UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

ESCUELA NACIONAL DE ESTUDIOS SUPERIORES, UNIDAD MORELIA

BIOLOGÍA EVOLUTIVA

Variación morfológica y filogeografía de *Quercus mexicana* (Fagaceae) en México

TESIS

PARA OPTAR POR EL GRADO DE:

DOCTORA EN CIENCIAS

PRESENTA:

VANESSA ELISABETH SÁNCHEZ ACEVEDO

TUTOR PRINCIPAL DE TESIS: DR. ALBERTO KEN OYAMA NAKAGAWA

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INSTITUTO DE INVESTIGACIONES EN ECOSISTEMAS Y SUSTENTABILIDAD, UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

MORELIA, MICHOACÁN, 2022

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COORDINACIÓN DEL POSGRADO EN CIENCIAS BIOLÓGICAS

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M. en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día **01 de agosto de 2022** se aprobó el siguiente jurado para el examen de grado de **DOCTORA EN CIENCIAS** de la estudiante **SÁNCHEZ ACEVEDO VANESSA ELISABETH** con número de cuenta **515015768** con la tesis titulada **"Variación morfológica y filogeografía de *Quercus mexicana* (Fagaceae) en México",** realizada bajo la dirección del **DR. ALBERTO KEN OYAMA NAKAGAWA** quedando integrado de la siguiente manera:

Presidente:	DRA. SUSANA VALENCIA AVALOS
Vocal:	DRA. MERCEDES ISOLDA LUNA VEGA
Vocal:	DR. CÉSAR ANDRÉS TORRES MIRANDA
Vocal:	DR. EDUARDO RUIZ SÁNCHEZ
Secretario:	DR. ANTONIO GONZÁLEZ RODRÍGUEZ

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

A T E N T A M E N T E
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Ciudad Universitaria, Cd. Mx., a 10 de noviembre de 2022

COORDINADOR DEL PROGRAMA

DR. ADOLFO GERARDO NAVARRO SIGÜENZA

POSGRADO EN CIENCIAS BIOLOGICAS
COORDINACIÓN

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Discusión general

Cuadro 1. Antecedentes filogeográficos en *Quercus* en México. Se muestra la referencia, tipo de marcador, estructura filogeográfica, diversidad genética, número de haplotipos, especie y región donde se distribuye la especie de estudio (FVT: Faja Volcánica Transmexicana; SMOr: Sierra Madre Oriental; SMS: Sierra Madre del Sur; SMOc: Sierra Madre Occidental; SMC: Sierra Madre de Chiapas; MC: Meseta Central).

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Resumen

Los encinos han sido considerados un modelo óptimo para el estudio evolutivo y ecológico debido a su abundancia, su alta diversidad de especies y su elevado endemismo en México. En particular, varios estudios filogeográficos han brindado información sobre los factores y procesos que podrían haber influido en la distribución espacial de los linajes genéticos de las especies de encino en México. Los objetivos de este estudio fueron: 1) determinar la variación morfológica a lo largo de gradientes ambientales en las poblaciones de *Q. mexicana,* y; 2) determinar la estructura genética y los patrones filogeográficos de un encino endémico, *Quercus mexicana*, en el área de su distribución en la Sierra Madre Oriental y zonas adyacentes. El Capítulo I, describe la variación intraespecífica de los caracteres morfológicos foliares en respuesta a gradientes ambientales (factores climáticos y geográficos) de *Q. mexicana*. Para realizar este estudio, se utilizaron muestras de 39 poblaciones recolectadas en toda el área de distribución a la especie. Se midieron ocho rasgos macromorfológicos en 5507 hojas y tres rasgos micromorfológicos en 228 hojas. Posteriormente, se analizó la diferenciación morfológica entre poblaciones y la relación entre la variación foliar y las variables ambientales. Se identificó que las poblaciones de *Q. mexicana* tienen diferenciación morfológica foliar a lo largo de su distribución. Se probaron relaciones significativas entre los caracteres foliares y las variables ambientales. Asimismo, los resultados muestran que las variables relacionadas con la temperatura y humedad permiten una adecuada discriminación y agrupamiento entre poblaciones. Hojas pequeñas y gruesas con baja densidad de tricomas y estomas pequeños se encontraron en poblaciones en sitios más áridos. En contraste, hojas más grandes y delgadas con mayor densidad de tricomas y estomas más grandes ocurrieron en sitios más húmedos. Estos resultados indican que *Q. mexicana* tiene tolerancia a un amplio intervalo de condiciones climáticas. Específicamente, este capítulo, proporciona

información sobre cómo los factores climáticos influyen en la variación de caracteres morfológicos en las poblaciones de *Q. mexicana*. Con respecto al Capítulo II, se muestran los principales resultados filogeográficos para *Q. mexicana* a lo largo de su área de distribución en la Sierra Madre Oriental, principalmente. Para ello, se utilizaron marcadores de microsatélites de ADN nuclear y de cloroplastos para determinar la diversidad genética y la estructura de 39 poblaciones de la especie. Además, se utilizaron modelos de nicho ecológico para evaluar las expansiones/contracciones demográficas de las poblaciones de esta especie durante el Holoceno Medio, Último Máximo Glacial y Último Máximo Interglacial. También, se compararon estos modelos de nicho con la distribución actual de *Q. mexicana* para proporcionar información sobre cómo las fluctuaciones climáticas podrían haber influido en la historia demográfica de esta especie. Los resultados sugieren fluctuaciones históricas en el rango de distribución de *Q. mexicana.* Se encontró que el rango de expansión histórico, la heterogeneidad del hábitat, el flujo de genes, las barreras geográficas, los cambios altitudinales y latitudinales en respuesta a los cambios climáticos en el pasado han dado forma a la estructura filogeográfica de *Q. mexicana.*

Abstract

The oaks have been considered a study model due to their abundance, high species diversity and a high rate of endemic species in Mexico. In particular, several phylogeographical studies have provided information on the factors and processes that could influence the spatial distribution of genetic lineages of oak species in Mexico. In this study, the aims were 1) To determine the morphological variation along environmental gradients of *Q. Mexicana* populations, and 2) To determine the genetic structure and phylogeographic patterns of the endemic oak, *Quercus mexicana*, which inhabits the Sierra Madre Oriental in northeastern Mexico. In the Chapter I, the intraspecific variations were analyzed in leaf morphological and functional traits in response to environmental gradients (climatic and geographic factors) of *Q. mexicana*. To perform this study, samples were used from 39 populations collected in the Sierra Madre Oriental and east of the Trans-Mexican Volcanic Belt. Thereby, eight macromorphological traits were measured in 5,507 leaves and three micromorphological traits in 228 leaves. Afterwards, this study evaluated the morphological differentiation among populations and the relationship between variation in leaf traits and environmental variables. The main results indicated that populations of *Q. mexicana* showed leaf morphological differentiation throughout its distribution. Also, this study found significant relationships between leaf traits and environmental variables. Likewise, the results revealed that temperature- and water-related variables discriminated and grouped populations. Smaller and thicker leaves with lower trichomes and smaller stomata were found in populations situated in the aridest areas. These results indicate that *Q. mexicana* is adapted to an extensive range of climatic conditions. To conclude, this chapter provide information about how abiotic factors can influence the morphological variation on an endemic oak. While, in the Chapter II describes the results of the phylogeographical study of *Q. mexicana* across the Sierra

Madre Oriental and adjacent regions. To this end, nuclear and chloroplast DNA microsatellite markers were used to determine the genetic diversity and structure of 39 populations of *Q. mexicana*. In addition, the ecological niche models were constructed to assess the demographic expansions/contractions of the populations of this oak species during the Mid Holocene, Last Glacial Maximum and Last Interglacial Maximum. Moreover, the niche models were compared to the current distribution of *Q. mexicana* to provide insights into how climatic fluctuations influenced the demographic history of this endemic oak. The results showed historical fluctuations in the distribution range of *Q. mexicana*. Also, this study suggests that the historical expansion range, habitat heterogeneity, gene flow, physical barriers, altitudinal, and latitudinal shifts in response to climate changes in the past have shaped the phylogeographic structure of *Q. mexicana.*

Introducción

El género *Quercus*

El género *Quercus* L. (Fagaceae) comprende aproximadamente 500 especies de árboles y arbustos, polinizados por viento, inflorescencias unisexuales y bellotas como frutos característicos (Nixon 1993b; Aldrich and Cavender-Bares 2011), Se divide en dos subgéneros *Cerris* (Oerst.) y *Quercus*; las especies del subgénero *Cerris* se distribuyen en la región Paleártica y cuenta con tres secciones: *Cyclobalanopsis* (Oerst.), *Ilex* (Loudon) *y Cerris* (Dumort.). El subgénero *Quercus* se divide en cinco secciones diferentes: *Quercus* (encinos blancos), *Protobalanus* (Trelease, encinos intermedios), *Lobatae* (Loudon, encinos rojos), *Ponticae* (Stefanoff) y *Virentes* (Loudon) (Denk *et al.* 2017; Manos and Hipp 2021). El subgénero *Quercus* tiene un origen Neártico y se distribuye ampliamente en el Neotrópico, así como en algunas regiones de los bosques templados, regiones tropicales y subtropicales del hemisferio norte (Valencia 2004; Denk *et al.* 2017).

Los encinos de las secciones *Quercu*s y *Lobatae* surgieron y se diversificaron simultáneamente en las zonas templadas de América del Norte, posteriormente, la tasa de especiación aumento al llegar a México (Hipp *et al*. 2018). Se propone que las secciones americanas *Lobatae, Protobalanus, Ponticae, Virentes,* y *Quercus* surgieron hace 33 millones de años (Hipp *et al.* 2018). Por otro lado, se ha documentado que los encinos blancos ingresaron a México durante el Oligoceno-Mioceno migrando del este de Estados Unidos hasta la Sierra Madre Oriental (Nixon 1993a). Se ha reportado que lograron una amplia distribución durante el Eoceno superior-Oligoceno inferior (Manos y Stanford 2001). También, se estima que la evolución de los encinos rojos ocurre hace 15 millones de años que acompaño su avance hacia el

sur de México, esto coincide con una rápida diversificación y altas tasas de especiación, como resultado de movimientos tectónicos, vulcanismo y oscilaciones climáticas (Manos y Hipp 2021)

Existen dos centros de diversidad para el género *Quercus,* el primero se localiza en el sureste de Asia y el segundo se localiza en México donde se encuentran aproximadamente 161 especies (Valencia 2004). Particularmente, México es considerado el mayor centro de diversificación y endemismo específico para el subgénero *Quercus* en América (Rzedowski 1978; Valencia 2004; Hipp *et al.* 2018). Factores como la diversidad topográfica, climática y de hábitat probablemente ejercen una influencia importante en los procesos de radiación evolutiva y en el mantenimiento de la alta diversidad de especies de encinos en México (Nixon 1993a; González-Rodríguez *et al*. 2004). De las 161 especies del subgénero *Quercus*, la sección *Lobatae* (encinos rojos exclusivos de América) agrupa 76 especies, de las cuales 61 son endémicas, la sección *Protobalanus* (encinos intermedios) con cuatro especies y una de ellas es endémica, y la sección *Quercus (*encinos blancos) cuenta con 81 especies, de ellas 47 son endémicas. Cabe resaltar que las 161 especies de *Quercus* en México, 109 son endémicas y 76 especies pertenecen a sección *Lobatae*, es decir que el 80% de encinos rojos presentes en nuestro país son endémicos (Valencia 2004).

El género *Quercus* es uno de los géneros de plantas leñosas más abundantes y económicamente importantes del hemisferio norte (Manos *et al.* 1999). En México, constituyen el principal tipo de plantas dominantes en el dosel de los sistemas montañosos (Rzedowski 1978). Los encinos juegan un papel ecológico importante en numerosas comunidades de plantas y animales (Tovar-Sánchez *et al.* 2004; Vitelli *et al.* 2017). Por ejemplo, son hábitat de una gran cantidad de plantas epífitas y de animales vertebrados e invertebrados (Valencia 2004). A pesar

de la importancia ecológica y económica de varias de las especies del género *Quercus*, aún existe relativamente poco conocimiento sobre su diversidad de especies (Valencia 2004); uno de los factores que dificulta el conocimiento sobre las especies del género *Quercus* es la variación morfológica presente dentro del mismo individuo (Valencia 2004). De hecho, la amplia variación morfológica intraespecífica podría atribuirse a la hibridación (González Rodríguez *et al*. 2004).

Por otra parte, la Sierra Madre Oriental (SMOr) se considera el área natural más diversa del país en especies del género *Quercus;* esta región parece ser un centro de confluencia de especies provenientes del norte y occidente del país, así como un posible puente de dispersión de encinos hacia el sur del país (Nixon 1993a). Torres-Miranda *et al*. (2011) reconocen la importancia de la Sierra Madre Oriental como un centro de endemismos de la sección Lobatae. Por último, considerando los patrones de distribución de la sección Lobatae, se menciona que la Sierra Madre Oriental podría estar dividida en dos sectores: norte (Sierra Plegada) y sur (Torres-Miranda *et al.* 2013).

En este estudio, se eligió a *Quercus mexicana* Bonpl, como una especie modelo para entender la historia evolutiva de la Sierra Madre Oriental. *Q mexicana* pertenece a la sección *Lobatae,* es una especie endémica de climas cálidos semiáridos a subhúmedos, característicos de bosques mixtos con bajas precipitaciones, en altitudes entre 1600 and 2890 m (Valencia 2004). Se distribuye desde Nuevo León hasta el centro de México, a lo largo de la Sierra Madre Oriental (SMO), principalmente, y en regiones adyacentes al sureste del Altiplano y este de la Faja Volcánica Transmexicana, a lo largo de más de 1000 km. *Q. mexicana* es una de las pocas especies de encinos rojos presentes en ambos sectores biogeográficos (i.e., norte y sur), en que se ha dividido a la Sierra Madre Oriental (Luna-Vega *et al*. 2004). Los factores anteriores justifican la elección de *Q. mexicana* como modelo para estudios filogeográficos y de variación

morfológica en la Sierra Madre Oriental debido a que esta es endémica. De esta manera, se obtendrá información relevante sobre la evolución y divergencia evolutiva de especies del noreste de México, una región que se caracteriza por su escaso conocimiento evolutivo.

Variación morfológica y su relación con los factores ambientales

La variación en la morfología foliar se ha estudiado ampliamente a nivel inter e intraespecífico (Pyakurel *et al.* 2014). Se ha documentado que los caracteres foliares influyen en las funciones de las hojas (Zhu *et al.* 2012). Asimismo, estos rasgos pueden variar a lo largo de gradientes ambientales (Uribe Salas *et al.* 2008; Hill *et al*. 2014). Por otro lado, se considera un rasgo funcional cualquier atributo que tenga una influencia significativa en el establecimiento, supervivencia y aptitud (Reich *et al.* 2003). Por lo tanto, distintos estudios se han centrado en identificar la relación entre los rasgos morfológicos asociados a cambios ambientales (Uribe-Salas *et al.* 2008; Tang & Ohsawa 1999). Este tipo de estudios son importantes porque nos ayudan a determinar las respuestas de las plantas ante nuevas presiones de selección, como pueden ser las producidas por el cambio climático global (Ramírez *et al.* 2015; Yan *et al.* 2017).

Estudios morfológicos muestran que las hojas han sido consideradas como el principal órgano blanco para evaluar el efecto de los cambios ambientales (Köner *et al.* 1989; Gifford, 1989; Uribe Salas *et al.* 2008). Las estructuras morfológicas más diversas en las plantas son las hojas (Parker 1982; Press *et al*. 1999), estas pueden variar en el tamaño foliar (Wright *et al.* 2017), densidad de venas foliares (Zhu *et al*. 2012), grosor foliar (Abrams *et al.* 1990; Warren *et al.* 2005), área foliar especifica (Rosbakh *et al*. 2015), longitud y grosor del pecíolo (González-Rodríguez *et al.* 2005; Xu *et al*. 2008), densidad y tamaños de estomas (Hill *et al.* 2015; Yan *et al.* (2017) y densidad de tricomas foliares (Benz y Martin 2006; Agrawal *et al.* 2004).

La variación de caracteres foliares ha permitido a las plantas desarrollarse en un amplio gradiente climático (Pyakurel *et al.* 2014). En este sentido, la variación en algunos atributos morfológicos y funcionales de las hojas se han asociado a diversos gradientes ambientales como la temperatura (Abrams 1990; Reich *et al.* 2004), elevación (Oleksyn-Francisca *et al*. 1998; Paridari *et al.* 2013), latitud (Tang y Ohsawa 1999; Uribe Salas *et al*. 2008), precipitación (Klich 2000), irradiación (Hovenden *et al*. 2006) disponibilidad de nutrientes en el suelo (Niinemets *et al.* 2001) y condiciones de sequía (McDonald *et al.* 2003; Warren *et al.* 2005; Nicotra *et al.* 2008; Sun *et al.* 2016). El análisis de la variación morfológica de los atributos foliares en especies que se distribuyen a lo largo de amplios gradientes climáticos, altitudinales y latitudinales, puede brindar información valiosa para entender las respuestas ecológicas y evolutivas de las especies en el contexto de la variación geográfica (Ogaya y Peñuelas 2007; Fajardo *et al.* 2011).

En particular, la temperatura y la precipitación son considerados los principales promotores de la variación foliar (Moles *et al*. 2014; Valladares *et al.* 2014, Wright *et al.* 2017), por ejemplo, plantas con hojas pequeñas se encuentran en sitios con menor precipitación media anual (Wright *et al.* 2017) siguiendo un gradiente latitudinal (Uribe-Salas *et al.* 2008) o altitudinal (Tang y Ohsawa 1999). Asimismo, el peso seco y grosor foliar ha mostrado una relación positiva con alta radiación solar, alta temperatura y menor precipitación (Niinemets 2001). También, se han observado variaciones entre las poblaciones para algunos variables foliares, por ejemplo, una mayor área foliar se ha asociado con una mayor precipitación (Gouveia y Freitas 2009) y se ha observado una mayor densidad de venas foliares en ambientes más secos (Zhu *et al.* 2012). Particularmente, estudios en encinos han encontrado que las hojas de menor tamaño se encuentran en ambientes secos, en especies como *Quercus acutissima* (Xu

et al. 2008), *Q. deserticola* (Rodríguez-Gómez *et al.* 2018); secos y fríos en *Q. suber* (Ramírez *et al.* 2009).

Algunos otros caracteres que se han documentado están asociados al pecíolo, una estructura importante en la captación de luz. Los peciolos largos favorecen mejores tasas de captación de luz para las hojas, al mantenerlas separadas entre sí (González-Rodríguez *et al.* 2005). Además, peciolos cortos son frecuentes en condiciones de estrés lumínico en *Q. acutissima* (Xu *et al.* 2008). Por otra parte, en distintas especies de encinos, el pecíolo es una de las características que consistentemente presenta patrones especiales de cambio a nivel de localidades, poblaciones e individuos, ya sea en longitud, forma o diámetro (Tovar-Sánchez *et al.* 2004; González- Rodríguez *et al.* 2005).

La respuesta hídrica de las plantas está asociada a rasgos macro y micro morfológicos. En el aspecto macro morfológico las venas foliares, se consideran estructuras esenciales para mantener el estado hídrico de las hojas y la capacidad fotosintética (Walls 2011). La densidad de venas muestra una alta variación interespecífica e intraespecífica y una respuesta muy diversa a una combinación de variables ambientales (Zhu *et al.* 2012).

Estudios previos nos muestran como los caracteres micromorfológicos funcionales de las plantas pueden variar en respuesta a una amplia gama de condiciones ambientales, por ejemplo, la densidad y tamaño de los estomas influyen en el equilibrio hídrico de las hojas, estas estructuras están asociadas con la disponibilidad de agua (Hill *et al.* 2015). En estudios previos, se ha observado una reducción de la densidad estomática a concentraciones más altas de CO_2, mientras que al aumento la intensidad de la luz aumenta el índice estomático en varias especies (Casson & Gray 2008; Franks & Beerling 2009). Asimismo, la densidad estomática tiende a

aumentar en las plantas que se encuentran en sitios con altas temperatura y sequía (Yan *et al*. 2017). Los rasgos micro morfológicos juegan un papel esencial en la regulación hídrica en las plantas (Hetherington y Woodward 2003; Casson y Gray 2008; Franks y Beerling 2009).

Mientras que estudios relacionados con la variación en los tricomas foliares han permitido analizar de manera detallada la amplia respuesta de las especies de plantas ante diferentes gradientes ambientales (Benz y Martin 2006). La pubescencia foliar se considera un mecanismo fisiológico para mitigar las condiciones ambientales extremas (Koner *et al.*, 2003). Destacando su papel en diversos procesos como su función en la regulación hídrica, además de actuar como una barrera protectora contra la alta radiación solar (Levizou 2004; Benz y Martin 2006), regulador de la difusión de gases (Brewer *et al.* 1994), resistencia a bajas temperaturas (Agrawal *et al.* 2004), y como un medio de defensa ante depredadores (Schilmiller *et al.* 2008). Protege a la planta contra insectos, debido a que los tricomas producen compuestos químicos tóxicos paraque envenena a los herbívoros (Duke 1994).

El género *Quercus* tiene una gran variabilidad morfológica (Valencia 2004) y recurrentes procesos de hibridación (Rzedowski 1978; Manos *et al.* 1999; Bruschi *et al.* 2000). Las especies del género *Quercus* son un buen modelo para estudiar la variación de los caracteres foliares a lo largo de gradientes ambientales. En este estudio se evaluó el efecto de los factores ambientales en la variación morfológica foliar en *Q. mexicana* a lo largo de un gradiente geográfico y ambiental de 1000 km. Se analizó la variación de características macro y micro morfológicas, especialmente las relacionadas con la tolerancia a la sequía, por ejemplo, grosor, venas y estomas, de una especie que se distribuye en ambientes con bajas tasas de precipitación. Por otro lado, se realizó un análisis filogeográfico de *Q. mexicana* en todo el rango de su distribución.

Adicionalmente, se relacionó la información genética con modelos de nicho ecológico para evaluar expansiones o contracciones demográficas de las poblaciones de *Q. mexicana* durante el Holoceno Medio, Último Máximo Glacial y Último Interglaciar.

Filogeografía

La filogeografía es el estudio de los principios y procesos que gobiernan la distribución geográfica de especies y grupos de especies cercanamente relacionadas (Avise 1998). Esta disciplina es el resultado de la unión del estudio de procesos microevolutivos y macroevolutivos (Avise 1998; Avise 2000). La filogeografía infiere los factores que determinan los niveles de diversidad genética, tomando en cuenta aspectos ecológicos, históricos y de distribución en el espacio geográfico (Avise 2004). De esta manera se puede inferir la distribución de los genes en tiempo y espacio, además de probar los procesos históricos que han moldeado la historia genética de las poblaciones como son: flujo génico, cuellos de botella, hibridación, expansión demográfica, estimar tiempos de divergencia, eventos de vicarianza, disyunción, especiación, migración y dispersión (Avise 1998; Avise 2000).

