a way of designing better conservation strategies.

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Data accessibility statement

Individual fastq data files are available at the SRA repository of NCBI under BioProject number PRJNA1076599.

Competing interests statement

None declared

Author contributions

VI-T, JAA-L and AG-R designed the study; VI-T and AG-R collected samples from natural populations; VI-T conducted genotyping and data analyses; MCL-C performed connectivity analysis; MCL-C and EYA conducted analysis of avocado expansion; VI-T, JAA-L and AG-R wrote the manuscript draft. All authors completed and approved the final manuscript.

ORCID

Vanessa Izaguirre-Toriz: <https://orcid.org/0009-0007-9894-020X>

Jonás A. Aguirre-Liguori: <https://orcid.org/0000-0003-1763-044X>

María Camila Latorre-Cárdenas: <https://orcid.org/0000-0002-9555-2769>

Eugenio Y. Arima: <https://orcid.org/0000-0003-3366-4287>

Antonio González Rodríguez: <https://orcid.org/0000-0002-6196-7288>

Figures and tables

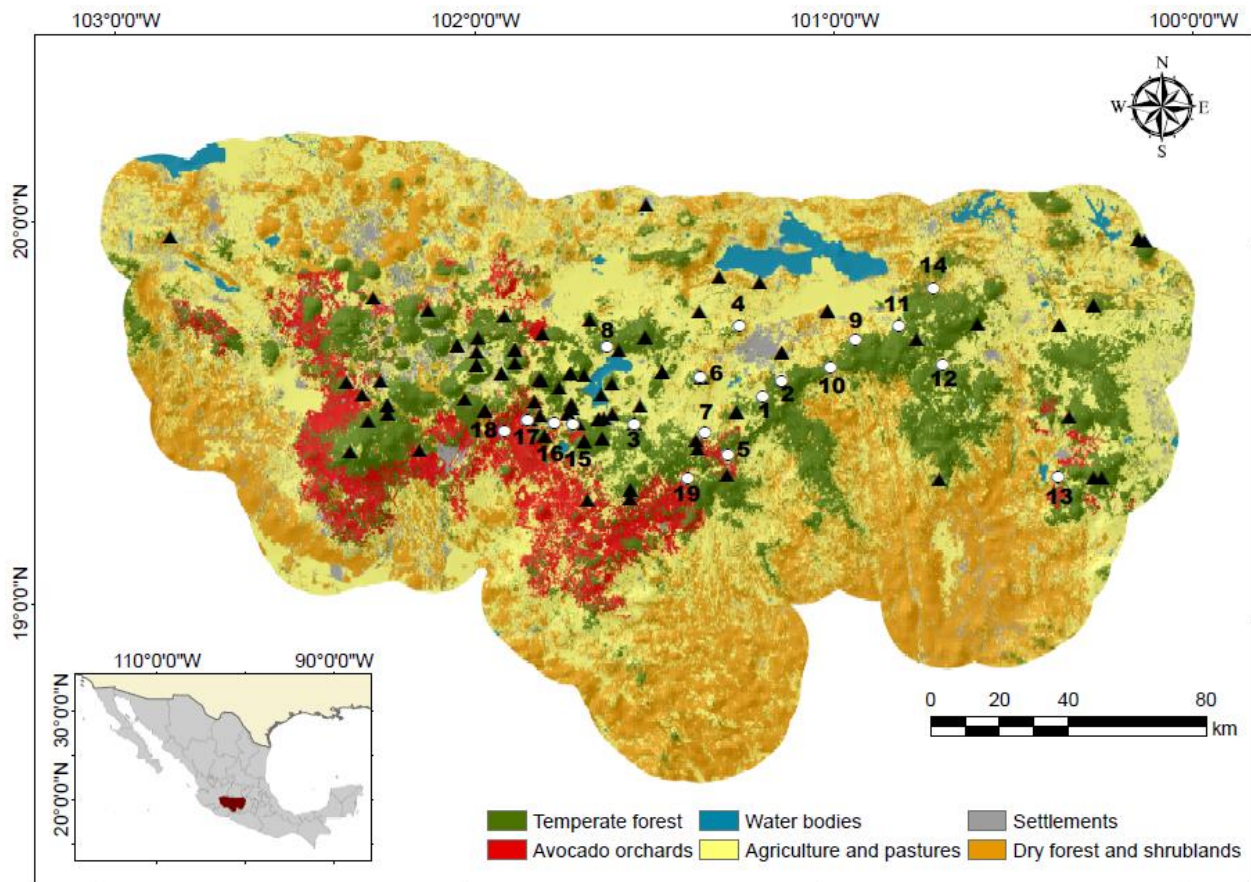


Figure 1. Land-use and land-cover map bases on 2019 satellite images of the Avocado Belt located in the state of Michoacán, Mexico, showing six land-use classes. White dots represent the 19 collected locations of *Pinus leiophylla* and black triangles show the location of presence records of the species from the National Herbarium of Mexico (MEXU) database.

