



UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE INVESTIGACIONES EN ECOSISTEMAS Y SUSTENTABILIDAD

ECOLOGÍA Y MANEJO INTEGRAL DE ECOSISTEMAS

GENÉTICA DEL PAISAJE DE *Quercus castanea* Née (Fagaceae)

EN LA CUENCA DE CUITZEO, MICHOACÁN.

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

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CENTRO DE INVESTIGACIONES EN GEOGRAFÍA AMBIENTAL, UNAM

MORELIA, MICHOACÁN, MARZO, 2021.



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M. en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
Presente

Me permito informar a usted que en la reunión ordinaria del Comité Académico, del Posgrado en Ciencias Biológicas, celebrada el día **09 de noviembre de 2020**, se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la estudiante **LARA DE LA CRUZ LIBNY INGRID**, con número de cuenta **508019490**, con la tesis titulada. "**Genética del Paisaje de *Quercus castanea* Née (Fagaceae) en la Cuenca de Cuitzeo, Michoacán.**", realizada 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.

ATENTAMENTE
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Cd. Universitaria, Cd. Mx., a 04 de febrero de 2021

COORDINADOR DEL PROGRAMA



DR. ADOLFO GERARDO NAVARRO SIGÜENZA



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-Isabel Allende

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RESUMEN

Los árboles son especies clave en la estructura y funcionamiento de muchos ecosistemas terrestres, por lo que su manejo y conservación es fundamental debido a la importancia ecológica y económica que tienen. Las especies arbóreas han desarrollado diferentes niveles de tolerancia a condiciones ambientales variables, lo cual se ve reflejado en adaptaciones fenotípicas (con bases genéticas) a gradientes ambientales en diferentes escalas espaciales. La genética del paisaje es un área de estudio que tiene como objetivo proporcionar información acerca de la interacción entre las características del paisaje (incluyendo variables ambientales como temperatura o precipitación y accidentes geográficos como montañas, ríos, cuencas, valles) y procesos micro evolutivos (flujo génico, deriva génica, selección natural) y su influencia sobre la cantidad y la distribución espacial de la variación genética. Actualmente, algunos grupos de árboles de bosque templado, en especial los géneros *Quercus*, *Fagus* y *Castanea*, han surgido como “clados modelo” para realizar estudios con un enfoque de genética del paisaje, debido a que muchas especies de estos grupos tienen áreas extensas de distribución en ambientes heterogéneos con diferentes presiones selectivas, lo que influye en su alta variación fenotípica y genética. México es un importante centro de diversificación del género *Quercus*, con alrededor de más de 160 especies (32-40% de las especies a nivel mundial), de las cuales más de 100 son endémicas. En el presente trabajo se analizó de qué manera las variables ambientales y las características topográficas afectan la variación en atributos fenotípicos funcionales, en la variación y diferenciación genética neutra y potencialmente adaptativa, así como en la conectividad funcional en el encino rojo mexicano *Quercus castanea* a una escala de paisaje. El estudio se realizó en la cuenca del

lago de Cuitzeo en Michoacán, que tiene un área de 4 026 km² y que se caracteriza por condiciones ambientales y topográficas altamente heterogéneas. Los resultados mostraron que existe una diferenciación significativa en los atributos funcionales estudiados entre las poblaciones, particularmente en el grosor foliar. Se encontraron niveles altos de diversidad genética y baja estructura genética, lo que sugiere que existen altas tasas de flujo génico entre las poblaciones. También se encontró que la estacionalidad de la precipitación, el intervalo anual de la temperatura y la precipitación anual son variables ambientales que influyen en los patrones de flujo génico, probablemente debido a su efecto sobre la fenología floral. Se identificaron 8 SNPs que presentaron señal de selección divergente y 194 SNPs asociados con la variación climática, algunos de estos SNPs se encuentran en genes que están relacionados con la respuesta al estrés por temperatura, estrés oxidativo, estrés osmótico, germinación y floración. En conjunto los resultados de este trabajo muestran evidencia de adaptación local, tanto a nivel fenotípico como genético a la escala estudiada.

ABSTRACT

Trees are key species in many terrestrial ecosystems and their management and conservation is essential due to their ecological and economic importance. Tree species have developed tolerance to different environmental variables, which can be observed through phenotypic adaptations (with genetic basis) to environmental gradients at various spatial scales. Landscape genetics is a research field that aims at providing information about the interaction between landscape characteristics (environmental variables such as temperature and precipitation or geographic accidents such as mountains, rivers, basins, valleys) and micro evolutionary processes (gene flow, genetic drift and natural selection) and their influence on the amount and spatial distribution of genetic variation. Recently, temperate forest trees, particularly tree genera such as *Quercus*, *Fagus* and *Castanea*, have emerged as “model clades” to carry out studies with a landscape genetics and landscape genomics approach. This is because many species have broad geographic distribution ranges with different environmental conditions and therefore, they are subjected to different selective pressures, which leads to a great phenotypic and genetic variation. Mexico is an important diversification center for the oaks (*Quercus*), with more than 160 species (32-40% of the species worldwide), of which more than 100 are endemic. In this study, it was analyzed how environmental variables and topographic features affect functional trait variation, neutral and potentially adaptive genetic variation, genetic differentiation and functional connectivity in the Mexican red oak *Quercus castanea* at a landscape scale. This study was carried out in the Cuitzeo lake basin in Michoacan, which has an area of 4 026 km² and is characterized by highly heterogeneous environmental and topographic conditions. The results showed there is

a significant differentiation in the studied functional traits between the populations, particularly in leaf thickness. High levels of genetic diversity and low genetic differentiation were found, suggesting that there are high rates of gene flow between populations. It was also found that precipitation seasonality, temperature annual range and annual precipitation are environmental variables that affect pollen movement and gene flow, probably because of their effect on flowering phenology. Eight F_{ST} outlier SNPs consistent with divergent selection and 194 SNPs associated with climatic variation were detected, some of these SNPs are found in genes that are associated to response to temperature stress, oxidative stress, osmotic stress, germination and flowering. Overall, the results of this study indicate evidence of local adaptation at both phenotypic and genetic levels at the spatial scale studied.

INTRODUCCIÓN GENERAL

Importancia de las especies arbóreas y su ambiente climático

Los árboles son organismos sésiles y longevos y constituyen un grupo funcional muy importante de plantas, ya que son organismos clave en muchos ecosistemas terrestres y tienen particular interés para el manejo y la conservación. Los árboles constituyen aproximadamente el 82% de la biomasa continental, proporcionan numerosos servicios ecosistémicos y son la base estructural de alrededor del 50% de la biodiversidad terrestre (Neale y Kremer, 2011). Por lo tanto, es muy importante entender el origen y mantenimiento de su diversidad genética y su potencial adaptativo (Kremer *et al.* 2012; Alberto *et al.* 2013; Sork *et al.* 2013; Kettle 2014; Porth *et al.* 2014; Roschansky *et al.* 2016).

La precipitación y la temperatura son consideradas las variables ambientales responsables de moldear la distribución y la diversidad de las plantas a una escala global. Ambas variables comúnmente presentan diferentes patrones de variación en forma de gradientes ya sea longitudinales, latitudinales o altitudinales (Clarke y Gaston, 2006; De Frenne *et al.* 2013). Por otra parte, una clina se puede definir como el cambio gradual de un rasgo fenotípico en un gradiente ambiental (Zobel y Talbert 1994, Morgenstern 1996). Para una especie dentro de su área natural de distribución puede haber una serie de clinas para diferentes características (Zobel y Talbert 1994) y tales clinas pueden ser concordantes o no. La variación adaptativa en los árboles frecuentemente está arreglada de manera clinal, principalmente en respuesta a gradientes climáticos (García-Gil *et al.* 2003). La diferenciación adaptativa entre poblaciones de árboles de la misma especie ha sido documentada a través del estudio de la variación clinal en rasgos fisiológicos, fenológicos, fenotípicos y genéticos en relación con gradientes climáticos (Manel *et al.* 2010).

Por lo mencionado anteriormente, es importante conocer y entender cómo las características del paisaje influyen en la riqueza genética de las poblaciones, para lo que se necesitan datos de diversidad y diferenciación genética, así como también son necesarios datos que evidencien una posible adaptación local. Actualmente se están combinando diversos enfoques (ecológico, genético, fisiológico, entre otros) para estudiar patrones de variación y adaptación a nivel de paisaje, surgiendo nuevas áreas de estudio como es la genética del paisaje.

La genética del paisaje

La genética del paisaje es un campo de estudio que puede considerarse relativamente reciente, ya que surgió formalmente en el año 2003 (Manel *et al.* 2003). Es un área de estudio interdisciplinaria, resultado de la fusión de la genética de poblaciones, la ecología del paisaje y la estadística espacial. Tiene como objetivo proporcionar información acerca de la interacción entre las características del paisaje y procesos micro-evolutivos como el flujo génico, la deriva génica y la selección natural, y su influencia sobre la cantidad y la distribución espacial de la variación genética. Tiene implicaciones para la ecología, la evolución y la biología de la conservación (Manel *et al.* 2003; Storfer *et al.* 2007; Holderegger y Wagner 2008; Manel *et al.* 2010; Sork *et al.* 2010; Schoville *et al.* 2012). El “paisaje” generalmente consiste en variables ambientales (temperatura, precipitación, altitud), accidentes geográficos (montañas, ríos, cuencas, valles), tipos de vegetación, así como elementos antrópicos como carreteras y zonas urbanas (Manel *et al.* 2003; Holderegger y Wagner 2008; Schoville *et al.* 2012; Garrido-Garduño y Vázquez-Domínguez 2013; Rico 2019). Dependiendo de la especie de estudio y la escala espacial a la que se llevan a cabo los

procesos de dispersión y flujo genético de la misma, el área de estudio puede ser muy grande (miles de km²) o pequeña (cientos de m²) (Holderegger *et al.* 2010).

La diversidad genética es un atributo muy importante de las poblaciones ya que determina la capacidad de las mismas para responder a los cambios ambientales (Frankham 2003; Reed y Frankham 2003). Un mecanismo específico para mantener la variación genética es el flujo de genes (Sork y Smouse 2006). El movimiento de genes dentro y entre las poblaciones puede influir de manera significativa en los patrones de diferenciación genética y en la evolución de adaptaciones locales (Sork 2016). Por lo tanto, la genética del paisaje nos ayuda a comprender el patrón espacial de la diversidad genética (Sork y Waits 2010). El objetivo más común de los estudios con este enfoque ha sido identificar características del paisaje o características ambientales que facilitan o limitan la conectividad funcional efectiva, es decir, el flujo génico (Storfer *et al.* 2010; Garrido-Garduño y Vázquez-Domínguez 2013). La capacidad de los investigadores para evaluar la conectividad funcional potencial y la resistencia del paisaje al flujo génico ha mejorado notablemente gracias a la obtención de datos de sistemas de información geográfica de alta resolución (Thomasen *et al.* 2010) y al uso de diferentes metodologías para estimar las superficies de costo basadas en las probabilidades de flujo génico en diferentes tipos de ambientes (McRae 2006). Los efectos del paisaje sobre la distribución de la variación genética neutra pueden ayudar a identificar corredores y barreras al flujo génico, pero la asociación entre los gradientes ambientales y genéticos puede proporcionar evidencia inicial del impacto de la selección natural (Holderegger *et al.* 2006; Manel *et al.* 2010; Sork y Waits 2010).

Algunos estudios han demostrado que la heterogeneidad ambiental tiene un efecto en la variación y diferenciación genética entre las poblaciones de especies arbóreas, lo que resulta en patrones geográficos de variación genética que son congruentes con rasgos fenotípicos (Savolainen *et al.* 2007; Sork *et al.* 2010). Al estudiar asociaciones de tipo genotipo-ambiente se pueden detectar variables ambientales específicas que están moldeando la estructura genética de las poblaciones (Foll y Gaggioti 2008), e incluso identificar loci específicos que pudieran estar bajo selección natural (Joost *et al.* 2007; Sork *et al.* 2010). La identificación de loci bajo selección puede ayudarnos a entender las bases genéticas de la adaptación local y la especiación (Manel *et al.* 2003). La genética de paisaje se encuentra en una fase de rápido desarrollo debido a que la producción de datos genéticos se incrementa exponencialmente y también a la adquisición de datos ambientales y métodos analíticos espaciales que pueden relacionar el paisaje y datos genéticos de forma significativa (Parisod *et al.* 2012; Sommer *et al.* 2013).

En los últimos años, la disponibilidad de análisis de todo el genoma mediante técnicas de secuenciación masiva ha permitido identificar un gran número de loci, incluyendo loci potencialmente adaptativos, incluso en especies no modelo, creando así la oportunidad para el surgimiento de la genómica del paisaje (Segelbacher *et al.* 2010; Sork *et al.* 2013). La genómica del paisaje está incluida dentro de la genética del paisaje. Es un campo de estudio, cuyo objetivo principal es identificar los factores ambientales que ejercen un efecto sobre la variación genética adaptativa y los loci asociados a la adaptación local (Luikart *et al.* 2003; Joost *et al.* 2007; Sork *et al.* 2013). Puede considerarse una fusión de la bioinformática, la genómica, la estadística espacial y la ecología del paisaje (Holderegger *et al.* 2006, 2008; Manel *et al.* 2010; Schoville *et al.* 2012; Bolliger 2014). El enfoque de la genómica del

paisaje se basa en la suposición de que la selección ha ocurrido a través de un período de tiempo suficientemente largo para establecer una relación detectable entre los loci y el ambiente (Joost *et al.* 2007).

Si los recursos y el tamaño del genoma no fuesen factores limitantes, secuenciar el genoma entero sería la mejor estrategia para identificar loci involucrados en la adaptación (Schoville *et al.* 2012; Schwartz *et al.* 2010). Las exploraciones del genoma (escaneos genómicos) son un método atractivo para identificar loci potencialmente adaptativos porque pueden ser fácilmente utilizados en organismos no modelo (Jones *et al.* 2013). Estos escaneos genómicos eliminan la necesidad de conocimiento previo del organismo a estudiar (Bonin *et al.* 2007; Talbot *et al.* 2016). Cuando un locus o un conjunto de loci en un determinado genoma tienen una diferenciación genética (en términos del parámetro F_{ST}) mayor o menor de lo esperado bajo neutralidad sugiere que podrían estar bajo selección y son conocidos como loci “outlier” (Vitti *et al.* 2013). Anteriormente se evaluaba la adaptación local de las especies arbóreas a través de la medición de atributos fenotípicos de individuos de poblaciones que provenían de regiones con climas diferentes en experimentos de jardín común (Savolainen 2007; De Kort *et al.* 2014). Actualmente estos experimentos pueden complementarse con estudios de todo el genoma con el objetivo de encontrar señales de diferenciación adaptativa y que no se confundan con la plasticidad fenotípica (De Kort *et al.* 2014; Lepais y Bacles 2014).

Los árboles, en especial los géneros de bosque templado como son *Quercus*, *Fagus* y *Castanea* están emergiendo como “clados modelo” para estudiar las bases genéticas de la adaptación mediante la integración de la genética, la ecología y la evolución. Muchas

especies de estos géneros tienen áreas de distribución extensas, con diferentes condiciones ambientales. Las diferentes presiones selectivas impuestas por los diversos ambientes ecológicos en los que se desarrollan estas especies, las conduce a presentar una gran variación dentro y entre poblaciones, por lo que se considera que son organismos ideales para develar la base molecular de la divergencia adaptativa en la naturaleza (González-Martínez *et al.* 2006; Petit *et al.* 2013; Cavender-Bares 2019).

El género *Quercus*

Los encinos (*Quercus*, Fagaceae) constituyen un grupo muy diverso que cuenta con aproximadamente 500 especies a nivel mundial. Los encinos se encuentran en casi todos los bosques templados del hemisferio Norte, así como en algunas regiones tropicales y subtropicales del mismo (Valencia 2004). Existen algunas especies que habitan en lugares más secos como el sureste de Asia y el noreste de África. En el continente americano se localizan desde Canadá hasta Colombia, incluyendo Cuba (Valencia 2004). Son el grupo de especies leñosas dominantes de América del Norte, en biomasa y riqueza de especies según los datos del inventario forestal DRYFLOR (Latin American and Caribbean Seasonally Dry Tropical Forest Floristic Network) y de la base de datos del Servicio Forestal de Estados Unidos (US Service) (Banda *et al.* 2016; Cavender-Bares 2016). Todas las especies de encinos tienen en común ciertas características biológicas como son hojas coriáceas, tallos leñosos, la presencia de bellotas, polinización por viento y un crecimiento lento (Nixon 1993). El estudio de los encinos es de gran interés debido a su alta diversidad y a su importancia ecológica (especies dominantes, almacenamiento de carbono, hábitat de una

gran cantidad de plantas epífitas y de animales vertebrados e invertebrados), económica (elaboración de recipientes, pisos, mangos para herramientas, muebles, postes, como carbón vegetal o leña) y cultural (uso medicinal, uso alimenticio) (Nixon 1993; Luna-José *et al.* 2003; Lowe *et al.* 2005; Valencia 2004; Curtu *et al.* 2007).

Se han descrito dos centros de diversificación para el género *Quercus*, el primero se encuentra en el sureste de Asia con 125 especies y el segundo se localiza en México (Nixon 1993, 2008). En nuestro país se encuentran representadas cuatro secciones del género *Quercus*, las cuales son *Quercus* (encinos blancos), *Lobatae* (encinos rojos), *Protobalanus* (encinos intermedios) y *Virentes* (Valencia 2004; Arizaga *et al.* 2009; Cavender-Bares *et al.* 2015; Denk *et al.* 2017). Se estima que la riqueza de especies de encinos en México se sitúa en más de 160 especies, de las cuales 109 son endémicas del país y 47 pertenecen a la sección *Quercus*, 61 a la sección *Lobatae*, cuatro a *Protobalanus* y tres a *Virentes*; por lo tanto, en nuestro país están representadas el 32-40% de las especies del género a nivel mundial (Valencia 2004; Cavender-Bares *et al.* 2015). Sin embargo, la riqueza total de especies de encinos en México ha sido difícil de precisar, debido a su gran variación morfológica, la escasez, carencia e inaccesibilidad del material tipo, además de la sobre descripción de muchas de las especies (Valencia 2004).

La notable heterogeneidad de hábitats y la variabilidad climática y edáfica en la que se desarrollan las especies y poblaciones de encinos, juegan un papel fundamental sobre la variación tanto fenotípica como genética que presentan, a través de su efecto en los procesos demográficos y mediante la selección natural, ya que el clima influye directamente sobre la expansión poblacional, la contracción y la migración, dando como resultado una gran

variación asociada a gradientes climáticos que contribuyen en la especiación, la evolución adaptativa y la divergencia en el género (Gailing *et al.* 2009).

Los encinos y su variación fenotípica y genética

De manera general, los árboles muestran patrones geográficos de variación tanto fenotípica como genética, los cuales están asociados a la adaptación local (Morgenstern 1996; Savolainen *et al.* 2007; Sork 2016). Trabajos con diferentes especies de encinos han mostrado evidencia de adaptación local mediante el estudio de la variación en atributos fenotípicos medidos en campo (Uribe-Salas *et al.* 2008; Albarrán-Lara *et al.* 2015; Lara-De La Cruz *et al.* 2020) y en experimentos de jardín común (Ramírez-Valiente *et al.* 2010, 2018; Yucedag *et al.* 2019). Otros trabajos reportan que los encinos presentan variaciones funcionales inter e intraespecíficas relacionadas con los rasgos foliares, la fenología y las tasas de crecimiento asociadas a la altitud, latitud, disponibilidad de agua o gradientes de temperatura (Bussotti *et al.* 2002; Uribe-Salas *et al.* 2008; Alberto *et al.* 2011; Koehler *et al.* 2012; Chai *et al.* 2015; Ramírez-Valiente *et al.* 2017). Se sugiere que las variaciones fenotípicas y funcionales encontradas en los encinos son el resultado de diferentes estrategias para evitar o tolerar diferentes tipos de estrés ambiental, lo que permite que las especies de encino se desarrollen en una amplia variedad de condiciones ambientales y disponibilidad de recursos.

La diversidad genética poblacional de especies de árboles templados, como los encinos, se encuentra espacialmente estructurada a diferentes escalas: local, de paisaje, regional y continental (Petit *et al.* 2002; Holderegger y Wagner 2008; Sork *et al.* 2010) aunque, en general, debido a la dispersión de polen por viento, las especies de encinos se caracterizan

por tener una alta variación genética dentro de las poblaciones y una baja diferenciación genética entre poblaciones (Albarrán-Lara *et al.* 2010; Aldrich y Cavender-Bares 2011; Peñaloza-Ramírez *et al.* 2010).

Previamente, se han realizado trabajos de genética del paisaje con algunas especies de encinos. Por ejemplo, Ortego *et al.* (2012) estudiaron la asociación entre variables climáticas y la variación y estructura genética espacial de poblaciones de un encino endémico del sur de California, *Q. engelmannii*. Su trabajo sugiere que las condiciones ambientales locales pueden moldear los patrones de estructura y variación genética incluso en especies con alto potencial de flujo génico y rangos de distribución relativamente pequeños.

Ashley y *et al.* (2015) estudiaron los patrones de conectividad genética entre poblaciones de *Quercus lobata*, una especie endémica de California, en Estados Unidos. Concluyeron que los gradientes topográficos y climáticos responsables de la distribución de *Q. lobata* también afectan sus patrones de flujo génico. También con *Q. lobata*, Sork *et al.* (2016) mediante un análisis de SNPs estimaron la diferenciación genética entre poblaciones y correlacionaron las frecuencias de los SNPs con gradientes climáticos. El análisis reveló que tres SNPs asociados a genes de floración y apertura de yemas apicales, y dos SNPs asociados a estrés ambiental, están significativamente correlacionados con la precipitación media anual, sugiriendo evidencia de variación genética adaptativa.

Homolka *et al.* (2013) analizaron la diversidad nucleotídica de ocho genes candidatos asociados a estrés por sequía y su correlación con variables ambientales en poblaciones de *Q. petraea* y *Q. robur* en Austria, encontrando SNPs con una fuerte correlación al régimen de

temperatura y precipitación locales, sugiriendo evidencia de una posible adaptación local a condiciones de aridez.

En México, Oyama *et al.* (2018) estudiaron la diversidad genética y la conectividad funcional de poblaciones de *Q. candicans*, *Q. crassifolia* y *Q. castanea* en el estado de Oaxaca, un estado caracterizado por su alta heterogeneidad climática y fisiográfica, llegando a la conclusión de que incluso en un entorno con una fisiografía tan heterogénea las especies de encinos aún tienen altos niveles de diversidad genética y conectividad entre las poblaciones.

Herrera-Arroyo *et al.* (2013) y Oyama *et al.* (2017) realizaron estudios de genética del paisaje de *Q. castanea* a una escala local en la cuenca de Cuitzeo, en el estado de Michoacán. En dichos trabajos se analizó el flujo génico por semillas y polen, respectivamente, de fragmentos de bosque, así como también árboles aislados de *Q. castanea*. Los autores concluyeron que la fragmentación del hábitat dentro de la cuenca está interrumpiendo el flujo de genes mediado por la dispersión de semillas entre las poblaciones existentes, y que el flujo génico por polen aún es extenso entre los fragmentos y los árboles aislados, enfatizando el alto valor de los árboles aislados para la conservación de la diversidad genética y la conectividad.

Por último, Martins *et al.* (2018) estudiaron poblaciones de *Q. rugosa* dentro de la Faja Volcánica Transmexicana, encontrando 97 SNPs asociados a la variación climática presente dentro de la faja. Ellos concluyen que los patrones espaciales de variación genética están fuertemente asociados con la estacionalidad de la precipitación y la distancia geográfica.