La filogeografía utiliza como herramienta marcadores citoplásmicos, como el ADNmt y el ADN de cloroplasto (ADNcp), heredados por vía materna, lo que permite detectar linajes matrilineales. Particularmente, en el caso de los encinos del género *Quercus*, los estudios filogeográficos están basados en el análisis de la variación de ADNcp, el cual se hereda maternalmente a través de las semillas (Dumolin *et al*. 1995; Petit *et al*. 2002; Grivet *et al.* 2006; Peñaloza-Ramírez *et al*. 2011; Albarrán-Lara *et al*. 2011; Chen *et al.* 2012; Liu *et al*. 2013). Sin embargo, el ADNcp solo puede revelar el flujo de genes de la semilla, sin proporcionar información sobre flujo de genes de polen. Con relación a esto, estudios previos han contrastado patrones con marcadores de herencia citoplasmáticos y nucleares para determinar y entender los

patrones de flujo génico histórico y contemporáneo (semillas y polen), así como conocer su historia evolutiva (Hare *et al.* 2001; Bai *et al.* 2010, Cavender-Bares *et al.* 2011, Pauwels *et al.* 2012; Ohtani *et al.* 2013).

Los Modelos de Nicho Ecológico (ENM) son una herramienta complementaria para comparar y robustecer los patrones filogeográficos (Waltari 2009). Los ENM constituyen una técnica que permite estimar áreas actuales o potenciales de distribución de una entidad (especies, poblaciones), usando principalmente registros de presencia y/o ausencia en el espacio de interés (Peterson 2004). En años recientes, los ENM han sido ampliamente utilizados para reconstruir áreas de distribución geográfica de las especies en el pasado durante el último máximo glacial (UMG hace aproximadamente 20,000 años) y el último interglacial (UIG, hace aproximadamente 140,000 años), lo cual ha ayudado a contrastar y complementar patrones filogeográficos obtenidos con marcadores moleculares (Jakob 2009).

Los estudios filogeográficos en encinos indican que los cambios geológicos y climáticos durante el último período glacial tuvieron una influencia significativa en la diversidad genética, estructura genética, diferenciación intra e interespecífica de los encinos. Por ejemplo, estudios previos en Europa encontraron que las especies de encinos blancos estaban restringidas a diferentes refugios durante la última glaciación (Petit *et al*. 2002; Grivet y Petit 2003; Bagnoli *et al*. 2016), y que después del Máximo Glacial, especies de encinos recolonizaron el continente siguiendo diversas rutas postglaciales. Estos procesos históricos dieron origen a una baja diversidad genética, fuerte estructura filogeográfica y genética en las diferentes especies de *Quercus (*Petit *et al*. 1993; Dumolin-Lapègue *et al*. 1997; Petit *et al.* 2002). Por el contrario, los encinos de América del Norte han mostrado una alta diversidad y poca estructura genética

(Grivet y Petit 2003). De hecho, se ha documentado que el movimiento de genes entre las poblaciones de encinos americanos ha sido más estable en respuesta a eventos climáticos y geológicos del pasado (Magni *et al.* 2005). Además, se han encontrado bajos niveles de diferenciación del ADN del cloroplasto en varias especies de encinos americanos (Magni *et al.* 2005; Grivet *et al.* 2006; Cavender-Bares *et al.* 2015) en comparación con los que se observan típicamente en los encinos europeos (Petit *et al.* 2002; Bagnoli *et al.* 2016).

Estudios han planteado que, en los trópicos, las condiciones de aridez y bajas temperaturas creadas durante los periodos glaciares del Pleistoceno ocasionaron contracciones y retracciones del área de distribución de las poblaciones (Wijninga *et al.*1995). Por lo tanto, se considera que las glaciaciones tuvieron un papel muy importante en la divergencia de las poblaciones y la especiación. Para el caso de los encinos mexicanos se ha documentado que es probable que poblaciones grandes hayan persistido durante varios episodios de cambio climático a lo largo del Pleistoceno, y que hayan experimentado contacto secundario con poblaciones de otras especies originando zonas de hibridación de especies (González-Rodríguez *et al.* 2004).

Particularmente, en México, los estudios filogeográficos en varias especies de encinos han mostrado una alta diversidad genética y baja estructura genética, además, desplazamientos altitudinales y patrones dinámicos de flujo de genes durante el Pleistoceno (González-Rodríguez *et al*. 2004; Tovar-Sánchez *et al*. 2008; Rodríguez-Correa *et al.* 2015; Ramos-Ortiz *et al*. 2016; Rodríguez-Gómez *et al*. 2018; McCauley *et al*. 2019; Albarrán-Lara *et al.* 2019; Peñaloza-Ramírez *et al.* 2020). Sin embargo, hasta el momento no se han realizado estudios suficientes sobre cómo las glaciaciones afectaron la composición genética y la estructura de especies endémicas de *Quercus* que habitan áreas de alta riqueza de especies como la Sierra Madre

Oriental (González-Rodríguez *et al*. 2004; Jaramillo-Correa *et al*. 2006).

Historia geológica, tectónica y cambios en el clima a través del tiempo, su repercusión en los patrones de la distribución de las especies en México

México es considerado un país megadiverso debido a que se ubica entre los cinco países con mayor número de especies en el mundo (Llorente *et al.* 2008). La alta diversidad en México se debe a su posición geográfica privilegiada, en un área donde se traslapan dos regiones biogeográficas (Neártica y Neotropical) (Challenger *et al.* 2008); caracterizada por un complejo relieve, con la presencia de sierras y montañas que se elevan más de 4500 metros sobre el nivel del mar, y una compleja historia geológica. Todas estas características hacen del territorio mexicano un entorno sumamente heterogéneo, con mosaicos de diversos climas y suelos (Rzedowski 1991; Luna *et al*. 2008).

Los taxones también se encuentran asociados a rasgos fisiográficos, los cuales a su vez son determinados por los movimientos de las placas tectónicas y la historia geológica de la Tierra (Torres *et al*. 2013). En México, se han dado cambios fisiográficos muy importantes, ya que el país se encuentra en la convergencia de cinco placas tectónicas; Norteamérica, Caribe, Cocos, Pacifico y Riviera (DeMets *et al.* 2001; Padilla 2007). Los cambios en la superficie de la Tierra producidos por el movimiento de las placas tectónicas han dado origen a la formación de grandes corredores y barreras geográficas que han favorecido la migración selectiva y el aislamiento de la biota (Graham 1999).

Diversos estudios filogeográficos han documentado que las barreras tienen un papel fundamental por qué promueven la estructura filogeográfica entre diferentes poblaciones de

plantas (Soto *et al*. 2007; Sosa *et al*. 2009; Zhang *et al.* 2015; Ochoa-Zavala *et al.* 2020). Ante la ausencia de flujo génico debido a barreras geográficas, las poblaciones separadas se diferencian, evolucionan y eventualmente dan origen a nuevas especies por vicarianza (Futuyma 1998; Morrone 2005). De tal manera que la estructura geográfica de las especies en México se relaciona con las principales barreras geográficas documentadas. En las especies montanas, las principales barreras identificadas en su distribución son el Istmo de Tehuantepec (IT), así como las tierras altas y secas del interior de México (Sánchez *et al*. 2007), el río Verde (Hernández 1995; Puebla *et al*. 2008), la cuenca Lerma-Santiago, la Depresión Pánuco (Ruiz *et al*. 2013), y la Depresión Central de Chiapas (Órnelas *et al*. 2013). Sin embargo, en México el Istmo de Tehuantepec es la barrera de mayor importancia en la distribución de especies montanas (Órnelas *et al.* 2013). Este se reconoce como una zona de alta complejidad biogeográfica, donde hubo varios eventos tectónicos, fluctuaciones en el nivel del mar y un levantamiento continental que unió a México con América Central durante el Plioceno-Pleistoceno (5ma- 2ma) (Ferrusquía 1993). Asimismo, para el caso de los encinos, el Istmo de Tehuantepec constituye la principal barrera geográfica a la distribución de la mayoría de las especies de la sección *Lobatae* (Torres-Miranda *et al*. 2013)

Por otra parte, el origen de la Faja Volcánica Transmexicana (FVTM) se ha explicado debido a la subducción de la placa de Cocos debajo de la Norteamericana (Aguayo 1996; Ferrari 2012). Con base a la edad, orogenia y características geológicas se ha dividido esta región en tres sectores principales (Gómez-Tuena *et al*. 2007), adicionalmente, estudios biogeográficos apoyan estos resultados (Torres- Miranda *et al*. 2013). La FVTM es un complejo de los más ricos topográficamente de América, además de que tiene una historia biogeográfica asociada a grandes eventos geológicos como la conexión de América del Norte y del Sur mediante el Istmo de

Panamá durante el Plioceno-Pleistoceno posibilitando el gran intercambio biótico americano, que permitió una gran diversificación y especiación (Futuyma 1986). Estudios filogeográficos en plantas han documentado que los cambios geológicos podrían haber influido en la diferenciación genética de las poblaciones en la FVTM, lo que podría haber causado fragmentación alopátrica (Ruiz- Sánchez *et al*., 2013), originando la división de las poblaciones en dos sectores principales a lo largo de la FVTM (Ruiz-Sánchez *et al*., 2012).

Por otro lado, la distribución de las especies se ha visto afectada por procesos atmosféricos como el cambio climático, y procesos biológicos como extinciones locales y dispersión. Durante el Plioceno-Pleistoceno, muchas especies sufrieron fluctuaciones en sus áreas de distribución debido a los cambios en los ciclos glaciales (Hewwit *et al*. 2004). Los patrones de cambio climático global fueron un factor implicado en la configuración de los patrones de variación genética en la biota templada y tropical. Como consecuencia de estos eventos, la biota ha experimentado cambios dramáticos en su riqueza, abundancia y distribución geográfica (Hu *et al.* 2009). De hecho, se ha documentado que, en los trópicos las condiciones de aridez y bajas temperaturas creadas durante los periodos glaciares del Pleistoceno ocasionaron contracciones del área de distribución de las poblaciones (Wijninga *et al*. 1995). Por lo tanto, se considera que las glaciaciones tuvieron un papel muy importante en la divergencia de las poblaciones y la especiación.

En México, las glaciaciones favorecieron más las migraciones altitudinales que latitudinales (González-Rodríguez *et al*. 2004; Caballero *et al.* 2010) fragmentando y/o ampliando los rangos de distribución de las especies (Caballero *et al.* 2010). En particular se ha documentado que el descenso de la vegetación de hasta 1000 m contribuyó a la diversificación de los *Pinus* en México (Caballero *et al.* 2010). Para el caso de los encinos mexicanos se ha documentado que es probable

que poblaciones grandes hayan persistido durante varios episodios de cambio climático a lo largo del Pleistoceno y hayan experimentado contacto secundario con poblaciones de otras especies originando zonas de hibridación de especies (González- Rodríguez *et al.* 2004; Caballero *et al.* 2010). Durante el Último Máximo Glacial, el clima parece haber causado modificaciones en la composición de las comunidades favoreciendo a las especies más resistentes a las nuevas condiciones ambientales (Caballero *et al*. 2010). Cabe mencionar que la vegetación no sólo responde al enfriamiento, sino también al cambio en la humedad, dado que hay evidencias de climas relativamente secos a pesar de la disminución en temperatura (Caballero *et al.* 2010).

La Sierra Madre Oriental: patrones y barreras

La Sierra Madre Oriental (SMOr) comprende parte de los estados de Nuevo León, Tamaulipas, San Luis Potosí, Hidalgo, Guanajuato, Querétaro, Veracruz y Puebla. Latitudinalmente la SMOr abarca desde el paralelo 20º hasta cerca del 30º, en un gradiente altitudinal de 200 a 3500 msnm, aunque con altitudes predominantes entre los 1500 y 2000 msnm (Luna *et al.* 2004). La SMOr es una zona de transición entre las regiones biogeográficas Neártica y Neotropical (Challenger *et al.* 2008). La formación del sistema orogénico de la Sierra Madre Oriental inició en el Paleoceno, promovido por un intenso proceso de plegamiento que dio origen a sus sierras y el cual finalizó en el Eoceno. La mayor parte de las serranías de esta provincia fueron formadas por el plegamiento, durante la Orogenia Laramide (INEGI 2002). Esta formación se desarrolla de forma paralela al Istmo de Tehuantepec y la Sierra Madre de Chiapas (Antuñano *et al.* 2000; López 2003; Zenteno *et al.* 2005).

Desde el punto de vista fisiográfico, la SMOr comprende un conjunto de serranías, cuya identidad geológica está dada por la dominancia de formaciones del Cretácico medio y superior,

aunque también existen rocas Jurásicas y Paleozoicas, y muy pocas generadas por el vulcanismo del Cenozoico. Las rocas predominantes son sedimentarias, metamórficas del Cretácico y Jurásico Superior (65 a 156 ma), entre las que predominan las calizas, las areniscas y las lutitas (rocas arcillosas) (Lugp *et al.* 2003).

Considerando tanto su origen geológico como sus los patrones del paisaje, la provincia fisiográfica de la SMOr ha sido subdividida en ocho regiones principales: 1) Sierras Transversales, 2) Pliegues de Saltillo-Parras, 3) Sierras y Llanuras occidentales, 4) Gran Sierra Plegada, 5) Sierra de San Carlos, 6) Sierra de Tamaulipas, 7) Carso Huasteco, y 8) Llanuras y Sierras de Querétaro e Hidalgo (Luna *et al.* 2004).

La heterogeneidad topográfica asociado a los sistemas atmosféricos más importantes, han dado como resultado un complejo mosaico climático en la SMOr, en donde se ubican algunas de las zonas con mayor precipitación del país, así como otras de las más secas (Luna *et al.* 2004). La SMOr posee el 99% de los climas existentes en México, es decir, se presentan ambientes muy áridos hasta cálidos húmedos. La temperatura media oscila entre 12° y 25°C. Mientras que la temperatura más baja oscila entre 5° y 12° y las más altas oscilan entre 22° y 26° (Luna *et al.* 2004). La cantidad de precipitación al año va de menos de 300 mm en el centro y norte a más de 4,000 mm en el extremo sur (Hernández 2004). El clima es más seco y extremo hacia el norte de la SMOr y sobre su fachada occidental, mientras que en las partes media y sur imperan zonas subhúmedas, la vegetación y la niebla, han contribuido a generar importantes sistemas fluviales que corren hacia el oriente (Challenger 2008). La SMOr juega un importante papel ecológico porque regula los procesos meteorológicos, la dinámica hidrológica y los ciclos biogeoquímicos del noreste de México (Luna *et al.* 2004).

Efecto de la Sierra Madre Oriental sobre la evolución y ecología de las especies

Algunos estudios han reportado el papel que han desempeñado los sistemas orográficos como corredores biológicos promoviendo una alta diversidad de especies. En este aspecto, se ha hipotetizado que, tanto la SMO como la Sierra Madre Occidental, conformadas entre el Eoceno tardío y el Oligoceno tardío (40-28 ma), funcionaron como corredores para muchos grupos de origen Neártico, por ejemplo, los encinos (Nixon 1993a) y pinos (Perry 1998). Una vez colonizado el norte de México, y terminado el desarrollo de la Faja Volcánica Transmexicana en el Mioceno Tardío (11 ma), fue posible la continuidad de la SMOr y la Sierra Madre Occidental con la Faja Volcánica Transversal, lo cual permitió ampliar las áreas de distribución de muchas especies que habitaban tierras altas en México hacia el sur del país y colonizar las zonas montanas de Centroamérica (Rzedowski 1993).

Estudios biogeográficos previos sugieren la división de la Sierra Madre Oriental (SMOr) en tres secciones, esta división resulta de la existencia de barreras naturales originando subprovincias, tales barreras son: la Depresión del Pánuco (González *et al.* 2007) y el sistema montano Saltillo-Monterrey (Del Conde 2009; Sanginés *et al* 2011). Lo anterior sugiere la existencia de barreras naturales que han permitido proponer un sistema de regionalización de la SMO con base en estudios previos para cactus (Del Conde 2009), helechos (Sanginés *et al*. 2011), mamíferos (León *et al*. 2004), aves (Navarro *et al.* 2004) y Asteraceae (González *et al.* 2007). Asimismo, estudios filogeográficos sustentan la Depresión del Pánuco como barrera geográfica promoviendo estructura filogeográfica en sus poblaciones (Sosa *et al*. 2009; Ruiz-Sánchez *et al.* 2012). Adicionalmente, existen estudios filogeográficos previos en la SMOr en diversas especies de plantas y animales como; *Hunnemannia fumariifolia* (Sosa *et al*. 2009;

Ruiz- Sánchez *et al.* 2012), *Podocarpus matudae* (Órnelas *et al*. 2010), *Palicourea padifolia* (Gutiérrez-Rodríguez *et al.* 2011), *Nolina parviflora* (Ruiz-Sánchez *et al.* 2013), *Alshopilla firma* (Ramírez-Barahona *et al.* 2014), *Mousonnia deppeana* (Órnelas *et al.* 2014), *Rhipsalis baccifera* (Órnelas *et al.* 2015), *Ephedra compacta* (Loera *et al.* 2015), *Crotalus triseriatus* (Bryson *et al.* 2011) y *Sceloporus scalaris* (Bryson *et al.* 2012).

Estructura de la tesis

La presente tesis se divide en dos capítulos. El primer capítulo se titula, "Variabilidad en caracteres morfológicos foliares de un encino endémico mexicano (*Quercus mexicana* Bonpl.) a lo largo de un gradiente ambiental." En este capítulo se analizó la variación morfológica foliar de *Q. mexicana* para identificar la respuesta de los rasgos foliares ante diferentes gradientes ambientales y evaluar si hay evidencia de diferenciación poblacional a lo largo de la distribución de la especie. Para este estudio, se colectaron 39 poblaciones en la Sierra Madre Oriental y zonas adyacentes. Posteriormente, se midieron ocho caracteres macromorfológicos en 5,507 hojas y tres caracteres micromorfológicos en 228 hojas. Se realizaron análisis estadísticos univariados, bivariados y multivariados para evaluar la diferenciación morfológica entre poblaciones, así como las relaciones entre la variación morfológica foliar y gradientes ambientales relacionadas con la temperatura y la disponibilidad hídrica. Los principales resultados indican que las poblaciones de *Q. mexicana* muestran diferenciación morfológica evidente a lo largo de su distribución. Se encontraron correlaciones significativas entre caracteres foliares y variables ambientales. Análisis multivariados mostraron que variables relacionadas con la temperatura y la humedad permiten la discriminación y agrupación de poblaciones. Hojas pequeñas y gruesas con baja densidad de tricomas y estomas pequeños se encontraron en poblaciones en sitios más áridos. En contraste, hojas más grandes y delgadas con mayor densidad de tricomas y estomas más grandes ocurrieron en sitios más húmedos.

El segundo capítulo, se titula "Nuclear and chloroplast DNA phylogeography reveals high genetic diversity and postglacial range expansion in *Quercus mexicana* (Fagaceae)". En este capítulo se describe el análisis genético y filogeográfico realizado de *Q. mexicana* a lo largo de

su área de distribución utilizando microsatélites de cloroplasto (cpSSR) y nucleares (nSSR) como marcadores moleculares de ADN. Posteriormente, relacionamos la información genética con modelos de nicho ecológico para evaluar expansiones o contracciones demográficas de poblaciones de *Q. mexicana* durante el Holoceno Medio, Último Máximo Glacial y Último Interglacial. Los resultados indican una alta diversidad genética asociada a una baja estructura genética en las poblaciones de *Q. mexicana*. Los modelos de nicho ecológico sugirieron fluctuaciones históricas en el rango de distribución de esta especie. El rango histórico de expansión, el flujo de genes y las barreras físicas parecen haber jugado un papel importante en la configuración de la estructura filogeográfica de *Q. mexicana*.

Hipótesis

1. El tamaño foliar estará asociado positivamente a gradientes de precipitación. Sin embargo, el grosor foliar estará asociado positivamente a ambientes secos. Mientras que la mayor densidad de tricomas estará asociado positivamente a ambientes con mayor temperatura. También, se espera encontrar una disminución en la longitud de la apertura estomática y el tamaño de los estomas en condiciones de sequía.

2. Se esperaría que la estructura filogeográfica esté influenciada por la Depresión del Pánuco considerada como una barrera geográfica fundamental que divide a la Sierra Madre Oriental en un sector norte y uno sur (Torres-Miranda *et al*., 2011; 2013). Esto sugiere que la Depresión del Pánuco podría representar una barrera al flujo génico entre poblaciones de *Quercus mexicana.*

Objetivo general de la tesis

Analizar los patrones de variación morfológica, genética y filogeográfica de *Q. mexicana* a lo largo de su distribución en la Sierra Madre Oriental y regiones adyacentes.

Objetivos específicos

Variación morfológica en *Q. mexicana*

1) Determinar la variación y el grado de diferenciación en los caracteres morfológicos foliares entre las poblaciones de *Q. mexicana*.

2) Evaluar la influencia de los factores climáticos y geográficos sobre la variación fenotípica a lo largo de gradientes ambientales y geográficos.

Patrones filogeográficos en *Q. mexicana*

1) Describir la diversidad y estructura genética de las poblaciones en *Q. mexicana*.

2) Determinar eventos históricos de contracción y expansión demográfica en *Q. mexicana*.

3) Modelar los rangos de distribución geográfica de *Q. mexicana* en tres períodos (Holoceno medio, Último Máximo Glacial y Último Interglaciar). Además, comparar los modelos de nicho ecológico con su distribución actual con el propósito de proporcionar información sobre cómo las fluctuaciones climáticas afectaron la distribución de *Q. mexicana* en México.

Capítulo I

Variability in leaf morphological traits of an endemic Mexican oak (*Quercus mexicana* Bonpl.) along an environmental gradient

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Ecology / Ecología

Variability in leaf morphological traits of an endemic Mexican oak (*Quercus mexicana* Bonpl.) along an environmental gradient

Variabilidad en caracteres morfológicos foliares de un encino endémico mexicano (*Quercus mexicana* Bonpl.) a lo largo de un gradiente ambiental

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Abstract

Background: Phenotypic and functional traits of plant populations vary with environmental conditions at local and regional scales. The analysis of these traits along environmental gradients provides information on the differential response of populations to climate changes.
Objective: We analyzed the leaf morphological variation of an endemic oak to identify the degree of population differentiation along an environmental gradient.
Study species: *Quercus mexicana* Bonpl. (Fagaceae).
Study site and dates: Samples were collected from 39 populations in the Sierra Madre Oriental and east of the Trans-Mexican Volcanic Belt from 2014 to 2016.
Methods: We measured eight macromorphological traits in 5,507 leaves and three micromorphological traits in 228 leaves. We performed univariate and multivariate statistical analyses to assess the morphological differentiation among populations, and the relationship between variation in leaf traits and environmental variables related to temperature and water availability.
Results: Populations of *Q. mexicana* showed leaf morphological differentiation along its distribution. Significant linear correlations were found between leaf traits and environmental variables. Smaller and thicker leaves with lower density of trichomes and smaller stomata were found in populations located in more arid regions. In contrast, larger and thinner leaves with higher trichome density and larger stomata occurred in more humid places.
Conclusions: Populations of *Q. mexicana* are adapted to a wide range of climatic conditions. Considering the predictive future climatic changes for the region (*i.e.*, warmer and drier conditions), *Q. mexicana* populations with traits better adapted to a more humid and cooler environments could be negatively affected.
Keywords: climate change, drought tolerance, endemic oaks, leaf variation, morfo-functional traits, Sierra Madre Oriental.