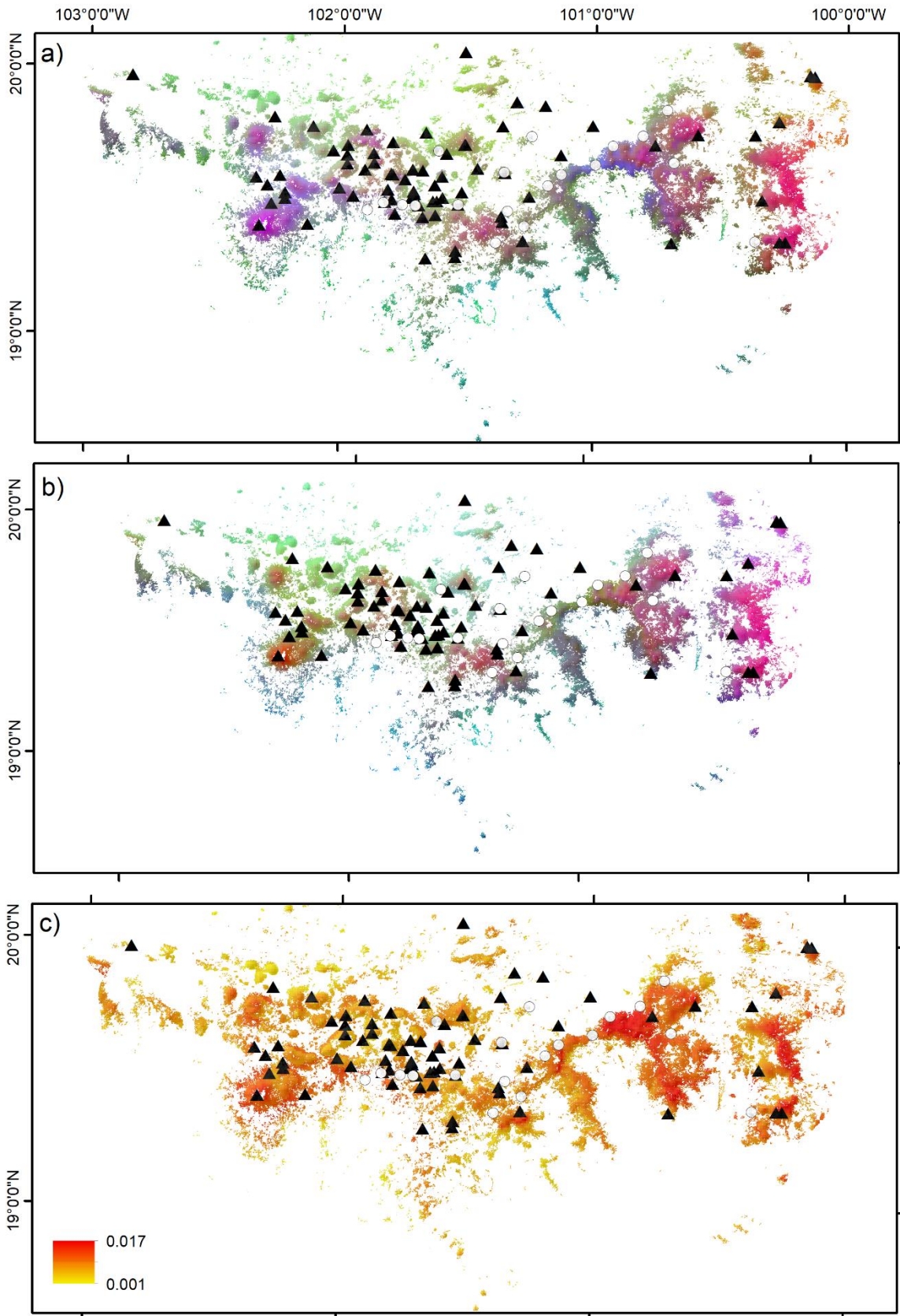


Figure 2. Predicted spatial allele turnover of *P. leiophylla* from Gradient Forest analysis for (a) full and (b) outlier set of SNPs within temperate forest patches. Colors in (a) and (b) represent the genetic turnover based on the modeled relationships with environmental and spatial variables. Similar colors represent localities with similar expected genetic composition (colors are not comparable between panels). (c) Procrustes residuals from comparison between the models from the outlier set and the full set of SNPs. Bright red indicates larger differences. White dots represent the 19 collected locations of *Pinus leiophylla* and black triangles show the location of presence records of the species from the National Herbarium of Mexico (MEXU) database.

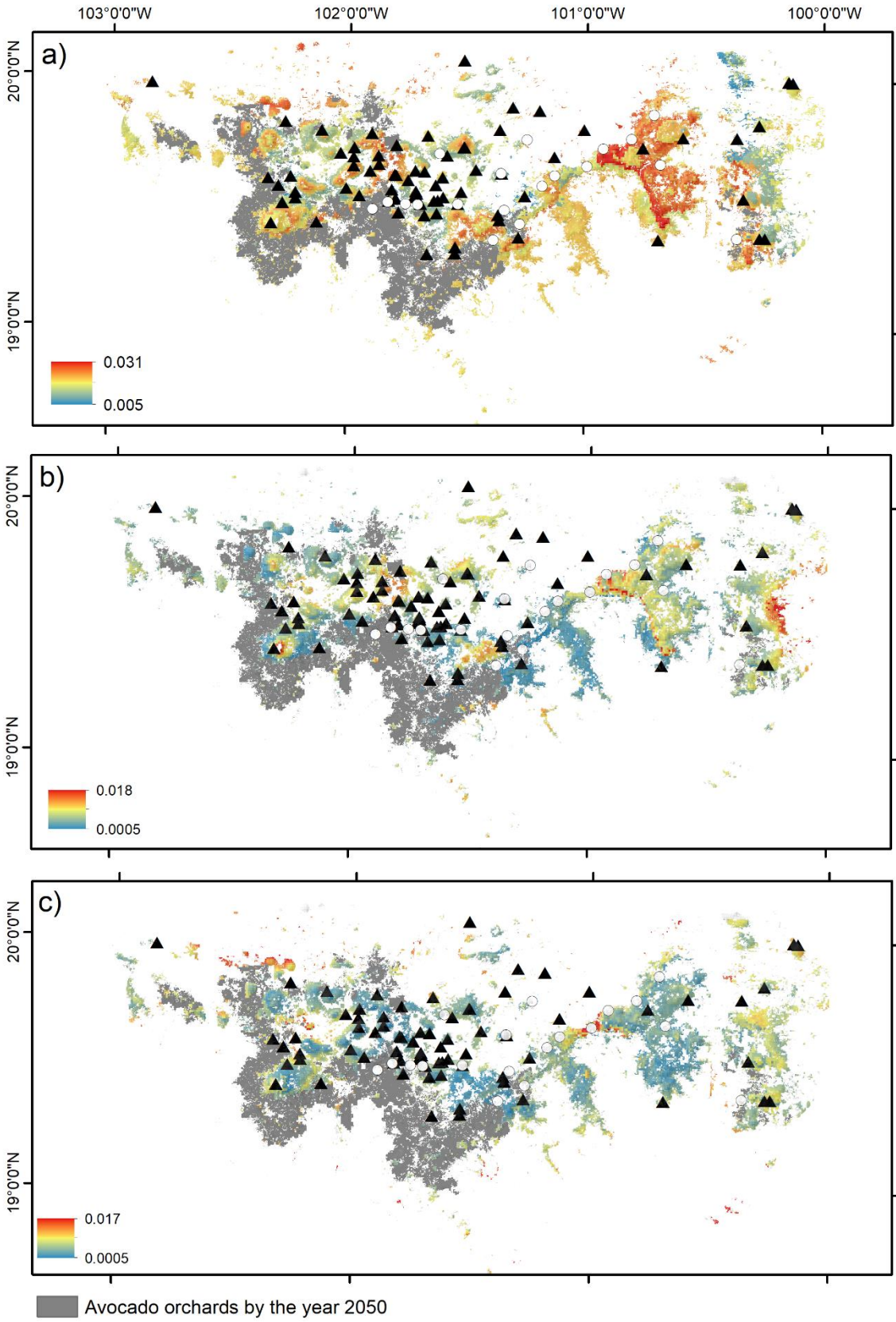


Figure 3. Local (a), forward (b), and reverse offset (c) of *Pinus leiophylla* estimated from the set of outlier SNPs within the predicted future temperate forest patches. The offset values were calculated for 2050 under the Miroc6_ssp370 climate scenario. Gray areas represent the projected extent of avocado orchards for the year 2050 according to our land use model. White dots represent the 19 collected locations of *Pinus leiophylla* and black triangles show the location of presence records of the species from the National Herbarium of Mexico (MEXU) database.