Es importante mencionar que la mayoría de los trabajos sobre la variación en rasgos funcionales y la variación genética de encinos se han realizado en áreas geográficas extensas; sin embargo, la adaptación de las poblaciones al ambiente climático puede ocurrir a escalas regionales (Eckert *et al.* 2015). Aún se sabe poco acerca de cómo varían los rasgos funcionales entre las poblaciones de árboles a escalas geográficas de paisaje o regionales. En este contexto de escala de paisaje, se ha prestado poca atención al papel que juegan las variables climáticas en la facilitación o restricción al flujo génico en ambientes heterogéneos, si existen patrones reconocibles de diferenciación genética, así como también al grado en el que puede o no estar ocurriendo adaptación local. Todo este tipo de información es importante para entender procesos evolutivos, pero también para un manejo adecuado y conservación de las poblaciones de árboles.

Especie y área de estudio

Quercus castanea Née (Lobatae) es un encino rojo de amplia distribución e importancia ecológica y económica en México (Figura 1). Es un árbol de hasta 18 m de altura, ocasionalmente puede ser un arbusto (Trelease 1924). Los adultos florecen de marzo a junio y producen bellotas maduras de octubre a diciembre (Herrera-Arroyo *et al.* 2013; Schondube *et al.* 2010). Como todas las especies de encinos, es polinizado por el viento, posee exocruzamiento obligado, la dispersión de las semillas se lleva a cabo por la gravedad, algunas aves y roedores (Schondube *et al.* 2010; Sork *et al.* 2002). Se encuentra ampliamente distribuido en la Sierra Madre Occidental, Faja Volcánica Transmexicana, Sierra Madre del Sur y en algunas localidades de la Sierra Madre Oriental (Valencia 2004). En Michoacán se

distribuye casi a todo lo largo de la Faja Volcánica Transmexicana, incluyendo fragmentos en la parte central y este de la depresión del Río Lerma, así como en la parte central de la Sierra Madre del Sur, en un intervalo altitudinal de los 1450 a 2600 msnm (Bello y Labat 1987).



Figura 1. *Quercus castanea*: a) individuo adulto representativo de la especie, b) tronco, c) inflorescencias masculinas, d) hojas y semillas, e) vista adaxial de las hojas.

La cuenca del lago de Cuitzeo, está situada dentro de la Faja Volcánica Transmexicana, entre los 19°30' y 20°05' de latitud Norte y 100°35' y 101°30' de longitud Oeste. Posee una superficie de 4026 km². El clima predominante es templado con lluvias de verano. En la cuenca existe un gradiente climático muy marcado, con el aumento de la precipitación y la disminución de la temperatura de norte a sur y con la altitud, es decir que, la parte norte es la

más seca y la parte sur la más húmeda (López-Granados *et al.* 2002; Mendoza *et al.* 2006; Leal-Nares 2010). Los encinares abarcan una superficie de 400 km² (10% del área de la cuenca) y se encuentran repartidos en más de 1000 fragmentos con diferentes áreas y formas (López *et al.* 2006). La especie de encino con la distribución más amplia en la cuenca, excepto en la parte noreste, es *Q. castanea* (Aguilar-Romero *et al.* 2012; Herrera-Arroyo *et al.* 2013).

Por lo mencionado anteriormente, se propone que *Q. castanea* es un excelente modelo para evaluar cómo las características ambientales presentes en la cuenca afectan la variación en atributos fenotípicos funcionales, la variación y diferenciación genética neutra y potencialmente adaptativa, y la conectividad funcional entre poblaciones.

La presente tesis está organizada en tres capítulos. En el primero se estudió cómo los gradientes de temperatura y precipitación dentro de la cuenca promueven la variación en atributos funcionales relacionados con el ajuste a la disponibilidad de agua. Se encontró que existe una diferenciación significativa en los atributos funcionales estudiados entre las poblaciones, particularmente en el grosor foliar. En el segundo capítulo, utilizando 8 microsátélites nucleares, se estudiaron los patrones de diversidad y estructura genética, y conectividad funcional potencial para comprender cómo las variables ambientales y las características topográficas influyen en el flujo génico a nivel de paisaje. Se encontraron niveles altos de diversidad genética y baja estructura genética, lo que sugiere que existen tasas altas de flujo génico entre las poblaciones. También se encontró que la estacionalidad de la precipitación, el intervalo anual de la temperatura y la precipitación anual son variables ambientales que afectan la dispersión del polen y el flujo génico, probablemente debido a su

efecto sobre la fenología floral. Finalmente, en el tercer capítulo utilizando polimorfismos de un solo nucleótido (SNPs) se evaluó si en *Q. castanea* existe evidencia molecular de la acción de la selección natural, en otras palabras, de la adaptación local a las condiciones ambientales presentes en la cuenca, y a pesar del flujo génico tan alto entre las poblaciones. Se identificaron 8 SNPs que presentan señal de selección divergente y 194 SNPs asociados con la variación climática; algunos de estos SNPs se encuentran en genes que están relacionados con la respuesta al estrés por temperatura, estrés oxidativo, estrés osmótico, germinación y floración.

CAPÍTULO I

ASSOCIATION OF FUNCTIONAL TRAIT

VARIATION OF *Quercus castanea* WITH

TEMPERATURE AND WATER AVAILABILITY

GRADIENTS AT THE LANDSCAPE LEVEL

Lara-De La Cruz LI, García-Oliva F, Oyama K, González-Rodríguez A. 2020. *Botanical Sciences* 98(1): 16-27.



ASSOCIATION OF FUNCTIONAL TRAIT VARIATION OF *QUERCUS CASTANEA* WITH TEMPERATURE AND WATER AVAILABILITY GRADIENTS AT THE LANDSCAPE LEVEL

RELACIÓN DE LA VARIACIÓN DE ATRIBUTOS FUNCIONALES DE *QUERCUS CASTANEA* CON GRADIENTES DE TEMPERATURA Y DISPONIBILIDAD DE AGUA A NIVEL DE PAISAJE

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Abstract

Background: Phenotypic variability of tree species is often associated to environmental factors. *Quercus castanea* is a Mexican red oak with a wide geographical and altitudinal distribution along contrasting environments. It is the most abundant oak species in the Cuitzeo basin, which is characterized by highly heterogeneous environmental conditions.

Hypothesis: We hypothesized that gradients in temperature, precipitation and soil characteristics across the distribution of *Q. castanea* within the Cuitzeo basin promote variability in functional traits related to the adjustment to differential water availability at a landscape level.

Studied species: *Quercus castanea* Née (Fagaceae).

Study site and years of study: Cuitzeo basin in Central Mexico. 2015-2016.

Methods: We quantified leaf chlorophyll concentration (CC), leaf area (LA), leaf thickness (LT), leaf mass per area (LMA) and the Huber value (HV) in 10 individuals from 22 populations of *Q. castanea* throughout the basin.

Results: Despite the relatively small geographical area (4,000 km²), our results revealed significant differentiation among populations in the studied functional traits. The strongest variation found was in LT, which was negatively correlated with precipitation seasonality. This pattern is opposite to previous reports on Mediterranean oaks but similar to tropical oaks and suggests that the combination with other traits such as leaf phenology is important in the response to water availability.

Conclusions: Significant functional differences exist among populations of *Q. castanea* separated by a few kilometers in the heterogenous landscape of the Cuitzeo basin. This species shows clearly sclerophyllous leaves, but leaf thickness varies to a considerable degree across populations.

Key words: climate gradient, leaf economic spectrum, plant morpho-physiological variation, *Quercus*.

Resumen

Antecedentes: La variación fenotípica de especies arbóreas con frecuencia se asocia a factores ambientales. *Quercus castanea*, un encino rojo mexicano de amplia distribución geográfica y altitudinal en ambientes contrastantes es el encino más abundante en la cuenca de Cuitzeo, caracterizada por presentar condiciones ambientales heterogéneas.

Hipótesis: Los gradientes de temperatura, precipitación y características del suelo en la cuenca de Cuitzeo promueven la variación en los atributos funcionales de *Q. castanea* relacionados con el ajuste a la disponibilidad de agua a nivel de paisaje.

Especie en estudio: *Quercus castanea* Née (Fagaceae).

Sitio de estudio y fechas: Cuenca de Cuitzeo, centro de México. 2015-2016.

Métodos: Cuantificamos la concentración de clorofila de la hoja (CC), el área foliar (LA), el grosor foliar (LT), la masa por área foliar (LMA) y el valor de Huber (HV) en 10 individuos de 22 poblaciones de *Q. castanea* distribuidas en la cuenca.

Resultados: A pesar del área geográfica pequeña (4,000 km²), nuestros resultados mostraron una diferenciación significativa en los atributos funcionales entre las poblaciones. La variación más marcada fue en LT, que se correlacionó negativamente con la estacionalidad de la precipitación. Este patrón es opuesto a resultados previos en encinos mediterráneos pero similar a encinos tropicales y sugiere que la combinación con otros atributos, como la fenología foliar, es importante en la respuesta a la disponibilidad de agua.

Conclusiones: Existen diferencias funcionales entre las poblaciones de *Q. castanea* separadas unos pocos kilómetros dentro de la cuenca. En particular el grosor de la hoja varía considerablemente.

Palabras clave: espectro económico foliar, gradiente climático, *Quercus*, variación en atributos funcionales.

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Variation in phenotypic traits is a fundamental attribute of natural populations and often reflects the linking of traits with geographical and climate variables across the range of a species (McKnow *et al.* 2014). In tree species, variation among populations in several fundamental functional traits is considerably influenced by water availability, which in turn depends on the amount and distribution of rainfall, soil physical properties, and the relationship between evaporation and transpiration (Souto *et al.* 2009, Ramírez-Valiente *et al.* 2010, Cooper *et al.* 2018). Therefore, differences in water availability along the distribution of a species are expected to set contrasting selection pressures leading to local adaptation of populations, as has been shown in many tree species (Ramírez-Valiente *et al.* 2010, Aranda *et al.* 2015, Lind *et al.* 2107). However, the extent of local adaptation at fine spatial scales in long-lived tree species characterized by high rates of gene flow has not been sufficiently studied yet (Cavender-Bares & Ramírez-Valiente 2017). Alternatively, such species may face environmental challenges by maintaining high levels of within-population genetic variation or through phenotypic plasticity, particularly when single individuals may experience a range of conditions throughout the course of a long lifespan (Cavender-Bares & Ramírez-Valiente 2017, Meireles *et al.* 2017).

The genus *Quercus* (oak species) is a very diverse group of tree species distributed in temperate, subtropical and tropical regions of the northern hemisphere, where it is considered one of the most important taxa of trees (Cavender-Bares 2016). This genus, along with others in the Fagaceae family are emerging as non-classical models to study adaptive variation, integrating ecology and evolution (Petit *et al.* 2013, Cavender-Bares 2019). Mexico is an important diversification center for the oaks, with about 161 species in total (32-40 % of the diversity in the world) and more than 100 endemics (Valencia-A 2004). Oaks constitute a very important group for understanding the factors that determine phenotypic variability, due to their particularly high levels of variation at different levels, that is, among species, among populations, within populations and within individuals (Uribe-Salas *et al.* 2008, Hernández-Calderón *et al.* 2014, García-Nogales *et al.* 2016).

Plant functional traits are measurable characteristics that influence performance or fitness and are assumed to reflect evolutionary responses to external conditions and can frequently refer to ecological factors changing along a gradient (Lavorel *et al.* 2007). Research on different oak species has revealed functional variation related to leaf traits, phenology, and growth rates associated to altitudinal, latitudinal, water availability or temperature gradients (Bruschi 2010, Hernández-Calderón *et al.* 2013, Ramírez-Valiente *et al.* 2017). This variation has been explained as resulting from strategies to avoid or tolerate different types

of environmental stress, allowing oak species to develop under a wide range of environmental conditions and resource availability. However, most of these studies have been conducted at broad geographical scales, and little is known about how functional traits vary among tree populations at finer spatial scales such as the landscape level.

Quercus castanea is a Mexican red oak with a wide geographical and altitudinal distribution that occupies contrasting environments, with populations in the Sierra Madre Occidental, the Central Plateau, the Trans-Mexican Volcanic Belt, and the Sierra Madre del Sur (Valencia-A 2004). It is a dominant element in temperate forests and mountain cloud forests and is frequently found in perturbed areas with a xerophytic shrub type of vegetation (Valencia-Cuevas *et al.* 2015). Therefore, *Q. castanea* represents a suitable species to study patterns of phenotypic variation in response to environmental factors at a landscape scale, despite potentially very high gene flow even in a fragmentation scenario (Herrera-Arroyo *et al.* 2013, Oyama *et al.* 2017).

In this study, we hypothesized that gradients in temperature, precipitation and soil characteristics across the distribution of *Q. castanea* within the Cuitzeo basin promote variability in functional traits related to the adjustment to differential water availability. We focused on leaf traits that have been considered easy and inexpensive to measure in a standardized manner (leaf chlorophyll concentration, leaf area, leaf mass per area, leaf thickness and the Huber value) but that capture the general patterns of plant functional responses to environmental gradients (Wright *et al.* 2004, Lavorel *et al.* 2007). According to these previous generalizations, we expected *Q. castanea* individuals to have a higher Huber value and smaller, thicker leaves with a higher mass per area in the parts of the basin with higher temperature and lower precipitation. Particularly, the objectives were: i) to evaluate the amount and patterns of variation in this set of functional traits across *Q. castanea* populations at a landscape level and ii) to determine significant associations between functional traits and environmental variables.

Materials and methods

Study site and sampling procedure. This study was conducted in the basin of Lake Cuitzeo. It has an area of 4,026 km² and is located at 19° 30' - 20° 05' N and 100° 35' - 101° 30' W in the Trans-Mexican Volcanic Belt in the northern part of Michoacan state and the southern part of Guanajuato state (Figure 1). Climate in the basin is temperate with a marked rainy season during summer months (June to September). There is a significant climate gradient, with precipitation increasing and temperature decreasing from north to south and with altitude (Mendoza *et al.* 2006). The topography and soils of the study area are

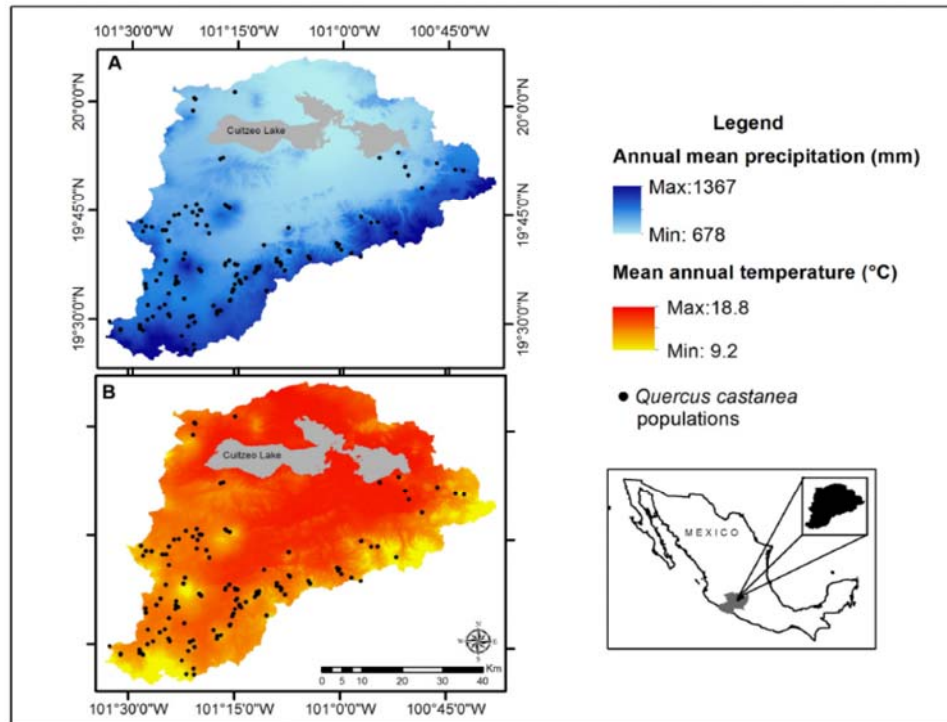


Figure 1. Climate gradients in the Cuitzeo lake basin and the distribution of the *Q. castanea* populations; a) annual mean temperature, b) annual mean precipitation.

product of the volcanic activity of the Quaternary. The dominant soil groups are vertisols, luvisols, leptosols, Acrisols and Andosols (Mendoza *et al.* 2006, Chávez-Vergara *et al.* 2014).

Quercus castanea grows throughout the Cuitzeo basin, except in the northeastern portion (Aguilar-Romero *et al.* 2016). In the study region, populations can be found between 2,000 and 2,800 m (Herrera-Arroyo *et al.* 2013). Twenty-two populations were chosen for sampling, mostly covering the distribution of the species within the Cuitzeo basin (Table S1, Figure 2). At each population, 10 randomly chosen adult trees separated by at least 20 m from each other were sampled by taking four haphazardly selected sun-exposed branches, with terminal twigs with at least 10 mature leaves with no visible damage, at heights between 2 and 5 m. Only in population 10, fewer trees were collected (6) because of their low availability.

Functional traits. In each individual tree, we measured the following functional traits: leaf chlorophyll concentration (CC), leaf area (LA), leaf mass per area (LMA), leaf thickness (LT) and Huber value (HV, the sapwood cross section area divided by the leaf area distal to the stem) (Tyree & Ewers 1991). Studying these functional traits is relevant because they are adaptive and vary along

environmental gradients in many plant species (Auger & Shipley 2013, Rosbakh *et al.* 2015, Cochrane *et al.* 2016). CC directly influences the photosynthetic capacity of plants (Croft *et al.* 2017). LA is important in the balance of water and energy of the plant and usually smaller leaves are associated with high radiation, heat-, cold- or drought-stress (Cornelissen *et al.* 2003). LMA is positively related to the investment in structural defenses and with leaf lifespan and usually higher values of LMA occur in environments with higher resources stress. LT is associated with photosynthetic rates per unit of leaf area and is usually correlated positively with mean temperature and with solar radiation (Niinemets 2001). Finally, HV is higher in more arid sites (Carter & White 2009).

Leaf chlorophyll concentration was measured in the field using the Minolta Soil Plant Analysis Development (SPAD-502) chlorophyll meter (Markwell *et al.* 1995) in three randomly chosen leaves per individual. For the other traits, fully developed leaves without damage were dried at 55 °C, weighed in an analytical balance, and lamina thickness measured in a portion of the leaf without veins using a Mitutoyo Absolute digital caliper (model 500-172-20) with a 0.01 mm precision. Leaves were then imaged with a flatbed high resolution scanner (Epson V700), and the area of each leaf was determined

Functional trait variation in *Quercus castanea*

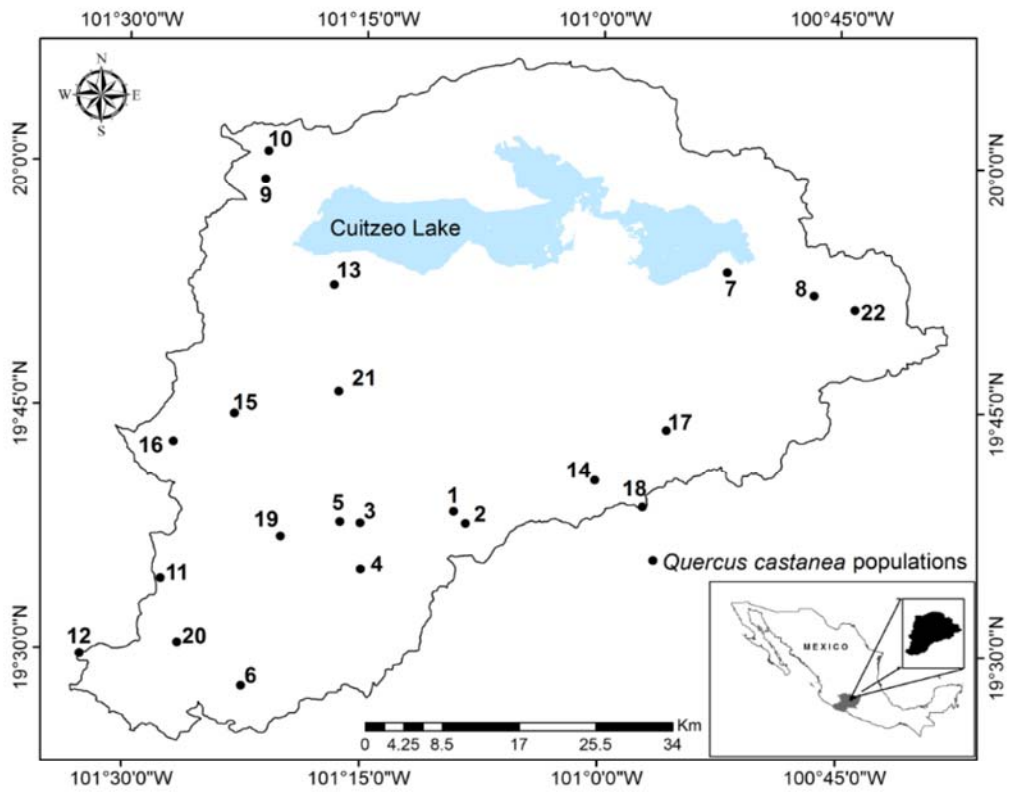


Figure 2. Cuitzeo lake basin and the distribution of the 22 *Q. castanea* populations sampled.

using the ImageJ software (Rasband 2010). Leaf mass per area was calculated as the ratio between dry mass and leaf area values. These traits were measured in ten randomly chosen leaves per tree. For the Huber value, three twigs per tree were selected. The leaf area was determined by digitalizing all the twig leaves with the high-resolution flatbed scanner and summing their individual areas. The area of the sapwood was determined from the diameter of cross sections of the leafless twigs measured with the digital caliper after removing the bark. This trait was determined for three twigs per individual.

Geographic and environmental variables. Spatial coordinates and altitude of each sampling population were recorded using a global positioning system (GPS) unit (Table 1S). Soil type for each population was extracted from a soil surface (Cabrera-González *et al.* 2010) using GIS Arc View ver. 3.3 (ESRI 1999). To determine soil water holding capacity, three soil samples were taken in different random directions (keeping a minimum angle of 70° between samples) one meter away from the main trunk of each oak tree sampled using a soil core sampler. In the laboratory, soil samples were dried in an oven at 70 °C for 3 days.

Subsequently, 10 g of dry soil from each sample were weighted, then wetted to field capacity and weighted again. Soil water holding capacity (WHC) was calculated as follows:

$$\text{WHC} = (\text{weight of wet soil} - \text{weight of dry soil} / \text{weight of dry soil}) \times 100$$

This variable was determined as an average of values of the three soil samples per oak tree and then an average value per population was obtained.

To characterize the climate at each population, 19 bioclimatic variables derived from monthly precipitation and temperature values (period 1910-2009) were extracted at 30 arc seconds and downscaled using a digital elevation model at 30 m of resolution (Cuervo-Robayo *et al.* 2014, Correa-Ayram *et al.* 2017). Nineteen climatic variables were finally obtained for each population using GIS Arc View v3.3 (ESRI 1999).

Data Analysis. The significance of the differences among populations for each functional trait were evaluated through one-way analyses of variance (ANOVA) using average values of individual trees. To determine how the total

variance for each functional trait is partitioned among populations, among trees within populations, and within trees, we estimated the variance components using the restricted maximum likelihood (REML) method. Residual variation was considered to correspond to differences among leaves within trees. In this analysis the full database with individual leaf values was used. A stepwise canonical discriminant analysis (CDA) using individual tree averages for each functional trait was performed to further determine which functional traits have the highest variation among the populations sampled. Finally, to evaluate the degree of association among the different functional traits, a pairwise correlation analysis was performed. These analyses were carried out in JMP 8 (SAS Institute, Cary, North Carolina).