Resumen

Antecedentes: Los caracteres fenotípicos y funcionales de las poblaciones de plantas varían a escalas local y regional. El análisis de estos caracteres a lo largo de gradientes ambientales proporciona información sobre la respuesta diferencial de las poblaciones a cambios climáticos.
Objetivo: Analizamos la variación foliar morfológica de un encino endémico para identificar el grado de diferenciación poblacional a un gradiente ambiental.
Especie de estudio: *Quercus mexicana* Bonpl. (Fagaceae).
Sitio de estudio y fechas: Se colectaron 39 poblaciones en la Sierra Madre Oriental y del este de la Faja Volcánica Transmexicana de 2014 al 2016.
Métodos: Medimos ocho caracteres macromorfológicos en 5,507 hojas y tres micromorfológicos en 228 hojas. Se realizaron análisis estadísticos univariados y multivariados para evaluar la diferenciación morfológica entre poblaciones, y las relaciones entre la variación foliar y variables relacionadas con la temperatura y la disponibilidad de agua.
Resultados: Las poblaciones de *Q. mexicana* están diferenciadas a lo largo de su distribución. Se encontraron correlaciones significativas entre caracteres foliares y variables ambientales. Hojas pequeñas y gruesas con baja densidad de tricomas y estomas pequeños se encontraron en sitios más áridos. En contraste, hojas más grandes y delgadas con mayor densidad de tricomas y estomas más grandes ocurrieron en sitios más húmedos.
Conclusiones: Las poblaciones de *Q. mexicana* están adaptadas a un amplio intervalo de condiciones climáticas. Considerando los futuros cambios climáticos para la región (*i.e.*, condiciones más secas y cálidas), las poblaciones de *Q. mexicana* con caracteres adaptados a ambientes más húmedos y fríos podrían afectarse negativamente.
Palabras clave: cambio climático, caracteres morfo-funcionales, encinos endémicos, tolerancia a la sequía, variación foliar, Sierra Madre Oriental.

Variation in climatic conditions at local and regional scales determines the phenotypic and physiological expression of plants and, at larger scales, the geographical distribution patterns of plant species (Pollock *et al*. 2012, Stahl *et al*. 2014, Riordan *et al*. 2016). Phenotypic variability in functional traits determines the limits of species distributions (Pollock *et al.* 2012, Stahl *et al.* 2014, Körner *et al.* 2016) through the intraspecific responses of plants to specific environmental factors (Albert *et al*. 2010, Laforest-Lapointe *et al.* 2014, Henn *et al.* 2018), in turn determining the ecological differentiation among populations (Albarrán-Lara *et al.* 2019, Martínez-Blancas & Martorell 2020). Identifying the relationships between morphological traits and environmental changes increases our understanding of the differential responses of populations, the potential shifts in species distributions, and the potential responses of plant species to climate change (Valladares *et al.* 2014, Henn *et al.* 2018).

Temperature and precipitation are two of the main environmental drivers of leaf variation at a global scale (Moles *et al.* 2014, Valladares *et al.* 2014, Wright *et al.* 2017). Patterns of foliar variation associated with gradients of temperature and precipitation have been identified for some leaf traits. For instance, plants with smaller leaves occur at sites with lower mean annual precipitation (Wright *et al.* 2017) along latitudinal (Uribe-Salas *et al*. 2008) or elevational (Tang & Ohsawa 1999) gradients. Similarly, leaf dry mass per area and leaf thickness have shown positive relationships with high solar radiation and temperature and negative relationships with rainfall at the multispecies level at a global scale (Niinemets 2001). However, these patterns vary at the intraspecific level at a regional scale. Variation among populations has been observed for some traits; for instance, a larger leaf area has been associated with elevated rainfall (Gouveia & Freitas 2009), and greater leaf vein density has been observed in drier environments (Zhu *et al.* 2012).

The density and type of trichomes also provide evidence of the functional responses of plants exposed to different environmental conditions. Under dry conditions, trichomes reduce the absorbance of solar radiation (Benz & Martin 2006), providing resistance to low temperatures (Agrawal *et al.* 2004) and UV ray protection (Schilmiller *et al.* 2008). Similarly, the importance of stomata in sensing and driving environmental change has been observed (Hetherington & Woodward 2003). Reduced stomatal density has been observed at higher CO_2 concentrations (Casson & Gray 2008), while stomatal density tends to increase in plants located at sites with elevated temperature and drought stress (Yan *et al.* 2017).

In this study, we analyzed the intraspecific variation in leaf morphological and functional traits in response to environmental gradients for an endemic red oak from northeastern Mexico: *Quercus mexicana* Bonpl. (Section *Lobatae*). The populations of *Q. mexicana* are distributed over a wide area, including subhumid regions, temperate forests, and xeric areas (Pérez-Mojica & Valencia-Á. 2017), in which conditions of temperature, precipitation and humidity display a large degree of variation. The objectives of this study were to determine the variation and degree of differentiation in leaf morphological traits among the populations of *Q. mexicana* and assess the relative influences of climatic and geographic factors on this phenotypic variation throughout the entire distribution of this species.

Materials and methods

Study species. Quercus mexicana is a tree species that grows to be 6 to 12 m tall. This species presents coriaceous leaves that are elliptic, or oblong to oblong-obovate, from 2.8 to 8 cm in length and 1.5 to 2.5 cm in width and bear 7 to 12 secondary veins. Leaves present stellate trichomes at the base of the adaxial side and fasciculate trichomes on the abaxial side (Pérez-Mojica & Valencia-Á. 2017). *Quercus mexicana* is distributed mainly in the Sierra Madre Oriental (eastern Mexico) and at the eastern boundaries of the Mexican Plateau and the Trans-Mexican Volcanic Belt (Figure 1). It has been recorded in pine forests, oak forests, and xeric shrublands at elevations ranging from 1,600 to 2,700 m in clay soils with rocky outcrops (Pérez-Mojica & Valencia-Á. 2017).

Study area. The Sierra Madre Oriental is a mountain chain with marked climatic and environmental variation and a notable difference in the composition of the biotas. Hernández & Carrasco (2004) reported arid to warm humid climates (temperature ranges between 12 and 25 °C, and annual precipitation ranges between 300 mm and 4,000 mm).

The northern and southern regions of the Sierra Madre Oriental are separated by the Pánuco Depression; a warm and humid climate dominates on the Gulf of Mexico slope, while it becomes arid and warm towards the western slope and the south and colder towards the north, particularly in the highlands (Hernández & Carrasco 2004, Figure 1). The climatic differences of the Sierra Madre Oriental with respect to the Trans-Mexican Volcanic Belt and Mexican Plateau are also striking, with colder temperatures with elevated humidity or aridity in the last two regions (Figure 1).

Sampling strategy. Leaf samples were collected from 39 populations of *Q. mexicana* throughout its geographic distribution (Figure 1, Appendix 1) (Valencia-Á. 2004). In each population, 10 individuals separated by at least 20 m apart were randomly selected. From each tree, 15 mature leaves were collected from the lower branches. The collected leaves were pressed and dried in an oven at 70 °C.

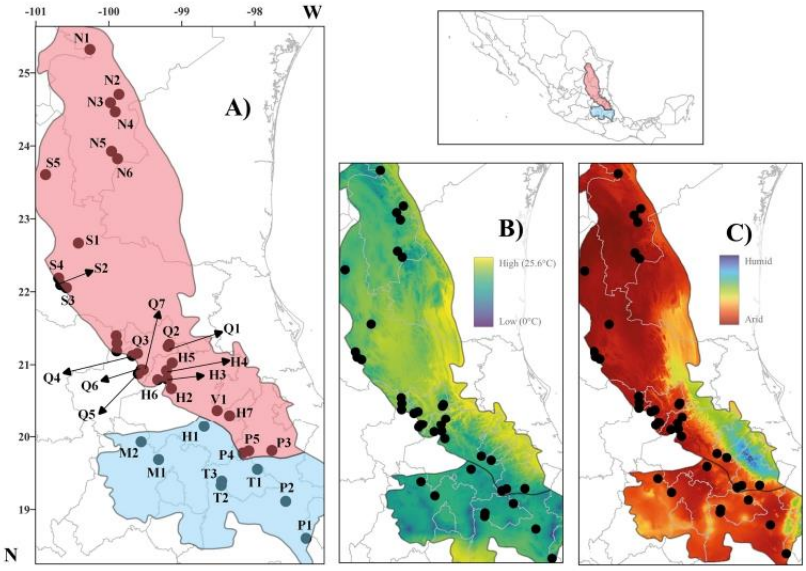

Figure 1. Study area. (A) Geographic locations of the 39 populations of *Quercus mexicana* across the Sierra Madre Oriental (SMO in red) and adjacent regions (in blue). Maps of (B) annual mean temperature and (C) aridity index in the study area. Population abbreviations are described in Appendix 1.

Leaf macromorphological traits. For each of the 5,507 sampled leaves, leaf length and thickness were measured with a digital Vernier caliper. Measurements of dry weight were performed with an analytical balance (precision = 0.001 g). Leaf area (LA, cm^2) was estimated through a quantitative evaluation of the area and leaf shape contours using elliptical Fourier descriptors with SHAPE ver. 3.1 software (Iwata & Ukai 2002). With this procedure, a matrix of eight macromorphological traits (M_{MAC}, Table 1) was constructed: leaf length, leaf width, leaf length/leaf width ratio, number of secondary veins, leaf thickness, specific leaf area, petiole length and petiole width. These traits were obtained from 5,507 leaves from 39 populations, with an average of 370 individuals.

Leaf micromorphological traits. We randomly selected four to six leaves per population of *Q. mexicana* for a total of 228 leaves to measure leaf trichomes and stomata using scanning electron microscopy (SEM). Three images corresponding to the X100, X200 and X500 fields for each leaf were taken; in total, 684 images were analyzed. For each population, trichome density (trichome number/area) was quantified using X100 field images and stomatal density (stomatal number/area) with X200 field images with the Digimizer software (http://www.digimizer.com). The X500

images were used to measure stomatal aperture length in µm. The three variables were integrated into a micromorphological matrix (M_{MIC}, Table 1) for further analysis.

Table 1. List of macro- and micromorphological leaf traits and climatic and geographic variables used to identify the differentiation among populations of *Quercus mexicana*.

Leaf morphological traits (M_{MOR})			
Macromorphological (M_{MAC}) traits		Micromorphological (M_{MIC}) traits	
LL	Leaf length	DTR	Density of trichomes
LW	Leaf width	STD	Stomatal density
LWr	Leaf (length / width) ratio	STL	Stomatal aperture length
PL	Petiole length		
PW	Petiole width		
SLA	Specific leaf area		
NV	Number of secondary veins		
LT	Leaf thickness		
Environmental variables			
Water-related variables		Temperature-related variables	
AI	Aridity index	AMT	Annual mean temperature
PET	Potential of evapotranspiration	TS	Temperature seasonality
AP	Annual precipitation	ATR	Annual temperature range
PS	Precipitation seasonality	MTC	Mean temperature of coldest quarter
PD	Precipitation of driest quarter		

Environmental variables. A matrix of nine environmental variables without collinearity that described the conditions of each sampled population was constructed (Table 1). Seven noncorrelated bioclimatic variables were obtained from WorldClim (Hijmans *et al.* 2005): annual mean temperature, temperature seasonality, annual temperature range, mean temperature of coldest quarter, annual precipitation, precipitation seasonality, and precipitation of driest quarter. Potential evapotranspiration and an aridity index were obtained from the Global Aridity Index and Potential Evapotranspiration Climate Database version 2.0 (Trabucco & Zomer 2009). Potential evapotranspiration relates the evapotranspiration processes and rainfall deficit, and aridity index represents the ratio between precipitation and potential evapotranspiration (high or low values of aridity index correspond to wet or arid conditions, respectively). Latitude was added as a geographic variable. Visualization and manipulation of climatic information was performed using ArcMap® v. 10.2 (ESRI). The overall topographic variables were calculated using Google Earth®.

Univariate analyses. Statistics of each morphological trait were calculated for each of the 39 populations. As a first step, to determine the differences in leaf morphological and functional traits among populations of *Q. mexicana*, we used ANOVA for each of the eight macromorphological and the three micromorphological variables, and a Tukey-Kramer test was conducted for *a posteriori* comparison among populations using R v. 3.6.1 software (R Core Team 2016). Then, data normality was tested using a Shapiro-Wilk test, and Pearson correlations were calculated with R software, using nine bioclimatic variables and latitude as independent variables and eight macromorphological (M_{MAC}) and three micromorphological traits (M_{MIC}) as dependent variables.

Multivariate analyses. We performed discriminant function analysis (DFA) and clustering analysis (CA) to identify leaf morphological groups among populations. First, a DFA was performed based on the morphofunctional traits (M_{MAC}, M_{MIC}, and M_{MOR}), and we used each of the 39 populations as a discriminant factor. Second, from the DFA results, the centroids of each population were obtained, the Euclidian distance among centroids was calculated, and the CA was performed with the Ward method (Ward & Hook 1963). The CA was performed to yield a dendrogram depicting the morphological relatedness among the means of individuals in each population.

From the previously identified groups (named morphogroups), DFA was performed based on the differences among environmental conditions between morphogroups. Three independent analyses were conducted following the previous procedure: the first considering macromorphological traits (DFA_{MAC}), the second with micromorphological traits (DFA_{MIC}) and the third considering both attribute sets (DFA_{MOR}). This procedure was performed with R software.

To evaluate the contribution of temperature and water availability to leaf morphological variation at a global scale, three redundancy analyses (RDAs) were performed considering the following data sets: a) a complete model including both sets of bioclimatic variables (RDA_{full}: space + climate), b) a partial model considering temperature-related variables controlling the water effect (pRDA1: climate|space), and c) a partial model considering water-related variables controlling the temperature effect (pRDA2: space|climate). Three independent analyses were performed: the first considering macromorphological traits (RDA_{MAC}), the second with micromorphological traits (RDA_{MIC}) and the third considering both traits (RDA_{MOR}). This procedure was performed with the vegan package in R software.

Results

Univariate analyses. All the morphological traits, except for stomatal density, showed significant differences among populations (Figure 2; Appendix 1). After Tukey's test (with at least $P < 0.05$), the morphological traits that had the greatest differences among populations were leaf width, leaf thickness, number of secondary veins and petiole length. In addition, all the morphological traits displayed gradual variation among all the populations (Figure 2).

Among the macromorphological traits, leaf length varied without a regional pattern, whereas leaf width was significantly smaller and the leaf length/width ratio was significantly larger in the northern populations of the Sierra Madre Oriental. Petiole length was significantly larger in some populations in the north of the Sierra Madre Oriental (N1, N2, S1), in Hidalgo (H1, H3), and in the Trans-Mexican Volcanic Belt in Estado de Mexico and Tlaxcala (T3). Significantly wider petioles were detected in a group of populations in the northern Sierra Madre Oriental (N1, N4, N6, S5) and in the southwestern Sierra Madre Oriental (H5, Q4, Q7); narrower petioles were found in the southeastern Sierra Madre Oriental (H7, V1, P4) and parts of the Trans-Mexican Volcanic Belt (M1, P2). The specific leaf area differed in only a couple of populations in the north, and some populations displayed smaller leaf areas. The number of veins was significantly greater in the northern Sierra Madre Oriental and smaller in the southeastern Sierra Madre Oriental (H1, H7, P5, V1) as well as in some populations of the Trans-Mexican Volcanic Belt (P1, T1). The thickest leaves were found in the northern Sierra Madre Oriental and in populations in the southwestern Sierra Madre Oriental (H3, H4, M2). Significantly thinner leaves were found in several populations, predominantly in San Luis Potosí, Puebla, and Querétaro (Appendix 2).

With respect to the micromorphological traits, the populations that displayed the highest density of trichomes were in Puebla (P2, P4), and the lowest density of trichomes was found in populations of the northern Sierra Madre Oriental (N2, N3, N5), as well as populations in Hidalgo (H2, H4). The stomatal aperture length was smaller in most of the populations from Querétaro, as well as some in the north (N3, S2); the largest stomatal aperture was found in populations from the Trans-Mexican Volcanic Belt, significantly differing only in Tlaxcala (T2) (Figure 2; Appendix 2).

In the linear correlation analyses (Table 3; Figure 3), leaf length and specific leaf area displayed similar correlations with the environmental variables; they were positively correlated with latitude, all the temperature variables, potential evapotranspiration, and precipitation of driest quarter and negatively correlated with the aridity index. Leaf

width followed a similar pattern except that it was correlated with precipitation seasonality, and no correlation with the aridity index was found. The leaf length/width ratio was only positively correlated with latitude and negatively correlated with precipitation seasonality. The number of veins and leaf thickness were positively correlated with latitude, temperature seasonality, potential evapotranspiration and precipitation of driest quarter, and the number of veins and leaf thickness were inversely correlated with the aridity index and precipitation seasonality. The number of veins was positively correlated with all the temperature variables. With regard to petiole size, petiole length was not correlated with temperature, but petiole width was negatively correlated with the annual temperature range; with respect to the precipitation variables, petiole length and width were negatively correlated with precipitation seasonality.

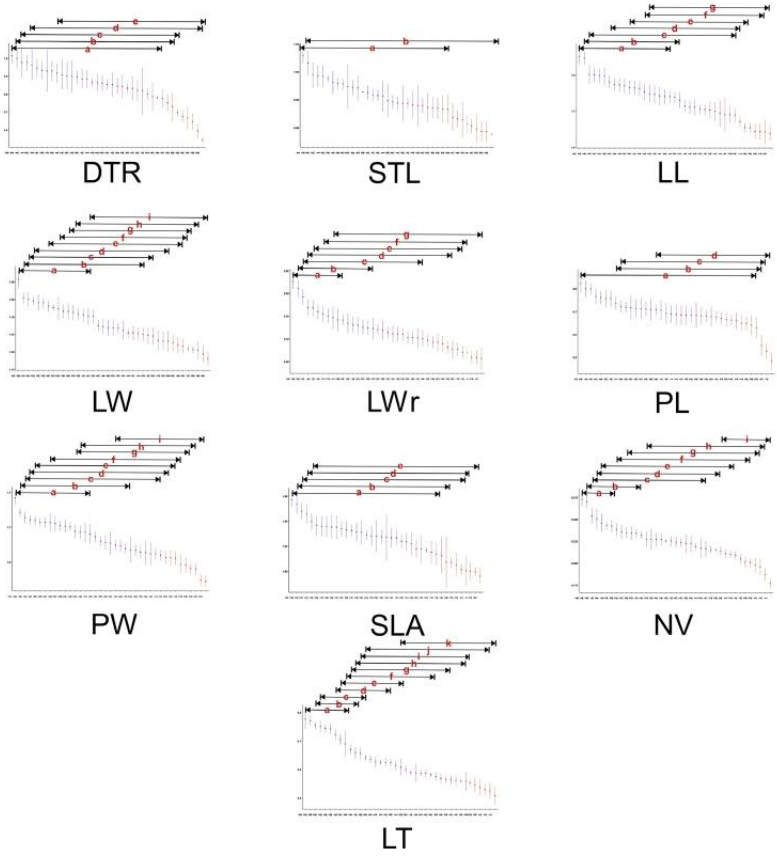

Figure 2. Summary of the Tukey-Kramer post-hoc tests for the differentiation of micro- and macromorphological traits among populations of *Quercus mexicana*. Lines above populations indicate no significant differences (at $P < 0.05$) and only the results with a significance $P < 0.001$ are presented.

Among the micromorphological characters, the density of trichomes showed a negative correlation with latitude, temperature seasonality, precipitation of driest quarter, and potential evapotranspiration but a positive correlation with the aridity index. Stomatal density was correlated only with potential evapotranspiration, while stomatal aperture length was negatively correlated with latitude, potential evapotranspiration, precipitation of driest quarter, and three of the temperature variables (Table 2).

Table 2. The results of ANOVA for the differentiation of leaf morphological traits among populations of *Quercus mexicana*. Abbreviations of leaf morphological traits as indicated in Table 1. Degrees of freedom (df), sum of squares (ss), mean squares (ms) and *F*-values are indicated (*** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$).

	df populations /df residuals	ss populations / ss residuals	ms populations / ms residuals	*F* value
DTR	38 / 126	1.953 / 1.741	0.05139 / 0.01381	3.72***
STD	38 / 126	0.471 / 1.026	0.01239 / 0.00814	1.522*
STL	38 / 126	0.194 / 0.241	0.0051 / 0.00191	2.671***
LL	38 / 329	1.088 / 1.358	0.02862 / 0.00412	6.933***
LW	38 / 329	1.018 / 1.286	0.02679 / 0.0039	6.855***
LWr	38 / 329	0.579 / 0.859	0.01524 / 0.00261	5.836***
PL	38 / 329	1.571 / 4.128	0.04134 / 0.01255	3.295***
PW	38 / 329	1.115 / 1.163	0.02933 / 0.00353	8.298***
SLA	38 / 329	0.512 / 1.422	0.01349 / 0.00432	3.121***
NV	38 / 329	0.145 / 0.147	0.00381 / 0.00044	8.515***
LT	38 / 329	2.047 / 1.178	0.05386 / 0.00358	15.04***

Table 3. Results of Pearson's correlations between environmental variables and leaf morphological traits. Abbreviations of leaf morphological traits as indicated in Table 1. Significant values: *** $P < 0.001$, ** $P < 0.01$ and * $P < 0.05$.

		Micromorphological traits					Macromorphological traits				
		DTR	STD	STL	LL	LW	LWr	PL	PW	SLA	NV	LT
	LAT	-0.41***	0.05	-0.18*	0.16**	-0.04	0.29***	0.12*	0.24***	0.12*	0.49***	0.44***
Precipitation	AI	0.26***	-0.10	0.23**	-0.15**	-0.09	-0.07	-0.10*	0.01	-0.13*	-0.36***	-0.18***
	PET	-0.36***	0.17*	-0.19*	0.21***	0.15**	0.08	0.15**	0.17**	0.18***	0.32***	0.31***
	AP	0	0	-0.11	0.04	0.09	-0.07	-0.15**	0.13*	0.07	-0.08	-0.02
	PS	0.20**	0.05	-0.02	0.07	0.21***	-0.23***	-0.11**	-0.18***	0.07	-0.19***	-0.31***
	PD	-0.29***	-0.02	-0.21**	0.16**	0.09	0.08	-0.05	0.30***	0.14**	0.22***	0.25***
Temperature	AMT	-0.14	0.09	-0.22**	0.39***	0.39***	-0.02	0.01	-0.04	0.20***	0.22***	0.04
	TS	-0.34***	0.1	-0.25***	0.31***	0.26***	0.07	0.07	0.13*	0.23***	0.39***	0.26***
	ATR	0.06	0.08	-0.05	0.11*	0.16**	-0.05	0	-0.19***	0.11*	0.17**	0.02
	MTC	-0.06	0.08	-0.20*	0.38***	0.39***	-0.05	0	-0.09	0.18***	0.14**	-0.03

The highest correlation scores with temperature variables were observed for annual mean temperature and temperature seasonality in relation to leaf length, leaf width, density of trichomes and number of veins. On the other hand, the water availability variable with the highest correlation scores was potential evapotranspiration in relation to the density of trichomes and number of veins.