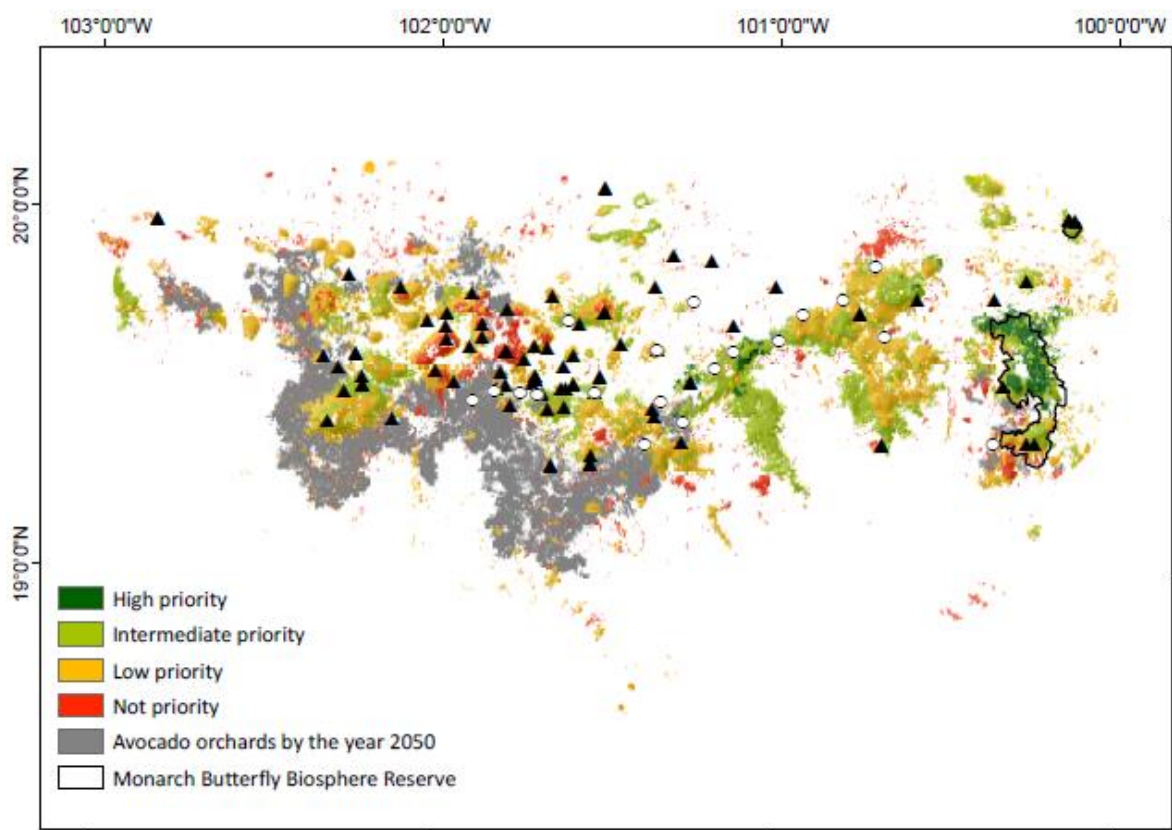
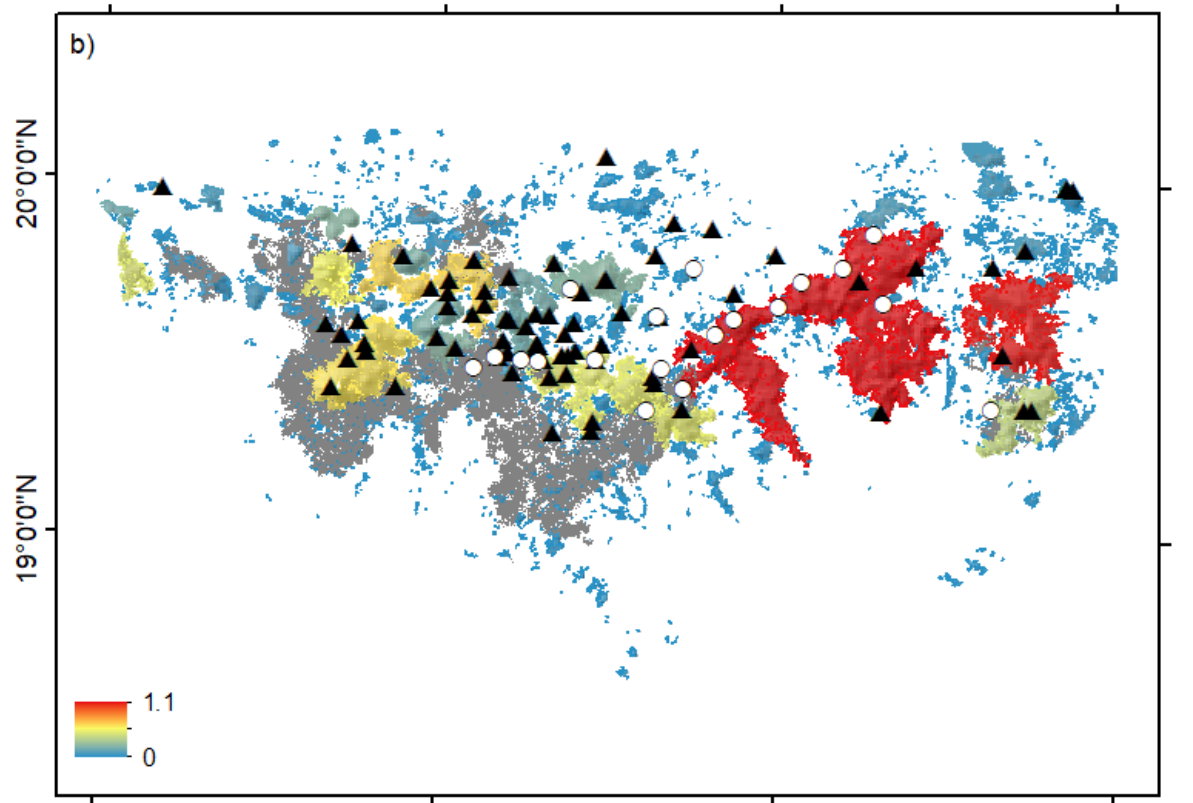
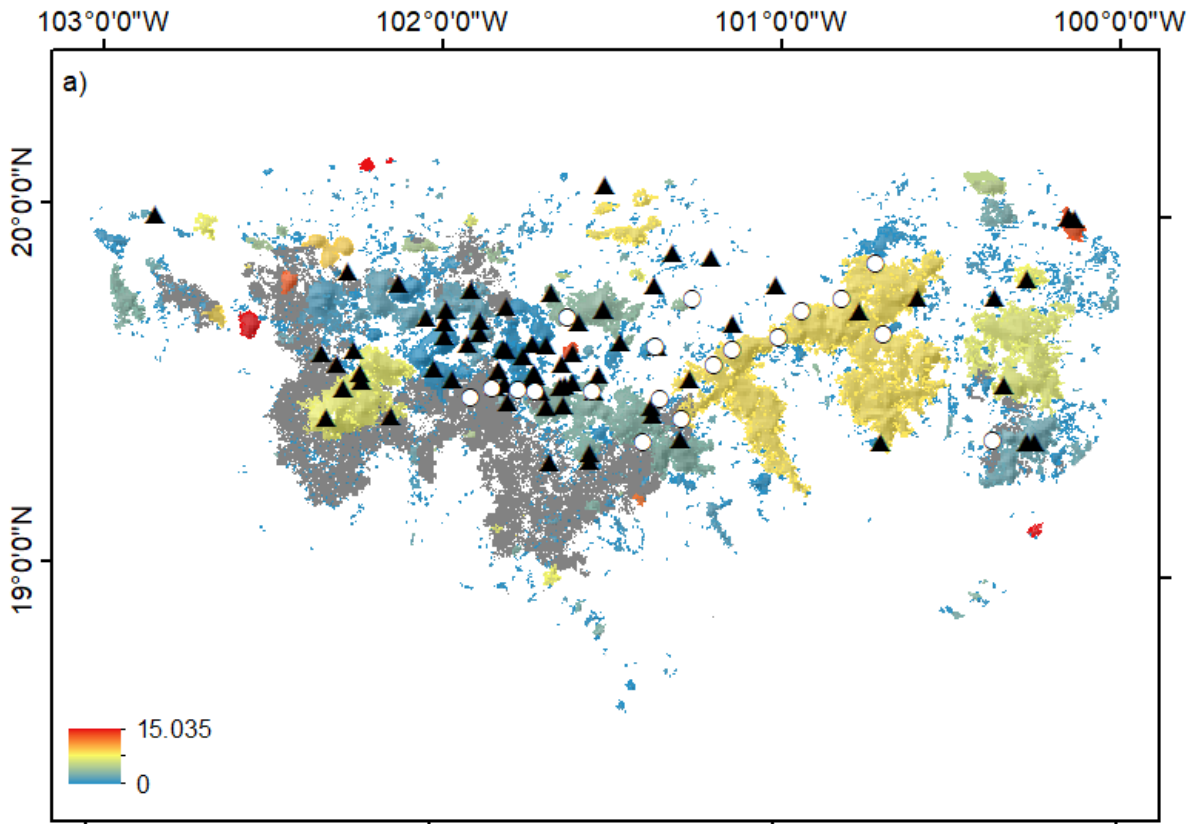