The associations between the population mean values of each functional trait and the environmental variables at each sampling population were evaluated with Spearman's correlation analyses. Before conducting the correlation analyses, redundancy was reduced among the environmental variables by assessing pairwise correlations and discarding the more specific variable in each pair of highly correlated variables ($r \geq 0.8$). The included environmental variables were soil water holding capacity (WHC), annual mean temperature (B1), mean diurnal range (B2), isothermality (B3), minimum temperature of the coldest month (B6), temperature annual range (B7), annual precipitation (B12), precipitation of the driest month (B14), precipitation seasonality (B15) and precipitation of the driest quarter (B17). The JMP 8 program was used in these analyses (SAS Institute, Cary, North Carolina).

To separate the effects of spatial distance and environmental variables on phenotypic variation we performed a Redundancy Analysis (RDA) (Van den Wollenberg 1977), which is a constrained ordination method analogous to linear regression for datasets with multiple dependent and multiple independent variables. Three separate RDAs were conducted: (1) a full model with environmental and geographic variables (latitude and longitude of the populations) as explanatory variables; (2) a partial RDA (pRDA1) where we removed geographic variables (*i.e.*, a pure environmental model) and (3) a partial RDA (pRDA2) where we removed environmental variables (*i.e.*, a pure geographic model). We then used variance partitioning to calculate the proportion of variation explained by the independent contributions of environmental and geographic variables and their joint effect (Borcard *et al.* 1992, Peres-Neto *et al.* 2006). The significance of each RDA model was calculated using a permutation test with 1,000 permutations. We calculated the adjusted coefficient of multiple determination (R^2_{adj}) for all models (Peres-Neto *et al.* 2006). Data of the five functional traits were used as the response variables in the three RDAs.

The five variables were \log_{10} -transformed to correct for skew, and then centered and standardized before the RDA analysis. The explanatory variables included eight of the environmental variables described above, but we excluded isothermality (B3) because centering and standardizing this variable resulted mostly into zero values. Explanatory variables also included spatial variables defined using principal coordinates of neighborhood matrices (PCNM), which were calculated from the geographic coordinates in decimal degrees (Dray *et al.* 2006). We retained half of the PCNM variables with positive eigenvalues, as has been suggested by some authors (Manel *et al.* 2012, Fitzpatrick & Keller 2015), which were seven in our case. Calculation of PCNM, RDA, test for the significance and adjusted coefficient of multiple determination of the models were performed in R (R Core Team 2016), using the 'vegan' 2.3-0 package (Oksanen *et al.* 2015).

Results

One-way ANOVAs indicated highly significant differences among populations for the five functional traits evaluated (Table 1). However, total variance was partitioned differently depending on the trait. LT showed the largest variation among populations (43.9 %) while for the other four traits variation among trees within populations and leaves within trees accounted for the largest proportion of the variance (Table 2).

Table 1. Results of the one-way ANOVAs for the comparison of five functional traits among 22 populations of *Q. castanea* in the Cuitzeo basin, Michoacan state, Mexico.

Trait	$F_{21,186}$	P
Chlorophyll concentration	5.33	< 0.0001
Leaf area	6.08	< 0.0001
Leaf mass per area	3.4	< 0.0001
Leaf thickness	13.94	< 0.0001
Huber value	4.44	< 0.0001

The stepwise canonical discriminant analysis indicated that the variable that contributes more importantly to differentiate populations is LT, followed by LMA, LA, CC and HV. The two first canonical discriminant functions allowed highly significant discrimination among populations (Wilks' lambda = 0.048; $P < 0.0001$) and explained 71.2 and 16.13 % of the variation, respectively (Table 3). The variables that contributed more strongly to the first canonical function were LT and LMA, while LA, CC and HV contributed to the second canonical function.

We found moderate but significant positive correlations of leaf mass per area with leaf thickness and with the Huber

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value (Table 4). Chlorophyll content had positive weak correlations with leaf mass per area and the Huber value. In contrast, negative correlations were observed between leaf mass per area and leaf area, between chlorophyll content and leaf thickness and between the Huber value and leaf area (Table 4).

According to the Spearman's correlation analyses, LMA had a significant negative correlation with annual mean temperature ($r_s = -0.5$; $P = 0.017$) and a positive correlation with isothermality ($r_s = 0.5$; $P = 0.016$). CC and HV were

also positively related to isothermality ($r_s = 0.48$; $P = 0.02$ and $r_s = 0.43$; $P = 0.04$, respectively), while LT was negatively correlated with precipitation seasonality ($r_s = -0.6$; $P = 0.003$).

The full RDA model for the combined effect of geographic and climatic explanatory variables on functional trait variation was significant ($P = 0.001$). The pRDA1 for the association between environmental variables and functional trait variation while controlling for geographic effects was also significant ($P = 0.001$, $R^2_{adj} = 13.9\%$).

Table 2. Restricted maximum likelihood (REML) variance components (as percentage of total variation) for five functional traits for *Q. castanea* in the Cuitzeo basin. Variation among populations, among individuals within populations and within individuals (residual variance) was considered.

Trait	Population	Individuals within populations	Residual
Chlorophyll concentration	24.25	43.74	32
Leaf area	20.7	34.73	44.56
Leaf mass per area	7.2	25.8	67
Leaf thickness	43.9	27.98	28.11
Huber value	16.4	30.53	53.06
Average	22.49	32.56	44.95

Table 3. Results of the stepwise canonical discriminant analysis for functional traits in *Q. castanea*.

	Canonical 1	Canonical 2
Eigenvalue	4.36	0.99
Percent (%)	71.2	16.13
Cumulative percent (%)	71.2	87.33
Functional trait	Scoring coefficients	
CC	0.02	0.14
LMA	767.15	-57.96
LA	0.07	-0.167
LT	-63.5	9.86
HV	-0.05	0.152

CC = chlorophyll concentration, LMA = leaf mass per area, LA = leaf area, LT = leaf thickness, HV = Huber value

Table 4. Pairwise correlations among functional trait variables.

Variables	CC	LA	LT	HV	LMA
CC	1.0000				
LA	-0.1232	1.0000			
LT	-0.1362*	0.0520	1.0000		
HV	0.1944**	-0.4651**	0.0368	1.000	
LMA	0.1570*	-0.2934**	0.5229**	0.4640**	1.0000

CC = chlorophyll concentration, LA = leaf area, LT = leaf thickness, LMA = leaf mass per area, HV = Huber value * $P < 0.05$; ** $P < 0.01$

(Table 5). In this analysis, the most important variables were annual precipitation (B12) and precipitation of the driest quarter (B17), followed by minimum temperature of the coldest month (B6), annual mean temperature (B1), mean diurnal range (B2), temperature annual range (B7) and precipitation of the driest month (B14) (Table S2). The pRDA2 indicated that geography alone explained a slightly higher proportion of functional trait variation ($P = 0.001$, $R^2_{adj} = 18.4\%$). Finally, joint climate and geography accounted for 71.9% of the variation (Table 5).

Discussion

Our analysis at a landscape scale showed significant variation among populations of *Q. castanea* in the functional traits studied. Furthermore, we identified the environmental variables with the largest influence on these functional traits at this spatial scale. It is widely acknowledged that leaf area, leaf mass per area, leaf thickness and Huber value are adaptive and vary according to the environment. These traits are related to water use efficiency and tolerance to temperature and water stress (Westoby *et al.* 2002, Wright *et al.* 2004, Lohbeck *et al.* 2013), and leaf mass per area and leaf thickness are functional traits within the leaf economic spectrum (LES) (De La Riva *et al.* 2016).

Variation among populations. In comparison to other oak species studied across the world, we found that *Q. castanea* showed a high leaf mass per area (range 134-183 g m⁻²), relatively low leaf areas (9.38-18.62 cm²) and intermediate leaf thickness (0.11-0.24 mm) (Table S3). The value of leaf mass per area is situated at the higher end of values reported for oaks, and comparable to values found in evergreen Mediterranean and evergreen arid species (Gil-Pelegrin *et al.* 2017). According to Flexas *et al.* (2014) a leaf mass per area value higher than 120 g m⁻² indicates a true sclerophyllous species. Leaf thickness was within the range of mean values reported for deciduous temperate and evergreen arid oak species. In turn, leaf area was higher than for Mediterranean and arid evergreen species, but

lower than for tropical and temperate evergreen and deciduous species (Gil-Pelegrin *et al.* 2017). These three traits (leaf area, leaf thickness, leaf mass per area) showed highly significant variation among the sampled populations of *Q. castanea* even though, except for leaf thickness, variation among trees within populations was higher, suggesting the importance of microenvironmental factors (or other unaccounted factors) in determining intraspecific functional trait variation.

Leaf mass per area has been considered a key leaf functional trait for plants (Wright *et al.* 2004, Poorter *et al.* 2009). In *Q. castanea* populations, leaf mass per area showed a positive correlation with leaf thickness and a negative correlation with leaf area (Table 4). However, leaf thickness was the trait that showed the lower intra-individual and intra-population variation and the higher among-population variation (Table 2), suggesting its importance in the adjustment of *Q. castanea* to the environment.

In a previous study within the Cuitzeo basin, Aguilar-Romero *et al.* (2017) had compared water-use strategies among nine oak species (including *Q. castanea*) distributed along an aridity gradient. In that study, species from more arid areas had more deciduousness and a higher instantaneous water-use efficiency, while their more humid counterparts had less deciduousness and a xylem that was more resistant to embolisms, suggesting a trade-off between the xylem vulnerability to embolism and deciduousness. In that study, *Q. castanea* was characterized as occupying an intermediate position along the aridity gradient, with a brevideciduous phenology and also intermediate values (in relation to the other eight species) of wood density, water use efficiency, Huber value and xylem resistance to embolism (Aguilar-Romero *et al.* 2017). However, the breadth of the environmental niche of each species and its correspondence with the degree of intraspecific variability in functional traits was not considered. As we have seen in this study, such evaluation is critical, since some traits may exhibit even higher intraspecific than interspecific variation. For example, both in greenhouse grown seedlings and in

Table 5. Results of redundancy analysis (RDA) for the association between functional trait variation, geography and climate variables for *Quercus castanea* populations. Two partial analysis are shown pRDA1 (effect of climatic variables while controlling for geographic effects) and pRDA2 (effect of geographic distribution while controlling for climatic variation). The proportion of variance explained by both geographic and climate effects is also indicated (joint climate/geography). Proportion constrained corresponds to the partitioned variance relative to the constrained variance of the full RDA model.

	Inertia	Proportion	R^2_{adj}	P
Full model: geography and climate (constrained variance)	5.000	1	0.28	0.001
Pure climate (pRDA1)	0.5917	0.1183	0.13	0.001
Pure geography (pRDA2)	0.8136	0.1627	0.18	0.001
Joint climate/geography	3.5947	0.7190	-0.04	NA

adult trees from the field, [Ramírez-Valiente et al. \(2015\)](#) found higher differences among populations of the tropical oak *Q. oleoides* than among species within the same clade (*Virentes*, the live oaks), for various functional traits in response to drought. Therefore, the extent and ratio of intra-versus interspecific variation, and their relationship with the niche breadth of the species and their coexistence within communities is still an open question ([Cavender-Bares et al. 2004](#)).

Association with environmental variables. Even at the relatively fine spatial scale of the Cuitzeo basin, we were able to define several significant associations of the traits measured with environmental variables. The strongest pairwise association was a negative correlation between leaf thickness and precipitation seasonality, meaning that leaves are thinner at populations where precipitation is more seasonal (i.e. there are more marked dry and wet periods). In turn, leaf mass per area had a positive correlation with isothermality and negative correlation with annual mean temperature, and the Huber value had a positive correlation with isothermality. Higher Huber values mean that the tree produces more wood per unit of leaf area, a characteristic that suggests an improvement in its water and nutrient storage capacity ([Callaway et al. 1994](#)) and that reduces the vulnerability to embolism of the xylem ([Tyree & Dixon 1986](#)).

In turn, the multivariate RDA identified annual precipitation (B12) and precipitation of the driest quarter (B17) as the most important environmental variables influencing functional trait variation of *Q. castanea* in the Cuitzeo basin, with also a considerable contribution of precipitation of the driest month (B14). Overall, these results indicate that leaves of *Q. castanea* are more sclerophyllous (i.e. have higher leaf mass per area) in sites that are cooler and less seasonal in precipitation and temperature variation, and less sclerophyllous in more seasonal and warmer sites. Previously, sclerophylly has been considered an adaptation to arid environments across biomes ([Niinemets 2001](#)). At the intraspecific level, leaf mass per area has been shown to increase with aridity in Mediterranean oaks such as *Q. ilex* and *Q. coccifera* ([Peguero-Pina et al. 2016](#)). However, the opposite pattern has been shown in populations of *Q. oleoides* that tended to have more sclerophyllous leaves in more mesic areas ([Ramírez-Valiente et al. 2015, 2017](#)). The authors suggested that this counterintuitive result is due to phenological differences: populations from mesic populations maintain leaves for longer time during the dry season and therefore maintain function with increasing water stress ([Cavender-Bares & Ramírez-Valiente 2017](#)). Therefore, selection has favored an increase in deciduousness (and less investment in tissue construction) with increasing dry season severity in

this tropical oak species, contrasting with Mediterranean species that are limited by both cold winters and dry summers ([Cavender-Bares & Ramírez-Valiente 2017](#)). Therefore, *Q. castanea* follows a pattern resembling more a tropical than a Mediterranean species. We hypothesize that phenological patterns (i.e. degree of deciduousness) in this species should be negatively associated with leaf mass per area along populations in the Cuitzeo basin, but this should be evaluated in further studies.

We conclude that, while previous studies have considered functional variation in oak species at a whole-range level, we have shown that significant functional differences exist among populations of *Q. castanea* separated by a few kilometers in the heterogenous landscape of the Cuitzeo basin. This species shows clearly sclerophyllous leaves but leaf thickness varies to a considerable degree across populations in association with precipitation seasonality, indicating that the temporality of water availability represents a significant environmental pressure for this oak species. Whether the observed functional variation is due to local adaptation, plasticity or a combination of both will be clarified in ongoing common garden and landscape genomics studies.

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Supplemental data

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Author contribution: LILC conceived the idea, designed the study, performed field work, analyzed the data and wrote the manuscript; FGO supervised the design of the study, the data analysis and the preparation of the manuscript; KO obtained funding and supervised the manuscript preparation; AGR supervised the design of the study, data analysis and manuscript preparation, participated in the field work and obtained funding; all authors contributed to the review and approval of the manuscript.

Supplementary material**Table S1.** Locality number and name, altitude, geographical coordinates, mean annual temperature (MAT), mean annual precipitation (MAP) and soil group for the collecting populations.

Population	Altitude (m)	Lat N	Lon W	MAT (°C)	MAP (mm)	Soil group
1. Jesús del Monte	2118	19.6443333	-101.153389	16.2	951	Acrisol
2. San Miguel del Monte	2097	19.6321944	-101.140583	16.4	949	Leptosol
3. Presa Cointzio	2038	19.6312222	-101.251361	16.7	889	Acrisol
4. Las Cruces	2060	19.5840833	-101.250417	16.6	936	Luvisol
5. Carretera Pátzcuaro	2007	19.6325278	-101.272917	16.6	896	Leptosol
6. Tamanguio	2457	19.4639444	-101.374806	14.3	1145	Andosol
7. San Bartolomé Coro	2138	19.8921111	-100.867833	16.4	856	Phaeozem
8. San José del Rincón	2050	19.869	-100.775972	17.1	824	Andosol
9. San Nicolás Tumbastatiro	2285	19.9830833	-101.355889	16.0	805	Acrisol
10. San Isidro	2233	20.0116111	-101.35325	16.7	766	Vertisol
11. Fontezuelas	2191	19.5728611	-101.461222	15.6	974	Luvisol
12. Mina Arena	2422	19.4949722	-101.545306	15.2	1032	Leptosol
13. Rosa de Castilla	2218	19.8750833	-101.282056	16.0	840	Leptosol
14. Carretera Mil Cumbres	2048	19.6781583	-101.005172	16.3	945	Luvisol
15. Tiristarán	2226	19.7428611	-101.38525	15.8	868	Leptosol
16. Capula	2341	19.7134167	-101.449444	15.4	924	Andosol
17. Las Huertas	2196	19.7300528	-100.930372	15.5	1038	Acrisol
18. El Temazcal	2207	19.6509167	-100.954622	15.6	1067	Luvisol
19. Cerro El Remolino	2523	19.6169444	-101.335278	14.2	1087	Luvisol
20. Las Tablas	2259	19.5073222	-101.442847	15.4	1026	Andosol
21. Cerro Quinceo	2341	19.7666222	-101.275744	15.4	933	Luvisol
22. Carretera Ucareo	2369	19.8548694	-100.732603	15.3	990	Andosol

Table S2. Loadings of climate variables into the first two axes of pRDA1 and their significance.

Variable	df	pRDA1			
		Axis 1	Axis 2	F	P
B1	1	-0.04	-0.41	4.3	0.006
B2	1	0.04	-0.44	3.9	0.005
B6	1	0.17	-0.29	4.5	0.002
B7	1	0.09	-0.39	3.3	0.021
B12	1	0.01	0.40	7.9	0.001
B14	1	0.14	0.18	3.3	0.013
B15	1	0.01	0.02	1.5	0.182
B17	1	-0.05	0.5	6.1	0.002
WHC	1	0.08	-0.05	1.8	0.108

Functional trait variation in *Quercus castanea*

Table S3. Mean and standard error (SE) values of the six functional traits in *Q. castanea* for the 22 populations sampled.

Population	Chlorophyll concentration (SPAD values)		Leaf area (cm ²)		Leaf mass per area (g·cm ⁻²)		Leaf thickness (mm)		Huber value	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	41.7	1.3424	16.24	1.3140	0.0164	0.0005	0.24	0.0087	3	0.4
2	41.6	1.0538	17	1.1835	0.0152	0.0004	0.2	0.0082	3	0.4
3	43.6	1.0453	14.86	1.4543	0.0145	0.0007	0.18	0.0101	3	0.5
4	45.5	0.8920	14.23	1.6471	0.0150	0.0003	0.22	0.0093	4	0.5
5	44.4	1.7506	14.06	1.1567	0.0155	0.0005	0.2	0.0064	3	0.3
6	44.5	1.8296	15.93	1.8593	0.0165	0.0006	0.19	0.0057	4	0.6
7	45.4	1.1111	14.58	1.2394	0.0148	0.0002	0.13	0.0033	3	0.6
8	45.8	1.0829	18.62	1.6216	0.0155	0.0007	0.13	0.0060	3	0.5
9	51	1.0631	13.49	1.5629	0.0150	0.0004	0.14	0.0066	3	0.3
10	48.8	0.9609	15.21	1.5229	0.0148	0.0015	0.11	0.0023	3	0.4
11	50.4	1.2816	11.96	1.5634	0.0167	0.0003	0.15	0.0059	6	0.4
12	49	0.8224	11.3	1.0165	0.0162	0.0004	0.15	0.0083	4	0.5
13	45.8	1.0814	9.58	1.0584	0.0171	0.0006	0.17	0.0127	5	0.6
14	50.3	0.9641	11.93	0.9246	0.0165	0.0006	0.16	0.0117	5	0.7
15	49.1	1.3095	9.38	0.4236	0.0183	0.0005	0.22	0.0086	6	0.8
16	48.7	1.2813	10.28	0.6541	0.0164	0.0006	0.16	0.0090	5	0.7
17	46.8	1.7164	14.92	2.1658	0.0161	0.0003	0.15	0.0076	4	0.7
18	43.5	1.2037	15.39	1.3439	0.0134	0.0005	0.13	0.0065	3	0.4
19	49.3	0.7264	24.71	2.1366	0.0155	0.0005	0.19	0.0096	3	0.3
20	45.9	1.8663	11.72	1.3522	0.0177	0.0009	0.15	0.0119	6	0.10
21	46.8	0.8652	10.88	1.4385	0.0177	0.0008	0.16	0.0120	7	0.15
22	42.8	1.0265	18.49	1.6714	0.0166	0.0007	0.15	0.0124	4	0.5

CAPÍTULO II

POPULATION CONNECTIVITY OF THE MEXICAN RED OAK *Quercus castanea* AT A LANDSCAPE SCALE IN CENTRAL MEXICO

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Population connectivity of the Mexican red oak *Quercus castanea* at a landscape scale in central Mexico.

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Abstract

Pollen is the main vector of gene flow in wind-pollinated trees due to its great potential dispersal over long distances. Therefore, pollen dispersal plays a key role in the maintenance of genetic diversity within populations and connectivity among populations in forest trees such as oaks. For this reason, it is important to identify the topographic and environmental features that promote or restrict gene flow. Using eight polymorphic nuclear microsatellites, we studied patterns of genetic diversity, structure and population connectivity in 22 populations of the Mexican red oak *Q. castanea* to understand how environmental variables and topographic features influence gene flow at the landscape scale. The study was carried out in the Cuitzeo basin in central Mexico, with an area of 4,026 km² and characterized by highly heterogeneous environmental conditions and topography. We found high levels of genetic diversity within populations and low genetic structure, suggesting extensive gene flow. ResistanceGA analysis provided evidence of isolation by environmental resistance, identifying precipitation seasonality, temperature annual range and annual precipitation as significant environmental variables that affect pollen movement, probably because of their effect on flowering phenology.

Key words: landscape genetics, genetic connectivity, *Quercus*, pollen, gene flow.

Introduction

Trees constitute a keystone functional group because of their ecological and economic importance. Many forest tree species are characterized by long generation times and the capacity for long dispersal distances through pollen, seeds or both. However, pollen is usually the most important vector of gene flow in wind pollinated species due to its great potential dispersal over long distances, playing a key role in the maintenance of genetic diversity within populations and connectivity among populations (Burczyk and Chybicki 2004; Hamrick 2004). In some forest tree species, pollen dispersal can occur at distances of 80 km or more (Buschbom *et al.* 2011).

One of the goals of landscape genetics is to understand the effect of landscape characteristics on genetic diversity and dispersal patterns of the species, becoming important for preserving ecological and evolutionary processes such as gene flow and functional connectivity (Manel *et al.* 2003; Storfer *et al.* 2007; Manel and Holderegger 2013). The correlation between genetic structure, geographic distance, and environmental heterogeneity allows to describe the dispersal characteristics and gene flow for a particular species (Lowe and Allendorf 2010; Wang and Bradburd 2014). Isolation by distance (IBD) and isolation by resistance (IBR) are two theoretical spatial models that have been proposed to explain patterns of genetic structure and variability in the landscape. IBD is the result of a decrease in gene flow between distant populations (Wright 1943); therefore, genetic differentiation increases with geographic distance regardless of environmental differences among populations. On the other hand, under IBR, there is a positive correlation among genetic

differentiation and resistance distance (cumulative cost of movement of an individual across the landscape) imposed by landscape attributes (McRae 2006).

In other words, IBR models estimate how landscape features influence gene flow by quantifying the resistance that different variables of interest exert on organismal dispersal (Spear *et al.* 2010). Commonly used approaches involve setting up spatial layers for the environmental variables that are considered relevant, which are then used to estimate cost or resistance distances between sampling locations of the study organism (Spear *et al.* 2016). Then, the relationship of gene flow among locations with these resistance distances is estimated. A resistance distance is a value that denotes the degree to which a landscape feature limits or facilitates connectivity across the landscape. Low resistance values are expected to be associated with high gene flow, while high resistance values are expected to restrict gene flow (Spear *et al.* 2016). Resistance surfaces can be used to identify subtle influences of environmental dissimilarities on gene flow that may not be identified using population genetic measures such as genetic diversity, divergence and structure, or inbreeding (Radespiel and Bruford 2014).