Multivariate analyses. DFA of micromorphological traits (DFA_{MIC}) explained 51.3 % of the variation for the first discriminant function (DF1), and 30.5 % for the second discriminant function (DF2, Figure 4A). For the DFA_{MAC}, DF1 explained 42.4 % of the variation, and DF2 explained 24.1 % (Figure 4B). In the combined dataset, DFA_{MOR}, the DF1

explained 30 % and DF2 explained 23.3 % of the differentiation among populations (Figure 4C). All the axes were significant according to Wilk's lambda (Figure 4). From the three analyses, the highest percentage of accurate classification was obtained with DFA_{MOR} (68.1 %). In the three DFAs, the populations from northern Sierra Madre Oriental together with others from Hidalgo formed a separate group (Figure 4). In the DFA_{MIC}, the density of trichomes was the trait that contributed most to explaining the DF1, while stomatal length aperture explained the variation in DF2. In DFA_{MAC} and DFA_{MOR}, leaf thickness and leaf length presented the highest scores in discriminating populations.

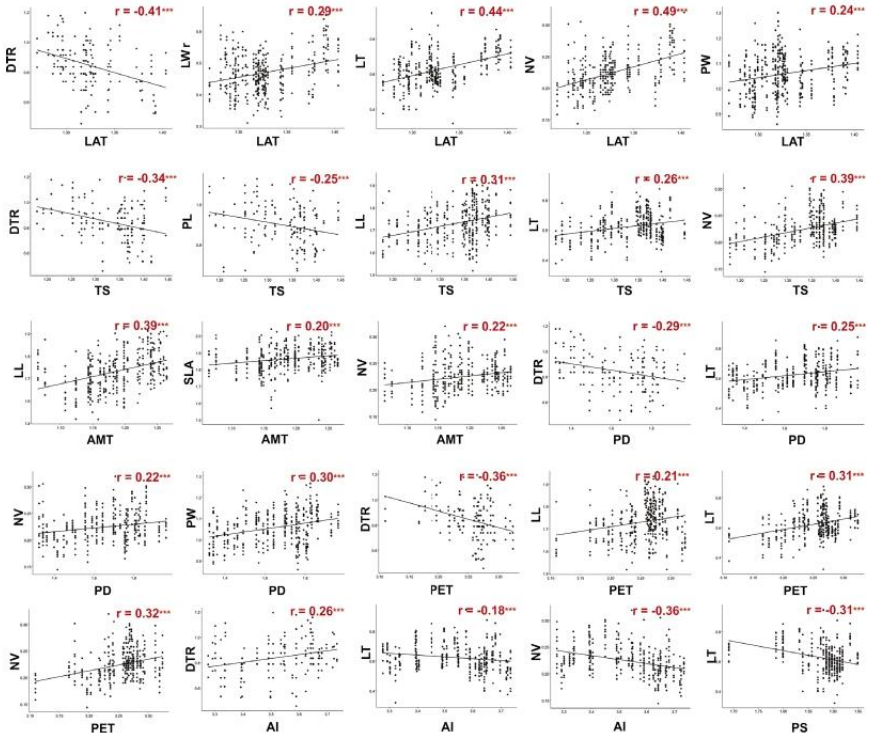

Figure 3. Graphs of Pearson's correlations between leaf morphological traits and environmental variables. Only significant correlations are presented. Abbreviations of leaf morphological traits as indicated in Table 1.

The cluster analyses allowed us to identify five groups for micromorphological traits and three groups for macromorphological traits and another three for all the morphological traits (Figure 4). The first group of the cluster with all the morphological traits comprised populations from the northern of Sierra Madre Oriental and Altiplano and southern Sierra Madre Oriental regions (Figure 4C). The traits that characterized this first group were thin leaves (range 0.17 to 0.22 mm) and wide petioles (1.31 to 1.58) (DFA_{MOR}, Figure 4C). The second group included populations from the Trans-Mexican Volcanic Belt, the southeastern Sierra Madre Oriental and one in the north. This group was characterized by high density of trichomes (8.4 to 10.56 per mm^2), large stomatal aperture (8.59 to 11.09 mm^2), and short (4.35 to 5.26 cm) and narrow (1.55 to 1.86 cm) leaves (DFA_{MOR}, Figure 4C). In the third group, most of the populations were located on the western slope of the southern Sierra Madre Oriental, in San Luis Potosí and some in the northernmost distribution (Figure 4C). The traits that characterized this group were long (5.67 to 7.29 cm) and wide (1.87 to 2.63 cm) leaves with low density of trichomes (3.5 to 7.49 per mm^2).

DFA based on environmental variables for DFA_{MIC}, explained 56.1 and 28.9 % of the variance via the first two discriminant functions, respectively; only the first function was significant (Figure 4A). The contribution of pre-

cipitation seasonality to the first discriminant axis was the most important for separating the populations; however, the groups were not recovered according to the morphofunctional traits, with 59 % of them correctly classified. For DFA_{MAC}, the first two discriminant functions explained 62.7 and 37.3 % of the variance, and neither of them was significant. The analysis that best explained the variance of the discriminant functions was DFA_{MOR}, with 78.8 and 21.2 % for the first two discriminant functions, respectively. However, only the first function was significant. The climatic variables of temperature seasonality and potential evapotranspiration were the most important for discriminating among populations according to DF1, and these variables characterized the group of populations of the western slope of the Sierra Madre Oriental. The northern Sierra Madre Oriental group was better explained by the aridity index; the Trans-Mexican Volcanic Belt + southeastern Sierra Madre Oriental group was better explained by negative scores of annual precipitation (Figure 4C).

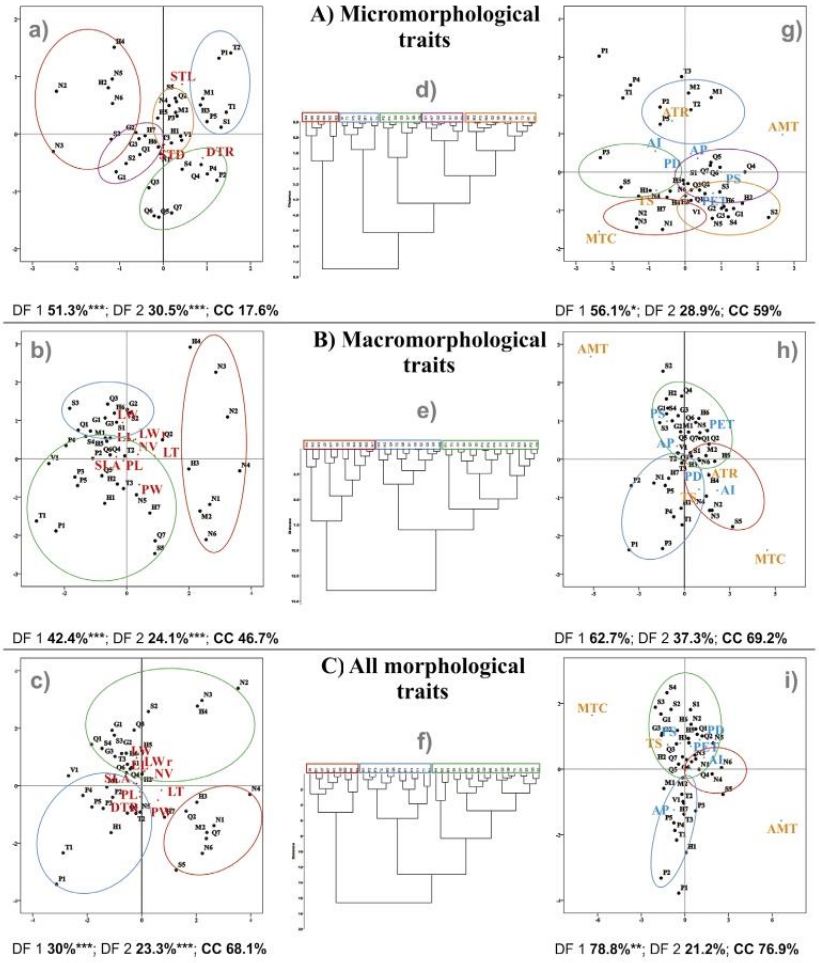

Figure 4. Discriminant function analyses (DFA) and clustering analyses (CA) for A) micromorphological traits, B) macromorphological traits and C) combined both datasets. DFA based on morphological traits (left: letters a, b, c), groups identified in the CA (center: d, e, f) and DFA based on environmental differences (right: g, h, i) are presented. Percentage of explained variation of the first two discriminant functions and CC represent the percentage of correctly classified cases in DFA. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

In the redundancy analyses, temperature-related variables explained 2.83 % (RDA_{MIC}: $P > 0.05$), 4.77 % (RDA_{MAC}: $P < 0.001$) and 5.45 % (RDA_{MOR}: $P < 0.001$) of leaf variation, with significance in the last two analyses. Water-related variables significantly explained 5.0 % (RDA_{MIC}; $P < 0.05$), 7.47 % (RDA_{MAC}: $P < 0.001$) and 9.84 % (RDA_{MOR}: $P < 0.001$) of the leaf variation. The interaction of temperature and water variables significantly explained 18.06 % (RDA_{MIC}; $P < 0.001$), 17.01 % (RDA_{MAC}: $P < 0.001$) and 20.83 % (RDA_{MOR}: $P < 0.001$) of the leaf variation (Appendix 3). For the full RDA, the most important and significant environmental variable in RDA_{MIC} was precipitation of driest quarter; for RDA_{MAC} and RDA_{MOR} the most important variables were precipitation seasonality, precipitation of driest quarter and the aridity index (Appendix 3). When plotting the full redundancy analysis results, precipitation seasonality and the aridity index were positively correlated with the density of trichomes. Potential evapotranspiration, precipitation of driest quarter, and temperature seasonality were negatively correlated with petiole width and leaf thickness (Figure 5).

Figure 5. Full redundancy analysis with the complete data set of morphological and environmental variables (RDA_{MOR}). Purple letters represent morphological traits, orange letters represent temperature variables and blue letters represent precipitation variables. Dots in green indicate the populations of *Quercus mexicana* (abbreviations of localities are described in Appendix 1). Percentage of explained variation of each axis at *** $P < 0.001$.

Discussion

Leaf morphological variability. Our results showed that the populations of *Q. mexicana* show variable leaf traits along their distribution, with groups of similar traits, although some populations with similar traits are not geo-

graphically proximal. Variation in leaf traits over a wide distribution area and along environmental gradients has been detected in *Q. rugosa* (Uribe-Salas *et al.* 2008), *Q. elliptica* (Maya-García *et al*. 2020) and *Q. deserticola* (Rodríguez-Gómez *et al*. 2018). Leaf morphological variation at the landscape level has also been observed in *Q. castanea* (Lara-De la Cruz *et al.* 2020).

Specifically, the northern Sierra Madre Oriental populations showed the greatest differences and were more consistent with the recovered groups; in the correlation analyses, these populations showed most of the significant differences in leaf trait variation. Indeed, latitude was positively correlated with most of the traits evaluated. A correlation with a latitudinal trend has also been found in *Q. rugosa* (Uribe-Salas *et al.* 2008), in which shorter leaves were present in its northern distribution. The most important traits for differentiating the northern Sierra Madre Oriental group from the rest of the populations were leaf thickness and petiole width. Leaf thickness was the most significantly different trait among the populations; this trait was also the most differentiated for *Q. castanea* populations at the landscape level (Lara-De la Cruz *et al.* 2020).

Relationships with environmental factors. In *Q. mexicana*, all the leaf traits showed correlations with variables of precipitation and temperature, except for stomatal density. In several studies, leaf variability and geographical differentiation of *Quercus* species have also been correlated with environmental variables (*e.g*., Uribe-Salas *et al.* 2008, Rodríguez-Gómez *et al*. 2018, Maya-García *et al.* 2020).

In this study, leaf thickness was correlated with temperature seasonality, indicating its association with extreme temperatures, which coincides with the northern Sierra Madre Oriental populations, and thinner leaves were associated with less arid and more seasonal environments, coinciding with the Trans-Mexican Volcanic Belt and southeastern Sierra Madre Oriental population climate (Figure 2). Lara-De la Cruz *et al.* (2020) also found thinner leaves at drier sites in *Q. castanea* at the landscape level. Contrary to our findings, in *Q. elliptica*, thickness was positively correlated with precipitation seasonality (Maya-García *et al*. 2020). Higher tissue density and thickness are correlated with higher water content (Vendramini *et al.* 2002). At sites with lower water availability, leaves are generally characterized by higher tissue density (de la Riva *et al.* 2016). Thus, a higher tissue density is associated with more successful performance under water-limited conditions (Harzé *et al*. 2016), which would explain the greater thickness and petiole width characterizing the northern Sierra Madre Oriental populations.

The largest specific leaf area of *Q. mexicana* was identified at drier sites with higher evapotranspiration and at higher latitudes. This result contrasts with the expectations of a correlation between specific leaf area and rainfall (Gouveia & Freitas 2009). Commonly, a decrease in specific leaf area has been associated with drier sites (Aranda *et al.* 2014) and an increase in temperature (Lee *et al.* 2005) in different oak species. The specific leaf area of *Q. mexicana* was also correlated with temperature variables. In other plant species, high temperature has been shown to decrease water use efficiency but increases specific leaf area (Meier & Leuschner 2008). Similarly, in *Q. elliptica*, specific leaf area was associated with drier conditions (Maya-García *et al.* 2020).

The number of secondary veins showed significant differentiation among populations and a significant correlation with all the environmental variables. Vein density shows a high inter- and intraspecific variation, and a very diverse responses to a combination of environmental traits (Zhu *et al.* 2012). For instance, higher vein density has been associated with higher sun exposure (Sack & Frole 2006) and drier soils for some species (Dunbar-Co *et al.* 2009). In other species, higher vein density is found under more humid conditions (Zhu *et al.* 2012). For *Q. mexicana*, the correlations suggested that leaves with a larger number of secondary veins were associated with humidity and less marked precipitation seasonality, but leaves with a larger number of secondary veins were also associated with higher potential evapotranspiration and more marked temperature seasonality.

Among the micromorphological traits, the density of trichomes was the trait that showed the highest discrimination coefficient among populations of *Q. mexicana*. Populations with the highest trichome density were identified at lower latitudes, at sites with marked precipitation seasonality and less evapotranspiration, characterizing the populations of the southern Sierra Madre Oriental and Trans-Mexican Volcanic Belt (Figures 2, 3). Patterns of trichome density observed in other plant species indicate that greater density is associated with drier conditions (Pérez-Estrada

et al. 2000, Benz & Martin 2006) or lower temperatures (Agrawal *et al.* 2004). Our results showed an inverse pattern of trichome density; for *Q. mexicana* lower trichome density characterized populations in the northern Sierra Madre Oriental located at sites with drier conditions and extreme high temperatures.

Although stomatal size and its density are relatively plastic traits (Richardson *et al.* 2001), we did not find significant differences among populations in stomatal density, nor did stomatal size or density show significant correlations with most of the environmental variables; only a significant correlation with potential evapotranspiration was found, as has been shown in other oak species (Nóbrega & Pereira 1992). Stomatal aperture length correlations with environmental variables indicate that larger stomata are associated with more humid conditions with less temperature seasonality. A small aperture length can provide a reduction in total pore area, facilitating faster closure in response to drier environments (Lawson & Blatt 2014), which would explain the presence of a larger stomatal aperture length in populations of the Trans-Mexican Volcanic Belt and southeastern Sierra Madre Oriental, where the conditions are more humid than those of the northern Sierra Madre Oriental.

On the other hand, the relationships that are established between micro- and macromorphological traits and environmental variables are very interesting and can be summarized by the RDA. First, the densities of trichomes and stomata are better explained by the seasonality of temperature and the availability of water. The size of the leaves seems to respond to changes in temperature; larger leaves and wider petioles are found in places with extreme temperature conditions, and the seasonality of temperature seems to be a key variable in the morphological variation and differentiation of *Q. mexicana* populations. Global trends indicate that leaves are generally smaller at drier and warmer sites, or at colder and wetter sites (Wright *et al.* 2017). The presence of larger leaves under drier or colder conditions is possible because of the thermoregulatory capability of leaves (Meier & Leuschner 2008, Michaletz *et al.* 2016): larger leaves can warm up fast on colder mornings, allowing better photosynthetic performance (Wright *et al.* 2017).

Even though we found that morphology was significantly correlated with environmental variables, the RDAs explained a relatively low, although significant percentage of the variation, specifically, when temperature and water variables were considered separately. This finding may be due to multiple factors, such as weak selection, extensive gene flow among populations (Riordan *et al.* 2016) or the effect of other environmental variables on populations, such as geomorphology or soil types (Wright *et al.* 2017). The global patterns of leaf change in relation to climatic variables also explain a small but significant amount of the variation, according to the evaluation of Wright *et al.* (2017).

To better understand the observed relationship between morphology and environmental variation and to assess if any of the aforementioned factors affect this relationship, it is necessary to assess the extent of genetic differentiation among the *Q. mexicana* populations. For example, in *Q. deserticola*, leaf variation is correlated with the environmental gradient of the Trans-Mexican Volcanic Belt but not with the genetic variation of the populations; phenotypic plasticity, common ancestry or adaptative differences could explain the environmental responses of the leaf variation (Rodríguez-Gómez *et al.* 2018).

Potential impacts of climate change. The morphological variability and differentiation among populations indicate species adaptability to environmental changes. Our results show a wide range of responses of *Q. mexicana* populations given the high environmental diversity of the distribution range of the species. Some trait variations seem to be restricted to specific climatic conditions or geographical areas, while others traits vary throughout the range without a specific pattern.

In Mexico, oak species have been moderately impacted by recurring climate changes caused by decreases in temperature coupled with conditions of moderated humidity throughout the Tertiary and Quaternary (Graham 1993). Palynological records indicated recurrent expansion–contraction changes in the communities of temperate plants during the Pleistocene (1.8 Ma to 10,000 years ago) in central Mexico (Lozano-García & Vázquez-Selem 2005). These outcomes suggested that species must have survived Pleistocene glaciations in areas with favorable climates at lower altitudes in the mountain major ranges of Mexico, and migration to higher elevations would have occurred during warm periods (Toledo 1982, Metcalfe *et al.* 2010). The effects of these climatic fluctuations on oak species

in Mexico lead to more diversity and less genetic structure but larger historical population sizes and a complex exchange among species (González-Rodríguez *et al.* 2004, Tovar-Sánchez *et al.* 2008).

A recent assessment of the climatic trends between 1910 and 2009 in Mexico (Cuervo-Robayo *et al.* 2020) indicates that warmer and drier conditions can be expected in the species distribution area in the future. The Mexican Transition Zone, which includes the Sierra Madre Oriental and the Trans-Mexican Volcanic Belt, showed an increase of 0.03 °C in the annual mean temperature between 1950 and 2009 and a decrease of 12 mm in annual rainfall. If these trends continue, it is probable that populations of *Q. mexicana* adapted to drought or extreme temperatures will respond better to these climate changes. However, populations with morphological traits best adapted to humid conditions could be negatively affected.

Morphological and functional leaf traits show a plastic response in plants. In *Q. mexicana*, apart from plasticity, other factors could influence the variation in these traits. In the case of this endemic species, populations could face pressures from future climate changes, and a reduction in phenotypic plasticity and, consequently the genetic diversity of the species can be expected. Integrating data on the genetic structure of the species could provide a complete perspective on the future outcome for this endemic oak.

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Appendix 1. Localities of populations of *Quercus mexicana* sampled for this study.

Population	Name of the locality	Elevation (m)	Latitude	Longitude	Aridity index
G1	Palomas, Guanajuato	1,860	21.39640	-99.89830	0.2239
G2	Xichú, Guanajuato	1,939	21.29510	-99.88830	0.2151
G3	Atarjea, Guanajuato	2,119	21.18930	-99.89180	0.3316
H1	Mineral del Chico, Hidalgo	2,806	20.14250	-98.69390	0.3923
H2	Cardonal, Hidalgo	2,364	20.67260	-99.14310	0.2873
H3	Cerro Prieto, Hidalgo	2,114	20.80370	-99.19970	0.3069
H4	Jacala, Hidalgo	1,921	20.91900	-99.20940	0.3641
H5	La Colorada, Hidalgo	1,677	21.02410	-99.13110	0.3906
H6	Zimapán, Hidalgo	2,132	20.79380	-99.32900	0.2483
H7	Apulco, Hidalgo	2,187	20.29770	-98.34530	0.3779
M1	Magú, Estado de México	2,396	19.68500	-99.31750	0.3043
M2	Jilotepec, Estado de México	2,512	19.92820	-99.55720	0.3487
N1	Laguna de Sánchez, Nuevo León	1,960	25.32250	-100.25840	0.2634
N2	Bosque Escuela, Nuevo León	1,619	24.70690	-99.86110	0.2232
N3	Pablillo, Nuevo León	2,131	24.59260	-99.97700	0.2592
N4	Puerto las Ánimas, Nuevo León	2,335	24.46710	-99.91170	0.2876
N5	San Josecito, Nuevo León	2,577	23.92670	-99.96240	0.2572
N6	Peña Nevada, Nuevo León	2,637	23.82380	-99.87980	0.2939
P1	Nicolás Bravo, Puebla	1,836	18.60450	-97.29620	0.3124
P2	El Seco, Puebla	2,480	19.11220	-97.57540	0.2399
P3	Tetela de Ocampo, Puebla	2,052	19.81000	-97.76480	0.3761
P4	Chignahuapan, Puebla	2,439	19.77470	-98.16360	0.2426
P5	Cuauhtémoc, Puebla	2,373	19.80540	-98.08140	0.2512
Q1	Landa de Matamoros, Querétaro	1,688	21.25060	-99.18750	0.4726
Q2	El Madroño, Querétaro	1,853	21.27930	-99.17000	0.5083
Q3	Pinal de Amoles, Querétaro	2,122	21.15440	-99.60800	0.3444
Q4	Cerro Pinguical, Querétaro	2,553	21.12510	-99.67830	0.4153
Q5	Cadereyta, Querétaro	2,289	20.87640	-99.59540	0.3917
Q6	San Joaquín, Querétaro	2,406	20.93230	-99.55560	0.4311
Q7	Cerro Boludo, Querétaro	2,255	20.92960	-99.52780	0.4379
S1	Guadalcázar, San Luis Potosí	1,999	22.66740	-100.41700	0.2014
S2	Valle de los Fantasmas, San Luis Potosí	2,340	22.09620	-100.65930	0.1411
S3	San Luis Potosí, San Luis Potosí	2,046	22.05520	-100.58100	0.1760
S4	Armadillos de los Infantes, San Luis Potosí	1,920	22.18840	-100.68790	0.1485
S5	Real de Catorce, San Luis Potosí	2,876	23.60610	-100.86710	0.1491
T1	Lázaro Cárdenas, Tlaxcala	2,460	19.55080	-97.96310	0.2489
T2	El Verde, Tlaxcala	2,296	19.33060	-98.45980	0.2634
T3	Atotonilco, Tlaxcala	2,389	19.38920	-98.45260	0.2766
V1	Agua Bendita, Veracruz	2,124	20.36910	-98.51540	0.4273

Appendix 2. Means and standard deviations of all morphological traits of the 39 populations of *Quercus mexicana*. Abbreviations of the populations as in Appendix 1 and for morphological traits as indicated in Table 1.