Figure 4. Prioritization of areas for conservation considering local offset, connectivity from seed and pollen dispersal and avocado orchards extent by 2050. Gray areas represent the projected extent of avocado orchards for the year 2050 according to our land use model. White dots represent the 19 collected locations of *Pinus leiophylla* and black triangles show the location of presence records of the species from the National Herbarium of Mexico (MEXU) database.



Avocado orchards by the year 2050

Figure 5. Composite Connectivity Index (CCI) values by the year 2050 for (a) seed dispersal scenario and (b) pollen dispersal scenario within the predicted temperate forest areas. Gray areas represent the projected extent of avocado orchards for the year 2050 according to our land use model. White dots represent the 19 collected locations of *Pinus leiophylla* and black triangles show the location of presence records of the species from the National Herbarium of Mexico (MEXU) database.

Table 1. Partitioning of genetic variation into environmental (env), geographic (geo) and their shared variance based on redundancy analysis (RDA). Adjusted R^2 are reported.

	Full set	Outlier set	$R^2 > 0$ set
Combined fractions			
F ~ geo	-0.005 ns	-0.003 ns	0.029 ns
F ~ env	0.014 ns	0.035 ns	0.182**
Individual fractions			
F ~ geo. env	0.014 ns	0.009 ns	0.053 ns
F ~ env geo	0.034 ns	0.048*	0.207**
Shared	-0.019	-0.013	-0.024
Residual	0.971	0.955	0.763

Note: Asterisks indicate statistical significance (* $p < .05$, ** $p < .01$, *** $p < .001$, ns nonsignificant).

F = dependent matrix of localities allele frequencies. RDA tests are of the form: F ~ dependent matrices | covariate matrices. Env = environment (PCs 1-3), geo = geography (x, y).

Table 2. Information from models based on the full and outlier SNP datasets used to perform the gradient forest analysis.

SNP sets	Number of SNPs	SNPs with $R^2 > 0$	Mean % R^2 [range]
Full set	3660	392	11.71 [0.016-48.76]
Outlier	130	15	10.94 [0.86-30.62]

SUPPLEMENTARY TABLES AND FIGURES

Supplementary table 1. Basic information of the 19 localities of *Pinus leiophylla* collected across the Avocado Belt in Michoacán, Mexico.