Oaks (genus *Quercus*) is one of the most important tree groups in terms of biomass and species richness in America (Cavender-Bares 2016; Cavender-Bares, 2019; Kremer and Hipp 2020). Mexico is an important diversification center, with about 161 species of oaks in total (32-40% of the species in the world) and more than 100 endemics (Valencia 2004). Previous landscape genetics studies of oaks have been conducted at broad geographical scales and have found that topographic features such as mountain and valleys affect the patterns of gene flow (Ashley *et al.* 2015; Gharehaghaji *et al.* 2017; Oyama *et al.* 2018; Li *et al.* 2019).

Nevertheless, less attention has been paid to the role of climatic variables at facilitating or restricting gene flow. However, for example, one of such few studies with another tree genus has recently suggested that precipitation variables influence population genetic diversity and connectivity in *Populus angustifolia*, a wind pollinated tree, through its full geographic range within the USA (Bothwell *et al.* 2017).

Quercus castanea is a Mexican red oak species that presents one of the broadest geographical and altitudinal distribution among Mexican oak species, occupying heterogeneous landscapes (Valencia 2004). It is a dominant constituent in temperate forests but is often found in other vegetation types (i.e. xerophytic scrub, cloud forests) and in perturbed areas (Rzedowski *et al.* 2001; Valencia-Cuevas *et al.* 2015). At a local scale in the basin of lake Cuitzeo located in the state of Michoacán, in central Mexico (Figure 1), it is the most abundant oak species (Aguilar-Romero *et al.* 2016). Previous studies showed that seed-mediated population connectivity has decreased as a result of recent forest fragmentation in the area (Herrera-Arroyo *et al.* 2013), but gene flow through pollen dispersal is still extensive (Oyama *et al.* 2017). However, that study included few forest fragments, and thus, a detailed characterization of gene flow patterns among *Q. castanea* populations within the basin was not obtained. Such characterization is important to identify the topographic and environmental variables that promote or restrict gene flow, which is fundamental for adequate management and conservation of remaining populations.

The main aim of this study was to understand how environmental heterogeneity, and particularly climate variables, determine the population connectivity of *Q. castanea* through its geographical distribution within the Cuitzeo basin. The specific objectives of this study

were: 1) to describe the genetic diversity and structure among populations; 2) to characterize the levels and patterns of population connectivity, and, 3) to evaluate which climate variables significantly influence gene flow.

Material and methods

Study site

The Cuitzeo Lake basin is located in the Trans-Mexican Volcanic Belt (TMBV) in the northern part of Michoacán state and the southern part of Guanajuato state at 19° 30' - 20° 05' N and 100° 35' -101° 30' W (Figure 1). It has an area of 4,026 km². The climate is temperate with a marked rainy season during summer months (June to September). There is also a clear climate gradient, with precipitation increasing and temperature decreasing from north to south of the basin and with altitude (Mendoza *et al.* 2006). The mean annual temperature ranges from 9 to 19 °C and the annual precipitation from 678 to 1367 mm (Lara-De La Cruz *et al.* 2020). The predominant vegetation type is oak forest, but with a high degree of fragmentation due to deforestation and forest degradation (Mendoza *et al.* 2011).

Study species and collecting procedure

Quercus castanea Née (*Lobatae*: red oaks) is a tree 10-18 m in height, wind-pollinated and widely distributed in Mexico along all the major mountains chains (Sierra Madre Oriental, Sierra Madre Occidental, Sierra Madre del Sur and Trans-Mexican Volcanic Belt; Valencia 2004). It is the most abundant oak species within the Cuitzeo basin, usually found at 2000 to 2800 m throughout the basin except in the northeastern portion (Aguilar-Romero *et al.* 2012;

Herrera-Arroyo *et al.* 2013). For this study, individuals from the same locality were considered as a population. Twenty-two populations, covering the distribution of the species within the basin, were sampled (Table 1, Figure 2; see also Lara-De La Cruz *et al.* 2020). Leaves from 10 haphazardly chosen adult trees separated by at least 30 m from each other were collected at each site. Only six individuals were collected in population 10 because of the small population size. Samples were frozen at -80°C until DNA extraction.

Microsatellite amplification and genotyping

Genomic DNA was isolated from 100 mg of frozen leaf tissue using the protocol proposed by Doyle and Doyle (1990) and applying a prewash protocol before the DNA extraction to remove secondary compounds (Gaddis *et al.* 2014). Eight nuclear microsatellite markers designed for *Q. rubra* (Aldrich *et al.* 2002) were amplified by multiplexing in three PCR sets. The first multiplex set included three loci, quru-GA-OM07, quru-GA-OM05 and quru-GA-OC11. The second multiplex also included three loci, quru-GA-IF07, quru-GA-IF02 and quru-GA-OI01. The third multiplex included quru-GA-OC19 and quru-GA-IC08. The multiplex PCR reactions were performed using the Applied Biosystems Multiplex PCR Kit, in a 6 µL volume including 1x multiplex PCR master mix, 2 µM of each primer, dH₂O and 10 ng of template DNA. Forward primers were fluorescently labeled. The thermal cycling conditions included an initial denaturalization at 94 °C for 4 min, then 30 cycles of 94 °C for 1 min, annealing at 48, 50 and 52 °C (respectively for the first, second and third multiplex mixes) for 1min, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were mixed with a GeneScan-600 LIZ size standard, and run in an 8-capillary

Applied Biosystems 3500 sequencer. GeneMarker ver. 2.6.4 (SoftGenetics LLC) was used for fragment analysis and final sizing in base pairs.

Geographic and environmental variables

Spatial coordinates were recorded for each population using a global positioning system (GPS) unit. A set of 19 bioclimatic variables were obtained from each coordinate from www.worldclim.com (Hijmans *et al.* 2005) with a spatial resolution of 0.0083 ° (~ 1km²) using Arc View ver. 3.3 (ESRI, 1999). Additionally, we obtained elevation from the digital elevation model “Continuo de Elevaciones Mexicano” (<https://www.inegi.org.mx/>). To reduce redundancy, we used the JMP 8 program (SAS Institute, Cary, North Carolina) to calculate pairwise correlations among variables and then dismissed the more specific variable (e. g. preferring annual mean temperature over maximum temperature of the warmest month) in each pair of highly correlated environmental variables ($r \geq 0.8$). The final set of environmental variables was constituted by annual mean temperature (Bio1), mean diurnal range (Bio2), isothermality (Bio3), minimum temperature of the coldest month (Bio6), temperature annual range (Bio7), annual precipitation (Bio12), precipitation of the driest month (Bio14), precipitation seasonality (Bio15), precipitation of the driest quarter (Bio17) and elevation.

Data analysis

Genetic diversity

Genetic variation was estimated for each population using the following parameters: the mean number of alleles per locus (N_a), the mean number of effective alleles per locus (N_e), the mean observed heterozygosity (H_o) and the mean unbiased expected heterozygosity (H_e), all of which were computed using GenAlEx 6.5 (Peakall and Smouse 2012). It is well known that microsatellites are susceptible to genotyping errors (Guichoux *et al.* 2011). Due to that, null allele frequencies and a corrected F_{ST} were estimated using the software FreeNA (Chapuis and Estoup 2007) with the number of replicates fixed to 2000. We performed this analysis using the ENA correction method to correct for the possible bias induced by the presence of null alleles on F_{ST} estimation (Chapuis and Estoup 2007). We also evaluated the effect of null alleles on the estimation of inbreeding levels in the populations with the software INest 2.0 (Chybicki and Burczyk 2009). This software allows the calculation of an unbiased inbreeding coefficient (F_{IS}) for multilocus data in presence of null alleles. The analysis evaluates the effects of null alleles (n), inbreeding (f), and genotyping errors (b) on the homozygosity values implementing the full model (nfb) and its comparisons with the alternative models (nf , nb , fb) using the Deviance Information Criterion (DIC).

Genetic structure

GenAlEx 6.5 (Peakall and Smouse 2012) was used to perform analyses of molecular variance (AMOVA) to partition genetic variation among and within populations by estimating F_{ST} and R_{ST} using 999 permutations for their significance. To estimate the number of differentiated genetic clusters in the data, we conducted two different Bayesian grouping analyses, one with Structure v.2.3.4 (Pritchard *et al.* 2000) and the second with Geneland v. 4.0.5 (Guillot *et al.*

2005). Structure was set to run using the admixture model, with values for the number of assumed genetic clusters (K) from 1 to 10, with 10 independent runs for each K . The length of the burn-in period was 50^4 followed by 10^6 Markov chain Monte Carlo repetitions. After running the program, the most likely value of K was determined based on the maximum value of ΔK (Evanno *et al.* 2005) as implemented in the Structure Harvester 0.6.9 software (Earl and von Holdt 2012).

The Geneland algorithm also identifies genetic clusters based on individual genotypes but includes geographic positions as an additional parameter in the analysis in order to identify spatial boundaries between genetic groups. In our case, we performed ten runs, each including 200 000 Markov chain Monte Carlo (MCMC) iterations with a thinning of 200, based on the correlated frequency model with an uncertainty of 100 m associated to spatial coordinates, and with the number of clusters (K) varying between one and 10. Excluding the first 200 values out of the 1000 saved iterations as a burn-in, MCMC convergence was assessed by comparing the number of clusters across replicate runs, with the highest mean posterior probability used as a criterion to choose the best run.

Migration rates among populations

We estimated recent migration rates among all populations using BayesAss 3.0.4 (Wilson and Rannala 2003). This software estimates migration rates over the last two or three generations through a Markov chain Monte Carlo procedure and does not assume that populations are in Hardy-Weinberg or migration-drift equilibria. A total of 10^7 MCMC

iterations (discarding the first 10^6 iterations as burn-in) were carried out, and samples were collected every 2000 iterations. The convergence and stability of the MCMC algorithm was examined by visualizing the plot of posterior parameter estimates using the software Tracer 1.6 (Rambaut *et al.* 2014). Delta values for migration rate, allele frequencies, and inbreeding values coefficients were set at 1.0, 0.7 and 1.0 respectively.

Landscape genetics analysis

We performed a landscape genetics analysis using the R package “Resistance GA” (Peterman 2018). This software generates landscape resistance surfaces that represent the cost of dispersal imposed by different landscape variables. ResistanceGA optimizes single and multiple surfaces without needing a priori resistance values based on expert opinion or ecological characteristics of the species, and in consequence, it eliminates potential biases due to inadequate knowledge of the species-specific cost of dispersal. It relies on a genetic algorithm to explore the parameter space to maximize the correlation between pairwise genetic distances and landscape resistance distances (i.e., landscape variables) without any a priori suppositions (Peterman 2018).

For this analysis, we used the previously described set of nine climate variables plus altitude. We optimized each environmental layer independently, using an eight neighbor joining scheme for measuring connectivity and the *commuteDistance* function, exploring resistance values up to 1,500. The *commuteDistance* function is equivalent to Circuitscape, with the advantage that it can be run in parallel; it calculates the time it takes for an individual

to move from point *a* to point *b* in a particular landscape (Kivimäki *et al.* 2014; vanEtten 2017; Peterman 2018). Our dependent variable was pairwise genetic distance measured as Slatkin's linearized F_{ST} ($D = F_{ST}/(1 - F_{ST})$) and our predictor variables were the resistance values of the environmental variables mentioned above. All of our environmental layers were continuous, and we tested all possible transformations for the resistance relation between both variables (i.e., linear, monomolecular, reverse monomolecular, inverse monomolecular, inverse-reverse monomolecular, Ricker, reverse Ricker, inverse Ricker, inverse-reverse Ricker). Besides, pair-wise geographic distances between samples were included as null model. To ensure results were robust, we carried out three independent optimization runs for each of the landscape variables. We identified the optimal resistance layer using the Akaike information criterion (AIC), which was determined from a linear mixed effects model with a maximum likelihood population effects parameterization (MLPE; Peterman *et al.* 2018). To evaluate the robustness of the model selection and optimization, we ran a bootstrap analysis in which 75% of the samples were randomly selected without replacement and each layer was fit to the subset of samples. For each layer, the model's average rank, average weight and percentage (top rank) were calculated with 10 000 iterations.

Results

Genetic diversity

The mean number of alleles per locus (N_a) ranged from 4.88 to 8.63 in the populations, the mean number of effective alleles per locus (N_e) ranged from 4.03 to 5.85, the observed

heterozygosity (H_o) ranged from 0.49 to 0.73 and the unbiased expected heterozygosity (H_e) ranged from 0.68 to 0.80 (Table 2). The mean value of the fixation index was 0.21. According to the FreeNA software, the mean frequencies of null alleles per locus across populations ranged from 0.02 (OI01) to 0.21 (OM05) (Table S1). The global F_{ST} values with and without the ENA correction were $F_{STENA} = 0.026$ and $F_{ST} = 0.027$, respectively, showing no significant effect of null alleles on the level of genetic differentiation (Table S2). According to the INEST software, the DIC value of the nfb model was lower than the DIC value of the nf model (12853.12 and 12855.03, respectively) indicating a contribution of null alleles to the inbreeding value. The revised estimated of F_{IS} was considerably lower (0.054) and consistent with the highly outcrossing nature of oak species.

Genetic structure

The AMOVA showed that the population genetic differentiation was low but significant ($F_{ST} = 0.041$, $p = 0.001$; $R_{ST} = 0.052$, $p = 0.001$). The analysis in Structure indicated that the most likely number of genetic groups was $K = 2$ (Figure 3a and 3b). Both genetic clusters are present in all the populations, but the red cluster shows a higher frequency in populations in the center and the northern part of the basin (Figure 3c). In turn, Geneland identified six genetic clusters, varying from 5-8 in different runs (Table 3). Eleven populations corresponded to the green cluster (11, 12, 14, 15, 16, 17, 18, 19, 20, 21 and 22), while five populations located in the south central part of the basin constituted another cluster (populations 1, 2, 3, 4 and 5, in brown in Figure 3d). The two northwestern populations 9 and

10 formed the red cluster and populations 8 and 13 the yellow cluster. Finally, the orange cluster corresponded to population 7 and the purple cluster to population 6 (Figure 3d).

Migration rates among populations

Results of BayesAss suggest asymmetric gene flow among the studied populations occurring mainly in the E-W (22 arrows), W-E (12 arrows) and N-S direction (11 arrows), and less gene flow in the S-N direction (9 arrows) (Figure 4). The proportion of individuals derived from their own population was high, ranging from 0.677 to 0.748 (Table S3). The highest migration rates, ranging from 0.021 to 0.079 were detected to be into populations 4, 7, 11, 13, 20 and 21, with migration in both directions between populations 11 and 20 and 11 and 21 (Figure 4, Table S1).

Landscape genetics analysis

From all the transformations tested, inverse Ricker function was the best fit for our genetic distance data (Figure 5). According to optimization and model selection results, the precipitation seasonality (Bio15) resistance layer was the best-supported model (24.27% of the times) based on 10,000 bootstrap replicates (Table 4). A value of 100 in precipitation seasonality corresponded to the lowest resistance to gene flow (Figure 5). The next best supported resistance layers were temperature annual range (Bio7, 22.30%), and annual precipitation (Bio12, 15.62%). In these cases, the lowest resistance values corresponded to a temperature annual range of 24 °C and an annual precipitation of 800 mm (Figure 5).

Furthermore, precipitation seasonality was a significant predictor of genetic distance on the generalized linear mixed-effects model, followed by temperature annual range and annual precipitation (Table 5). The climatic spatial resistance maps showed low resistance (Figure 6), suggesting significant connectivity among populations.

Discussion

Two important but not completely well understood processes in plant ecology and evolutionary biology are pollen and seed dispersal, which are critical for successful reproduction, gene flow and maintenance of genetic diversity (Sork *et al.* 2006; Cruzan *et al.* 2020). Nevertheless, a small number of landscape genetics studies have been carried out on plants (Cruzan *et al.* 2020). Given the importance of dispersal, it is crucial to understand the ecological drivers that impact the patterns of gene flow and its effects at different spatial scales (Begue *et al.* 2017; Cruzan *et al.* 2020).

Quercus castanea has high ecological importance and has been the subject of studies focusing on phylogeography (Peñaloza-Ramírez *et al.* 2019), hybridization (Valencia-Cuevas *et al.* 2015), population genetics (Valencia-Cuevas *et al.* 2014), community genetics (Tovar-Sánchez *et al.* 2013), gene flow estimation in a landscape with heterogeneous physiography (Oyama *et al.* 2018) and gene flow estimation in fragmented landscapes (Herrera-Arroyo *et al.* 2013; Oyama *et al.* 2017). In this study we focused on understanding in detail the distribution of genetic variation and gene flow patterns within the Cuitzeo basin and in evaluating the role of climatic variables at facilitating or restricting connectivity at this scale.

Our results evidenced high genetic variation. The high genetic diversity in terms of allele richness and heterozygosity is comparable to those values reported for *Q. castanea* by Valencia-Cuevas *et al.* (2014) and Oyama *et al.* (2017). Also, this high genetic diversity is similar to values found in other oak species (Gugger *et al.* 2013, Ashley *et al.* 2015; Ramos-Ortiz *et al.* 2016; Shi *et al.* 2017; Oyama *et al.* 2018; Lupini *et al.* 2019). Inbreeding values (F_{IS}) were significant and are probably due to mating among relatives as detected by Oyama *et al.* (2017).

Gene flow strongly influences the genetic diversity, connectivity and spatial genetic structure of forest trees populations. In nature, geographic and environmental factors are not mutually exclusive and can jointly affect gene flow. In this study, we found weak population genetic structure among populations of *Q. castanea* which represented 4-5% of the total genetic variation. The difference between results regarding the number of genetic groups identified can be attributed to the fact that the analysis in Structure did not consider the spatial location of the populations, while Geneland uses geographical coordinates as additional information to aid the clustering procedure and usually results in the detection of finer genetic structure (Guillot *et al.* 2005).

Our estimates of migration rates showed high gene flow in the E-W, W-E and N-S directions, and less gene flow in the S-N direction across the basin, and support the previously suggested high gene flow levels for this species in the Cuitzeo basin (Oyama *et al.* 2017). It is interesting that recent gene flow crossing the basin was inferred between populations separated by more than 50 km (i.e., 22 and 13, 10 and 20 and 10 and 7) and even by 82 km (populations 22 and 11) (Tables S3 and S4). This is congruent with previous evidence

showing that effective long distance pollen dispersal in oaks can occur at distances larger than 80 km (Buschbom *et al.* 2011). However, it is possible that pollen has not directly crossed the basin, but rather gene flow has occurred through chains of populations or through isolated trees (as evidenced by Oyama *et al.* 2017) not sampled in this study. Our results indicate that all *Q. castanea* populations show genetic connectivity with at least one other population.

According to the ResistanceGA analysis, resistance values associated to the environmental variables evaluated were in general low, which means that there is little restraint to gene flow between all populations (Figure 6, Table S5). However, this analysis identified precipitation seasonality, temperature annual range and annual precipitation, rather than geographical distance, as significant environmental drivers of gene flow across the basin for this oak species. Even though the precise mechanism through which these variables could influence gene flow may not be easy to identify, it can be speculated that this probably occurs because of their effect on flowering phenology. Temperature and precipitation are considered relevant climatic drivers of reproductive phenology (e.g., flowering, seed release; Caignard *et al.* 2017) but their effects could be different between tree species and ecosystems (Koenig *et al.* 2014). Populations of the same tree species can respond in the same way to climatic signals resulting in synchronized flowering (Bogdziewicz *et al.* 2017); and, it has been suggested that effective pollen-mediated gene flow largely depends on the synchrony of flowering among populations (Whittet *et al.* 2017; Rousi *et al.* 2019). In oaks, flowering phenology is influenced by climate, i.e. wet and cold climate produces heterogeneous microclimatic conditions and the desynchronization of plants, resulting in long pollen

seasons and reproductive failure (Koenig *et al.* 2015). On the other hand, dry and warm climate during pollen seasons leads to synchronous pollen release (Koenig *et al.* 2015). Because landscape genetics studies aim to understand patterns of gene flow, reproductive phenology becomes highly relevant in the case of plants.

While previous studies have considered spatial models to explain patterns of genetic variation and genetic structure in forest tree species at a whole-range level (Ortego *et al.* 2012; Temunovic *et al.* 2012; González-Díaz *et al.* 2018; Toth *et al.* 2019) we have shown that there is a significant IBR effect, even with high gene flow, among populations of *Q. castenea* in a relatively small geographical area (4000 km²). We have also previously shown that there is evidence of significant associations of functional trait variation with temperature and water availability gradients among populations of *Q. castenea* in the Cuitzeo basin (Lara-De La Cruz *et al.* 2020). Overall, these results indicate that IBR and functional differentiation can occur as a result of climate variability at relatively small geographic areas.

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Table 1. Locality number and name, altitude, geographical coordinates, mean annual temperature (MAT) and mean annual precipitation (MAP) for the collecting sites.

Population	Lat N	Lon W	MAT (°C)	MAP (mm)	Altitude (m)
1. Jesús del Monte	19.6443333	-101.153389	16.2	951	2118
2. San Miguel del Monte	19.6321944	-101.140583	16.4	949	2097
3. Presa Cointzio	19.6312222	-101.251361	16.7	889	2038
4. Las Cruces	19.5840833	-101.250417	16.6	936	2060
5. Carretera Pátzcuaro	19.6325278	-101.272917	16.6	896	2007
6. Tamanguio	19.4639444	-101.374806	14.3	1145	2457
7. San Bartolomé Coro	19.8921111	-100.867833	16.4	856	2138
8. San José del Rincón	19.869	-100.775972	17.1	824	2050
9. San Nicolás Tumbastatiro	19.9830833	-101.355889	16.0	805	2285
10. San Isidro	20.0116111	-101.35325	16.7	766	2233
11. Fontezuelas	19.5728611	-101.461222	15.6	974	2191
12. Mina Arena	19.4949722	-101.545306	15.2	1032	2422
13. Rosa de Castilla	19.8750833	-101.282056	16.0	840	2218
14. Carretera Mil Cumbres	19.6781583	-101.005172	16.3	945	2048
15. Tiristarán	19.7428611	-101.38525	15.8	868	2226
16. Capula	19.7134167	-101.449444	15.4	924	2341
17. Las Huertas	19.7300528	-100.930372	15.5	1038	2196
18. El Temazcal	19.6509167	-100.954622	15.6	1067	2207
19. Cerro El Remolino	19.6169444	-101.335278	14.2	1087	2523
20. Las Tablas	19.5073222	-101.442847	15.4	1026	2259
21. Cerro Quinceo	19.7666222	-101.275744	15.4	933	2341
22. Carretera Ucareo	19.8548694	-100.732603	15.3	990	2369

Table 2. Values of genetic variation of *Q. castanea* using eight microsatellite loci. Sample size, mean number of alleles per locus (Na), mean number of effective alleles per locus (Ne), observed heterozygosity (Ho), unbiased expected heterozygosity (He) and the fixation index (F) are shown.