Pop	DTR (No/mm²)	STD (No/mm²)	STL (mm)	LL (cm)	LW (cm)	LWr	PL (cm)	PW (cm)	SLA (g/cm²)	NV	LT (mm)
G1	5.794 / 1.275	392.604 / 66.872	8.028 / 1.808	5.5921 / 1.1148	2.0354 / 0.4142	2.846 / 0.422	0.4825 / 0.1332	0.1087 / 0.0404	69.823 / 7.842	13.156 / 1.021	0.28 / 0.048
G2	5.998 / 0.57	311.902 / 73.401	8.381 / 1.687	6.0126 / 1.0175	2.0986 / 0.3844	2.982 / 0.498	0.4863 / 0.1173	0.1125 / 0.0329	75.895 / 16.7	13.36 / 1.588	0.241 / 0.044
G3	5.907 / 1.812	316.264 / 62.076	8.887 / 1.706	6.313 / 1.0589	2.2476 / 0.3502	2.861 / 0.396	0.5968 / 0.1608	0.1302 / 0.0436	77.638 / 12.211	12.889 / 1.655	0.265 / 0.044
H1	8.009 / 1.704	343.529 / 50.763	9.004 / 1.47	5.2668 / 1.0665	1.6786 / 0.2913	3.273 / 0.471	0.697 / 0.2023	0.1263 / 0.0487	80.94 / 16.298	11.64 / 1.258	0.279 / 0.076
H2	3.992 / 2.098	344.308 / 80.844	9 / 1.909	5.6735 / 1.1733	1.9035 / 0.2981	2.916 / 0.422	0.5443 / 0.1892	0.1333 / 0.0368	77.037 / 18.435	12.14 / 1.212	0.256 / 0.039
H3	7.907 / 1.329	407.872 / 79.619	9.749 / 2.017	5.6151 / 1.1343	1.9089 / 0.4095	2.899 / 0.349	0.5906 / 0.1501	0.1289 / 0.0481	71.087 / 12.748	12.778 / 1.223	0.183 / 0.03
H4	4.544 / 1.113	321.717 / 52.415	9.718 / 1.608	7.2954 / 1.5236	2.631 / 0.5565	2.789 / 0.343	0.5626 / 0.2217	0.1216 / 0.0513	78.499 / 24.887	12.88 / 1.662	0.174 / 0.05
H5	6.543 / 2.955	318.446 / 62.363	9.084 / 1.897	6.3986 / 1.1821	2.2187 / 0.4054	3.055 / 0.472	0.5506 / 0.2044	0.1422 / 0.0327	79.438 / 14.978	11.98 / 1.237	0.279 / 0.053
H6	7.634 / 2.281	381.698 / 75.949	8.704 / 1.45	6.1099 / 1.0355	2.1641 / 0.3368	2.926 / 0.355	0.499 / 0.1399	0.1197 / 0.0408	78.478 / 12.523	12.6 / 1.784	0.246 / 0.036
H7	6.362 / 1.113	356.252 / 118.8	8.541 / 1.764	5.1383 / 1.0352	1.7956 / 0.3941	3.049 / 0.455	0.5152 / 0.1709	0.1353 / 0.0317	71.937 / 14.969	11.422 / 1.469	0.225 / 0.039
M1	8.452 / 3.752	359.887 / 27.804	9.79 / 2.097	5.2381 / 0.8897	1.6285 / 0.2252	3.337 / 0.51	0.6068 / 0.1813	0.0945 / 0.0359	76.664 / 14.762	12.6 / 2.06	0.241 / 0.037
M2	7.157 / 0.94	376.246 / 76.106	9.361 / 1.455	4.7339 / 0.7143	1.6869 / 0.3392	3.086 / 0.501	0.4739 / 0.1391	0.131 / 0.038	83.758 / 14.952	13.72 / 1.565	0.182 / 0.039
N1	7.225 / 1.413	403.51 / 42.936	8.713 / 1.908	5.2196 / 0.9576	1.6703 / 0.3362	3.324 / 0.481	0.5582 / 0.1353	0.1378 / 0.0349	72.426 / 31.906	14.174 / 2.224	0.2 / 0.033
N2	3.522 / 0.513	336.258 / 54.492	8.713 / 1.525	6.4937 / 1.423	1.8954 / 0.3869	3.516 / 0.468	0.46 / 0.1452	0.1303 / 0.0322	87.066 / 15.736	14.378 / 1.898	0.173 / 0.032
N3	3.976 / 0.797	327.17 / 31.608	8.838 / 1.759	7.1483 / 1.4268	2.0479 / 0.3813	3.681 / 0.602	0.6563 / 0.1943	0.1265 / 0.0332	79.016 / 17.047	15.26 / 1.882	0.192 / 0.034
N4	6.816 / 0.862	327.17 / 60.13	9.221 / 1.797	5.5534 / 0.9871	1.5976 / 0.3244	3.789 / 0.564	0.4939 / 0.1277	0.1354 / 0.0421	78.437 / 13.922	15.58 / 1.885	0.185 / 0.034
N5	4.771 / 0.862	303.541 / 50.41	9.185 / 1.87	4.6277 / 0.7657	1.5542 / 0.2805	3.076 / 0.326	0.5206 / 0.1175	0.0995 / 0.0274	66.186 / 8.777	13.275 / 1.485	0.234 / 0.033
N6	4.998 / 0.704	310.812 / 51.5	8.716 / 1.475	4.4915 / 0.9527	1.6378 / 0.3608	3.014 / 0.588	0.4575 / 0.193	0.1343 / 0.0368	76.611 / 10.096	13.471 / 1.689	0.179 / 0.032
P1	8.634 / 1.862	239.925 / 45.752	11.095 / 2.518	4.3556 / 1.1594	1.721 / 0.304	2.642 / 0.298	0.3193 / 0.1053	0.1143 / 0.0407	69.257 / 11.535	10.629 / 1.477	0.307 / 0.068

Pop	DTR (No/mm²)	STD (No/mm²)	STL (mm)	LL (cm)	LW (cm)	LWr	PL (cm)	PW (cm)	SLA (g/cm²)	NV	LT (mm)
P2	10.565 / 2.683	341.711 / 59.199	8.642 / 2.259	5.0959 / 0.6614	1.7479 / 0.4473	3.186 / 0.603	0.4592 / 0.1244	0.1038 / 0.0383	67.532 / 18.844	12.88 / 2.201	0.291 / 0.058
P3	7.498 / 1.879	287.183 / 76.702	9.197 / 1.663	5.1335 / 1.2579	1.8028 / 0.4058	2.97 / 0.331	0.4702 / 0.1653	0.1159 / 0.0568	76.323 / 19.804	12.218 / 1.031	0.301 / 0.076
P4	10.224 / 2.769	285.729 / 93.624	8.59 / 2.134	5.0986 / 0.6526	2.0504 / 0.3115	2.582 / 0.349	0.48 / 0.1326	0.0996 / 0.0306	63.133 / 6.629	11.86 / 1.107	0.283 / 0.04
P5	9.031 / 3.402	370.793 / 32.105	9.591 / 2.099	4.9722 / 1.0411	1.8652 / 0.415	2.703 / 0.423	0.5033 / 0.1458	0.1075 / 0.0347	64.033 / 8.813	11.222 / 1.46	0.275 / 0.047
Q1	6.702 / 1.323	261.736 / 41.955	8.233 / 1.319	5.896 / 1.2316	2.2709 / 0.4319	2.646 / 0.33	0.5075 / 0.1531	0.1191 / 0.036	77.892 / 15.627	12.24 / 1.08	0.281 / 0.055
Q2	6.816 / 1.879	287.183 / 46.61	9.502 / 1.834	5.9082 / 1.0694	2.2256 / 0.4285	2.773 / 0.388	0.516 / 0.1956	0.1322 / 0.0413	71.042 / 13.507	12.675 / 1.095	0.214 / 0.067
Q3	7.384 / 1.799	338.076 / 112.281	8.274 / 1.787	6.3867 / 1.3424	2.1795 / 0.5791	3.018 / 0.437	0.5319 / 0.1508	0.1169 / 0.0389	80.274 / 14.235	13.311 / 1.474	0.264 / 0.049
Q4	8.52 / 2.683	334.441 / 45.578	8.731 / 1.624	5.8818 / 1.0165	2.1336 / 0.296	2.863 / 0.398	0.5375 / 0.1541	0.1376 / 0.055	70.084 / 14.625	12.275 / 1.664	0.271 / 0.047
Q5	8.588 / 4.246	390.423 / 37.303	8.046 / 1.46	4.4205 / 0.8421	1.7337 / 0.4223	2.693 / 0.358	0.3776 / 0.1395	0.092 / 0.0296	74.837 / 7.653	12.86 / 1.485	0.235 / 0.036
Q6	7.952 / 2.184	399.875 / 45.578	7.749 / 1.366	5.4635 / 1.0438	1.8706 / 0.2935	3.004 / 0.425	0.4703 / 0.0997	0.1114 / 0.0532	74.726 / 11.791	13.267 / 1.195	0.262 / 0.044
Q7	8.747 / 2.086	359.887 / 81.319	8.007 / 1.832	4.9914 / 1.007	1.8092 / 0.306	2.917 / 0.436	0.3523 / 0.1191	0.1585 / 0.0442	75.112 / 16.535	12.709 / 1.329	0.242 / 0.041
S1	10.769 / 3.388	355.525 / 47.911	9.106 / 1.818	5.9741 / 1.2052	1.909 / 0.4515	3.235 / 0.478	0.6536 / 0.1943	0.1129 / 0.0443	75.046 / 13.3	13.76 / 1.117	0.263 / 0.043
S2	6.134 / 1.493	359.887 / 68.974	8.529 / 1.774	6.0559 / 1.3826	2.1064 / 0.4883	2.8 / 0.482	0.5327 / 0.1677	0.1134 / 0.0475	89.336 / 14.586	13.92 / 2.078	0.225 / 0.048
S3	5.725 / 2.486	296.634 / 42.518	8.452 / 1.359	5.7303 / 1.3163	2.2946 / 0.443	2.731 / 0.424	0.5046 / 0.1856	0.112 / 0.044	83.732 / 12.361	12.78 / 1.183	0.291 / 0.086
S4	8.52 / 2.467	361.705 / 73.455	8.62 / 1.695	5.7973 / 1.2058	2.1037 / 0.4714	2.817 / 0.385	0.5975 / 0.1783	0.1134 / 0.0333	73.031 / 11.168	12.44 / 1.248	0.277 / 0.048
S5	7.157 / 2.101	321.717 / 61.595	9.493 / 1.686	4.4635 / 0.8355	1.7054 / 0.3208	2.863 / 0.476	0.533 / 0.1079	0.1384 / 0.0461	62.188 / 8.621	11.4 / 1.074	0.217 / 0.033
T1	9.656 / 2.338	332.623 / 27.373	9.767 / 1.769	4.556 / 0.688	1.8031 / 0.33016	2.582 / 0.338	0.5165 / 0.1886	0.1096 / 0.0372	63.739 / 9.719	10.06 / 1.132	0.324 / 0.087
T2	10.338 / 3.842	287.183 / 57.982	10.584 / 1.733	5.5196 / 0.8632	1.8148 / 0.2922	3.189 / 0.4	0.5074 / 0.1496	0.1142 / 0.0265	72.943 / 9.799	11.92 / 1.259	0.242 / 0.044
T3	7.498 / 1.181	351.163 / 74.527	9.068 / 1.584	5.1321 / 1.6556	1.8176 / 0.3127	3.145 / 0.544	0.5361 / 0.1211	0.1246 / 0.0332	75.752 / 28.49	12.31 / 1.906	0.257 / 0.073
V1	8.406 / 2.538	270.824 / 92.13	8.401 / 1.57	5.0751 / 1.2306	2.0387 / 0.4175	2.58 / 0.395	0.5141 / 0.1558	0.1025 / 0.0326	64.196 / 11.755	11.114 / 1.623	0.301 / 0.054

Appendix 3. Partial redundancy analyses for micromorphological traits (RDA_{MIC}), macromorphological traits (RDA_{MAC}) and both set of traits (RDA_{MOR}) including temperature-related variables (pRDA1) and water availability-related variables (pRDA2); and full redundancy analysis (fullRDA) including all environmental variables. Total and constrained variance (λ), and *F* statistic ($P < 0.001$***, $P < 0.01$** and $P < 0.05$*) of axes 1 and 2 and environmental variables included in the analysis and respective R^2adj. Percentage of constrained variance (λ%) indicates the proportion of morphological variation explained by the environmental variables. Those variables or axes with higher statistical significance are in bold. Abbreviations of morphological traits as indicated in Table 1.

		RDA_{MIC}			RDA_{MAC}			RDA_{MOR}		
		λ	**%**	***F***	**λ**	**%**	***F***	**λ**	**%**	***F***
pRDA1 (Temperature)	Total	0.0343	100		0.0534	100		0.0864	100	
	Constrained	0.001	2.83	1.3384	0.0025	**4.77**	**6.45*****	0.0047	**5.45**	**2.63*****
	R^2adj	0. 007			0.038			0.035		
	Axis 1	0.0008	87.17	4.69	0.0018	**72.29**	**14.88*****	0.0037	**78.08**	**8.22*****
	Axis 2	0.0001	0.97	0.52	0.0006	22.13	4.56*	0.0007	15.81	1.67
	Cumulative	0.0009	96.85		0.0024	94.42		0.0044	93.89	
	AMT	0.0001	12.41	0.66	0.0012	**48.14**	**9.91*****	0.0026	**54.99**	**5.79*****
	TS	0.0003	30.44	1.63	0.0004	17.01	3.50**	0.0006	12.26	1.29
	ATR	0.0004	44.93	2.40	0.0005	21.64	4.45**	0.001	21.67	2.28*
	MTC	0.0001	12.21	0.65	0.0003	13.21	2.72*	0.0005	11.07	1.17
pRDA2 (Water availability)	Total	0.0343	100		0.0534	100		0.0864	100	
	Constrained	0.0017	**5.00**	**1.89***	0.004	**7.47**	**6.45*****	0.0085	**9.84**	3.80***
	R^2adj	0.024			0.063			0.074		
	Axis 1	0.0011	62.51	5.99	0.0027	**66.21**	**21.35*****	0.0059	**69.55**	**13.23*****
	Axis 2	0.0005	28.94	2.77	0.001	24.33	7.85**	0.0015	17.8	3.38
	Cumulative	0.0016	91.45		0.0037	90.55		0.0074	87.35	
	AI	0.0005	27.21	2.58	0.0014	**34.53**	**11.13*****	0.0032	**38.08**	**7.24*****
	PET	0.0007	**39.36**	**3.72***	0.0007	**17.54**	**5.66*****	0.0016	18.7	3.56**
	AP	0.0005	28.3	2.67	0.0009	**23.65**	**7.63*****	0.0019	22.95	4.36**
	PS	0.0000	1.71	0.16	0.0006	14.36	4.63**	0.0006	7.52	1.43
	PD	0.0000	3.39	0.32	0.0004	9.88	3.19*	0.0011	1.27	2.43*

		RDA_{MIC}			RDA_{MAC}			RDA_{MOR}		
		λ	**%**	***F***	**λ**	**%**	***F***	**λ**	**%**	***F***
Full RDA (Temperature + Water)	Total	0.0343	100		0.0534	100		0.0864	100	
	Constrained	0.0062	**18.06**	**3.80*****	0.0091	**17.01**	**8.15*****	0.018	**20.83**	**4.47*****
	R^2adj	0.133			0.149			0.162		
	Axis 1	0.0051	**83.01**	**29.46*****	0.0047	**51.25**	**37.71*****	0.0104	**58.02**	**23.36*****
	Axis 2	0.0007	11.79	4.18	0.0026	**28.96**	**21.31*****	0.0031	17	6.85**
	Cumulative	0.0058	94.8		0.0073	80.22		0.0135	75.03	
	AMT	0.0001	1.94	0.66	0.0012	*13.51*	9.91***	0.0026	14.4	5.79***
	TS	0.0001	1.91	0.65	0.0004	4.77	3.5**	0.0006	3.21	1.29
	ATR	0.0004	7.04	2.40	0.0005	6.07	4.45**	0.001	5.67	2.28*
	MTC	0.003	4.77	1.63	0.0003	3.71	2.71*	0.0005	2.89	1.17
	AI	0.0005	8.78	3.00	0.0014	**15.58**	**11.43*****	0.0031	**17.04**	**6.86*****
	PET	0.001	15.63	5.34**	0.0008	8.56	6.28***	0.0022	12.35	4.97***
	AP	0.0000	0.49	0.17	0.0006	6.13	4.50***	0.0008	4.61	1.86
	PS	0.0011	17.82	6.09**	0.002	**21.82**	**16.01*****	0.03	**16.65**	**6.7*****
	PD	0.0026	**41.59**	**14.21*****	0.0018	**19.84**	**14.56*****	0.0042	**23.16**	**9.33*****
WATER ∩ TEMP		**0.0035**	**10.23**		**0.0025**	**4.76**		**0.0048**	**5.53**	

Capítulo II

Nuclear and chloroplast DNA phylogeography reveals high genetic diversity and postglacial range expansion in *Quercus mexicana* (Fagaceae)

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Abstract

Premise: Phylogeographical studies are fundamental for understanding factors that influence the spatial distribution of genetic lineages of species. Population expansions and contractions, gene movement, and climate changes are among the most important factors shaping the genetic compositions of populations.

Methods: This study investigated the phylogeography of an endemic oak, *Quercus mexicana*, which has a restricted distribution in northeastern Mexico along the Sierra Madre Oriental (eastern Sierra Madre) and adjacent areas. Nuclear and chloroplast DNA microsatellite markers were used to describe the genetic diversity and structure of 39 populations of *Q. mexicana* along its entire distribution area. We tested whether population expansion or contraction events influenced the genetic diversity and structure of the species. Finally, this study modeled the historical distribution range of *Q. mexicana* (i.e., Mid Holocene, Last Glacial Maximum, and Last Interglacial) to estimate the extent to which climate fluctuations have impacted the current distribution of this oak species.

Results: The results revealed high genetic diversity and a low genetic structure in *Q. mexicana* populations. Ecological niche models suggested historical fluctuations in the distribution range of *Q. mexicana*. The historical expansion range, gene flow, and physical barriers seem to have played an important role in shaping the phylogeographic structure of *Q. mexicana.*

Conclusions: This study indicates that the genetic structure of *Q. mexicana* populations may have been the result of responses of oak trees not only to heterogeneous environments present in the Sierra Madre Oriental and adjacent areas but also to altitudinal and latitudinal shifts in response to climate changes in the past.

KEYWORDS

Ecological niche modeling, genetic diversity and structure, historical demography, oaks, phylogeography, *Quercus mexicana*

Introduction

Phylogeography has provided significant insights into the historical factors that influence the distribution and composition of species gene lineages, allowing us to elucidate how geological and climatic changes have impacted evolutionary processes such as gene flow, demographic expansions and contractions, genetic bottlenecks and hybridization (Avise, 2004). Chloroplast DNA (cpDNA) and nuclear microsatellite markers (SSRs) have been widely used in phylogeographic studies. While cpDNA retains strong signatures of population history due to its maternal mode of transmission, slow evolution and lack of recombination, nuclear microsatellite markers can reveal the patterns of genetic diversity and structure and population history at biparentally inherited loci (Dumolin et al., 1995; Grivet and Petit, 2003; Cavender-Bares et al., 2009; Ohtani et al., 2013).

Phylogeographic studies in oak trees (Fagaceae; *Quercus*) indicate that geological and climatic changes during the last glacial period have had a significant influence on their population genetic diversity and differentiation. For example, previous studies in Europe found that white oak species were restricted to different refugia during the last glaciation (Petit et al., 2002; Grivet and Petit, 2003; Bagnoli et al., 2016) and that after the glacial maximum, oak species recolonized the continent following diverse postglacial routes. These historical processes produced reduced diversity and strong genetic and phylogeographic structure in different oak species (Petit et al., 1993; Dumolin-Lapègue et al., 1997; Petit et al., 2002). In contrast, North American oaks have shown higher genetic diversity and a patchy genetic structure (Grivet and Petit, 2003). Indeed, gene movement among American oak populations has been more stable in response to past climatic and geological events (Magni et al., 2005). Furthermore, comparatively moderate levels of chloroplast DNA differentiation in several American oak species have been

found (Magni et al., 2005; Grivet et al., 2006; Cavender-Bares et al., 2015) in contrast to those typically observed in European oaks (Petit et al., 2002; Bagnoli et al., 2016).

In Mexico, several oak species have shown high genetic diversity but a low genetic structure, reflecting historical population processes characterized by considerable range stability, altitudinal displacements, and dynamic gene flow between populations (González-Rodríguez et al., 2004; Tovar-Sánchez et al., 2008; Rodríguez-Correa et al., 2015; Ramos-Ortiz et al., 2016; Rodríguez-Gómez et al., 2018; McCauley et al., 2019; Albarrán-Lara et al., 2019; Peñaloza-Ramírez et al., 2020).

Quercus mexicana Bonpl. (Fagaceae, *Lobatae* section) is an endemic species mainly distributed in northeastern Mexico in temperate forests of the Sierra Madre Oriental, with some populations in the Mexican Plateau and the Trans-Mexican Volcanic Belt at elevations between 1600 and 2890 m asl (Valencia-A., 2004). The Sierra Madre Oriental is a geologically and topographically complex physiographic province that was established at the end of the Cretaceous period (100-65 mya) with altitudinal gradients between 200 and 3500 m asl (Lugo-Hubp and Córdova, 1992; Morrone et al., 2002; García-Arizaga and Lugo-Hubp, 2003) and high biological richness as the result of the convergence of the Nearctic and Neotropical biotas (Rzedowski, 2006). The climate of the Sierra Madre Oriental is the result of a unique atmospheric system with a high-pressure subtropical belt, trade winds, and cold fronts in winter and tropical storms in summer (García-Arizaga and Lugo-Hubp, 2003). This climatic and topographic system has created very complex and heterogeneous environments with humid and dry areas along an altitudinal and latitudinal gradient on both sides of the Sierra Madre Oriental and adjacent areas (Lugo-Hubp and Córdova, 1992; García-Arizaga and Lugo-Hubp, 2003), providing an ideal scenario to evaluate the impacts of climate changes on the genetic diversity

and structure of tree species.

Here, this study conducted a phylogeographic analysis of *Quercus mexicana* across its entire distribution range using *chloroplast (*cpSSR) and nuclear (nSSR) microsatellites as DNA molecular markers. In addition, we related the genetic information with ecological niche models to evaluate demographic expansions or contractions of *Q. mexicana* populations during the Mid Holocene, Last Glacial Maximum and Last Interglacial. This research hypothesized that *Q. mexicana* populations distributed throughout warmer habitats (toward tropical latitudes) during glacial periods. Then, once the temperature started to increase, *Q. mexicana* was able to recolonize habitats toward higher latitudes and altitudes along the Sierra Madre Oriental. Thus, it expects that past climatic changes and the complex topography and physiography along the Sierra Madre Oriental have shaped the distribution and genetic structure of *Q. mexicana*. In particular, the objectives of this study were to (1) describe the genetic diversity and structure of *Q. mexicana* populations as revealed by two different neutral molecular markers; (2) evaluate the historical demographic events in populations of *Q. mexicana*; (3) identify the geographical barriers that could lead genetic discontinuities among populations of *Q, mexicana*; (4) asses if isolation by distance could promote genetic structure among populations of *Q. mexicana*; and (5)model the geographic distribution ranges of *Q. mexicana* in three periods (*i.e*., Mid Holocene, Last Glacial Maximum and Last Interglacial) for comparison with its current distribution to provide insights into how climatic fluctuations affected the distribution of this endemic oak species in México.