Id.	Locality	No. of individuals	Latitude	Longitude	Elevation (m)	Annual mean temperature (°C)	Annual precipitation (mm)
1	Atécuaro	3	19.5786	-101.1866	2283	15.85	1045
2	San Miguel del Monte	4	19.621	-101.134	2179	16.05	1010
3	Cerro los Lobos	4	19.503	-101.541	2502	15.23	1018
4	Umécuaro	4	19.7627	-101.2522	2483	15.61	947
5	La Paloma	5	19.42623	-101.2796	2122	16.15	1032
6	El Remolino	3	19.6269	-101.3594	2877	14.16	1074
7	Tamanguío	5	19.4816	-101.345	2132	16.47	975
8	Coeneo	5	19.71839	-101.619955	2244	16.23	829
9	Las Huertas	2	19.730053	-100.930372	2213	15.45	1151
10	Pontezuelas	4	19.658813	-100.999695	2122	16.35	1100
11	Pueblo viejo	5	19.769209	-100.811401	2251	15.45	1075
12	Cruz de caminos	5	19.668909	-100.688751	2178	15.26	1044
13	Cerro de la campana	5	19.3751	-100.3662	2168	15.55	1003
14	Ucareo	5	19.866472	-100.716644	2384	14.68	980

15 Ajuno	5	19.497982 -101.709480	2183	16.38	995
16 Huiramangaro	3	19.500492 -101.761032	2269	15.93	1011
17 Tingambato	4	19.506851 -101.836060	2159	16.46	1032
18 Zurumucapio	4	19.479477 -101.899239	1819	17.80	965
19 Cruz del plato	2	19.361502 -101.390411	2423	14.80	1100

Supplementary table 2. Observed heterozygosity (H_o), gene diversity (H_s), inbreeding index (F_{IS}) and number of private alleles (PA) for the 19 localities of *Pinus leiophylla*.

Id.	Locality	H_o	H_s	F_{IS}	PA
1	Atécuaro	0.154	0.141	0.027	0
2	San Miguel del Monte	0.157	0.15	0.012	1
3	Cerro los Lobos	0.154	0.138	0.055	0
4	Umécuaro	0.145	0.134	0.033	0
5	La Paloma	0.157	0.142	0.05	0
6	El Remolino	0.156	0.143	0.032	0
7	Tamanguío	0.156	0.138	0.065	0
8	Coeneo	0.153	0.145	0.021	3
9	Las Huertas	0.148	0.127	0.065	0
10	Pontezuelas	0.145	0.14	0.003	3
11	Pueblo viejo	0.163	0.157	0.012	0
12	Cruz de caminos	0.154	0.147	0.024	5
13	Cerro de la campana	0.16	0.154	0.014	0
14	Ucareo	0.147	0.14	0.024	0
15	Ajuno	0.156	0.147	0.031	0
16	Huiramangaro	0.153	0.14	0.034	0
17	Tingambato	0.152	0.14	0.012	0

18	Zurumucapio	0.142	0.151	-0.061	6
19	Cruz del plato	0.154	0.154	-0.08	0
	Global	0.144	0.153	0.06	

Supplementary table 3. Summary of AMOVA results among and within 19 localities of *P. leiophylla*.

Source of variation	Degree of freedom	Sum of squares	of Variance component	Percentage of variation
Among localities	18	5781.987	7.84836	2.95
Within localities	135	34806.117	257.82309	97.05
Total	153	40588.104	265.67144	

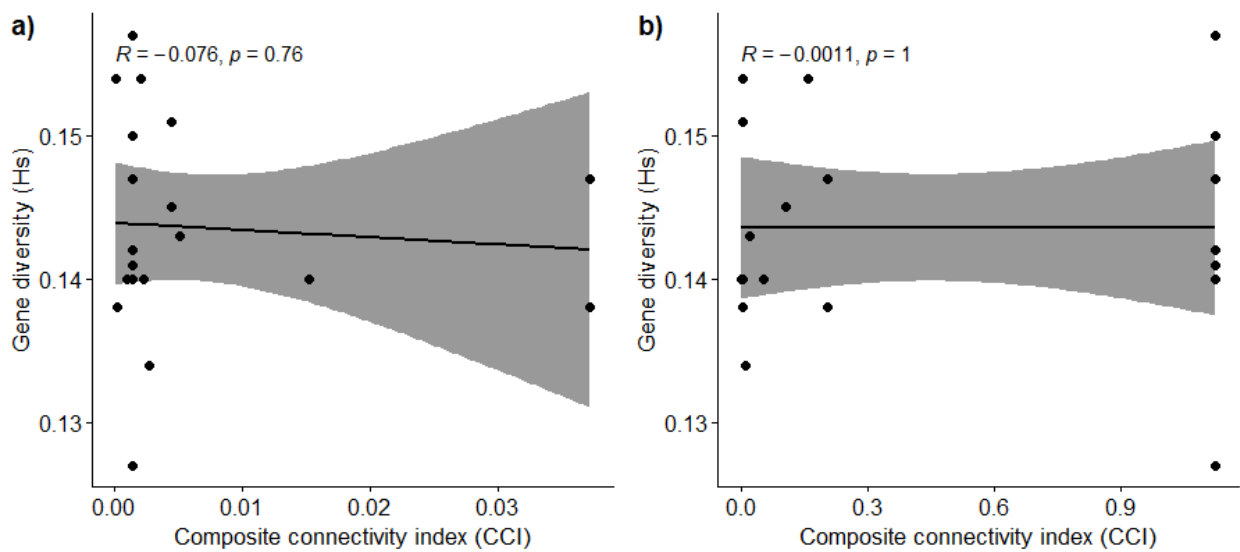
Supplementary table 4. Number of SNPs associated with each environmental variable with the LFMM analysis.

Environmental variable	Number of SNPs associated
Isothermality (BIO 3)	14
Minimum temperature of coldest month (BIO 6)	33
Temperature annual range (BIO 7)	30
Mean temperature of driest quarter (BIO 9)	35
Precipitation seasonality (BIO 15)	18
Precipitation of wettest quarter (BIO 16)	27
Precipitation of driest quarter (BIO 17)	22
Precipitation of warmest quarter (BIO 18)	33
Precipitation of coldest quarter (BIO 19)	26