Population	Sample size	Na	Ne	Ho	He	F
1. Jesús del Monte	10	6.88	4.35	0.54	0.74	0.24
2. San Miguel del Monte	10	7.38	5.23	0.55	0.79	0.30
3. Presa Cointzio	10	7.25	5.24	0.60	0.74	0.16
4. Las Cruces	10	7.50	4.46	0.59	0.73	0.18
5. Carretera Pátzcuaro	10	7.38	5.68	0.60	0.79	0.24
6. Tamanguio	10	6.88	4.89	0.61	0.75	0.17
7. San Bartolomé Coro	10	6.25	4.30	0.49	0.71	0.29
8. San José del Rincón	10	7.63	5.20	0.53	0.76	0.31
9. San NicolásTumbastatiro	10	7.38	5.30	0.56	0.79	0.28
10. San Isidro	6	4.88	4.03	0.52	0.68	0.20
11. Fontezuelas	10	7.25	5.36	0.58	0.74	0.18
12. Mina Arena	10	7.75	5.39	0.66	0.77	0.20
13. Rosa de Castilla	10	6.50	4.81	0.58	0.73	0.23
14. Carretera Mil Cumbres	10	7.50	4.93	0.60	0.72	0.16
15. Tiristarán	10	6.88	4.52	0.64	0.75	0.16
16. Capula	10	8.63	5.05	0.68	0.77	0.13
17. Las Huertas	10	6.88	5.05	0.59	0.73	0.18
18. El Temazcal	10	7.13	5.05	0.60	0.73	0.16
19. Cerro El Remolino	10	6.88	5.03	0.56	0.75	0.28
20. Las Tablas	10	7.25	5.42	0.58	0.74	0.31
21. Cerro Quinceo	10	7.38	5.44	0.58	0.77	0.22
22. Carretera Ucareo	10	8.00	5.85	0.73	0.80	0.08
Mean	9.81	7.15	5.03	0.59	0.75	0.21

Table 3. Multiple run for inferring the number of population using Geneland software.

Run	Modal number	% of model number	Mean of probability density
1	6	29.5	-4284.26
2	7	35.7	-3804.99
3	5	32.2	-3989.94
4	6	24	-3760.66
5	7	23.1	-3934.41
6	8	24.9	-3200.89
7	7	30.7	-3446.21
8	6	41.2	-2959.85
9	7	38.1	-3771.59
10	8	25.9	-4338.92

Table 4. Model selection results for the generalized linear mixed-effects models optimized on genetic distance (Slatkin's linearized F_{ST}) for *Q. castanea*. K is the number of parameters for each model.

Layer	K	Equation	AIC	Average weight	Average rank	Top model (%)
Temperature annual range	4	Inverse-Reverse Ricker Transformation	-762.97	0.078	4.287	22.30
Annual precipitation	4	Inverse Ricker Transformation	-762.86	0.073	4.693	15.62
Precipitation seasonality	4	Inverse Ricker Transformation	-762.67	0.089	6.298	24.27
Annual mean temperature	4	Inverse-Reverse Ricker Transformation	-762.27	0.060	6.917	8.49
Mean diurnal range	4	Reverse Ricker Transformation	-761.80	0.048	8.028	7.17
Precipitation of the driest month	4	Inverse Ricker Transformation	-761.61	0.045	8.448	8.46
Elevation	4	Inverse Ricker Transformation	-761.41	0.039	9.298	4.42
Isothermality	4	Inverse Ricker Transformation	-760.99	0.026	10.548	0.14
Precipitation of the driest quarter	4	Inverse Ricker Transformation	-760.75	0.022	11.300	0.16
Minimum temperature of the coldest month	4	Inverse Ricker Transformation	-760.49	0.021	12.154	0.52
Distance	2	NA	-759.66	0.049	5.932	8.40

Table 5. Results of parameter estimates from mixed effects models fit to optimized resistance surfaces. Models that performed better are in bold.

Layer	Parameter	B	SE	t-value
Annual mean temperature (Bio1)	Intercept	0.049978	0.002451	20.39138
	Bio1	-0.00292	0.001675	-1.74584
Mean diurnal range (Bio2)	Intercept	0.049978	0.002619	19.08555
	Bio2	0.002312	0.001197	1.930825
Isothermality (Bio3)	Intercept	0.049978	0.002555	19.55854
	Bio3	-0.00194	0.00157	-1.23783
Minimum temperature of the coldest month (Bio6)	Intercept	0.049978	0.002668	18.73296
	Bio6	0.000866	0.000818	1.059072
Temperature annual range (Bio7)	Intercept	0.049978	0.002426	20.60417
	Bio7	-0.00359	0.001697	-2.11795
Annual precipitation (Bio12)	Intercept	0.049978	0.002385	20.95264
	Bio12	-0.00319	0.001562	-2.04002
Precipitation of the driest month (Bio14)	Intercept	0.049978	0.002541	19.67146
	Bio14	-0.00259	0.001609	-1.60978
Precipitation seasonality (Bio15)	Intercept	0.049978	0.002599	19.22641
	Bio15	0.002862	0.001268	2.257097
Precipitation of the driest quarter (Bio17)	Intercept	0.049978	0.002649	18.86876
	Bio17	0.001888	0.001379	1.36771
Elevation	Intercept	0.049978	0.002743	19.06537
	Elevation	0.001755	0.001132	1.550288
Distance	Intercept	0.049978	0.002741	18.23155
	Distance	0.000496	0.000774	0.641432

Figure 1. Climate gradients in the Cuitzeo lake basin and the distribution of the *Q. castanea* populations; a) annual mean temperature, b) annual precipitation.

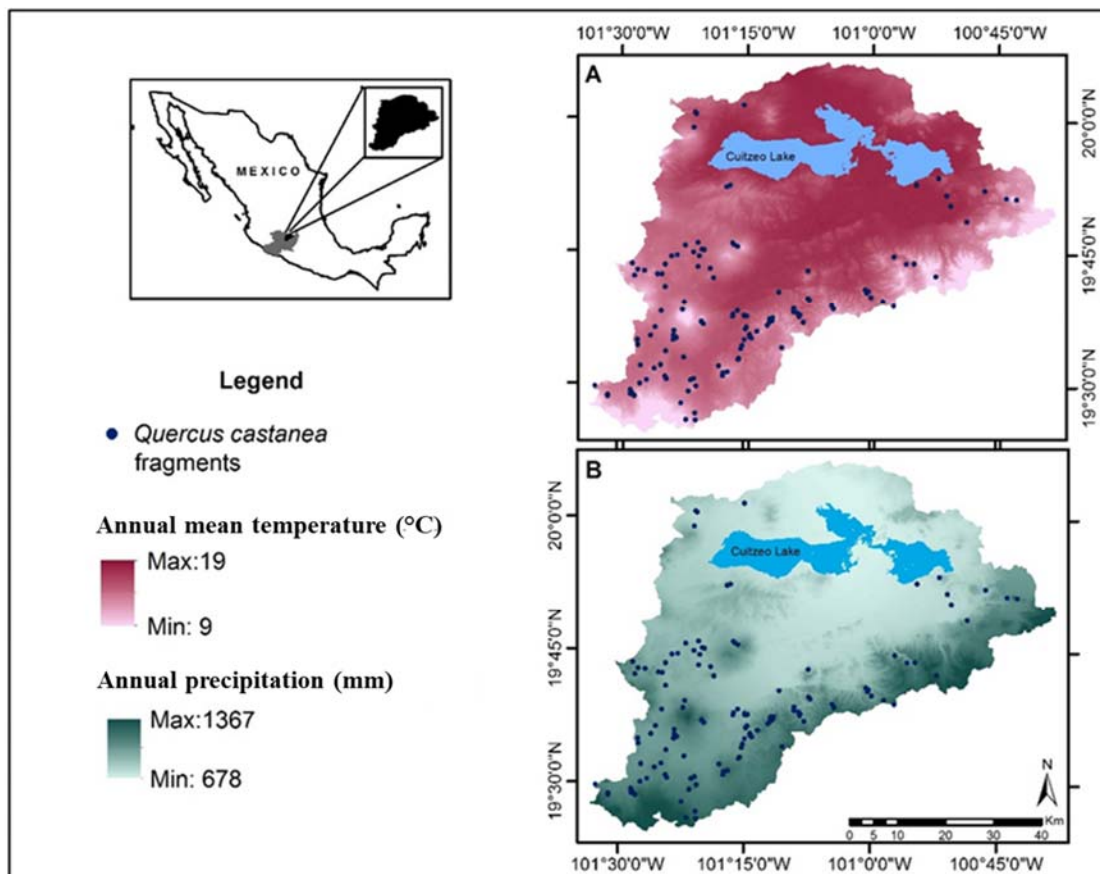


Figure 2. Cuitzeo lake basin and the distribution of the 22 *Q. castanea* populations sampled.

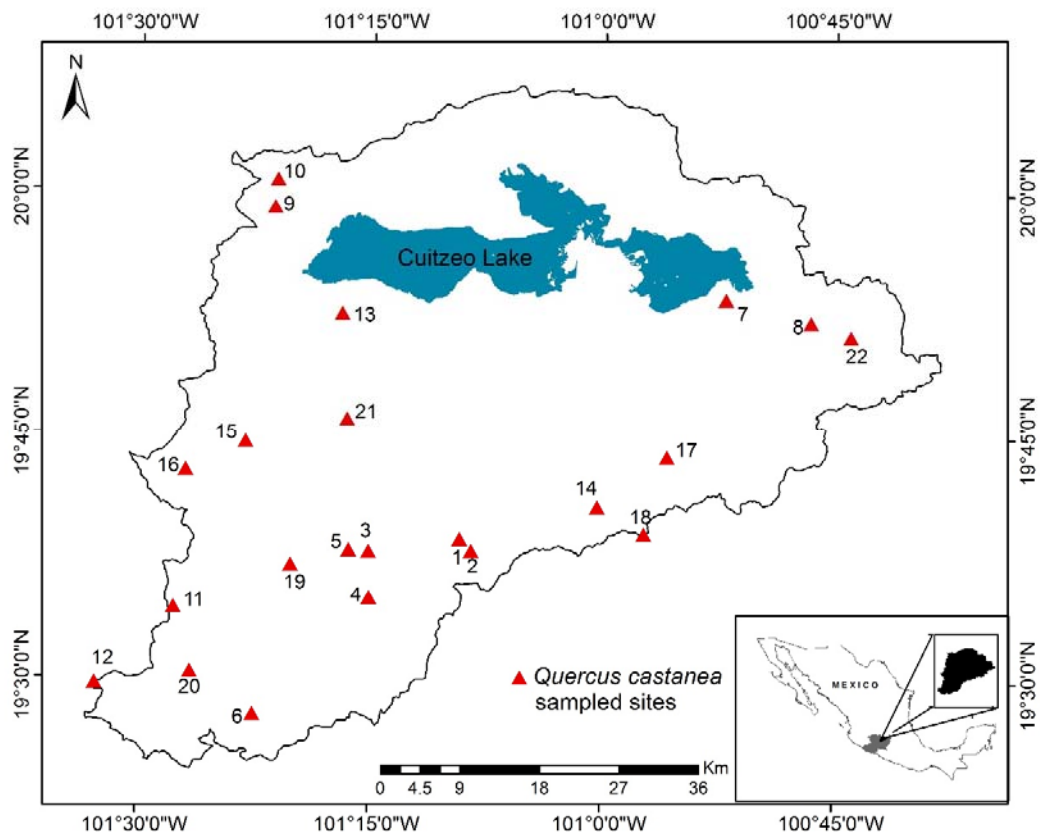


Figure 3. Results of genetic assignment analysis. a) Delta K analysis of the STRUCTURE results. b) Genetic assignment of individuals and populations according to the Bayesian method implemented in the program STRUCTURE, each vertical line represents an individual and the proportion of each color is the proportion of ancestry derived from each of the two genetic groups ($K = 2$) inferred. Populations are separated by black lines. c) Distribution of genetic ancestry proportion groups corresponding to *Q. castanea* within the Cuitzeo Basin. d) Geneland clustering results for *Q. castanea*. Each circle represents a sampled population and each color a different cluster membership.

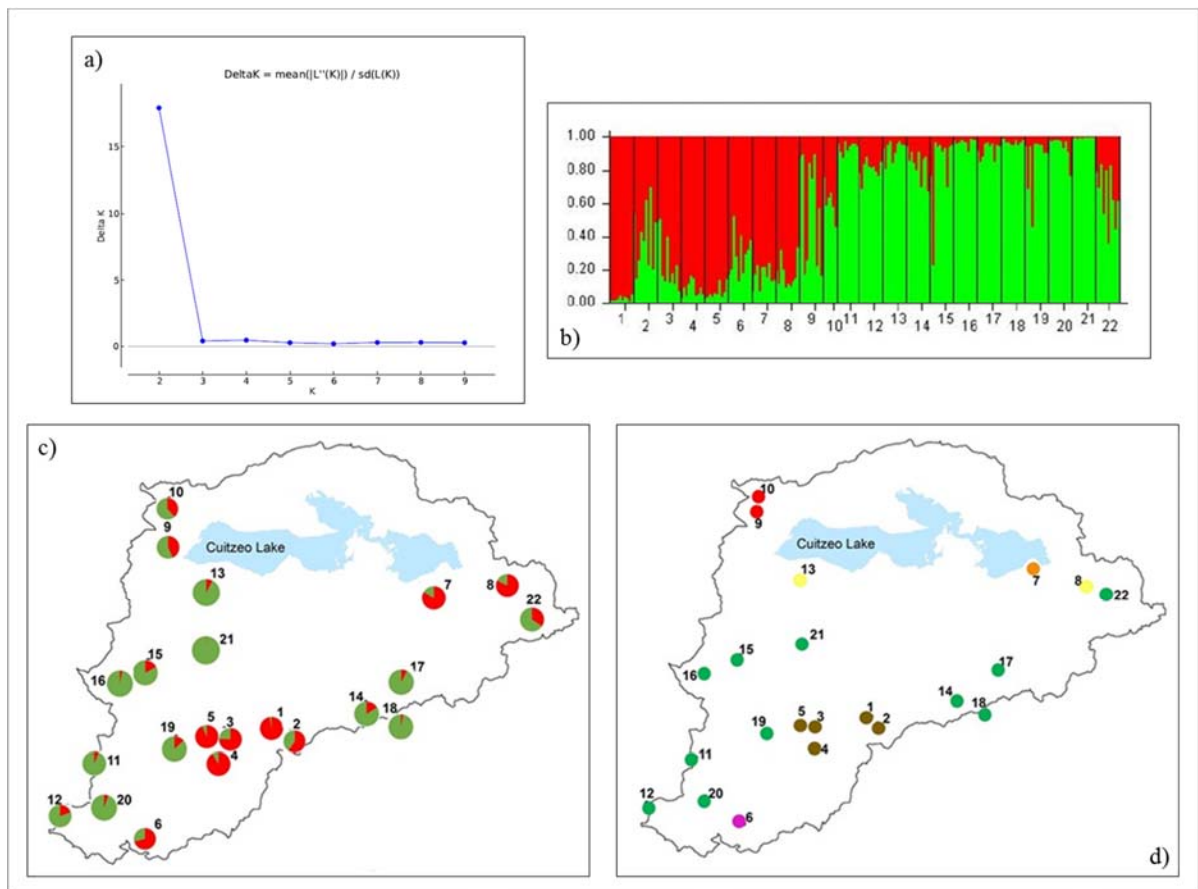


Figure 4. Recent migration rates (m) between populations estimated using BAYESASS. Arrows indicate direction of gene flow among populations and represent values of m higher than 0.02.

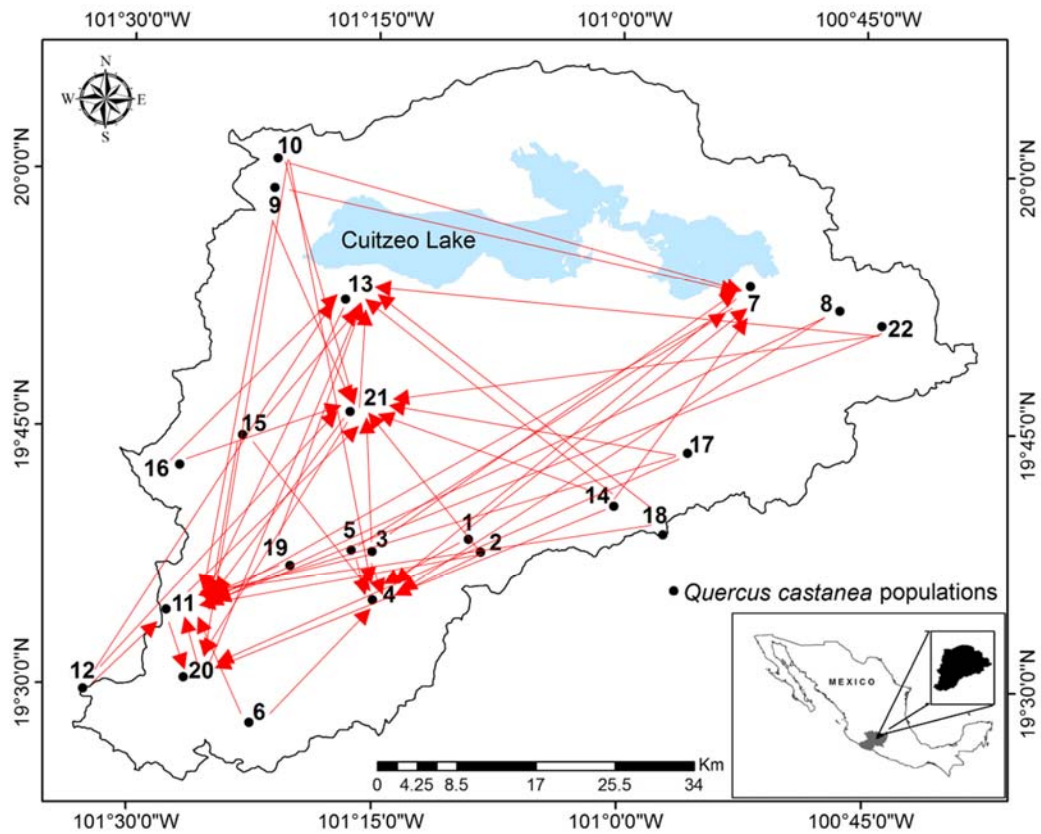


Figure 5. Single surface optimization response curves for the best supported resistance models: a) precipitation seasonality (Bio15, Inverse Ricker), b) temperature annual range (Bio7, Inverse-Reverse Ricker), and c) annual precipitation (Bio12, Inverse Ricker).

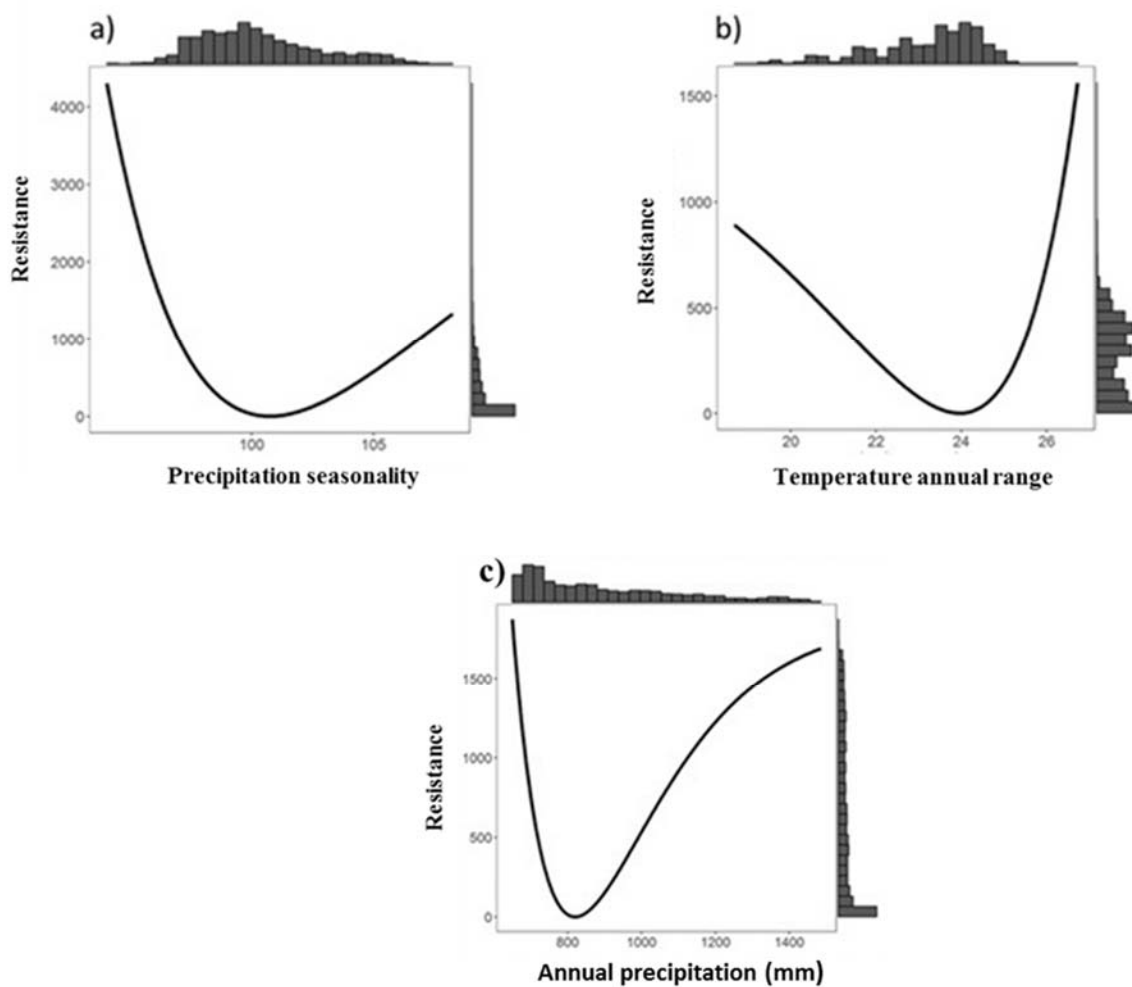
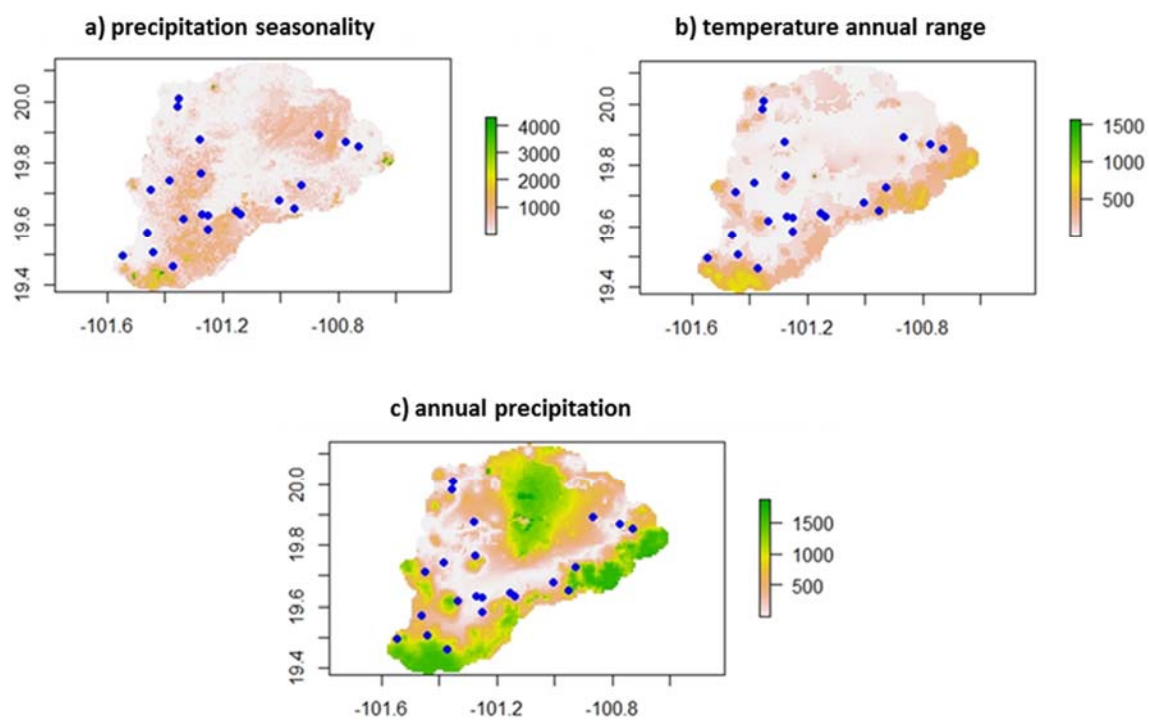


Figure 6. Surface from our best models projected on the study area: a) precipitation seasonality (Bio15), b) temperature annual range (Bio7), and c) annual precipitation (Bio12). Lighter areas represent areas that offer less resistance and therefore facilitate gene flow.