MATERIALS AND METHODS

DNA extraction, microsatellite amplification, and genotyping

Samples were collected from 39 populations of *Q. mexicana* encompassing the complete area of its distribution, ranging from Nuevo León to Puebla states, Mexico (Table 1, Fig. 1). They were randomly sampled nine or ten individuals with at least 20 m of separation between them in each population. From each individual, young leaves were collected and stored at -80 °C in the laboratory for genetic extraction. Genomic DNA was extracted from 100 mg of foliar tissue of each individual using the protocol designed by Lefort and Douglas (1999). The purified DNA concentration was quantified in a spectrophotometer at an absorbance of 260-280 nm. Twelve nuclear DNA microsatellite loci (nSSRs) were selected (Steinkellner et al., 1997; Kampfer et al., 1998; Aldrich et al., 2002) and amplified in multiplex polymerase chain reactions (PCR). PCR was performed using the QIAGEN Multiplex PCR kit (QIAGEN) in a volume of 5 µl containing 1X Multiplex PCR Master Mix, 2 µl of each primer, dH_2O, and 20 ng template DNA. Thermal cycling conditions consisted of 40 cycles, each at 95 °C for 1 min, annealing at 50 °C for 1 min, and extension at 72 °C for 2 min, and a final extension at 72 °C for 10 min was included. Four multiplex reactions, each including three loci, were set: i) quru-GA2M04, quru-GA0I01 (Aldrich *et al*., 2002) and QrZAG4 (Steinkellner et al., 1997); ii) quru-GAIC08, quru-GA2F05 and quru-GAOC19 (Aldrich et al., 2002); iii) quru-GAIF02, quruGAOA09 (Aldrich et al., 2002), and QpZAG 15 (Steinkellner et al., 1997); and iv) Qpzag 119, QpZAG 3/64, (Steinkellner et al., 1997) and QrZAG 96 (Kampfer et al., 1998). PCR products were combined with a GeneScan-500 LIZ size standard, and the analyses were performed using an ABI-PRISM 3100 Avant sequencer (Applied Biosystems). Fragments were analyzed and recorded using Gene Marker software (Holland et al., 2011).

For chloroplast DNA, seven microsatellite loci (cpSSRs) were selected (Weising et al., 1999; Deguilloux et al., 2003; Sebastiani et al., 2004) grouped according to allele size and fluorescent labels, which correspond to Cmcs10, Cmcs12, Cmcs14 (Sebastiani et al., 2004), udt4, ukk3 (Deguilloux et al., 2003), and ccmp2 and ccmp6 (Weising et al., 1999). PCR was performed using the QIAGEN Multiplex PCR kit (QIAGEN) in a volume of 5 μl containing 1X Multiplex PCR Master Mix, 2 μl of each primer, dH_2O, and 20 ng template DNA. The thermal cycling program consisted of one cycle at 95 °C for 4 min and then 40 cycles, each at 94 °C for 1 min, annealing for 1 min 30 s, and extension at 72 °C. The annealing temperature was 60 °C for the first multiplex (including loci Cmcs10, Cmcs12 and Cmcs14) and 58 °C for the second multiplex (ukk3, Udt4, ccmp2 and ccmp6). A final extension at 72 °C for 15 min was included. After amplification, the PCR products were combined with a GenScan-500 LIZ size standard and then run in an ABI-PRISM 3100-Avant Genetic Analyzer (Applied Biosystems). Fragments were analyzed, and size was recorded with Peak Scanner 1.0 (Applied Biosystems).

Genetic diversity and structure with nuclear (nSSR) and chloroplast (cpSSR) microsatellite markers

First, the CREATE program was used (Coombs et al., 2008) to facilitate input file preparation for all the programs used here for microsatellite data analysis. For all the *Q. mexicana* populations, this study tested for the presence of null alleles, large allele dropout or small genotyping errors mainly due to stutter in the SSR data with the Micro-Checker v2.2.3 program (Oosterhout et al., 2004). Micro-Checker was run with 10^2 bootstrap simulations and a 95% confidence interval (Oosterhout et al., 2006).

Using nSSR markers, the mean number of alleles per locus (*Na*), the mean effective

number of alleles (*Ne*), the mean observed heterozygosity (H_O) and the mean expected heterozygosity (H_E) were calculated for each population in GENETIX v4.02 (Belkhir et al., 2004). The fixation index (F_{IS}) was calculated using ARLEQUIN v3.5.1.2 (Excoffier and Lischer, 2010).

Polymorphisms of cpSSR markers were scored and used to define chloroplast haplotypes according to different combinations of length variants. Thus, the observed number of haplotypes *(H)*, the number of private haplotypes *(P)*, allelic richness *(Ar)* and haplotype diversity *(hs*) were determined using HAPLOTYPE ANALYSIS v1.05 (Eliades and Eliades, 2009).

Population genetic differentiation was estimated through analyses of molecular variance (AMOVA) using F_{ST} (based on the infinite alleles mutation model, IAM) and R_{ST} (based on the stepwise mutation model, SMM) for both the nSSR and cpSSR markers. Analyses were performed with 10^4 permutations in ARLEQUIN v3.5.1.2 (Excoffier and Lischer, 2010) without defining a priori groups of *Q. mexicana* populations. In addition, to identify groups of populations that are geographically homogeneous and maximally differentiated from each other, we performed a spatial analysis of molecular variance (i.e., SAMOVA; Dupanloup et al., 2002). We tested values for K in the range of 2-10, and the initial condition was set to 100 with 10 000 iterations. The configuration with the largest associated F_{CT} value after the 100 independent simulated annealing processes was retained as the best grouping of populations.

To test for isolation-by-distance (IBD) in the *Q. mexicana* populations for both nSSR and cpSSR loci, we performed Mantel correlation tests between the pairwise geographical distances among populations and the corresponding pairwise genetic distances (linearized F_{ST} values). Mantel tests were performed in GenAlex v6.5 (Peakall and Smouse, 2006) with pairwise genetic distances calculated in ARLEQUIN v3.5.1.2 (Excoffier and Lischer, 2010).

A clustering analysis using a Bayesian approach was conducted with the nSSR markers in STRUCTURE v2.3.3 (Pritchard et al., 2000; Falush et al., 2007; Hubisz et al., 2009). In this analysis, individuals are probabilistically assigned to one of the predefined K populations (gene pools) to identify the optimal number of genetic groups (Evanno et al., 2005). The optimal number of groups (*K*) was determined by testing different *K* values, from 1 to 10, and running the analysis ten times per *K* value to determine the maximum value of posterior likelihood [LnP (D)]. Each run was performed using 10^5 burn-in periods and 10^6 Markov chain Monte Carlo (MCMC) repetitions after burn-in. We used a model to allow admixture with correlated allelic frequencies without any prior information. Additionally, it was determined the most likely value of *K* using the maximum value of ΔK according to Evanno et al. (2005) implemented in the program Structure Harvester 0.6.1 (Earl and vonHoldt, 2012). Then, the CLUMPAK software was used (Kopelman et al., 2015) to obtain summary statistics and plots. Geographic distributions of clusters in each population were displayed using QGIS v3.12.0 (http://qgis.osgeo.org).

Additionally, to identify probable geographic and gene flow breaks among populations of *Q. mexicana* along the Sierra Madre Oriental, the Monmonier's maximum difference algorithm was performed using the software BARRIER v2.2 (Manni et al., 2004). This analysis creates a map of sampling locations from their geographical coordinates (Manni et al., 2004). Barriers are then characterized on the map by identifying the maximum values within the population pairwise genetic distance matrix (Manni et al., 2004). We employed a delta mu square distance (DMS) matrix for 39 populations of *Q. mexicana* (Goldstein et al., 1995; Slatkin 1995). To achieve statistical significance for the predicted barriers, 100 bootstrap replicate distance matrices were calculated by resampling loci with the MSA program (Dieringer and Schlötterer, 2003). For the

cpSSRs, a similar procedure was employed, except that a pairwise matrix of linearized F_{ST} values was calculated in Arlequin v3.5.1.2 (Excoffier and Lischer, 2010), and bootstrap support was not calculated since cpSSRs constitute linked loci.

Phylogeographical structure at cpSSRs

To depict the genealogical relationships among cpSSR haplotypes, a haplotype network was constructed using the median-joining algorithm (Bandelt et al., 1999). This method combines the topology of a minimum spanning tree with a parsimony-based maximum parsimony search to identify and remove unnecessary links between haplotypes. These methods were performed in NETWORK v4.51.6 (Bandelt et al., 1999). The geographic distribution of haplotypes in each population was displayed using ArcMap v9.3. The presence of phylogeographic structure was inferred by testing for significant differences between G_{ST} and N_{ST} in SPAGeDi v1.1 (Hardy and Vekemans, 2002) using a permutation test with 10,000 permutations. A significant $N_{ST} > G_{ST}$ indicates the presence of a phylogeographical structure with greater numbers of closely related haplotypes in the same populations than less closely related haplotypes (Pons and Petit, 1996)

Historical population demography

Fu's F_S statistic (Fu, 1997) and Tajima´s D statistic (Tajima, 1989) were implemented to detect signals of historical demographic fluctuations using ARLEQUIN v3.5.1.2 (Excoffier and Lischer, 2010). To this end, the cpSSR data were binary coded following Navascués and Emerson (2007). The number of repeats was coded as "1", and shorter alleles were coded by filling the differences in repeats as "0". The significance of *Fs* values was evaluated with 10^4 bootstraps (Excoffier et al., 2009). Additionally, a mismatch distribution analysis was performed

to detect historical demographic fluctuations (Harpending, 1994). We tested for deviations of the observed mismatch distributions from those expected under the model of Schneider and Excoffier (1999) with 10^4 bootstrap replicates. The validity of the sudden expansion assumption was determined using the sum of squares differences (SSD) (Harpending, 1994) between the observed and expected distributions and the raggedness index (Rogers and Harpending, 1992).

Ecological niche modeling

A database of 393 occurrence records was compiled from herbaria collections (MEXU, IEB, XAL, ENCB, UNL), taxonomic monographs and field sampling. To diminish the spatial autocorrelation effects of the aggregation in the modeling procedure (e.g., Zorrilla-Azcué et al. 2020), all the records except one within 10 km^2 were deleted, leaving a database with 150 occurrence records that was used in further analysis.

Nineteen bioclimatic variables for the locations of each sampled population were obtained from the WorldClim Global v1.0 database (Hijmans et al., 2005) with a 0.8° resolution. Bioclimatic variables with high correlations (> 0.8) were eliminated to avoid collinearity, resulting in eight variables that were used in the subsequent analysis: the annual mean temperature (amt), mean diurnal range of temperature (mdrt), mean temperature of the warmest quarter (mtwq), mean temperature of the coldest quarter (mtcq), annual temperature range (atr), annual precipitation (ap), precipitation in the driest month (pdq) and precipitation in the warmest quarter (pwq).

To model the ecological niche, we used a random partition method to split the available data into training and test sets. Twenty randomized subsets containing 60% of the records (90 occurrence records) were run as independent analyses using MaxEnt 3.3.3 (Phillips and Dudik,

2008). We obtained a model for each subset (considered replicates) using a default convergence threshold with a maximum number of 1 000 iterations. Then, the obtained models were binarized using the probabilities at which 90% of the input occurrence points were included in each prediction as a threshold (Zhu et al., 2016). Model validation was performed using the kappa index (Cohen, 1960). We selected six models with the highest kappa index (> 0.75) as the best subset of models that were subsequently used to project the distribution to past climate scenarios. A jackknife test was performed to identify the most important variables for generating the best subsets of models. The overlapping area of the six best models was considered the current distribution.

The best subsets of current niche models were projected into the reconstructed climatic conditions during the Mid-Holocene (MH, 6 ka), Last Glacial Maximum (LGM; ~21 ka) and Last Interglacial (LIG; ~120 ka) periods. For the Mid-Holocene and Last Glacial Maximum periods, we used three general circulation models: the Community Climate System Model (CCSM), Model for Interdisciplinary Research on Climate (MIROC), and Max Planck Institute for Meteorology Earth System Model (MPI-ESM) (*e.g.*, Cooper et al., 2016; Zhu et al., 2016); and for the Last Interglacial period, only the CCSM was used (Otto-Bliesner et al. 2006). Before projection into the past, we identified whether nonanalogous climatic conditions were present between the past scenarios with respect to our current model (climate anomaly analysis proposed by Silva et al. (2020)); amt and mtwq variables were identified to have anomalous behavior in the Last Interglacial period, and both variables were therefore excluded in this projection. The intersection area of the replicates with the highest kappa index was considered the distribution range of the species in each time horizon.

The maps of each period were intersected with a digital elevation model to determine the

potential coverage of the niche in its elevation range in each period. We obtained boxplots representing the likely changes in the elevational distribution, the number of pixels (0.08°) was counted, and the minimum and maximum elevation values and the first, second and third quartiles of the data were determined, which were used to cut a digital elevation model to calculate the elevation change.

RESULTS

Genetic diversity and structure using nuclear microsatellites (nSSRs)

The values of the genetic diversity parameters estimated with the nuclear markers for the 39 populations of *Q. mexicana* are reported in Table 2. No evidence of null alleles was found in any of the sampled-loci combinations. The tests for genotyping errors due to stuttering and large-allele dropout yielded negative results in all cases. Among the populations, the mean number of different alleles (*Na*) ranged from 4.917 to 7.333, the mean number of effective alleles (*Ne)* ranged from 3.384 to 5.198, the observed heterozygosity (*Ho*) ranged from 0.640 to 0.735, and the expected heterozygosity (*He*) ranged from 0.661 to 0.771. The inbreeding coefficient within populations (*F*) showed positive values in 24 populations and negative values in 15 populations. However, in all cases, the values were not significant. Significant deviations from the Hardy-Weinberg test were not found in any population after correcting for multiple testing using the Bonferroni procedure.

The results of the AMOVAs for the nuclear markers are presented in Table 3. When the populations were not grouped a priori, our results revealed that 94.26% (F_{ST}) and 91.45% (R_{ST}) of the total variation of *Q. mexicana* was partitioned within populations, whereas 5.74% (F_{ST}) and 8.55% (R_{ST}) of the variance was partitioned among populations. The analysis in SAMOVA identified four groups of populations with maximum differentiation, although this differentiation

was low. Three of these groups included single populations (5, 10 and 13), while the fourth group included all remaining populations. The amounts of genetic variation among these four groups were 2.04% (F_{ST}) and 3.96% (R_{ST}), while amounts of 5.34% (F_{ST}) and 7.66% (R_{ST}) were found among populations within groups. The rest of the variation was found within populations (Table 3).

The correlation between genetic distance and geographic distance (r = 0.66, p = 0.0001) was significant, indicating an isolation by distance pattern in the populations. Bayesian clustering analysis with STRUCTURE revealed three possible genetic groups for *Q. mexicana* along the Sierra Madre Oriental (Fig. 2). The genetic ancestry evidence (admixture proportion by population) and the distribution of ancestry proportions for *Q. mexicana* in each population are shown in Fig. 2.

The Monmonier's analysis revealed several barriers to gene flow with bootstrap support > 80 along the distribution of *Q. mexicana* (Fig. 3a). The first barrier separated populations 7, 8 and 9 from the remaining populations. The second barrier separated population 5 from the rest. A third barrier defined a southern group formed by populations 10 and 11, which was separated from populations 12 and 13 to the east and from the more northern populations. Finally, other barriers indicated some genetic structuring among populations in the central part of the distribution of the species (i.e., states of Hidalgo, Querétaro and Guanajuato).

Genetic diversity and structure using chloroplast microsatellites (cpSSRs)

In total, 81 haplotypes were found in the 39 sampled populations (Fig. 4, Table 1). The number of haplotypes (*A*) per population ranged from one to eight, and 29 populations had private haplotypes (*P*) (Table 4). Five populations [Tetela de Ocampo (9), El Chico (11), Apulco (12),

Zimapán (15) and Cerro Prieto (16)] did not share haplotypes with other populations. The highest haplotypic richness (*Rh*) and genetic diversity (*He*) were observed in Valle de los Fantasmas (population 30) (*Rh* = 6.40; *He* = 0.95), Landa de Matamoros (25) (*Rh*: 6.0; *He*: 9.44), Apulco (12) (*Rh*: 6.30; *He*: 0.93), Armadillos de los Infantes (31) (*Rh*: 5.50; *He*: 9.11), and Lázaro Cárdenas (5) (*Rh*: 4.60; *He*: 0.84) populations (Tables 1 and 4).

The AMOVAs based on cpSSRs are presented in Table 5. When the populations were not grouped a priori, the results showed that 77.88% (F_{ST}) and 85.39% (R_{ST}) of the total variation of *Q. mexicana* was partitioned among populations, whereas 22.12% (F_{ST}) and 14.61% (R_{ST}) were found within populations (Table 4). The analysis in SAMOVA identified nine groups of populations with maximum differentiation. The first group included five northern populations, including one from San Luis Potosí (33) and four from Nuevo León (34, 36-38). The second and the largest group was formed by 23 populations with southern and central distributions (populations 1-4, 7-9, 12-13, 16, 18-20, 22-23, 25-32). The third group consisted of five populations also with southern/central distributions (6, 10-11, 14-15). Finally, the remaining six groups contained a single population (4), (18), (21), (24), (35) and (39). The amounts of genetic variation among these nine groups were 34.39% (F_{ST}) and 75.29% (R_{ST}), while the amounts of 46.26% (F_{ST}) and 14.10% (R_{ST}) were found among populations within groups. Finally, 19.35% (F_{ST}) and 10.60% (R_{ST}) of the variation was within populations (Table 5).

The Mantel test showed a significant and high correlation between genetic and geographic distances (r = 0.8; p = 0.0001) indicating isolation by distance pattern among populations.

The haplotype network showed that in most cases, haplotypes were separated by only one mutational step (Fig. 5). The analysis showed three frequent (H3, H68 and H69), 54 unique and

27 shared haplotypes (Fig. 4). Haplotype H68 had the highest frequency (42 individuals), followed by haplotypes H3 (33 individuals) and H69 (32 individuals). In the haplotype network, the colors denote the nine groups identified by SAMOVA analyses (Fig. 5). The largest group included the haplotypes found in the 23 populations mentioned above and is indicated by gray color (Fig. 5).

Barrier analysis revealed genetic discontinuities between *Q. mexicana* populations. The first five barriers are shown in Figure 3b. The first two barriers separated the two populations at the extreme north [Laguna de Sánchez population (39)] and south [Nicolás Bravo (1)] from all of the populations. The third barrier separated Peña Nevada (34), San Jocesito (35), Puerto Animas (36), El Pablillo (37) and Bosque Escuela (38) populations from the rest of the populations. The fourth barrier divided Cerro Pinguical (22), and the fifth barrier divided Chignahuapan (7), Cuauhtémoc (8) and El Chico (11) populations from the rest of the populations (Fig. 3b).

Phylogeographical structure

The differentiation of cpSSRs between populations was high (N_{ST} = 0.512, p = 0.01; G_{ST} 0.443, p = 0.01). The N_{ST} values were significantly higher than G_{ST}, indicating a phylogeographic structure among populations of *Q. mexicana*.

Historical population demography

Fu's test indicated that some populations of *Q. mexicana* might have experienced demographic expansion (*Fs* = -24.03990, p = 0.00270). However, Tajima´s test was not significant (*D* = 2.14589, p = 0.9650). In addition, the raggedness index and the sum of squared deviations between observed and expected values were not significant (SSD = 0.00868, p = 0.6045; HRag =

0.00482, p = 0.87, respectively). Values on historical demography for each population are summarized in Table 6. Overall, Tajima's D statistic was significant in only two populations [Chignahuapan (7) and Cerro Prieto (16)] (Table 6). Fu's (*Fs*) values were negative and significant in six [Atotonilco (4), Chignahuapan (7), Apulco (12), Jacala (18), Cerro Boludo (19) and San Joaquín (20)] among the 39 populations, indicating a signal of demographic expansion in these populations (Table 6). Likewise, nonsignificant values were detected for SSD and HRag analyses, except for the Magú (6), Agua Bendita (13), Cerro Prieto (16) and Pinal de Amoles (23) populations (Table 6).

Ecological niche modeling

The kappa values of the six best replicates for *Q. mexicana* ranged between 0.75 and 0.7833, indicating good performance for the six models contrasting the current distribution of *Q. mexicana*. The most important climatic variables were mean temperature in warmest quarter, annual mean temperature, mean temperature of coldest quarter and annual temperature range. The current distribution area of *Q. mexicana* is larger than in the Mid-Holocene, Last Glacial Maximum and Last Interglacial periods (Fig. 6). The models revealed that *Q. mexicana* populations might have experienced a marked expansion in distribution since the Mid-Holocene period to the present, and during the Last Glacial Maximum period had its more restricted distribution area (Fig. 6). The distribution of the species increased when temperatures were warmer as in the Last Interglacial period.

The models did not reveal notable changes in the altitudinal distribution of *Q. mexicana* populations during the Mid-Holocene and Last Interglacial periods, but *Q. mexicana* did show changes in *elevation ranges* in the Last Glacial Maximum period (i.e., widening the altitudinal

range) or in the current distribution compared to its past distribution (Fig. 6). Currently, *Q. mexicana* is located at higher altitudes compared to the Mid-Holocene and Last Interglacial periods. The most important climatic variables in the best ecological niche models were the maximum annual temperature, annual temperature range and isothermality (Fig. 6).

DISCUSSION

Evolutionary processes such as range expansion, gene flow and seed dispersal or long-distance migration leave their imprint on the distribution of genetic variation within and among populations (Hamrick et al., 1992; Gugger et al., 2018; Ochoa-Zavala et al., 2019). In Mexico, the geographical location, complex topography, and dynamics of the tectonic and climatic history of the Mexican highlands have influenced the evolutionary history of oak species. Some phylogeographic studies have provided important evidence on species demographic history and population structure in Mexican oaks (González-Rodríguez et al., 2004; Ramos-Ortiz, 2016; Hipp et al., 2018; 2020, Rodríguez-Gómez et al., 2018; McCauley et al., 2019; Albarrán-Lara et al., 2019; Peñaloza-Ramírez et al., 2020). Here, the results indicate a genetic and phylogeographical structure of *Q. mexicana*. Also, this study found demographic expansion in some populations of *Q. mexicana,* and according to the ecological niche models, the current distribution of *Q. mexicana* was more extensive than that in the past. Likewise, *Q. mexicana* retains high levels of genetic diversity that can be the result of the expansions and effective gene flow of its populations through historical climatic fluctuations over time.