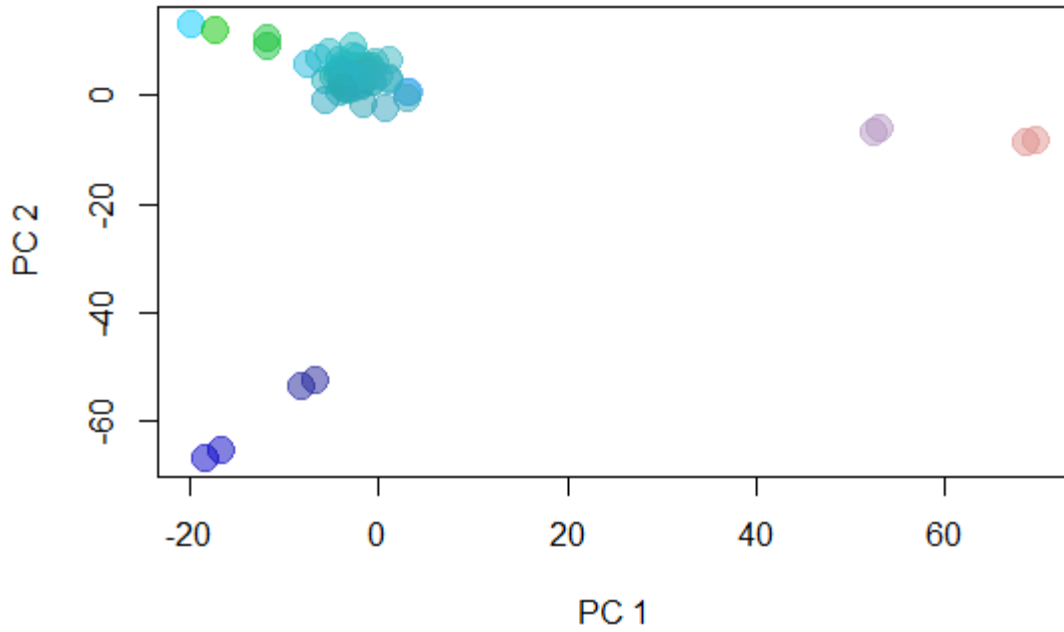
Supplementary table 5. Area in hectares of each patch for each locality for the present and by the year 2050, amount of area that are loss due to avocado expansion and the remaining percentage of the total area.

Id.	Locality	Area in the present (ha)	Area by 2050 (ha)	Amount of area loss (ha)	Remaining percentage of total area by 2050
1	Atécuaro	182562.66	181645.92	916.74	99.50
2	San Miguel del Monte	182562.66	181645.92	916.74	99.50
3	Los Lobos	62343.81	57660.3	4683.51	92.49
4	Umécuaro	1386.81	1386.81	0	100
5	La Paloma	182562.66	181645.92	916.74	99.50
6	El Remolino	2670.12	2670.12	0	100
7	Tamanguío	46.17	22.86	23.31	49.51
8	Coeneo	22565.7	22565.7	0	100
9	Las Huertas	182562.66	181645.92	916.74	99.50
10	Pontezuelas	182562.66	181645.92	916.74	99.50
11	Pueblo viejo	182562.66	181645.92	916.74	99.50
12	Cruz de caminos	182562.66	181645.92	916.74	99.50
13	Cerro de la campana	23554.08	22211.01	1343.07	94.30
14	Ucareo	23.49	23.49	0	100
15	Ajuno	62343.81	57660.3	4683.51	92.49

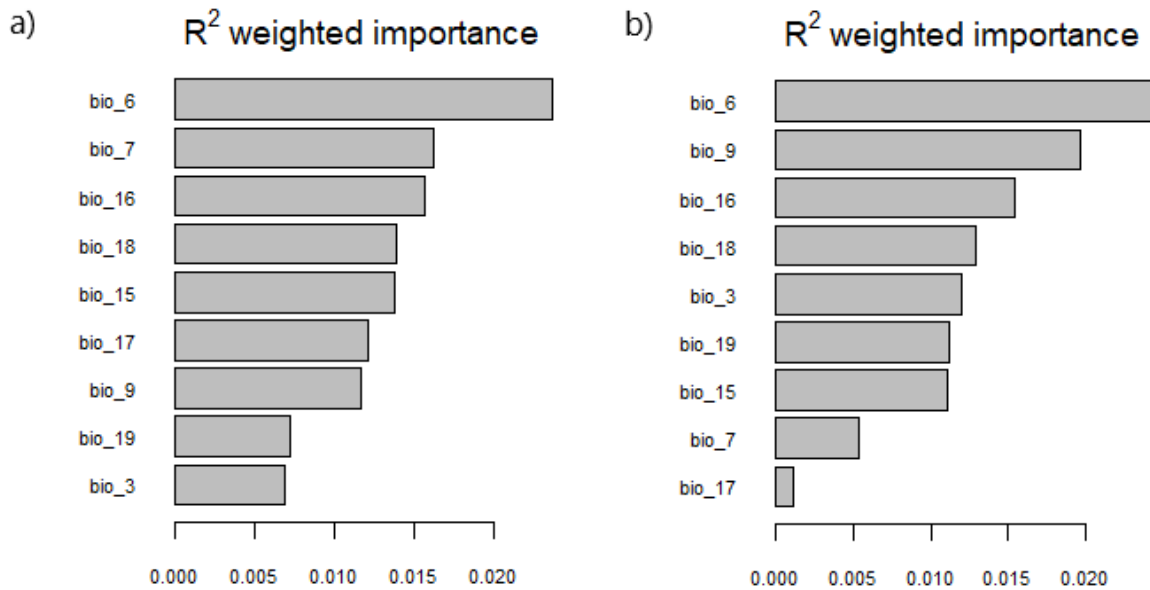
16	Huiramangaro	25.83	14.13	11.7	54.70
17	Tingambato	4481.1	221.31	4259.79	4.94
18	Zurumucapio	25.02	0	25.02	0.00
19	Cruz del Plato	31.14	28.35	2.79	91.04



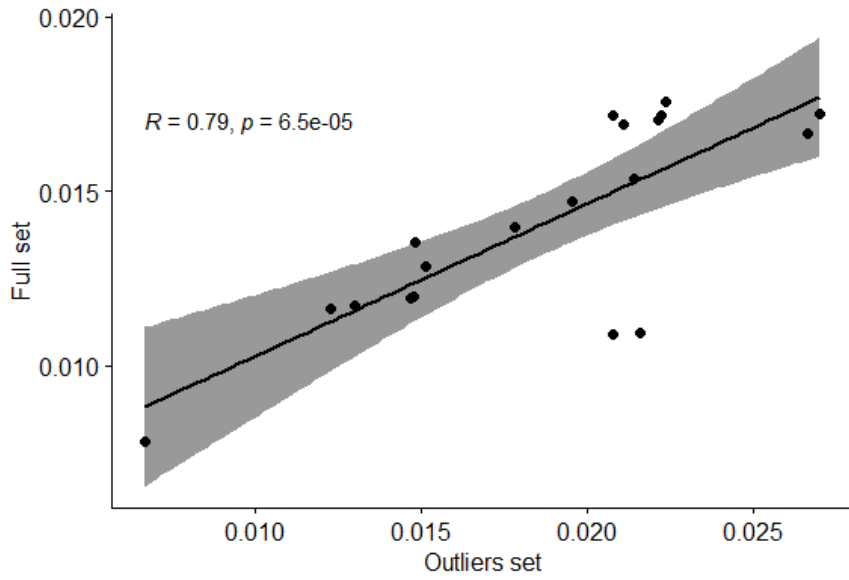
Supplementary figure 1. Correlation between gene diversity (H_s) values and composite connectivity index values for the 19 localities of *Pinus leiophylla* under (a) seed dispersal and (b) pollen dispersal scenarios.