Supplementary material

Table S1. Estimated frequencies of null alleles at 8 nSSR loci in 22 populations of *Quercus castanea* in the Cuitzeo basin.

Population	Locus							
	IF02	OM07	OC19	OI01	IC08	OM05	OC11	IF07
1. Jesús del Monte	0.28	0.12	0	0.13	0.13	0.12	0	0.12
2. San Miguel del Monte	0.10	0.33	0	0	0.09	0.18	0.05	0.29
3. Presa Cointzio	0.19	0.10	0.11	0.05	0.12	0.09	0	0
4. Las Cruces	0.09	0.24	0.06	0	0.12	0	0	0.18
5. Carretera Pátzcuaro	0.23	0.19	0.05	0.01	0.09	0.10	0.15	0.10
6. Tamanguio	0.14	0.07	0	0	0.22	0.32	0	0
7. San Bartolomé Coro	0.18	0.04	0.05	0.15	0	0.32	0	0.07
8. San José del Rincón	0.22	0.12	0.11	0.06	0.13	0.34	0	0
9. San Nicolás Tumbastatiro	0.21	0.25	0.03	0	0	0.34	0.08	0.18
10. San Isidro	0.41	0	0	0	0.34	0.08	0	0.08
11. Fontezuelas	0.08	0.25	0	0	0.02	0.13	0	0.23
12. Mina Arena	0.01	0.14	0	0	0.09	0.21	0	0.06
13. Rosa de Castilla	0.10	0.24	0	0	0	0.32	0.11	0.15
14. Carretera Mil Cumbres	0.23	0.23	0.16	0	0	0.13	0	0
15. Tiristarán	0.06	0.08	0.08	0	0	0.34	0.04	0
16. Capula	0.11	0.06	0.05	0	0.01	0.21	0	0
17. Las Huertas	0.17	0.12	0	0	0.12	0.41	0	0
18. El Temazcal	0.24	0.03	0	0	0.08	0.32	0	0.01
19. Cerro El Remolino	0.10	0.29	0.08	0	0.06	0.13	0.10	0.09
20. Las Tablas	0.08	0.13	0.12	0	0	0.24	0.20	0.01
21. Cerro Quinceo	0.33	0.15	0.03	0.04	0.09	0.09	0	0.07
22. Carretera Ucareo	0.23	0	0	0	0.14	0.10	0	0
Mean	0.17	0.14	0.04	0.02	0.08	0.21	0.03	0.07

Table S2. Estimated F_{ST} values for *Quercus castanea* with and without the ENA correction for null alleles according to FreeNA software.

Locus	Fst not using ENA	Fst using ENA
IF02	0.025	0.020
OM07	0.030	0.030
OC19	0.022	0.023
OI01	0.006	0.005
IC08	0.028	0.026
OM05	0.062	0.054
OC11	0.024	0.030
IFO7	0.019	0.020
Total	0.027	0.026

CAPÍTULO III

GENOTYPING-BY-SEQUENCING REVEALS THAT TEMPERATURE AND PRECIPITATION SEASONALITY VARIABLES DRIVE FINE-SCALE LOCAL ADAPTATION IN THE MEXICAN RED OAK *Quercus castanea*

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Antonio González-Rodríguez.

Genotyping-by-sequencing reveals that temperature and precipitation seasonality variables drive fine-scale local adaptation in the Mexican red oak *Quercus castanea*

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Abstract

There is evidence that climate is one of the main factors that determines local adaptation in forest tree species. Some studies have shown that environmental heterogeneity at various scales influences genetic differentiation between tree populations, creating geographic patterns of genetic variation that are consistent with phenotypic trait variation. By analyzing these associations, specific climate variables that are shaping the genetic structure of the populations can be detected, and even, it is possible to identify loci that could be under natural selection. The Cuitzeo basin, in Michoacán, has an area of 4,026 km² and is characterized by highly heterogeneous environmental conditions and topography. In general, precipitation increases, and temperature decreases from north to south of the basin, and with altitude. The most abundant oak species within the basin is *Quercus castanea*. Using genotyping-by-sequencing (GBS) we identified 5325 single nucleotide polymorphisms (SNPs) genotyped from 184 individuals from 46 populations that represent the environmental heterogeneity and the distribution of *Q. castanea* within the basin. We found 9 outlier SNPs and 194 SNPs associated with climate variation. Some of these SNPs are found in genes that are associated to response to temperature stress, oxidative stress, osmotic stress, germination and flowering.

Our results suggest that isothermality and precipitation seasonality represent important environmental pressures at both levels, genetic and phenotypic, for this oak species.

Key words: *quercus*, local adaptation, SNPs, landscape genomics.

Introduction

The forest landscape can be heterogeneous over short geographical distances. Studies about local adaptation of forest tree populations often cover wide geographical scales (Lu *et al.* 2019; Zhang *et al.* 2019; Martins *et al.* 2018; Brousseau *et al.* 2016), but adaptation to the environment by populations can be at local scales (Eckert *et al.* 2015). Local adaptation results from the balance between gene flow and many factors of natural selection (that could be the result of environmental pressures) among populations and habitats, taking place on genetically controlled fitness differences between individuals (Savolainen *et al.* 2013). The environment has an effect on many characteristics at the same time, and these adaptive characteristics are influenced by a wide number of alleles of small effects (Pritchard and Di Rienzo 2010). Identifying loci with significant genotype-environment associations represents a molecular perspective of the effects of local adaptation that complements research of fitness and phenotypic variation in common garden experiments (Sork *et al.* 2013). The study of patterns of local adaptation at fine spatial scales are very important to comprehend how evolution acts; and are also important to the effective management of forest tree species (Lind *et al.* 2018).

Landscape genomics is a research field whose main objective is to identify the environmental factors that shape adaptive genetic variation and the loci that underly it. It is becoming a powerful and important tool to study local adaptation, especially in long-lived species such as trees (Joost *et al.* 2013; Sork *et al.* 2013; Rellstab *et al.* 2015). Its approach is based on the assumption that selection has taken place over a long enough period of time to establish a detectable and significant relationship between the loci and the environment

(Joost *et al.* 2007). Because of this, landscape genomics provides important insights for conservation biology and evolution (Schwartz *et al.* 2009).

Landscape genomics employs next-generation sequencing approaches, such as GBS (Genotyping By Sequencing) (Elshire *et al.* 2011) to identify genomic regions associated with environmental variables. This approach is ideal for non-model organisms because a priori knowledge of traits or underlying candidate genes is not necessary (Stapley *et al.* 2010; Rellstab *et al.* 2015). GBS is a technique of genome analysis that makes it possible to find neutral and potentially adaptive single nucleotide polymorphisms (SNPs). SNPs represent the most common form of genetic variation throughout the genome, being found in both noncoding and coding regions. They are suitable genetic marker for studying adaptive variation, as they could be associated with functional genes and phenotypic traits on which the selection can act (Morin *et al.* 2004; Gailing *et al.* 2009).

There are two important approaches that are being used to identify genetic variation under selection. The first approach is F_{ST} outlier analysis that relies on the assumption that neutral processes have the same effect on all loci, while selection would affect only certain loci in the genome. Accordingly, loci with genetic differentiation (in terms of F_{ST} parameter) higher or lower than expected under neutrality are considered to be under positive or balancing selection, respectively (Lewontin and Krakauer 1973; Vitti *et al.* 2013). The second approach is environmental association analysis (EAA) whose objective is to identify associations between environmental variables and allele frequencies, relying on the assumption that alleles in a certain locus under selection caused by a particular environmental factor might exhibit a change in allele frequency following environmental change (Holderegger *et al.* 2010; Rellstab *et al.* 2015). Both are complementary approaches for the

detection of genetic variation under selection and have been applied in many organisms including forest trees (De Mita *et al.* 2013; de Villemereuil *et al.* 2014).

Oak species (*Quercus*) are ecologically and economically important in northern temperate forest and are ideal for adaptation studies because some species show extensive geographic distribution range that also includes a wide range of landscapes, environmental and soil conditions (Gailing *et al.* 2009; Petit *et al.* 2013; Cavender-Bares 2019). Research on different oak species has shown evidence of local adaptation through phenotypic data from surveys of trait variation in the field (Uribe-Salas *et al.* 2008; Albarrán-Lara *et al.* 2015, Lara-De La Cruz *et al.* 2020) and common garden experiments (Ramírez-Valiente *et al.* 2010, 2018; Yucedag *et al.* 2019). Candidate genes in different oak species that correlate with climatic variables resulting important for local adaptation have been found through association studies (Alberto *et al.* 2013; Leroy *et al.* 2019), landscape genomic analyses (Sork *et al.* 2016; Martins *et al.* 2018; Pina-Martins *et al.* 2019), and gene expression experiments (Derory *et al.* 2010; Gugger *et al.* 2017). Also, reference genome sequences and annotations have become available (Sork *et al.* 2016; Plomion *et al.* 2018; Ramos *et al.* 2018) offering an opportunity to develop comparative genomic studies between *Quercus* species from a similar perspective, representing valuable insights on whether the same or different loci are associated with climatic variables in related species.

Mexico represents an important diversification center for the oaks, with around 161 species in total (32-40% of the diversity in the world) and more than 100 endemics (Valencia 2004). Understanding the molecular basis of local adaptation in Mexican oaks species is an important aim for the understanding of their evolution, as well as for their effective conservation management. In this study, we generated a single-nucleotide polymorphism

(SNP) data set using genotyping-by-sequencing (GBS; Elshire *et al.* 2011) of samples of the Mexican red oak *Quercus castanea* within the Cuitzeo basin, in central Mexico. This basin is characterized by highly heterogeneous environmental conditions and topography (Leal-Nares *et al.* 2010, Lara-De La Cruz *et al.* 2020). A previous study identified significant associations of functional trait variation with temperature and water availability gradients in populations of *Q. castanea* in the basin (Lara De La Cruz *et al.* 2020) and it was also found that precipitation seasonality, temperature annual range and annual precipitation are environmental variables that affect pollen movement and gene flow, probably because of their effect on flowering phenology (Lara-De La Cruz *et al.* in prep.). In this study, we aimed at assessing if there is molecular evidence of the action of natural selection and hence local adaptation to environmental conditions in this oak species even at this fine geographical scale and despite very high levels of gene flow (Oyama *et al.* 2017; Lara-De La Cruz *et al.* in prep.). The specific goals were: 1) to identify candidate loci (SNPs) potentially related to local adaptation; and, 2) to assess which are the most relevant environmental variables involved in selection pressures.

Materials and methods

Study site

This study was carried out in the basin of Lake Cuitzeo, located in the Trans-Mexican Volcanic Belt in the northern part of Michoacán state and the southern part of Guanajuato state at 19° 30' – 20 ° 05' N and 100° 35' - 101° 30' W. It has an area of 4,026 km². The climate present in the basin is temperate with a marked rainy season from June to September

(summer months) and there is a significant climate gradient with precipitation increasing and temperature decreasing from north to south and with altitude (Mendoza *et al.* 2006) (Figure 1).

Study species and sampling procedure.

Quercus castanea Née is a Mexican oak species that belongs to section *Lobatae*, the red oaks. It has a wide geographic and altitudinal distribution, with populations in the Sierra Madre Occidental, the Central Plateau, the Trans-Mexican Volcanic Belt, and the Sierra Madre del Sur (Valencia 2004). It is a wind-pollinated, monoecious and relatively large tree, 10-18 m in height, but sometimes a shrub (Trelease 1924). The species can be found throughout the Cuitzeo basin, except in the northeastern portion, between 2000 and 2800 m (Herrera-Arroyo *et al.* 2013; Aguilar-Romero *et al.* 2016). For this study, individuals from the same locality were considered as a population. Forty-six locations were chosen for sampling, mostly covering the distribution of the species within the Cuitzeo basin and including the populations previously analyzed for functional traits by Lara-De La Cruz *et al.* (2020) and for genetic structure and connectivity with microsatellites (Lara-De La Cruz *et al.* in prep.) (Table 1, Figure 2). We collected leaves from 4 randomly chosen adult trees separated by at least 30 m from each other at each site. According to Willing *et al.* (2012), this sample size is enough to assess genetic differentiation when thousands of biallelic SNPs markers are used. Leaves were placed in labeled plastic bags, kept in a cooler with ice during transport to the laboratory, and then stored at -80°C until DNA extraction.

Laboratory procedures

Genomic DNA from each tree was extracted from frozen leaf tissue using the Doyle and Doyle (1990) protocol. To remove secondary compounds, a prewash protocol was applied before the DNA extraction (Gaddis *et al.* 2014). DNA samples were genotyped at UCLA Broad Stem Cell Research Center. A total of 184 samples were sequenced on an Illumina HiSeq2000 v3 (100 bp, single-end reads). Sequencing libraries were constructed using the *ApeKI* enzyme, largely following the original GBS protocol, but we pooled 48 samples per preparation instead of 96. Adapters were added during the ligation step not before the restriction digest. To ensure a consistent distribution of fragment sizes among all preps (between 200 -500 bp including adapters) we added AMPure XP bead-based size selection/purification steps after the ligation step and again after the PCR step. The number of PCR cycles was reduced from 18 to 16. Finally, the adequate size distribution of libraries was checked on an Agilent BioAnalyzer with the High Sensitivity DNA assay and quantified using a Qubit fluorometer.

Genomic data processing

The following commands were used in STACKS 1.28: “process_radtags” for quality filtering and demultiplexing of the Illumina reads, “adapter_mm” to remove adapter sequence with up to two mismatches, “r” to recover barcodes with up to one mismatch to the expected barcodes, “c” for removing any read with an uncalled base, “q” to discard low-quality reads as defined by default settings and “t” to trim all reads to 92 bp (Catchen *et al.* 2011; Catchen *et al.* 2013). The filtered reads were aligned to the *Quercus lobata* reference genome v0.5 (NCBI accession #ID 308314, also available at <http://valleyoak.ucla.edu>) (Sork *et al.* 2016) using BWA 0.7.12 (Li and Durbin, 2010). To identify SNPs in each aligned sample using a

minimum phred-scaled confidence threshold of 30 we used GATK 3.7 (DePristo *et al.* 2011). Afterwards, “VariantFiltration” and “SelectVariants” tools were used in GATK to exclude low-quality variants. The following filters were applied: QD < 20.0, MQ < 40.0, MQ RankSum < 12.5, and ReadPosRankSum < -8.0. VCFTools 0.1.15 (Danecek *et al.*, 2011) was used to filter the SNPs to include only diallelic sites, present in at least 95% of individuals, with minimum coverage depth of 5, and minor allele frequency (MAF) ≥ 0.2 . We used this MAF limit to reduce the likelihood of false-positive results due to spurious correlations. Statistics of coverage depth per sample were also performed in VCFTools. After assembly and SNP discovery, the final data set included 184 individuals from 46 populations (four individuals per population), and 5325 SNPs. Samples did not have missing data. On hundred and eighty-three samples had a mean depth greater than 10 X, only one sample had a mean depth of 5.

Geographic and climatic variables.

For each site, altitude and geographic coordinates were recorded using a global positioning system (GPS) unit (Table 1). Nineteen bioclimatic variables derived from monthly precipitation and temperature values (period 1910-2009) were obtained at 30 arc seconds and downscaled using GIS Arc View ver. 3.3 (ESRI, 1999) and a digital elevation model at 30 m of resolution for the Cuitzeo basin (Cuervo-Robayo *et al.* 2014; Correa-Ayram *et al.* 2017). Redundancy within the environmental variables was reduced by the evaluation of pairwise correlations and the dismissal of the more specific variable (e. g. preferring annual mean temperature over maximum temperature of the warmest month) in each pair of highly correlated variables ($r \geq 0.8$). The selected environmental variables were annual mean

temperature (Bio1), mean diurnal range (Bio2), isothermality (Bio3), minimum temperature of the coldest month (Bio6), temperature annual range (Bio7), annual precipitation (Bio12), precipitation of the driest month (Bio14), precipitation seasonality (Bio15) and precipitation of the driest quarter (Bio17). The JMP 8 program was used in these analyses (SAS Institute, Cary, North Carolina).

Genetic diversity and structure

Pairwise population differentiation was estimated using Weir and Cockerham's F_{ST} (Weir and Cockerham, 1984). We also calculated observed heterozygosity (H_o), gene diversity (H_s) and inbreeding coefficient (F_{IS}) per population in R 3.4.1 (R CoreTeam, 2015) using "hierfstat" package (Goudet 2005). Genetic structure was estimated with Admixture 1.3.0. The "optimal" number of genetic clusters was chosen based on the K with the lowest cross-validation error, as recommended by the developers (Alexander *et al.* 2009).

Outlier detection and environmental association analysis

Bayescan 2.1 (Foll and Gaggiotti 2008) was used to identify genomic regions under spatially divergent selection. This bayesian method assumes that populations diverged from an ancestral gene pool, and that their allele frequencies have different degrees of differentiation from it. We tested 5325 SNPs using the following parameter values: prior odds for the neutral model was set to 100, 5000 of outputted iterations, thinning interval size of 10, 20 pilot runs, pilot runs of 5000 iterations, burn-in length of 50,000 iterations. To decrease the chance of

false positives due to multiple testing, we adopted the false discovery rate (FDR) criterion (Benjamini and Hochberg 1995). Using “qvalue” package (Storey 2015) in R 3.4.1 (R CoreTeam, 2015) Q-values were calculated. SNPs with $q < 0.05$ ($-\log_{10} q > 1.3$) were considered as outliers. Some studies suggest that BayeScan has the best performance under departure from the island model in comparison to the other population differentiation methods (Narum and Hess 2011; DeMita *et al.*, 2013).

A second approach for detecting SNPs potentially under natural selection for local adaptation is to test for associations between SNPs and climatic gradients; for this purpose, we used a latent factor mixed model implemented in LFMM 1.3 (Frichot *et al.* 2013). This approach calculates allele-environment correlations between each SNP and each selected environmental variable at a time, while correcting for background population structure using latent factors. Environmental variables are tested independently and settled into each model as fixed effects, the number of K (latent factors) is incorporated in the model as a covariate to control for demographic history and environmental gradients not included in the study (Frichot *et al.* 2013). The majority of EAA methods are susceptible to false negatives when demography and environment are correlated. LFMM is less susceptible to both false negatives and false positives than other methods, such as Bayenv2 (Gunther and Coop 2013), because it is not dependent on a specific demographic model when accounting for population structure (Frichot *et al.* 2013; de Villemereuil *et al.* 2014; Lotterhos and Whitlock 2015). We inferred K (number of genetic clusters) using the program Admixture and using the function “snmf” in the LEA package for R (Frichot and Francois 2015). We did five independent LFMM runs using 10000 iterations and burn-in of 5000. Adjusted p- values (q) were

calculated using the genomic inflation factor (λ) procedure (Devlin and Roeder 1999). We checked the histograms of adjusted p -values to confirm that the confounding effects of population structure were under control. Histograms were very similar, indicating an adequately control for genetic structure. We also carried out these analyses in R using the LEA package.

Genomic contexts of candidate SNPs

The software SnpEff (Cingolani *et al.* 2012) was used to annotate and predict the effect of putative SNP loci under selection with respect to the reference genome of *Q. lobata* (Sork *et al.* 2016). SnpEff annotates SNPs based on their genomic locations, such as intronic, untranslated region (5'UTR or 3'UTR), upstream, downstream, splice site or intergenic regions. It also predicts coding effects such as synonymous or nonsynonymous amino acid replacement, start codon gains or losses, stop codon gains or losses, or frame shifts. Predicted effects are with respect to protein coding genes (Cingolani *et al.* 2012). We also did a search in the NCBI database to found information about the genes for which candidate SNPs fell within, followed by a search in the UniProtKB/Swiss-Prot database to identify homologs and know the protein names and protein function descriptions.

Results

Genetic diversity and structure

The mean genetic differentiation across populations was $F_{ST} = 0.02$, with pairwise F_{ST} among populations ranging from 0.007 to 0.065 (Table 2). Mean observed heterozygosity (H_o) was

0.173, mean gene diversity (H_s) was 0.152, with a mean inbreeding coefficient (F_{IS}) of -0.109. Population number 36, which is located in the southern part of the basin (Figure 2), showed the lowest gene diversity ($H_s = 0.139$) and the highest mean pairwise F_{ST} (0.065). The “optimal” number of genetic clusters inferred from Admixture is $K=1$, suggesting the lack of noticeable genetic structure among populations.

Outlier detection and environmental association analysis

Nine SNPs (0.17% of 5325) with elevated F_{ST} were identified with BayeScan (Figure 3). Mean F_{ST} of these outlier SNPs was 0.139 (SD = 0.029) ranging from 0.105 to 0.183. BayeScan did not identify significantly low outlier F_{ST} values that would be indicative of balancing or purifying selection. Histograms of adjusted p-values were uniformly distributed indicating that $K=1$ appropriately controlled for the potentially confounding effects of population structure.

Through LFMM, we found 194 SNPs (3.6%) that were significantly associated with climatic variables; these SNPs were different than those identified by BayeScan (Figure 4, 5, 6). We considered only the climate variable with the strongest association (i.e. highest $|z|$ -score). Out of the 194 SNPs, 86 were associated with temperature variables (mean $|z|$ -score = 3.98), with isothermality (Bio3) having the highest number of associated SNPs (29). In turn, 28 SNPs were associated with precipitation (mean $|z|$ -score = 3.86), being precipitation seasonality (Bio15) the variable with more associations (14 SNPs). Eighty SNPs were associated with two or more climatic variables (temperature and precipitation combined)

(Table 3). Climate-associated SNPs ($n = 194$) had mean $F_{ST} = 0.038$ (range: 0 – 0.16, SD = 0.034). Combined, BayeScan and LFMM identified 203 candidate SNPs.

Genomic contexts of candidate SNPs

The 203 candidate SNPs fell within 133 genes. Based on their genomic locations we found: 39 intronic, 16 within untranslated regions (5'UTR and 3'UTR), 16 intergenic, 18 within downstream regions, 14 within upstream regions and one within a splice site region; 44 nonsynonymous, 53 synonymous and 2 start gained (Supporting information, Table S1). Based on gene orthology, the identified proteins representing a broad range of biological processes, as DNA replication (i.e., helicase), transcription (i.e., transcription factors and regulatory proteins), metabolism (protein kinases, proteins involved in lipid catabolic processes, proteins involved in ubiquitination, proteases), cell differentiation, receptor proteins, and ion and protein transport. Furthermore, 47 of the 203 SNPs fell within 46 protein coding genes that in *Arabidopsis* are thought to be involved in important plant processes; two of these SNPs were also identified by BayeScan (Supporting information, Table S2). For example, laccase activity (protein Lacasse-15, Liang et al. 2006), auxin genes (gene ABCB4, Santelia et al 2005), response to drought stress (gene MYB60, Cominelli et al 2005; LEA proteins, Hong-Bo et al 2005), response to salt stress (gene ABCC5, Lee et al, 2004), response to oxidative stress (gene NCER1, Li et al 2015).

Discussion

Genetic diversity and structure

Like other oak species, *Q. castanea* is an outcrossing wind pollinated tree species with potentially long distance pollen flow. The low population differentiation ($F_{ST} = 0.02$) and the absence of genetic structure ($K = 1$) suggest high rates of gene flow among populations that would enable the spread of adaptive genetic variation across the basin creating a genetic gradient, instead of a number of differentiated genetic clusters. Furthermore, high level of gene flow was expected in *Q. castanea* within the Cuitzeo Basin based on a previous research (Oyama *et al.* 2017, Lara-De La Cruz *et al. in prep.*). Gene flow has an important role in the homogenization of allele frequencies in both neutral and adaptive loci, resulting in a decrease in population differentiation (Holderegger *et al.* 2006).