All the populations of *Q. mexicana* revealed high values for genetic diversity in all its parameters in both nuclear and chloroplast microsatellite neutral markers. The high genetic diversity of *Q. mexicana* reported here is consistent with that reported in other studies of oaks

distributed in North America (Craft et al., 2002, Craft and Ashley, 2007; Cavender-Bares et al., 2011; Abraham et al., 2011; Ortego et al., 2012; Ramos-Ortiz et al., 2016; Oyama et al., 2018; Peñaloza-Ramírez et al., 2020) and Asia (Ohsawa et al., 2011; San Jose-Maldia et al., 2017; Jiang et al., 2019). In contrast, low genetic diversity for European oaks has been reported (Soto et al., 2007; Neophytou *et al*., 2010). The high genetic diversity of *Q. mexicana* may be related to its long-term stable distribution with local migration and large effective population sizes for a long period. Habitat stability has been positively associated with high genetic diversity in other plant species (Gugger et al., 2013; Loera et al., 2017; Peñaloza-Ramírez et al., 2020). In *Q. mexicana*, the historical persistence and effective gene flow between populations have led to the maintenance of high levels of genetic diversity within this species.

Although isolation by distance and some genetic barriers might promote genetic structures among different plant populations (Soto et al., 2007; Sosa et al., 2009; Zhang et al., 2015; Ochoa-Zavala et al., 2020), our results also revealed that most of the genetic variation is within rather than among populations of *Q. mexicana*. This pattern seems to be common in several *Quercus* species of North America (Grivet et al., 2008; Cavender-Bares et al., 2011; Ashley et al., 2015; Oyama et al., 2018; McCauley et al., 2019; Albarrán-Lara et al., 2019; Peñaloza-Ramírez et al., 2020). As this study mentioned above, extensive pollen movement by wind has been reported to be the main factor in gene flow between populations of North American *Quercus* species (Kremer et al., 2012), and this extensive gene flow exchange between *Q. mexicana* populations seems to be related to its low to moderate genetic differentiation. In fact, our results suggested admixing of multiple lineages between clusters, as indicated in the structure analysis.

Our results also revealed that ancestral haplotypes are widely represented across the *Q.*

mexicana distribution range. This study also detected many private haplotypes for *Q. mexicana* populations (67%). Isolation by distance and physical barriers promote the phylogeographic structure in other plant species (Ruíz-Sánchez et al., 2012; Ramos-Ortiz et al., 2016; Sork et al., 2016; Rodríguez-Gómez et al., 2018; Ochoa-Zavala et al., 2019; Peñaloza-Ramírez et al., 2020).

The haplotype network did not show a star-like shape, which is indicative of a recent expansion. However, the historical demography results and the ecological niche models indicate a demographic expansion in several *Q. mexicana* populations. The patchy distribution of *Q. mexicana* haplotypes may also reflect expansion and migration processes of this species, at least since the LMG period, as our results indicated. During the Pleistocene, periods of climate change occurred; deglaciation processes in the mountains in the tropics occurred (Lachniet and Vázquez-Selem, 2005; Vázquez-Selem and Lachniet, 2013), climate and precipitation regimes changed, and climatic fluctuations molded the distribution of plant and animal species (Metcalfe et al., 2000). In Mexico, the glacial cycles influence the genetic diversity of species by generating contact zones due to periodic altitudinal and latitudinal migrations (Ramírez-Barahona and Eguiarte, 2013). The ice masses that covered mountains modified the upper limits of the elevational distribution of many tree species, which descended approximately 800 m asl from their original distributions (Graham et al., 1993). For example, in the Iztaccíhuatl volcano in central Mexico, pollen data indicate that during the early Holocene, the wooded line was established from 500 to 700 m asl below its current position at 4020 m asl (Lozano-García and Vázquez-Selem, 2005), and a similar process may have occurred in the Sierra Madre Oriental (Vázquez-Salem and Heine, 2005). In general, the ecological niche models suggest the expansion of *Q, mexicana* populations from the LIG period to the present. Indeed, the present distribution of *Q. mexicana* is wider than in the past. In the LGM scenario (~21 ka BP), the niche

amplitude was substantially reduced in the three climatic scenarios; however, the MIROC scenario showed the most drastic reduction in the *Q. mexicana* distribution along the Sierra Madre Oriental. The ecological niche of *Q. mexicana* seems to have had a greater reduction in the north of its distribution, while in the south, its reduction was not so drastic. The Mid-Holocene scenario showed an increase in the potential niche of *Q. mexicana*. Thus, when the temperature increased, *Q. mexicana* may have recolonized the northernmost part of the Sierra Madre Oriental. Nevertheless, the ecological niche models also suggest the prevalence and connectivity of the *Q. mexicana* populations throughout the glacial periods. Previous studies of cpDNA in European oaks showed strong genetic structure patterns, which may be the result of postglacial recolonization through different isolated routes (Petit et al., 2002). Therefore, our evidence reveals that range expansion and effective gene flow in *Q. mexicana* populations may have played an important role in shaping the genetic diversity and structure of this oak species along the Sierra Madre Oriental in both altitudinal and latitudinal gradients.

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Table 1. Locality, geographical coordinates, elevation for the 39 *Quercus mexicana* populations in Mexico. Haplotypes in each population are also indicated.

Locality	Sample size	Elevation (m)	Latitude	Longitude	Haplotypes
1. Nicolás Bravo	10	2531	18.6045	-97.29647	H68, H77, H79
2. El Seco	10	2498	19.1125	-97.57522	H51, H52, H69
3. El Verde	10	2304	19.33653	-98.45792	H65, H68, H79, H81
4. Atotonilco	10	2389	19.38989	-98.45581	H54, H58, H68
5. Lázaro Cárdenas	10	2460	19.55028	-97.96322	H44, H57, H59, H60, H64, H69
6. Magú	10	2410	19.66561	-99.31683	H4, H14
7. Chignahuapan	10	2456	19.77703	-98.16553	H40, H68, H69, H74
8. Cuauhtémoc	10	2373	19.80669	-98.08239	H22, H25, H41, H66, H69
9. Tetela de Ocampo	10	2005	19.80931	-97.76289	H36
10. Jilotepec	10	2512	19.92475	-99.55486	H4, H2
11. El Chico	10	2806	20.14253	-98.69389	H1, H6, H7
12. Apulco	10	2187	20.29736	-98.34492	H17, H18, H19, H45, H46, H47, H48, H49
13. Agua Bendita	10	2151	20.36928	-98.51639	H55, H58, H65, H68
14. Cardonal	10	2364	20.67261	-99.14314	H12, H13, H14
15. Zimapán	10	2132	20.79375	-99.32897	H11
16. Cerro Prieto	10	2114	20.80369	-99.19967	H28, H73
17. Cadereyta	10	2289	20.87667	-99.59536	H31, H35, H40, H42
18. Jacala	10	1921	20.91903	-99.20944	H3, H4, H5
19. Cerro Boludo	9	2312	20.92958	-99.52808	H64, H69, H70
20. San Joaquín	10	2398	20.93231	-99.55539	H65, H67, H68
21. La Colorada	10	1677	21.02408	-99.13106	H3
22. Cerro Pinguical	10	2553	21.12506	-99.67833	H56, H61, H68
23. Pinal de Amoles	10	2122	21.15044	-99.60711	H1, H3, H6
24. Atarjea	10	2180	21.18697	-99.89139	H16, H25
25. Landa de Matamoros	9	1718	21.25689	-99.18756	H24, H26, H33, H35, H38, H39, H76
26. El Madroño	10	1811	21.27931	-99.16967	H59, H62, H69, H75, H80
27. Xichú	9	1939	21.29525	-99.88903	H77, H79
28. Palomas	9	1860	21.39547	-99.89931	H15, H20, H29, H30
29. San Luis Potosí	10	2073	22.05522	-100.5822	H20, H29, H41, H66, H78
30. Valle de los Fantasmas	10	2062	22.09533	-100.9918	H21, H22, H23, H24, H31, H33, H39, H63
31.Armadillos de los Infante	10	1930	22.18839	-100.6879	H21, H24, H50, H58, H63, H65, H68
32. Guadalcázar	10	2018	22.66592	-100.417	H69
33. Real de Catorce	10	2876	23.60622	-100.8671	H27
34. Peña Nevada	10	2637	23.82583	-99.87883	H30, H37, H43, H53, H71
35. San Josecito	10	2577	23.92669	-99.96239	H3, H8, H9, H10
36. Puerto las Animas	10	2335	24.46706	-99.91167	H71, H72
37. Pablillo	10	2191	24.59183	-99.97931	H71
38. Bosque Escuela	9	1619	24.70889	-99.86244	H27
39. Laguna de Sánchez	10	1981	25.31975	-100.258	H3

Table 2. Number of different alleles *(Na)*, number of effective alleles (N_e), mean observed heterozygosity (H_O), mean expected heterozygosity (H_E), and inbreeding coefficient (F_{IS}) for the 39 populations of *Quercus mexicana* using nuclear microsatellites (nSSRs).

Locality	***Na***	***Ne***	***Ho***	H_E	F_{IS}
1. Nicolás Bravo	7.333	5.149	0.714	0.771	0.199
2. El Seco	6.083	4.005	0.701	0.717	0.131
3. El Verde	6.417	4.127	0.690	0.720	0.092
4. Atotonilco	6.167	4.095	0.670	0.700	-0.047
5. Lázaro Cárdenas	5.667	3.786	0.697	0.710	0.053
6. Magú	6.583	4.492	0.674	0.734	0.100
7. Jilotepec	6.250	4.518	0.708	0.729	0.157
8. Chignahuapan	5.500	3.849	0.715	0.725	-0.091
9. Cuauhtémoc	5.583	3.413	0.640	0.685	-0.031
10. Tetela de Ocampo	5.750	3.855	0.676	0.699	-0.031
11. El Chico	6.500	4.843	0.675	0.753	0.090
12. Apulco	6.333	4.598	0.700	0.746	-0.017
13. Agua Bendita	6.583	4.069	0.651	0.698	-0.043
14. Cardonal	6.750	4.600	0.727	0.742	0.037
15. Zimapán	6.750	4.226	0.681	0.731	0.089
16. Cerro Prieto	7.083	5.005	0.695	0.749	0.118
17. Cadereyta	6.750	4.608	0.709	0.740	-0.037
18. Jacala	7.167	5.198	0.735	0.755	0.131
19. Cerro Boludo	4.917	3.384	0.690	0.661	-0.086
20. San Joaquín	6.750	4.613	0.674	0.751	0.084
21. La Colorada	6.417	4.300	0.717	0.732	-0.064
22. Cerro Pinguical	6.333	4.357	0.720	0.734	-0.054
23. Pinal de Amoles	7.250	4.826	0.719	0.756	-0.139
24. Atarjea	6.917	4.611	0.711	0.760	0.008
25. Landa de Matamoros	6.750	4.614	0.701	0.718	-0.027
26. El Madroño	5.500	3.784	0.708	0.681	-0.149
27. Xichú	6.000	4.322	0.640	0.707	0.137
28. Palomas	6.167	4.387	0.707	0.739	-0.245
29. San Luis Potosí	6.500	4.308	0.665	0.724	0.189
30. Valle de los Fantasmas	6.167	3.978	0.701	0.726	-0.038
31.Armadillos de los Infantes	7.083	4.870	0.704	0.729	0.000
32. Guadalcázar	6.583	4.294	0.679	0.719	0.100
33. Real de Catorce	6.500	4.819	0.720	0.765	0.053
34. Peña Nevada	6.167	4.279	0.675	0.727	0.100
35. San Josecito	5.917	4.240	0.674	0.734	0.135
36. Puerto las Animas	6.500	4.290	0.702	0.741	0.188
37. Pablillo	6.250	4.224	0.709	0.732	0.010
38. Bosque Escuela	6.083	4.357	0.689	0.751	0.140
39. Laguna de Sánchez	6.083	4.331	0.717	0.733	0.147

Table 3. Hierarchical analysis of molecular variance (AMOVA) based on nuclear microsatellites (nSSRs) for *Quercus mexicana* using F_{ST} and R_{ST} models. (a) The populations were not grouped a priori, and (b) the populations were grouped according to SAMOVA results. Asterisks indicate statistically significant values ($p < 0.01$). Tests were based on 10^4 random permutations.

(a) Not grouped *a priori*	**d.f.**	**SS**	**Variance components**	**% of variation**	**Fixation index**
No. of different alleles (F_{ST})					
Among populations	38	329.568	0.24	5.74	Φ_{ST} =0.057**
Within populations	733	2881.3	3.93	94.26	
Total	771	3210.86	4.17		
Sum of squared size difference (R_{ST})					
Among populations	38	9104.23	7.86	8.55	Φ_{ST} =0.085**
Within populations	733	61614.86	84.06	91.45	
Total	771	5703.00	7.42		
(b) Grouped according to SAMOVA	**d.f.**	**SS**	**Variance components**	**% of variation**	**Fixation index**
No. of different alleles (F_{ST})					
Among groups	3	35.21	0.086	2.04	Φ_{CT} =0.02**
Among populations within groups	35	294.35	0.226	5.34	Φ_{SC} =0.054**
Within populations	733	2881.3	3.93	92.63	Φ_{ST} =0.07**
Total	771	3210.87	4.24		
Sum of squared size difference (R_{ST})					
Among groups	3	1117.56	3.76	3.96	Φ_{CT} =0.039**
Among populations within groups	35	7986.68	7.29	7.66	Φ_{SC} =0.079**
Within populations	733	61614.87	84.06	88.38	Φ_{ST} =0.116 **
Total	771	70719. 1	95.11		

Table 4. Estimates of genetic diversity using chloroplast microsatellites (cpSSRs) in 39 populations of *Quercus mexicana*. Number of haplotypes in each population (*A*), number of private haplotypes (*P*), effective number of haplotypes (*Ne*), haplotypic richness (*Rh*) and genetic diversity (*He*) are reported.

Locality	Genetic diversity				
	N	***A/P***	***Ne***	***Rh***	***He***
1. Nicolás Bravo	10	3/0	1.85	1.90	0.51
2. El Seco	10	3/2	1.51	1.80	0.37
3. El Verde	10	4/1	2.38	2.80	0.644
4. Atotonilco	10	3/1	1.51	1.80	0.37
5. Lázaro Cárdenas	10	6/3	4.16	4.60	0.84
6. Magú	10	2/0	1.92	1.00	0.53
7. Chignahuapan	10	4/1	1.92	2.70	0.53
8. Cuauhtémoc	10	5/0	2.50	3.60	0.66
9. Tetela de Ocampo	10	1/1	1.00	0.00	0.00
10. Jilotepec	10	2/1	1.22	0.90	0.20
11. El Chico	10	3/3	1.85	1.90	0.51
12. Apulco	10	8/8	6.25	6.30	0.93
13. Agua Bendita	10	4/1	1.92	2.70	0.53
14. Cardonal	10	3/2	2.17	1.90	0.60
15. Zimapán	10	1/1	1.00	0.00	0.00
16. Cerro Prieto	10	2/2	1.22	0.90	0.20
17. Cadereyta	9	4/1	2.07	3.00	0.58
18. Jacala	10	3/1	1.51	1.80	0.37
19. Cerro Boludo	9	3/1	1.58	2.00	0.41
20. San Joaquín	10	3/1	1.51	1.80	0.37
21. La Colorada	10	1/0	1.00	0.00	0.00
22. Cerro Pinguical	10	3/2	1.51	1.80	0.37
23. Pinal de Amoles	10	3/1	2.17	1.90	0.60
24. Atarjea	10	2/1	1.47	1.00	0.35
25. Landa de Matamoros	9	7/3	6.23	6.00	0.94
26. El Madroño	10	5/3	2.50	3.60	0.66
27. Xichú	9	2/0	1.52	1.00	0.38
28. Palomas	9	4/2	2.07	3.00	0.58
29. San Luis Potosí	10	5/1	3.12	3.70	0.75
30. Valle de los Fantasmas	10	8/1	7.14	6.40	0.95
31.Armadillos de los Infante	10	7/1	5.55	5.50	0.91
32. Guadalcázar	10	1/0	1.00	0.00	0.00
33. Real de Catorce	10	1/0	1.00	0.00	0.00
34. Peña Nevada	10	5/4	3.57	3.70	0.80
35. San Josecito	10	4/3	2.77	2.80	0.71
36. Puerto las Animas	10	2/1	1.22	0.90	0.20
37. Pablillo	10	1/0	1.00	0.00	0.00
38. Bosque Escuela	9	1/0	1.00	0.00	0.00
39. Laguna de Sánchez	10	1/0	1.00	0.00	0.00

Table 5. Hierarchical analysis of molecular variance (AMOVA) based on chloroplast microsatellites (cpSSRs) for *Quercus mexicana* using infinite allele (F_{ST}) and stepwise (R_{ST}) mutation models. (a) The populations were not grouped a priori, and (b) the populations were grouped according to SAMOVA results. Asterisks indicate statistically significant values ($p < 0.01$). Tests were based on 10^4 random permutations.

(a) Not grouped *a priori*	**d.f.**	**SS**	**Variance components**	**% of variation**	**Fixation index**
No. of different alleles (F_{ST})					
Among populations	38	709.01	1.84	77.88	Φ_{ST}= 0.78*
Within populations	345	180.48	0.52	22.12	
Total	383	889.50	2.36		
Sum of squared size difference (R_{ST})					
Among populations	38	4016.63	10.55	85.39	Φ_{ST}= 0.85**
Within populations	345	622.95	1.80	14.61	
Total	383	4639.58	12.35		
(b) Grouped according to SAMOVA	**d.f.**	**SS**	**Variance components**	**% of variation**	**Fixation index**
No. of different alleles (F_{ST})					
Among groups	8	325.16	0.92	34.39	Φ_{CT} =0.34**
Among populations within groups	30	383.89	1.25	46.26	Φ_{SC} =0.70**
Within populations	345	180.48	0.52	19.35	Φ_{ST} =0.81**
Total	383	889.48	2.70		
Sum of squared size difference (R_{ST})					
Among groups	8	3255.46	12.82	75.29	Φ_{CT} =0.75**
Among populations within groups	30	761.16	2.40	14.10	Φ_{SC} = 0.57**
Within populations	345	622.95	1.80	10.60	Φ_{ST} =0.89**
Total	383	4639.58	17.02		

Table 6. Summary statistics of historical demography analyses of *Quercus mexicana*. D = Tajima's D and FS = Fu's Fs. Significant negative values (**$p < 0.01$ and *$p < 0.05$ in bold) indicate historical demographic expansion events. SSD = differences in the sum of squares or mismatch distribution. Significant ($p < 0.05$ in bold) SSD values indicate deviations from the sudden expansion model.

Locality	Demographic history			
	D	Fs	SSD	HRag
1. Nicolás Bravo	-0.69	-0.59	0.02	0.18
2. El Seco	-1.24	0.39	0.03	0.28
3. El Verde	-0.50	-1.07	0.00	0.08
4. Atotonilco	-1.40	**-1.16***	0.00	0.18
5. Lázaro Cárdenas	-1.33	-1.89	0.02	0.11
6. Magú	2.22	7.40	**0.56****	0.78
7. Chignahuapan	**-1.66***	**-1.34***	0.00	0.06
8. Cuauhtémoc	-0.46	0.19	0.05	0.09
9. Tetela de Ocampo	0.00	0.00	0.00	0.00
10. Jilotepec	-1.11	-0.33	0.33	0.40
11. El Chico	-0.69	-0.59	0.02	0.18
12. Apulco	1.14	**-2.97***	0.01	0.04
13. Agua Bendita	-0.82	-0.65	**0.25****	0.14
14. Cardonal	1.33	0.47	0.05	0.24
15. Zimapán	0.00	0.00	0.00	0.00
16. Cerro Prieto	**-1.87***	3.33	**0.05***	0.72
17. Cadereyta	-0.51	0.97	0.13	0.27
18. Jacala	-1.40	**-1.16***	0.00	0.18
19. Cerro Boludo	-1.36	**-1.08***	0.00	0.16
20. San Joaquín	-1.40	**-1.16***	0.00	0.18
21. La Colorada	0.00	0.00	0.00	0.00
22. Cerro Pinguical	-0.32	1.48	0.10	0.42
23. Pinal de Amoles	0.47	0.83	0.23	**0.88***
24. Atarjea	0.01	0.41	0.00	0.20
25. Landa de Matamoros	0.87	-1.81	0.02	0.06
26. El Madroño	0.62	-1.05	0.01	0.04
27. Xichú	0.15	0.47	0.00	0.20
28. Palomas	-1.14	-0.92	0.03	0.16
29. San Luis Potosí	0.91	1.16	0.06	0.08
30. Valle de los Fantasmas	1.15	-2.61	0.02	0.05
31.Armadillos de los Infantes	2.01	-0.91	0.04	0.07
32. Guadalcázar	0.00	0.00	0.00	0.00
33. Real de Catorce	0.00	0.00	0.00	0.00
34. Peña Nevada	2.23	0.86	0.08	0.23
35. San Josecito	2.06	1.69	0.09	0.22
36. Puerto las Animas	-1.11	-0.33	0.33	0.40
37. Pablillo	0.00	0.00	0.00	0.00
38. Bosque Escuela	0.00	0.00	0.00	0.00
39. Laguna de Sánchez	0.00	0.00	0.00	0.00

Figures legends

Figure 1. The geographic location of 39 populations of *Quercus mexicana* in northeastern Mexico along the Sierra Madre Oriental (SMO) and adjacent areas (Table 1). The three major geographic regions of the populations are outlined also showed (SMO, ChD, TMVB). Gradient color represents variation in elevation from low (light brown) to high (in dark brown). ChD = Chihuahuan dessert, TMVB = Trans-Volcanic Mexican Belt. Red circles depict the location of each sampled population of *Q. mexicana*. Gradient color represents variation in elevation from low (light green) to high (in dark green).

Figure 2. Distribution of the genetic ancestry groups of *Quercus mexicana* populations. a) Distribution and genetic groups (haplotypes) within and between the 39 populations of *Q. mexicana* across the Sierra Madre Oriental (SMO) using SSR markers. b) Values of ΔK plotted against K, the peak indicates the most probable number of genetic groups provided by Structure Harvester. c) Distribution of the three genetic groups and their proportion assigned by STRUCTURE in all the populations population (blue, red, and green colors = genetic groups). The population number is given in Table 1 is also shown for each genetic group.

Figure 3. Geographic breaks (barriers) identified across the distribution of *Quercus mexicana* in northeastern Mexico using Monmonier's algorithm based on (a) nuclear microsatellites (nSSRs) and (b) chloroplast microsatellites (cpSSRs). The barriers are outlined in red for cpSSRs and blue for nSSRs. Original names of the population numbers are given in Table 1. The percentage of bootstraps support is also provided for each barrier. Red circles depict the location of each sampled population of *Q. mexicana*.

Figure 4. Geographic distribution and frequency of 81 haplotypes of the 39 populations of *Quercus mexicana* in Mexico.

Figure 5. a) The chloroplast DNA haplotype network constructed using NETWORK v4.51.6 (Bandelt *et al*., 1999). The network displays the 81 haplotypes identified in 39 populations of *Quercus mexicana* across the Sierra Madre Oriental (SMO) in México using cpSSRs (Table 1). Each circle represents an individual chloroplast haplotype, and size of the circles is scaled to haplotype frequency (i.e., the largest circles representing the most abundant haplotypes). Lines represent a single mutational change and unsampled haplotypes are indicated by small black circles. Branch length does not represent distance among haplotypes. Chloroplast haplotypes are colored to match the nine groups identified by SAMOVA. b) The map shows the distribution of the 39 populations of *Q. mexicana* colored by the nine SAMOVA groups (Table 1). Original names of the population numbers are provided in Table 1. b) Gradient color represents variation in elevation from low (light green) to high (in dark green).