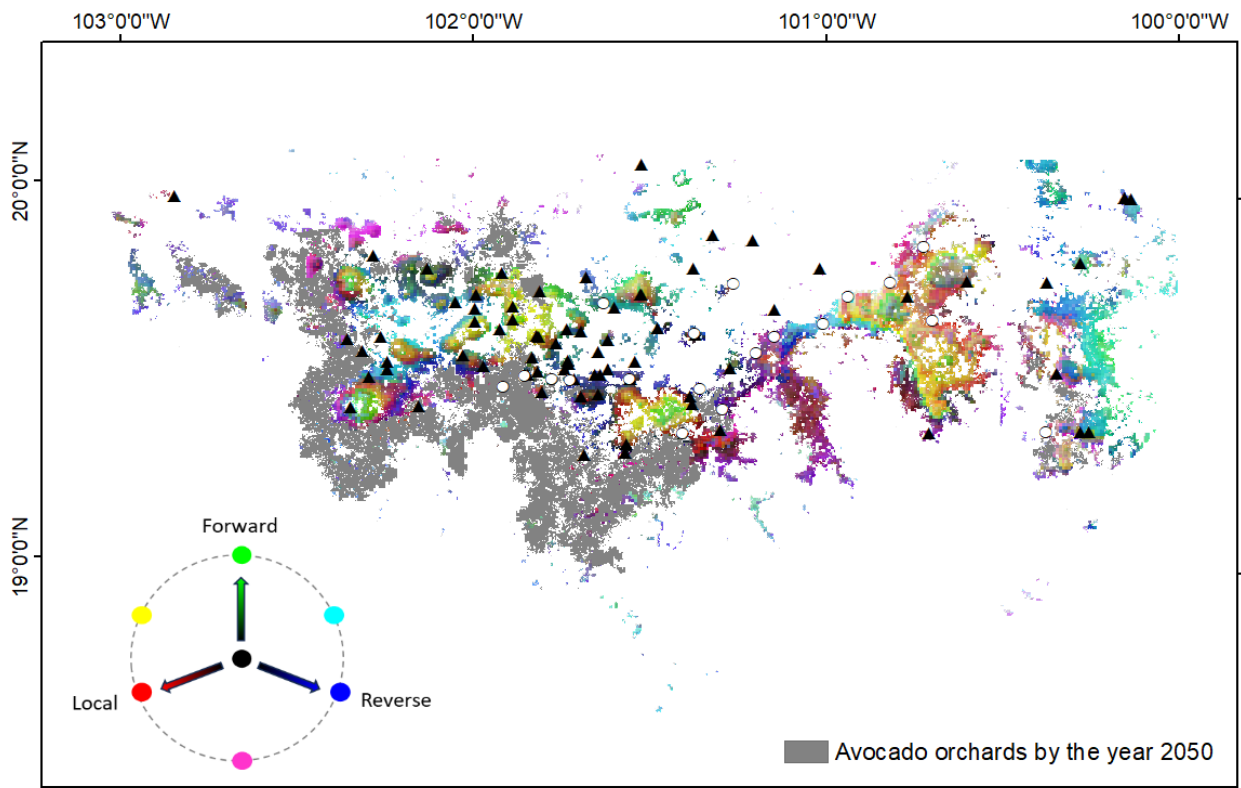
Supplementary figure 2. Principal component analysis (PCA) of *P. leiophylla* based on the 3660 SNPs. The first two principal components accounted for the 3.12 and 2.9% of the total variance, respectively.



Supplementary figure 3. Rank variable importance for (a) full and (b) outlier sets used in the gradient forest analysis.



Supplementary figure 4. Correlation between local genomic offset values between the full and outliers set models.



Supplementary figure 5. RGBplot of local, forward and reverse offset within the predicted temperate forest areas, darker colors mean lowest values, while brighter colors (closer to white) mean highest values. White dots represent the location of the 19 collected localities of *P. leiophylla* and black triangles show the location of the populations identified in the National Herbarium of Mexico (MEXU) database.

Discusión general y conclusiones

Discusión general

El cambio climático y el cambio de uso de suelo son dos de las principales causas de la pérdida de biodiversidad a nivel global; sin embargo, los efectos de ambos disturbios raramente se han modelado juntos. En este trabajo, se evaluó la vulnerabilidad climática de 19 poblaciones de *Pinus leiophylla* considerando simultáneamente escenarios de cambio climático y la creciente expansión de cultivos de aguacate a través de la franja aguacatera en el estado de Michoacán. Se encontró que algunas poblaciones localizadas en la parte centro y este de la región de estudio serán las más afectadas por los efectos del cambio climático, requiriendo una mayor cantidad de cambio genético para poder tener la misma asociación genotipo-ambiente que en la actualidad. Por otro lado, las poblaciones localizadas en la parte oeste del área de estudio serán las más vulnerables a los efectos de la fragmentación y pérdida de hábitat, dado que en esta zona se proyecta una mayor expansión del cultivo de aguacate para el año 2050, provocando la completa extinción de poblaciones como Zorumucapio y disminuyendo la conectividad entre las mismas. Bajo este escenario, la diversidad genética de las poblaciones de *P. leiophylla* dentro de la zona de estudio se encontrará amenazada por ambos disturbios antropogénicos, provocando una mayor susceptibilidad ante los nuevos cambios ocurridos en el ambiente al disminuir la capacidad evolutiva de las poblaciones, el tamaño efectivo (N_E) y aumentar los efectos negativos de la endogamia y la deriva génica. Para evitar el riesgo de extinción, será necesario realizar estrategias de flujo génico asistido a aquellos sitios donde se tiene predicho un menor valor de GO para poder conservar la diversidad genética que estas poblaciones albergan. Otra estrategia de conservación que podría implementarse sería promover con los agricultores el mantener franjas de bosque dentro de sus huertas de aguacate, permitiendo de este modo la subsistencia de aquellas poblaciones de *P. leiophylla* que se encuentren amenazadas por la expansión de este cultivo.