Outlier detection and environmental association analysis

The BayeScan analysis identified 9 outliers SNPs. We found less outliers SNPs in comparison with *Q. suber* (29 SNPs, Pina-Martins *et al.* 2019) and *Q. rugosa* (74 SNPs, Martins *et al.* 2018). This result could be due to: 1) the geographic scale of our study, 2) the high gene flow within the basin, and 3) small sample size (4 individuals in this study). It is suggested that outlier tests, such as BayeScan, have limited sensitivity to detect subtle changes in allele frequencies when there is high gene flow counteracting selection (Narum and Hess, 2011). The power of outlier tests depends also to a great extent on sample size, a higher proportion of outlier SNPs can be identified with a larger number of individuals (Lotterhos and Whitlock 2015; Ahrens *et al.* 2018). BayeScan tends to produce fewer false positives than other genetic differentiation methods (De Mita *et al.* 2013; Lotterhos and Whitlock, 2014), but is considered more conservative in identifying outlier SNPs than other

methods (Narum and Hess, 2011). The F_{ST} values of the outlier SNPs obtained (range = 0.105 - 0.183) were considerably higher than the background overall population differentiation ($F_{ST} = 0.02$) providing evidence of divergent selection. Even though gene flow can counteract the effects of selection, it has been suggested that adaptation in forest trees can be maintained despite high rates of gene flow (Kremer *et al.* 2012; Tigano and Friesen 2016).

EAA analyses, such as LFMM are more sensitive to subtle changes in allele frequencies even in small sample sizes (De Mita *et al.* 2013; Stephan 2016; Ahrens *et al.* 2018). This could be the explanation of the higher number of SNPs potentially under selection detected by EAA in this study (194 SNPs). The advantage of this approach is that the environmental variables can be identified, and for the Cuitzeo basin populations, the temperature variables were more significant than precipitation variables.

Studies of other temperate and subtropical tree species have also identified a greater proportion of SNPs associated with temperature than with precipitation (Jaramillo-Correa *et al.* 2015; Mosca *et al.* 2016, Gugger *et al.* 2018; Lu *et al.* 2019). In oaks, the number of SNPs associated with temperature and precipitation variables varies among species. In *Q. suber* (Pina-Martins *et al.* 2019), *Q. pubescens* and *Q. robur* (Rellstab *et al.* 2016) most of the SNPs are associated with precipitation variables; but in *Q. rugosa* (Martins *et al.* 2018), *Q. lobata* (Gugger *et al.* 2016) and *Q. petraea* (Rellstab *et al.* 2016), temperature variables had most of the associations. In general, in this study both temperature and precipitation seem important selective agents within *Q. castanea* populations in the Cuitzeo Basin and confirms that

isothermality represents an important environmental pressure at both levels, genetic and phenotypic, for this oak species (Lara-De La Cruz *et al.* 2020).

Genomic contexts of candidate SNPs

The GBS methodology protocol examines only a small portion of the genome, but our objective was not to identify all the adaptive genetic variation. This study identified 203 candidate SNPs potentially under selection, annotated in the *Q. lobata* reference genome, implied in a variety of biological processes. It is worth to highlight that 47 out of 203 SNPs, have orthologs in *A. thaliana* within functional genes involved in important plant processes (i.e. response to drought stress), and that two of them were also identified by the outlier test detection.

Our results evidence that synonymous, non-synonymous and noncoding SNPs showed signatures of selection. Nonsynonymous SNPs cause amino acid replacements resulting in a change in protein sequence, due to this, they have been mostly thought to be the main target of natural selection. However, there are studies indicating that synonymous substitution may affect mRNA splicing, stability and translation kinetics (Chamary *et al.* 2006; Komar 2007), and that could also affect the production of the final protein (Pagani *et al.* 2005). Likewise, SNPs in noncoding regions may also be implicated in the regulation of gene expression (Barrett *et al.* 2012). Hence, synonymous and noncoding SNPs can show signatures of selection due to a strong linkage with selected loci and because they can be under natural selection directly (Morin *et al.* 2004; Fyon *et al.* 2015).

For example, we identified an SNP putatively associated with a laccase locus. Laccases are known for their role in the lignification process (Berthet *et al.* 2011; Lu *et al.* 2013). Laccase activity can have an impact on tree growth and tolerance to various biotic and abiotic stresses (i.e., pathogens, water deficit, temperature) because lignin quantity and quality affects cell wall fragility (Peter and Neale 2004; Moura *et al.* 2010).

We also found SNPs associated with auxin genes. Auxin is an important plant hormone, that regulates many aspects of plant growth, development and responses to biotic and abiotic stress (Wolters and Jürgens 2009; Korver *et al.* 2018). Adaptive variation in auxin genes has been studied in oaks in response to drought (Homolka *et al.* 2013; Madrisch *et al.* 2019), bud dormancy (Lesur *et al.* 2015) and bud burst (Derory *et al.* 2006). Adaptive variation in auxin genes has also been demonstrated in other temperate tree species as *Picea glauca* (Liu *et al.* 2015) and *Populus trichocarpa* (McKknown *et al.* 2014). We also identified an SNP putatively associated with a late embryogenesis abundant (LEA) proteins, which are proteins involved in drought tolerance in oaks (Madrisch *et al.* 2019; Homolka *et al.* 2013)

Conclusions

We used a landscape genomic approach to investigate adaptive genetic variation in *Q. castanea* at a regional scale. We combined outlier detection tests and environmental association analysis, and we identify loci showing signatures of selection. The environmental drivers that have an impact on adaptive genetic variation in this study are the same that represent an environmental pressure for functional traits variation for *Q. castanea* within this basin. This represents new opportunities for future research to understanding the genetic basis

of adaptation of *Q. castanea* and other Mexican oak species to different environmental conditions at different scales. In the near future, more studies integrating genomic, phenotypic and environmental data are required to gain further insights into the mechanism of oak adaptation.

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Table 1. Locality number, altitude, geographical coordinates, mean annual temperature (MAT) and mean annual precipitation (MAP) for the sampling sites.

Population	Lat N	Lon W	MAT (°C)	MAP (mm)	Altitude (m)
1	19.6443333	-101.153389	16.2	951	2118
2	19.6321944	-101.140583	16.4	949	2097
3	19.6312222	-101.251361	16.7	889	2038
4	19.5840833	-101.250417	16.6	936	2060
5	19.6325278	-101.272917	16.6	896	2007
6	19.4639444	-101.374806	14.3	1145	2457
7	19.8921111	-100.867833	16.4	856	2138
8	19.869	-100.775972	17.1	824	2050
9	19.9830833	-101.355889	16.0	805	2285
10	20.0116111	-101.35325	16.7	766	2233
11	19.5728611	-101.461222	15.6	974	2191
12	19.4949722	-101.545306	15.2	1032	2422
13	19.8750833	-101.282056	16.0	840	2218
14	19.6781583	-101.005172	16.3	945	2048
15	19.7428611	-101.38525	15.8	868	2226
16	19.7134167	-101.449444	15.4	924	2341
17	19.7300528	-100.930372	15.5	1038	2196
18	19.6509167	-100.954622	15.6	1067	2207
19	19.6169444	-101.335278	14.2	1087	2523
20	19.5073222	-101.442847	15.4	1026	2259
21	19.7666222	-101.275744	15.4	933	2341
22	19.8548694	-100.732603	15.3	990	2369
23	19.4733722	-101.362894	15.4	1078	2220
24	19.6700333	-101.157911	16.3	912	2087
25	19.6522805	-101.121833	16.5	914	2154
26	19.6659638	-101.136786	16.6	886	2166
27	19.6372972	-101.222366	16.2	932	2063
28	19.6156194	-101.428022	15.6	958	2266
29	19.6177222	-101.198463	15.1	1053	2271
30	19.5735222	-101.224744	15.5	1048	2203
31	19.5493333	-101.261938	16.2	1000	2106
32	19.4996583	-101.345691	15.5	1079	2206
33	19.7664611	-101.349283	16.1	857	2151
34	19.7199777	-101.343419	16.2	865	2108
35	19.4502944	-101.343347	15.2	1110	2272
36	19.5341527	-101.309572	16.3	999	2076
37	19.5112777	-101.407702	15.7	1022	2174
38	19.5398111	-101.420777	15.8	994	2182
39	19.6540583	-101.081316	15.5	1034	2179
40	19.5449833	-101.250725	15.8	1040	2080
41	19.5882277	-101.392238	15.5	991	2315
42	19.5311666	-101.450788	15.8	989	2162
43	19.4814694	-101.516863	14.9	1070	2400
44	19.5151111	-101.293302	15.3	1078	2270
45	19.6597805	-101.366352	15.5	957	2211
46	19.6833388	-101.406272	15.9	900	2153

Table 2. Diversity parameters based on 46 populations of *Q. castanea* within the Cuitzeo basin. Mean pairwise F_{ST} per population, observed heterozygosity (H_o), gene diversity (H_s) and inbreeding coefficient (F_{IS})

Population	Sample size	Mean F_{ST}	H_o	H_s	F_{IS}
1	4	0.011	0.177	0.158	-0.095
2	4	0.018	0.178	0.157	-0.108
3	4	0.018	0.177	0.157	-0.100
4	4	0.018	0.173	0.152	-0.111
5	4	0.028	0.177	0.151	-0.135
6	4	0.014	0.172	0.154	-0.092
7	4	0.033	0.177	0.153	-0.131
8	4	0.025	0.176	0.153	-0.122
9	4	0.015	0.168	0.150	-0.093
10	4	0.040	0.180	0.151	-0.163
11	4	0.015	0.177	0.156	-0.110
12	4	0.016	0.178	0.158	-0.100
13	4	0.015	0.170	0.150	-0.105
14	4	0.013	0.179	0.160	-0.099
15	4	0.025	0.178	0.155	-0.120
16	4	0.024	0.176	0.154	-0.119
17	4	0.013	0.174	0.154	-0.106
18	4	0.013	0.174	0.156	-0.099
19	4	0.018	0.175	0.155	-0.108
20	4	0.008	0.171	0.158	-0.072
21	4	0.011	0.173	0.158	-0.080
22	4	0.018	0.174	0.153	-0.111
23	4	0.013	0.165	0.155	-0.058
24	4	0.012	0.173	0.155	-0.098
25	4	0.011	0.164	0.151	-0.070
26	4	0.019	0.163	0.148	-0.084
27	4	0.042	0.170	0.149	-0.121
28	4	0.027	0.155	0.143	-0.076
29	4	0.028	0.163	0.147	-0.093
30	4	0.027	0.169	0.147	-0.121
31	4	0.010	0.170	0.155	-0.085
32	4	0.036	0.170	0.148	-0.123
33	4	0.020	0.172	0.153	-0.103
34	4	0.035	0.171	0.150	-0.119
35	4	0.036	0.174	0.149	-0.140
36	4	0.065	0.178	0.140	-0.234
37	4	0.020	0.171	0.150	-0.114
38	4	0.023	0.176	0.153	-0.125
39	4	0.023	0.176	0.151	-0.131
40	4	0.040	0.175	0.147	-0.155
41	4	0.019	0.178	0.153	-0.128
42	4	0.026	0.171	0.150	-0.120
43	4	0.016	0.173	0.155	-0.095
44	4	0.021	0.145	0.146	-0.016
45	4	0.028	0.178	0.154	-0.127
46	4	0.022	0.172	0.151	-0.117

Table 3. SNPs associated with temperature and precipitation variables, in bold the climatic variable with the highest number of associated SNPs.

Environmental variable	Exclusive SNPs
Annual mean temperature (Bio1)	8
Mean diurnal range (Bio2)	14
Isothermality (Bio3)	29
Minimum temperature of the coldest month (Bio6)	22
Temperature annual range (Bio7)	13
Annual precipitation (Bio12)	2
Precipitation of the driest month (Bio14)	11
Precipitation seasonality (Bio15)	14
Precipitation of the driest quarter (Bio17)	1
Total	114

Figure 1. Climate gradients in the Cuitzeo basin and the distribution of the *Q. castanea* populations; a) annual mean temperature, b) annual mean precipitation.

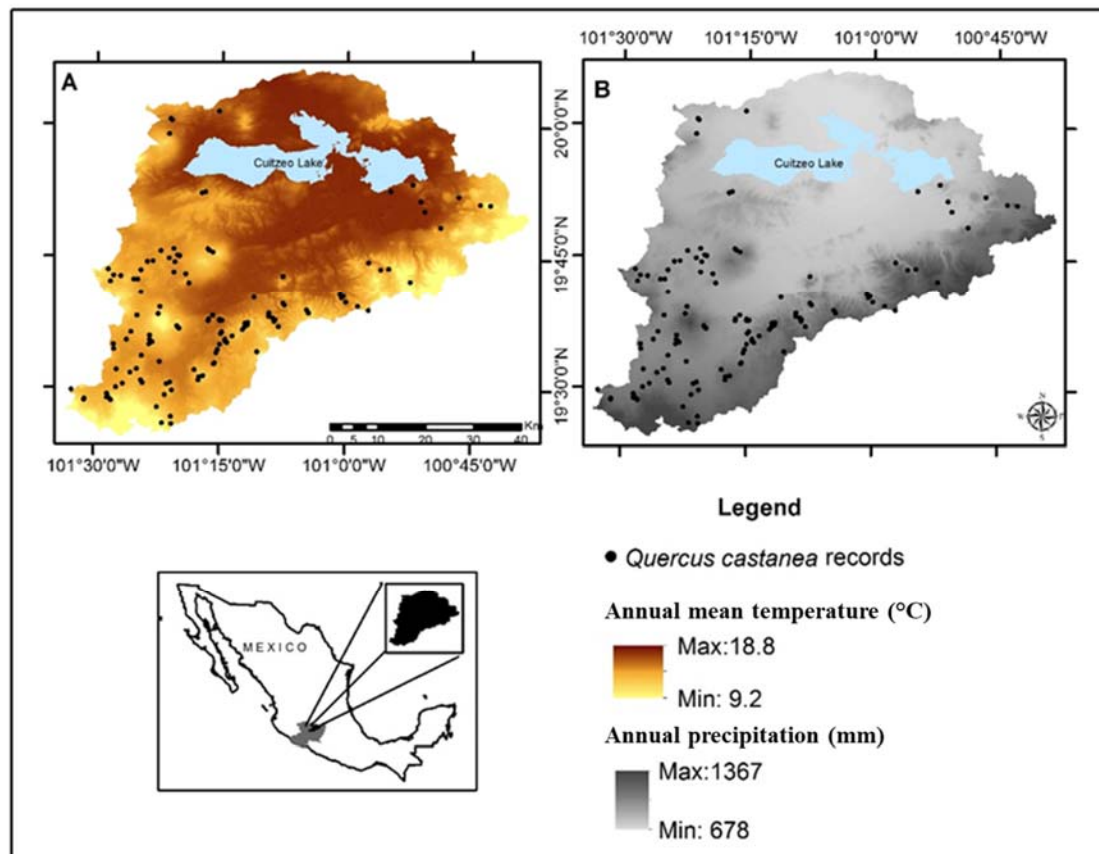


Figure 2. Cuitzeo basin and the distribution of the 46 *Q. castanea* populations sampled.

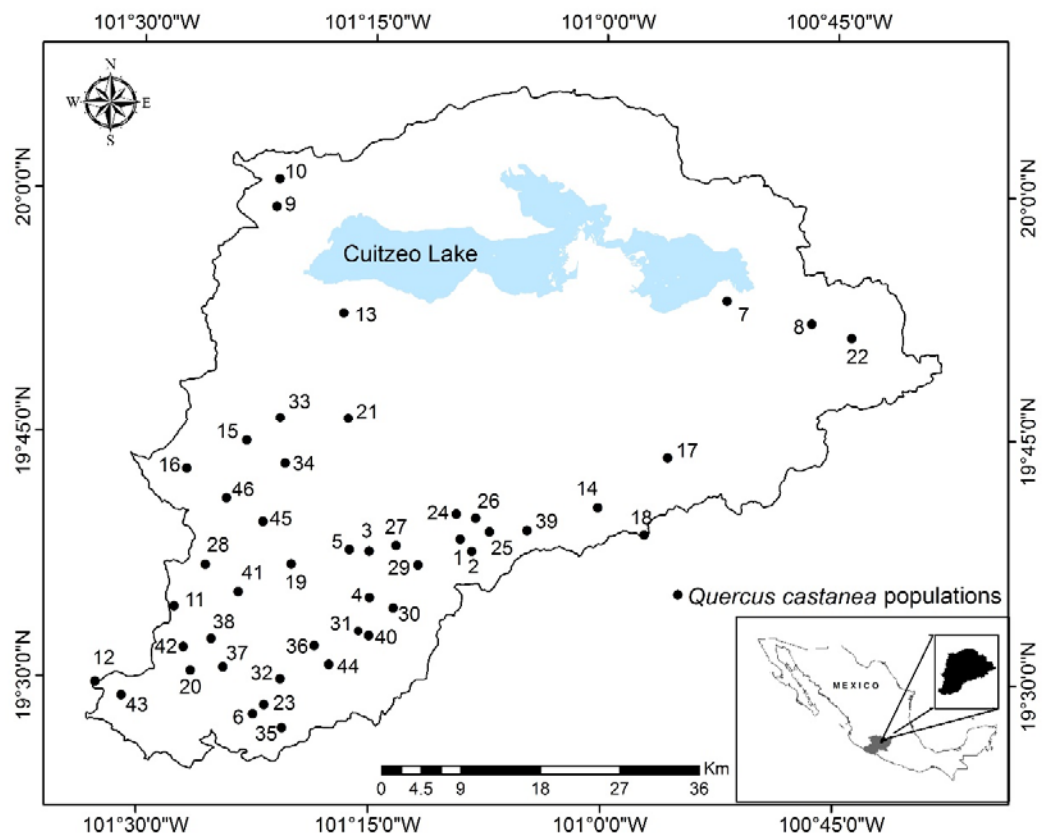


Figure 3. Results for the outlier F_{ST} test based on 46 populations of *Quercus castanea*. SNPs exceeding $\log_{10}q < -1.3$ are classified as outliers.

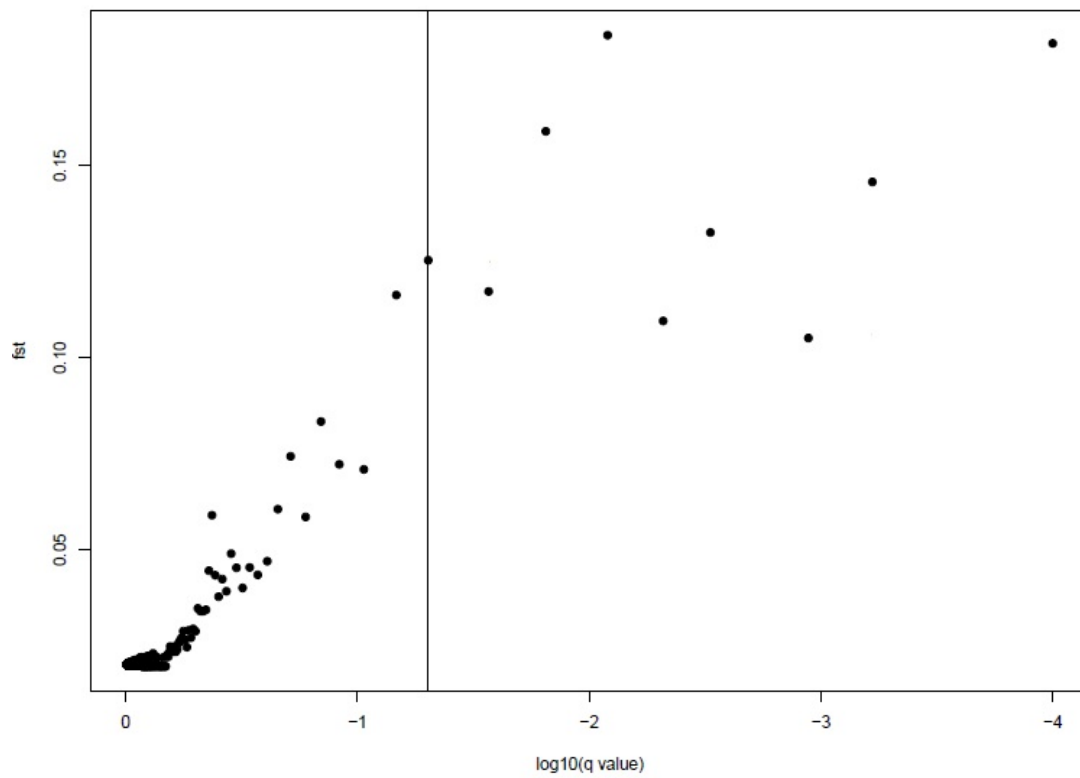


Figure 4. SNPs associated with annual mean temperature (Bio1), mean diurnal range (Bio2), isothermality (Bio3) in Latent Factor Mixed Models (LFMM) analysis in *Quercus castanea*.

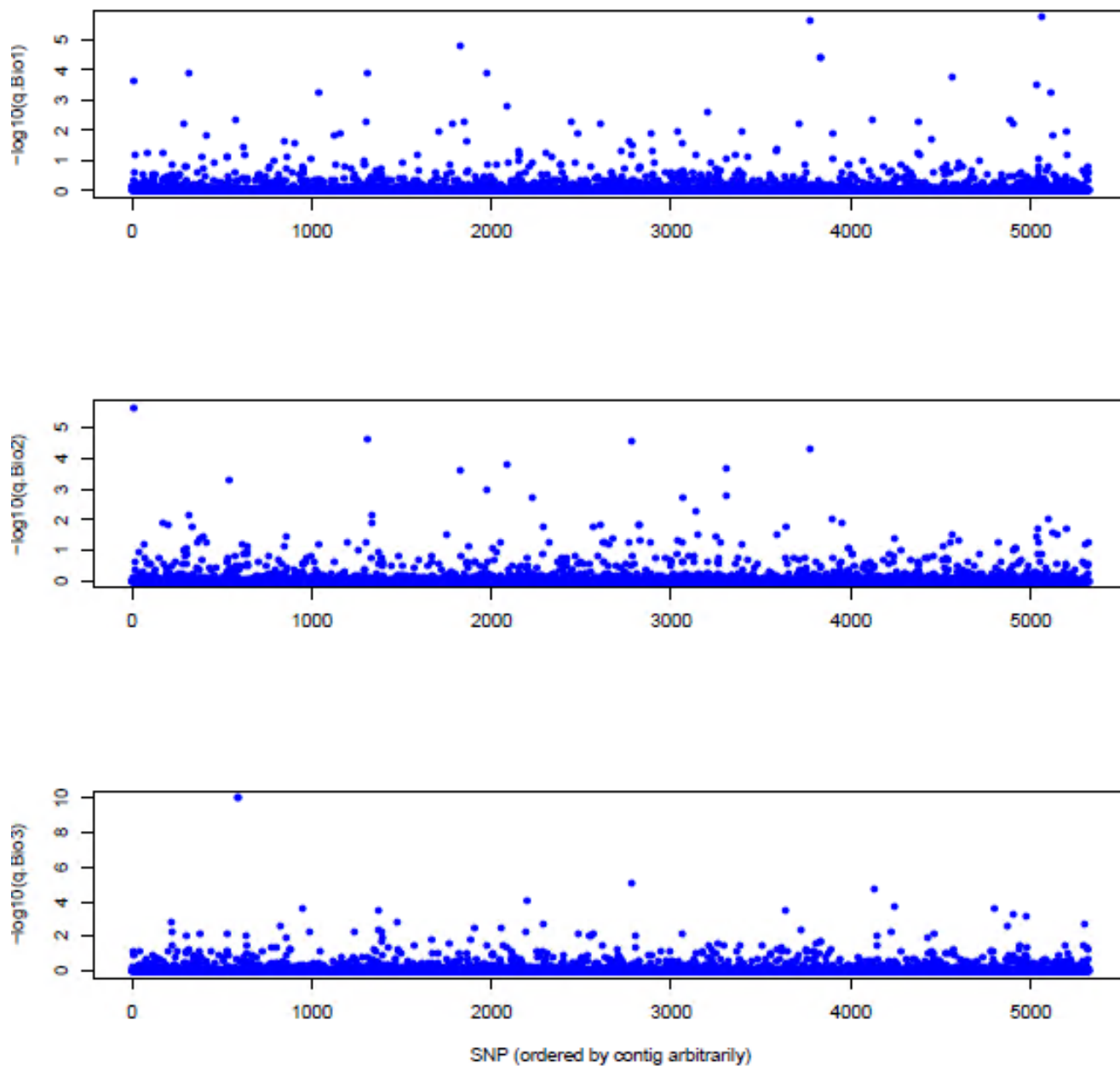


Figure 5. SNPs associated minimum temperature of the coldest month (Bio6), temperature annual range (Bio7), annual precipitation (Bio12) in Latent Factor Mixed Models (LFMM) analysis in *Quercus castanea*.