También encontramos que la zona este de la distribución será aquella con mayor prioridad de conservación tomando en cuenta bajos valores de GO, altos niveles de conectividad y

que sean menos afectadas por la pérdida de hábitat y fragmentación a causa del cultivo de aguacate. Esta zona es de gran relevancia dado que abarca la Reserva de la Biósfera de la Mariposa Monarca (RBMM), la cual es un área de gran importancia tanto ecológica, por las interacciones biológicas que alberga, como socioeconómica al proveer de numerosos recursos ecosistémicos a los municipios aledaños. Sin embargo, es fundamental preservar también las áreas adyacentes que permitan la conectividad entre las mismas y evitar el aislamiento de las poblaciones provocando efectos negativos por deriva génica y endogamia, además de establecer leyes que eviten la deforestación a causa de la tala ilegal y la sobreexplotación de especies arbóreas en estas zonas.

Así mismo, en futuros estudios también será importante medir el efecto tanto del cambio climático como de la fragmentación en conjunto ya que estos no actúan por separado, por el contrario, existe un efecto sinérgico, donde el impacto de los estresores es mayor que la suma de cada uno actuando individualmente (Zala & Penn, 2004). Mientras que el cambio climático provoca adaptaciones debido a distintos estresores como puede ser cambios en temperatura y precipitación, la pérdida y fragmentación del hábitat también ejercen presiones sobre las poblaciones, como el efecto de borde, extinciones locales y pérdida de diversidad genética, comprometiendo o reforzando la adaptación climática (Cheptou et al., 2017; Van Deale et al., 2023). Para entender la interacción entre ambos componentes es necesario estudiar cómo las especies responderán ante el cambio climático en paisajes fragmentados y cómo esta interacción afectará su éxito de supervivencia (Opdam & Wascher, 2004). Para esto se debe tomar en cuenta los efectos sinérgicos de ambos disturbios sobre la cantidad y distribución de la diversidad genética neutral y adaptativa, la cual nos podría informar sobre las presiones selectivas ejercidas en conjunto en las poblaciones a causa de ambos disturbios y su influencia en su potencial adaptativo. Además, también es necesario incorporar sus dinámicas poblacionales, como es el grado de conectividad entre las mismas lo que es vital para su futura conservación. Por ejemplo, en un trabajo realizado por Van Daele et al. (2023), en el que se evaluó la influencia conjunta de la adaptación climática y la fragmentación del hábitat sobre una especie herbácea del sotobosque (*Primula elatior*), se encontró un conjunto único de SNPs atípicos en áreas con

un mayor grado de fragmentación, en comparación con los SNPs característicos de áreas más conectadas, lo cual sugiere distintas presiones de selección en hábitats más fragmentados, además de encontrar una menor cantidad de diversidad genética relacionada con el clima, confiriéndoles un menor potencial adaptativo y haciéndolas más vulnerables a las presiones ocasionadas por el cambio climático.

De igual modo, es importante validar rigurosamente aquellos métodos utilizados para medir el efecto del cambio climático sobre las poblaciones, como es el método de Gradient Forest (Ellis et al., 2012) a través del GO. El GO nos indica la cantidad de cambio genético necesario para mantener la misma asociación genotipo-ambiente de la población ante escenarios futuros de cambio climático. No obstante, se deben tener consideraciones al momento de aplicar este método en nuestros estudios. Primero, se suele asumir que un mayor valor de GO resultará en un declive en la adecuación, no obstante, para una mejor aproximación es necesario estimar la relación entre las frecuencias alélicas, el fitness poblacional y los efectos del cambio climático, asegurándonos de conocer la magnitud de dichas predicciones sobre la supervivencia de las poblaciones (Ahrens et al., 2023). Para esto, es necesario realizar experimentos de jardín común en los que se tengan identificados aquellos genes sujetos a selección por los efectos del cambio climático y estimar el umbral de respuesta de las poblaciones, sin embargo, realizar en especies de vida larga como los pinos puede ser complicado debido al largo tiempo generacional de este grupo, así como de los recursos necesarios para llevarlos a cabo. Otro aspecto importante es que la supervivencia de los individuos no está mediada por un único gen, al contrario, la adaptación está determinada por múltiples genes con efectos que varían de pequeños a considerables, formando una red de interacción entre distintos componentes de la adecuación (Savolainen et al., 2013), por lo que evaluar esto es aún más complicado, en especial para especies no modelo donde muchas veces no se tiene la información necesaria para estudiar dichas interrelaciones entre los genes.

A pesar de esto, las validaciones que se han realizado a partir de las estimaciones del GO utilizando experimentos de jardín común, loci de rasgos cuantitativos y características fenotípicas, han demostrado una correlación entre valores altos de GO y un declive en la

supervivencia de las poblaciones, demostrando la confiabilidad de este método para predecir el riesgo de mal-adaptación de las poblaciones (Capblancq & Forester, 2021; Fitzpatrick et al., 2021; Lachmuth et al., 2023), brindando una mayor confiabilidad en la aplicación de estos modelos. En este trabajo nosotros recomendamos realizar futuros estudios que analicen a detalle la teoría detrás de la aplicación del GO, para tener una mayor comprensión del significado biológico de estos modelos y evitar la malinterpretación de estos. Además, recomendamos ampliar el estudio realizando experimentos de jardín común que nos permitan evaluar el éxito reproductivo de trasladar a los individuos exponiéndolos a condiciones ambientales similares que se predice tendrán en el clima futuro en el sitio receptor, evaluando su éxito de supervivencia y reproducción.

Conclusión general

En este trabajo incorporamos la información genética de las poblaciones, el cambio de uso de suelo en la franja aguacatera en el estado de Michoacán, así como métricas de conectividad con el objetivo de evaluar la respuesta de las poblaciones de *P. leiophylla* ante el cambio climático y el cambio de uso de suelo. Los resultados mostraron que las poblaciones ubicadas en la zona centro-este de la distribución serán las más afectadas por los efectos del cambio climático, mientras que las poblaciones en la zona oeste se verán más perturbadas por el cambio de uso de suelo a causa de la expansión del cultivo de aguacate para el año 2050. Así mismo, encontramos que la zona que abarca la Reserva de la Biósfera de la Mariposa Monarca será la menos afectada por ambos disturbios, además de presentar los valores más altos de conectividad, por lo que sugerimos enfocar las acciones de conservación hacia esta zona y en los fragmentos de bosques aledaños, permitiendo que ocurran los procesos ecológicos que estos sitios albergan. En este trabajo incorporamos distintas fuentes de información para evaluar el grado de vulnerabilidad de las poblaciones de *P. leiophylla* incorporando estrategias de conservación que permitan la futura persistencia de la especie y de los bosques de pino-encino dentro del estado de Michoacán.

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