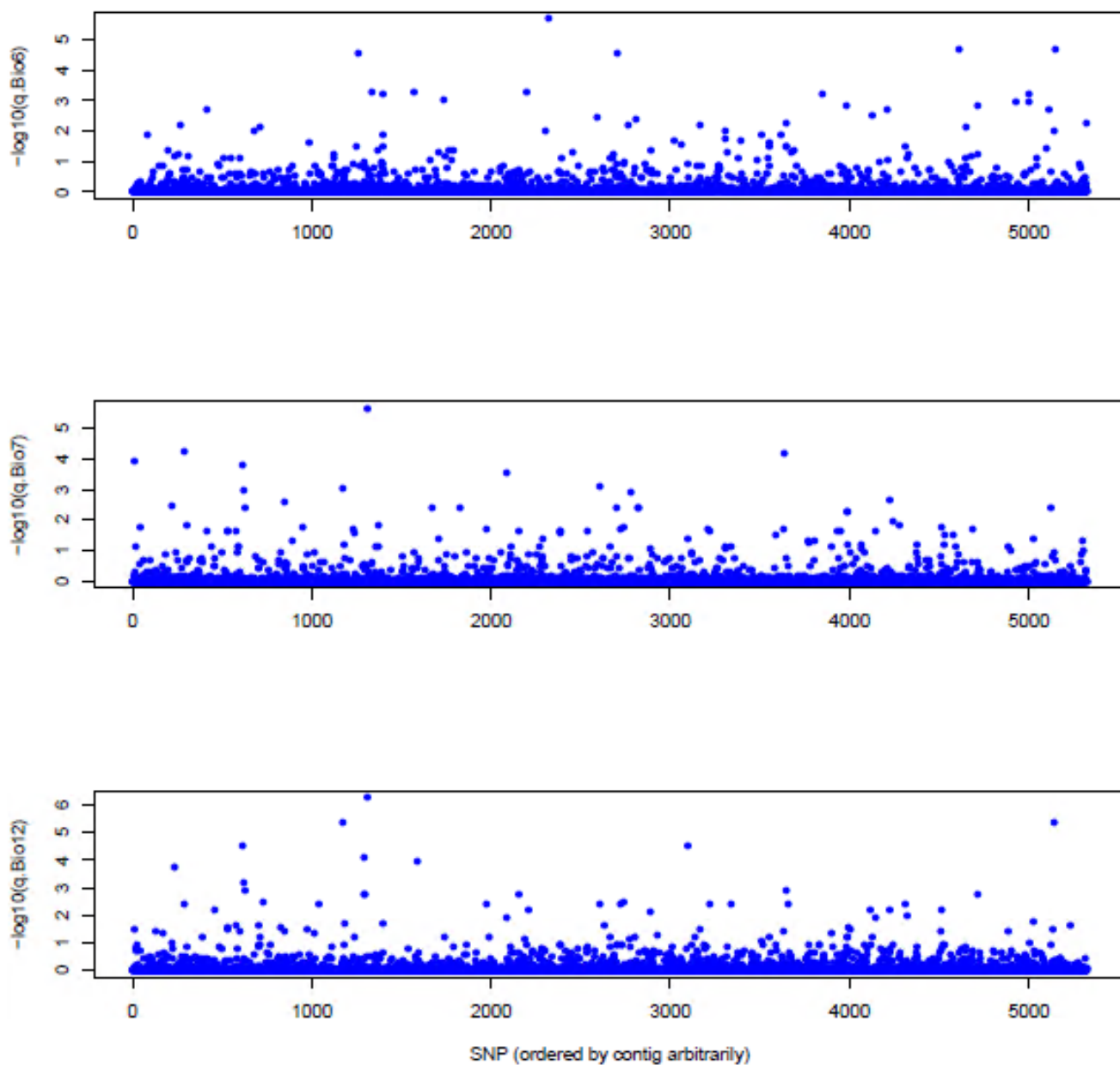
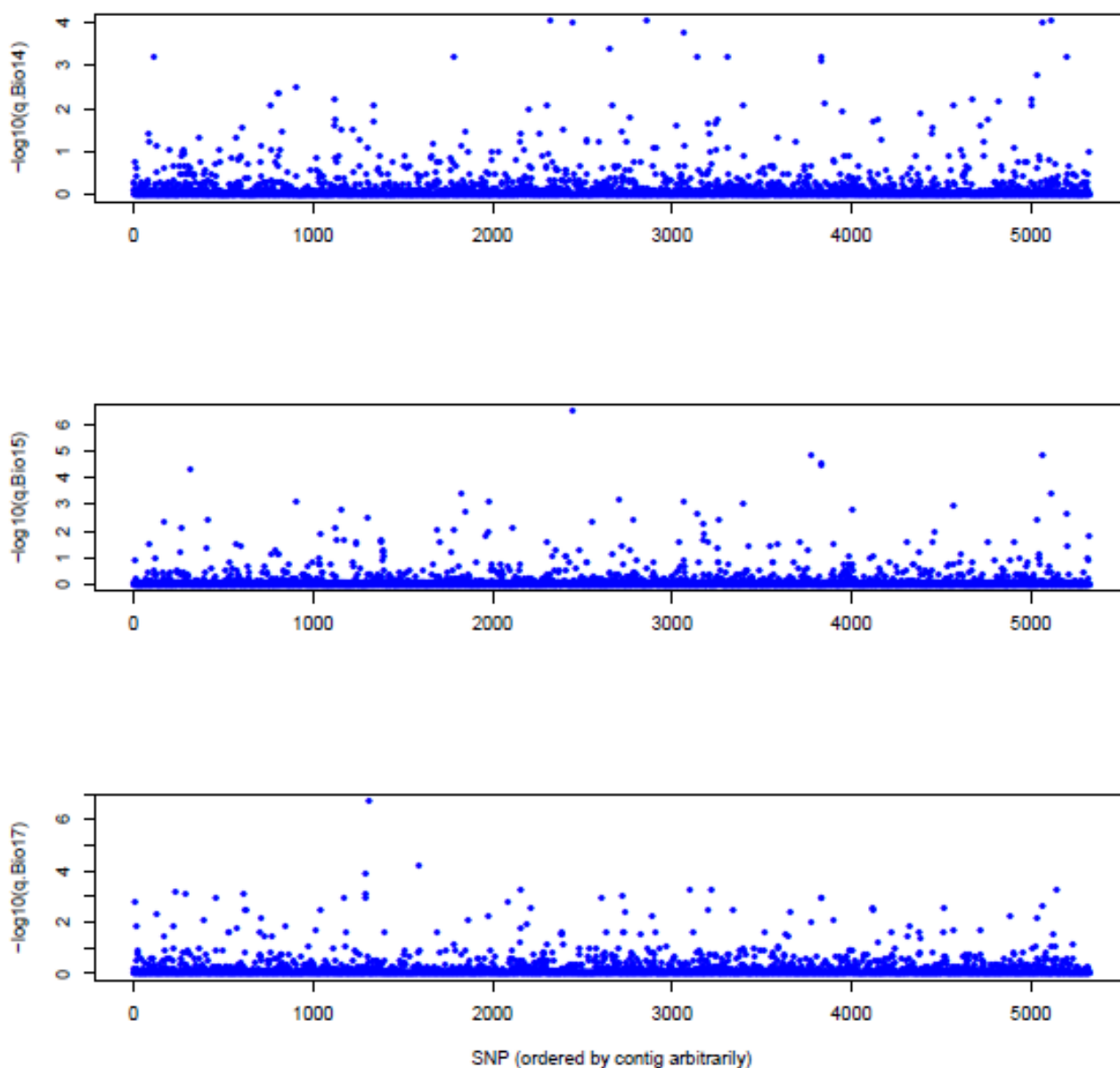


Figure 6. SNPs associated precipitation of the driest month (Bio14), precipitation seasonality (Bio15) and precipitation of the driest quarter (Bio17 in Latent Factor Mixed Models (LFMM) analysis in *Quercus castanea*.



DISCUSIÓN GENERAL

El clima es el principal factor que determina la composición y distribución de los biomas (Walther *et al.* 2002). La heterogeneidad ambiental a diversas escalas influye en la distribución de la variación genética y fenotípica de las poblaciones de especies arbóreas; estas condiciones ambientales tan cambiantes, inducen a los organismos a hacer uso de su plasticidad fenotípica, migrar o adaptarse para evadir la extinción (Sork *et al.* 2013).

En la actualidad, debido al surgimiento de la secuenciación de nueva generación, se cuenta con varias herramientas moleculares que permiten analizar el genoma completo y que producen una gran cantidad de datos genéticos. Por lo cual, la genética del paisaje ha tenido grandes avances en cuanto a sus metodologías para analizar, en múltiples especies, la variación genética y de atributos eco-fisiológicos en asociación a su distribución geográfica y climática para así entender la interacción entre las fuerzas micro-evolutivas, como son el flujo génico y la selección natural, en la estructuración de la variación genética y fenotípica. Sin duda, toda esta información puede ser evaluada de manera muy fina tanto a escalas locales como regionales, como nunca antes se había hecho, de manera que es posible explicar mejor los procesos ecológicos y evolutivos que mantienen a las poblaciones de especies. Sin embargo, es interesante mencionar que a la fecha se han realizado pocos estudios en plantas (Cruzan *et al.* 2020).

Atributos funcionales

Numerosos trabajos tanto de jardín común como estudios en campo demuestran que diferentes especies de árboles, incluidos los encinos, presentan una gran variación en

atributos funcionales como resultado de la adaptación local en respuesta principalmente a las condiciones de temperatura y precipitación de los ambientes donde habitan (Fotelli *et al.* 2000; Valladares *et al.* 2000; Aranda *et al.* 2007; Montserrat-Martí *et al.* 2009; Ramírez-Valiente *et al.* 2010, 2014, 2015; Riordan *et al.* 2016; Aguilar-Romero *et al.* 2017). El área, peso, grosor, contenido de materia seca, son atributos funcionales de las hojas que tienen una gran variación asociada a gradientes ambientales en un gran número de especies de plantas (Wilson *et al.*, 1999; Westoby *et al.*, 2002; Wright *et al.*, 2004; Mesier *et al.* 2010; Auger and Shipley 2013; Lohbeck *et al.* 2013; Rosbak *et al.* 2015; Cochrane *et al.* 2016), lo que evidencia que las plantas ajustan su morfología foliar en respuesta a las condiciones climáticas (Roa-Fuentes *et al.* 2015).

A pesar del área geográfica pequeña (4000 Km²) en comparación con otros trabajos, nuestros resultados demuestran que existe una fuerte diferenciación de los atributos funcionales estudiados entre las poblaciones. El grosor foliar mostró la menor variación a nivel de individuo y dentro individuos de la misma población, pero la mayor variación entre poblaciones, lo que sugiere su importancia en el ajuste de *Q. castanea* al ambiente climático. Se identificó una fuerte correlación negativa entre la estacionalidad de la precipitación y el grosor foliar, lo cual significa que las hojas de *Q. castanea* son más delgadas en las poblaciones donde la precipitación es más estacional (períodos muy marcados de secas y de lluvias). La esclerofilia (contenido de masa seca por unidad de área foliar) es considerada como una adaptación de las plantas a largos períodos de sequía y calor (Niinemets 2001). Nuestros resultados también indicaron que las hojas de este encino son más esclerófilas en sitios con bajas temperaturas y poca estacionalidad de precipitación y temperatura; y menos

esclerófilas en sitios más cálidos y estacionales. Esta variación en los atributos funcionales puede ser el resultado de plasticidad fenotípica, diferenciación genética adaptativa (adaptación local) o ambos; por lo tanto, este trabajo podría complementarse a futuro con experimentos de jardín común.

Conectividad funcional

Se encontró una alta variación genética en términos de riqueza alélica (en promedio 7.15 alelos por locus) y heterocigosidad (media de 0.59), la cual es comparable con valores reportados previamente para *Q. castanea* por Valencia-Cuevas *et al.* (2014) y Oyama *et al.* (2017), y con valores encontrados en otras especies de encinos (Gugger *et al.* 2013, Ashley *et al.* 2015; Ramos-Ortiz *et al.* 2016; Shi *et al.* 2017; Oyama *et al.* 2018; Lupini *et al.* 2019). La alta diversidad genética encontrada puede estar asociada al extenso flujo génico que hay entre las poblaciones.

El flujo génico determina en gran medida la diversidad genética, la estructura genética y la conectividad en las poblaciones de especies arbóreas (Wang *et al.* 2013; Sexton *et al.* 2014). Nuestros resultados mostraron que todas las poblaciones estudiadas están conectadas entre sí y que ocurre flujo génico a largas distancias (la mayor siendo de 82 km). Aunque se ha reportado que en encinos la dispersión de polen puede ser de más de 80 km (Buschbom *et al.* 2011), en este caso particular, es probable que el polen no haya viajado directamente los 82 km, sino que más bien la dispersión se haya dado a través de cadenas de poblaciones

o bien a través de árboles aislados (como lo menciona Oyama *et al.* 2017) y con migración reciente, que no fueron muestreados en este trabajo.

La dispersión de semillas y polen es un proceso muy importante para las plantas ya que es crítico para la reproducción, el flujo y la diversidad genética (Sork *et al.* 2006, Cruzan *et al.* 2020). Es por ello, que es muy importante conocer y entender los factores ecológicos que influyen en los patrones de flujo génico y sus efectos a diferentes escalas espaciales (Begue *et al.* 2017; Cruzan *et al.* 2020). Factores geográficos y ambientales no son mutuamente excluyentes y pueden, o no, en conjunto afectar el flujo génico. En este contexto, se conoce poco acerca del papel que juegan las variables climáticas al facilitar o restringir el flujo génico entre poblaciones. Un estudio reciente, sugiere que la precipitación influye de manera significativa en la diversidad genética y la conectividad en poblaciones de *Populus angustifolia* a lo largo de todo su rango geográfico de distribución en Estados Unidos (Bothwell *et al.* 2017).

El flujo génico juega un papel importante en la homogeneización de las frecuencias alélicas tanto en loci adaptativos como neutrales, dando por resultado una disminución en la diferenciación genética (Holderegger *et al.* 2006). En este estudio, probablemente debido al extenso flujo génico, se encontró una estructura genética débil entre las poblaciones de *Q. castanea* (4-5% del total de la variación genética). Se identificó que la estacionalidad de la precipitación, el intervalo anual de la temperatura y la precipitación anual son variables ambientales que afectan la dispersión del polen, probablemente debido al efecto que tienen sobre la fenología floral. Se conoce que en encinos la fenología floral está determinada por el clima (Koenig *et al.* 2015) y se ha sugerido que el flujo génico efectivo a través del polen

depende en gran medida de la sincronización de la fenología floral entre las poblaciones (Whittet *et al.* 2017; Rousi *et al.* 2019).

Adaptación local

La adaptación local es el resultado del equilibrio entre el flujo génico y diversos factores de selección natural (que pueden ser el resultado de la presión de factores ambientales) entre las poblaciones y hábitats, que se presentan en regiones genéticas particulares de los individuos (Savolainen *et al.* 2013). El estudio de los patrones de adaptación local a escalas de paisaje o regionales finas es muy importante para comprender procesos evolutivos y también para el manejo adecuado de las especies de árboles forestales (Lind *et al.* 2018).

Se conoce que existen dos formas para identificar variación genética bajo selección. La primera es mediante la medición de la diferenciación genética con el parámetro F_{ST} , es decir, loci con valores muy altos o muy bajos con respecto a lo esperado por neutralidad se considera están bajo selección (Lewontin y Krakauer 1973; Vitti *et al.* 2013). La segunda es a través de análisis de asociación ambiental, cuyo objetivo es identificar correlaciones significativas entre variables ambientales y frecuencias alélicas (Holderegger *et al.* 2010; Rellstab *et al.* 2015). Ambos enfoques son complementarios (De Mita *et al.* 2013; de Villemereuil *et al.* 2014). Utilizando estos dos enfoques, se identificaron en *Q. castanea*, SNPs potencialmente bajo selección e implicados en una gran variedad de procesos biológicos importantes. Ocho SNPs presentan señal de selección divergente y 194 SNPs

están asociados con la variación climática, la mayoría relacionados con la isothermalidad. Es importante resaltar que 47 del total de estos SNPs son ortólogos en *Arabidopsis thaliana* y están presentes en genes funcionales con respuesta a diferentes tipos de estrés, germinación y floración. Algunos trabajos con otras especies de árboles templados y subtropicales también han identificados una mayor proporción de SNPs asociados a variables de temperatura con respecto a variables de precipitación (Jaramillo-Correa *et al.* 2015; Mosca *et al.* 2016, Gugger *et al.* 2018; Lu *et al.* 2019); sin embargo, en el caso de los encinos, esto es versátil, dependiendo de la especie. En *Q. suber* (Pina-Martins *et al.* 2019), *Q. pubescens* y *Q. robur* (Rellstab *et al.* 2016) la mayoría de los SNPs identificados están relacionados con variables de precipitación; mientras que en *Q. rugosa* (Martins *et al.* 2018), *Q. lobata* (Gugger *et al.* 2016) y *Q. petrea* (Rellstab *et al.* 2016), están en su mayoría asociados a variables de temperatura.

La combinación de enfoques que analizan la información genotípica y fenotípica a nivel poblacional y que al mismo tiempo integran información ambiental, da como resultado estudios que aportan mayor información, permitiendo entender de manera más precisa y clara las bases genéticas y las características ambientales que conducen a la selección divergente y los rasgos fenotípicos que confieren adecuación a determinados ambientes, en otras palabras, permiten entender el proceso que con lleva la adaptación local. Finalmente, el conocimiento de la variación genética adaptativa puede servir de base para futuras estrategias de manejo y conservación de los bosques.

CONCLUSIONES GENERALES Y PERSPECTIVAS

Conclusiones generales

Se encontraron diferencias significativas en los atributos funcionales estudiados entre las poblaciones de *Quercus castanea*. El grosor foliar presentó mayor variación, lo cual está asociado con la estacionalidad de precipitación.

Se detectaron niveles altos de diversidad genética, baja estructura genética y un extenso flujo génico entre las poblaciones. El análisis de genética del paisaje, mostró que dentro de la cuenca la estacionalidad de la precipitación, el intervalo anual de la temperatura y la precipitación anual son las variables ambientales que afectan el flujo de polen, probablemente debido al efecto que tienen estas variables sobre la fenología floral.

A pesar de las tasas altas de flujo génico existente, se identificaron 8 SNPs que presentan señal de selección divergente y 194 SNPs asociados con la variación climática, de los cuales 29 están relacionados con la isothermalidad y 14 con la estacionalidad de la precipitación. Algunos de estos SNPs se encuentran en genes que están relacionados con la respuesta al estrés por temperatura, estrés oxidativo, estrés osmótico, germinación y floración.

En conjunto los resultados obtenidos en este trabajo muestran evidencia de adaptación en esta área geográfica pequeña (4000 Km²) asociada al gradiente de temperatura y precipitación presente en la cuenca. Se observa que tanto a nivel fenotípico como genético la estacionalidad de la precipitación, es decir, la temporalidad de la disponibilidad de agua, representa una fuerte presión ambiental para esta especie de encino.

Perspectivas

La extracción de madera, la producción de carbón y la expansión de la frontera agrícola son las principales causas de la disminución de poblaciones de diferentes especies de encino. Así mismo, los cambios climáticos que se están presentando a nivel global representan una amenaza latente para la persistencia, tanto de las especies de encino como de sus interacciones ecológicas a largo plazo.

Producto de esta tesis, para *Q. castanea* se encontraron SNPs asociados a la variación climática presente en la cuenca. La identificación de estos SNPs se hizo utilizando el genoma de referencia de *Q. lobata* (Sork *et al.* 2016).

Actualmente existen tres genomas de referencia de 3 especies de encinos, *Q. robur* (Plomion *et al.* 2018), *Q. suber* (Ramos *et al.* 2018) y el mencionado anteriormente para *Q. lobata*. *Q. lobata* es un encino endémico de California y *Q. robur* es un encino europeo. Ambas especies pertenecen a la sección *Quercus* (encinos blancos). *Q. suber* se distribuye en el suroeste de Europa y noroeste de África y pertenece a la sección *Cerris*.

México es un centro de diversificación para el género *Quercus*, con 32-40% de especies a nivel mundial (Valencia 2004; Cavender-Bares *et al.* 2015). Los encinos mexicanos cuentan con historias evolutivas interesantes de estudiar, diferentes a las de *Q. lobata*, *Q. robur* y *Q. suber*, debido a los eventos geológicos y paleoclimáticos que ha experimentado el territorio nacional y a que se encuentra en una zona de transición entre la región Neártica y Neotropical (Mastretta-Yanes *et al.* 2015).

Por lo tanto, sería muy importante generar un genoma de referencia de un encino mexicano y que mejor candidato que *Q. castanea* que es una de las especies de encino rojo de mayor amplia distribución geográfica y altitudinal.

Otra futura línea de investigación con *Q. castanea* y otras especies de encinos mexicanos, podría centrarse en realizar estudios de transcriptómica, ya que hasta el momento no se han realizado trabajos de este tipo con especies nacionales. Un gran número de trabajos sobre el transcriptoma de encinos se ha enfocado en caracterizar la respuesta de los árboles a estrés biológico y ambiental. Se han realizado estudios de transcriptómica en relación al estrés hídrico, estrés por temperatura, salinidad, estrés oxidativo para *Q. lobata*, *Q. suber* y *Q. robur* (Pereira-Leal *et al.* 2014; Gugger *et al.* 2016; Le Provost *et al.* 2016; Magalhaes *et al.* 2016; Gugger *et al.* 2017; Schmid-Siegert *et al.* 2017; Usié *et al.* 2017). Para *Q. suber* también se caracterizó su transcriptoma durante cuatro etapas de embriogénesis somática para identificar genes relevantes en este proceso con la visión de utilizar esta información para propagación clonal (Capote *et al.* 2019). En *Q. rubra* se estudió su transcriptoma en respuesta a la exposición al ozono (Soltani *et al.* 2020).

El tener caracterizado el transcriptoma de encinos durante el desarrollo embrionario sería información muy importante para desarrollar programas de reproducción y reforestación, ya que permitiría almacenar por varios años embriones viables y de calidad de diferentes especies.

Toda esta información biológica aunada a información de tipo económico-social permitirá elaborar planes de manejo y conservación de los remanentes de bosque de encino.

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