Figure 6. Ecological niche modeling for *Quercus mexicana* in México at present (1950-2000), Mid Holocene (MH; ~ 6 kya), Last Glacial Maximum (LGM; ~ 21 kya), and Last Interglacial periods (LIG; ~ 120 kya) (Top panel). The colors represent the probability of a species occurring in an area in each period. Stronger colors show a higher probability of occurrence of the species. The red dots represent the points of 150 records obtained from herbaria collections (MEXU, IEB, XAL, ENCB, UNL). Boxplots and its error bars of the shift in elevation of the forest ranges between the present and past (MH, LGM, and LIG) distribution of *Q. mexicana* (left bottom panel). c) Bar plot displays the distribution range of *Q. mexicana* for each period. masl = meters above the sea level (right bottom panel).

Figure 1

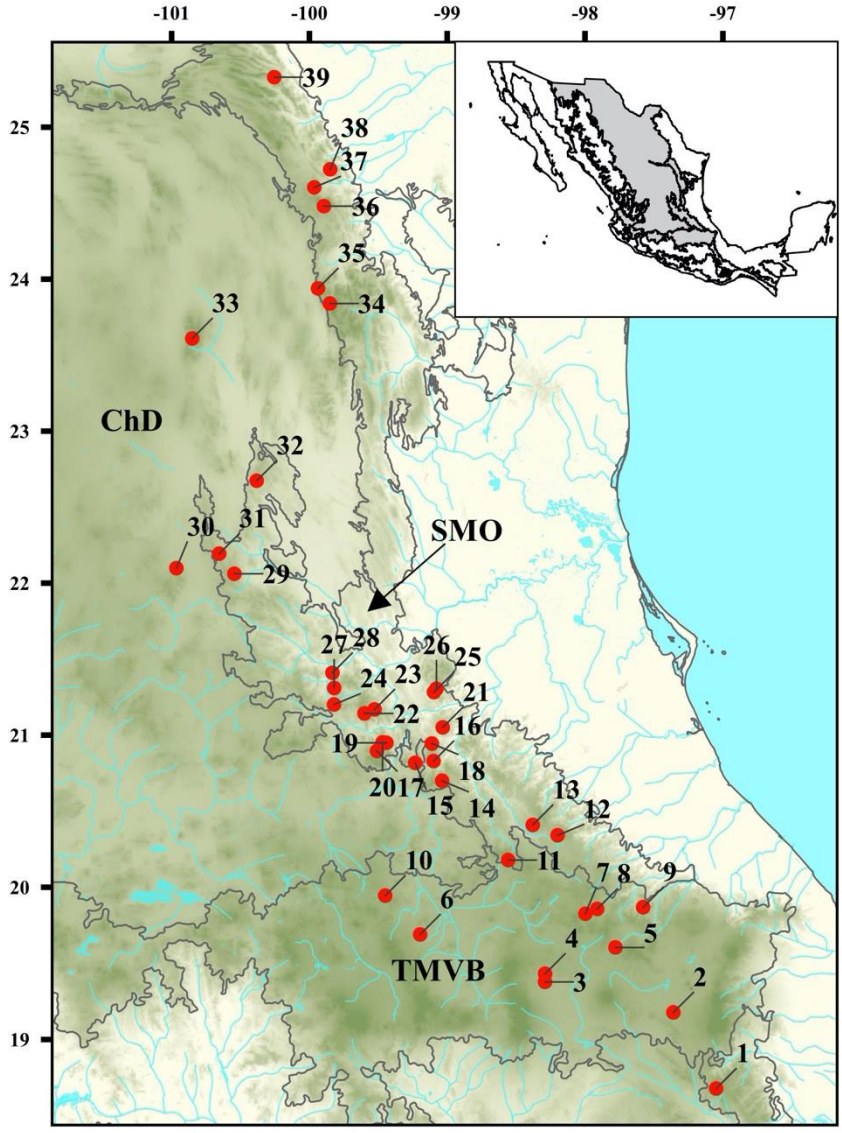
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Figure 2

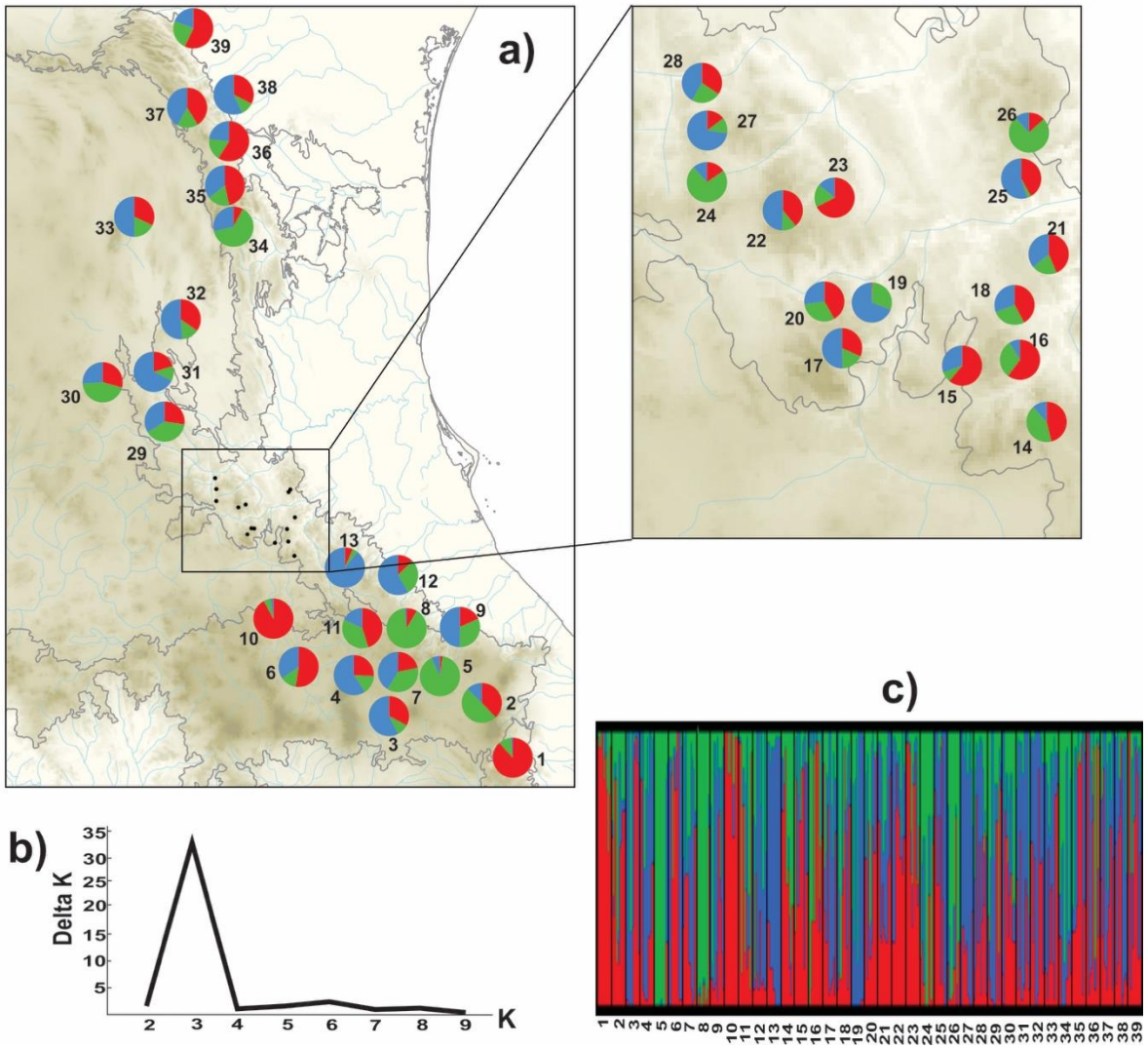


Figure 3

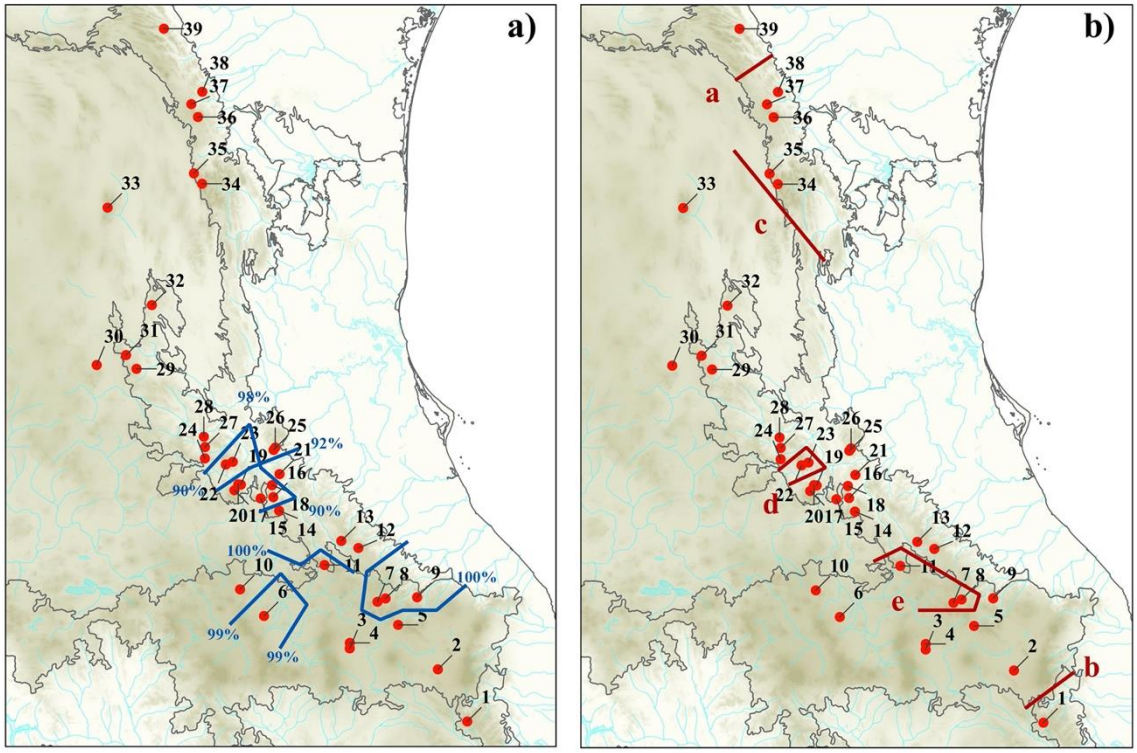
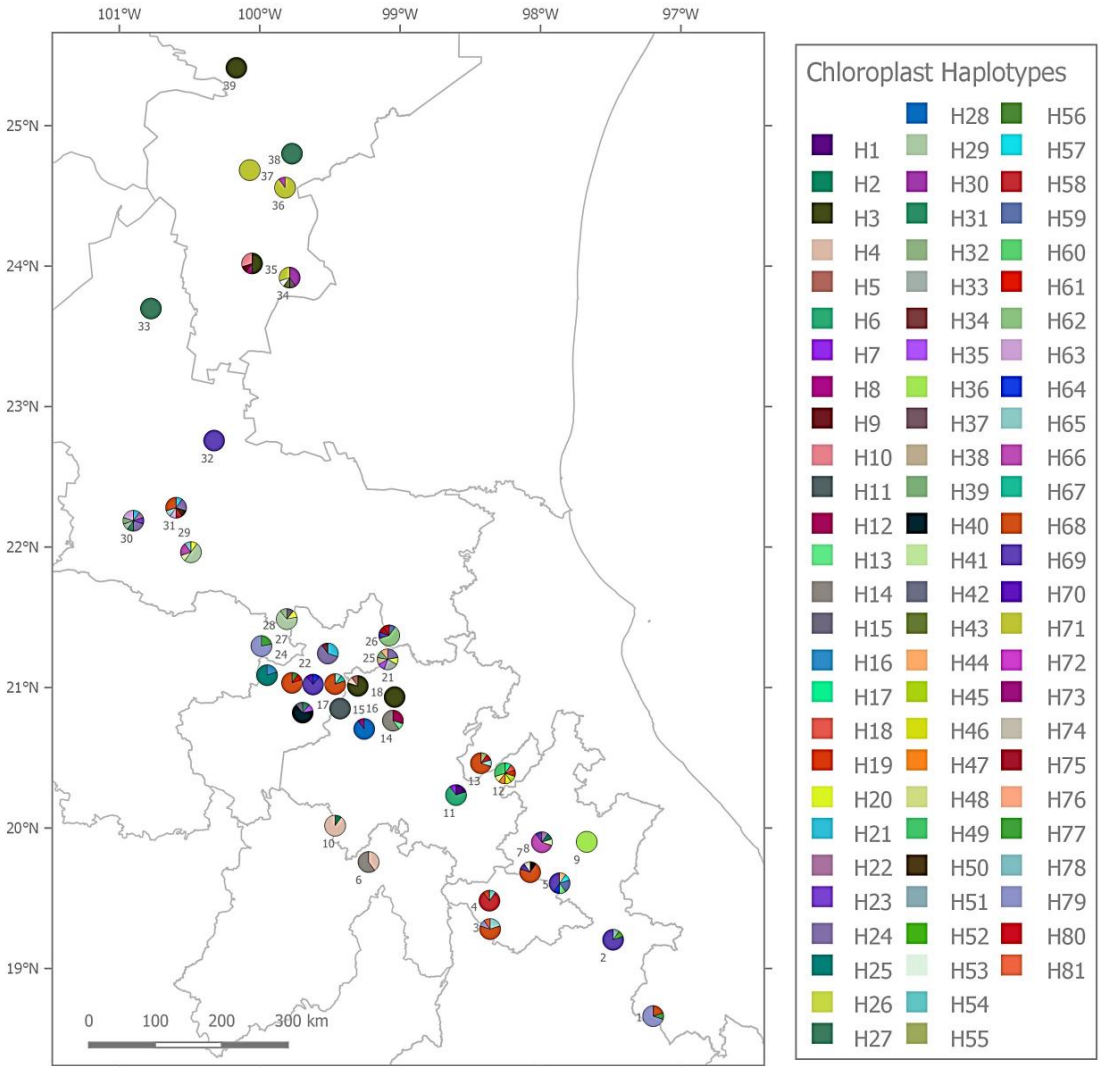


Figure 4


101°W
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98°W
97°W
25°N
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23°N
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0
100
200
300 km
Chloroplast Haplotypes
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Figure 5.

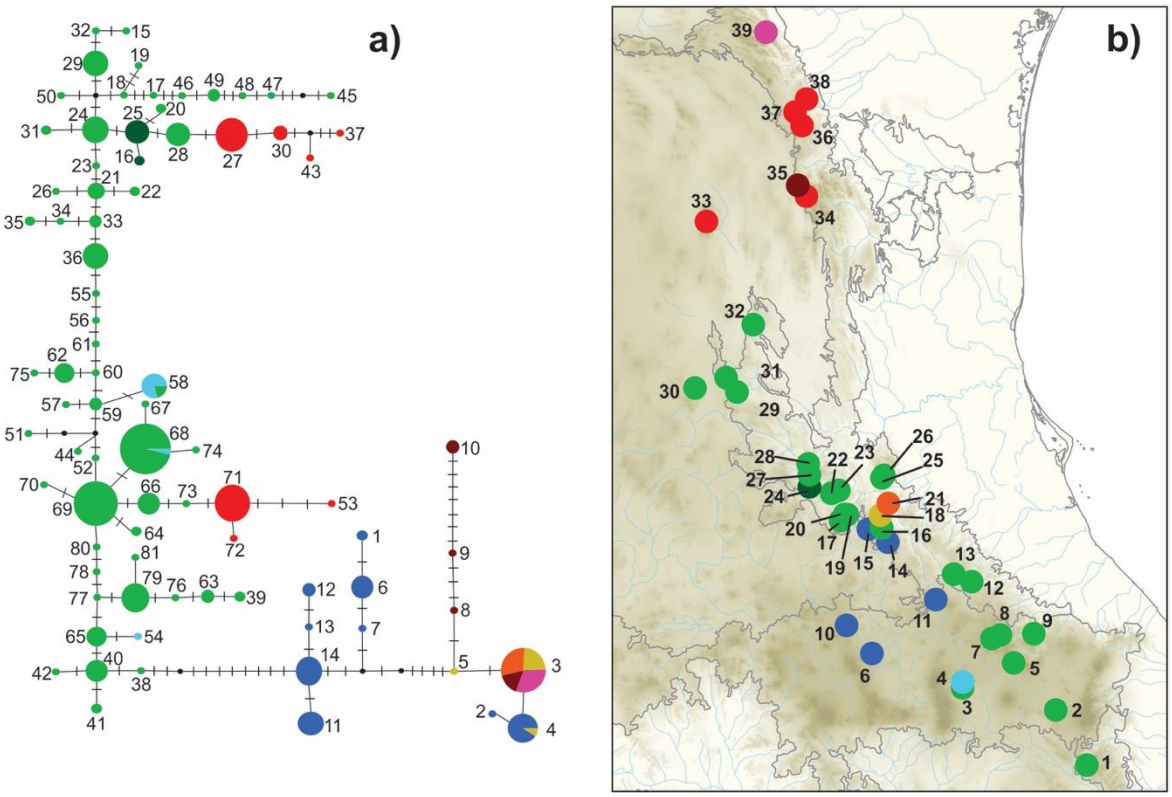
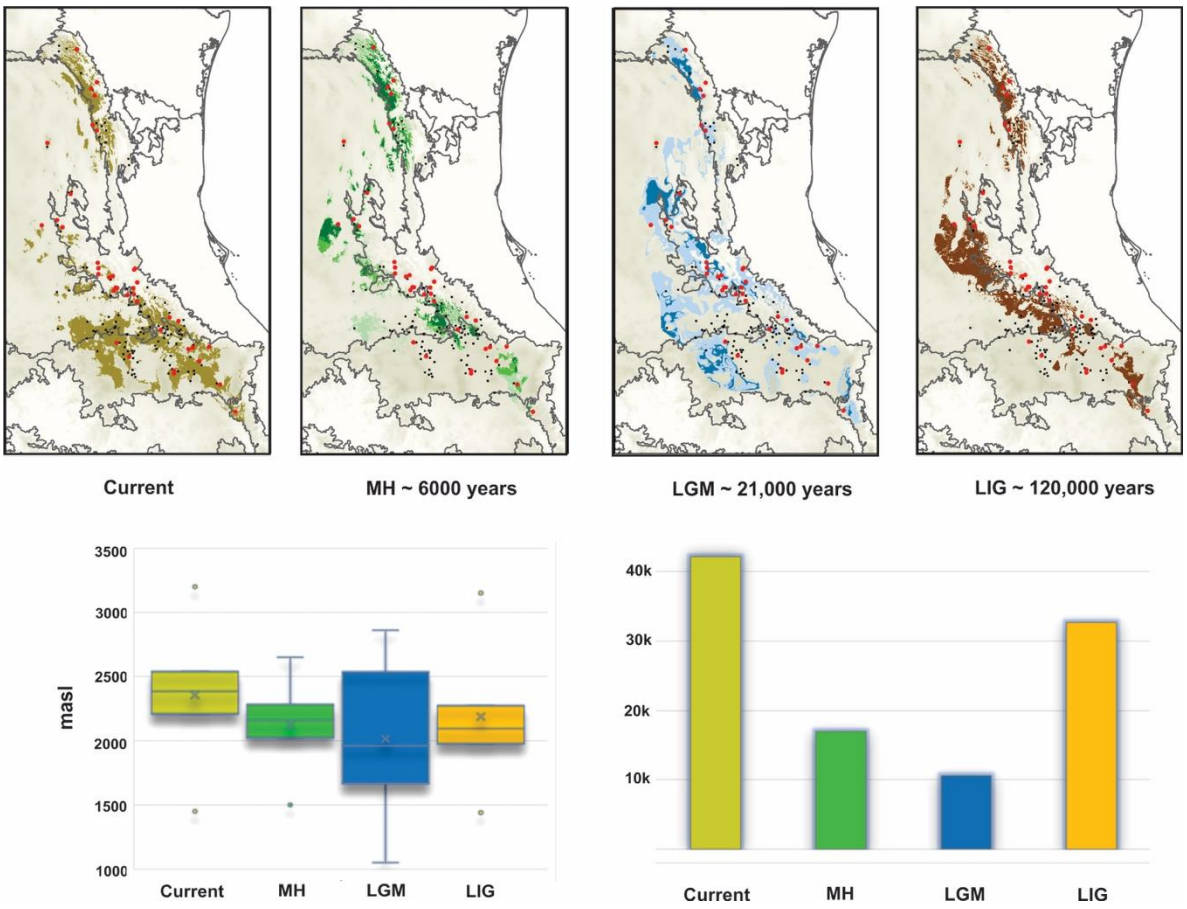


Figure 6


Current
MH ~ 6000 years
LGM ~ 21,000 years
LIG ~ 120,000 years
masl
3500
3000
2500
2000
1500
1000
Current
MH
LGM
LIG
40k
30k
20k
10k
Current
MH
LGM
LIG

Discusión general

Desde el punto de vista evolutivo, los encinos se consideran un buen modelo de estudio ya que México es uno de los principales centros de diversificación y endemismo, por su amplia variación fenotípica y sus niveles altos de flujo genético. Los encinos además son especies dominantes y abundantes en los bosques templados de México. La ubicación geográfica, la tectónica, la topografía compleja y la dinámica climática han influido en la historia evolutiva de las especies de encinos. *Quercus mexicana* es una de las pocas especies cuya distribución es casi endémica a la Sierra Madre Oriental por lo que se considera como un taxón idóneo para entender los procesos evolutivos de sus biotas. Algunos estudios filogeográficos han proporcionado evidencia importante sobre la historia demográfica y la estructura de las poblaciones de encinos mexicanos (Rodríguez-Gómez *et al*. 2018; Peñaloza-Ramírez *et al*. 2020). Asimismo, se ha corroborado una alta variación morfológica asociada a gradientes ambientales en encinos mexicanos (Uribe Salas *et al.* 2008; Maya-García *et al*. 2020).

En el capítulo I, se analizó la variación de rasgos foliares de *Q. mexicana*, los resultados sugieren que la variación tanto de caracteres micro y macromorfológicos entre poblaciones a lo largo de su distribución están asociados a gradientes ambientales. La variación foliar en las especies de *Quercus* es bien conocida (González-Rodríguez & Oyama 2005) y se ha asociado ampliamente a la variabilidad de las condiciones ambientales (Bruschi *et al.* 2003; Ramírez-Valiente *et al.* 2009; Riordan *et al.* 2016). Generalmente, la variación morfológica se refleja en cambios fenotípicos y fisiológicos en atributos morfológicos (Uribe Salas *et al.* 2008; Pyakurel *et al.* 2014), estos cambios generalmente les confieren mayor adecuación para el establecimiento y supervivencia (Pyakurel *et al.* 2014). En otras especies de plantas, se ha documentado

ampliamente el papel fundamental que juegan los factores climáticos en la variación morfológica de las especies (Nicotra *et al.* 2011). Incluso, algunos estudios han determinado que los factores ambientales son elementos clave en la distribución de muchas especies (Monge 1999). Se han realizado este tipo de estudios con la finalidad de conocer los ajustes que presentan las especies de plantas ante nuevas presiones de selección, como son las producidas por el cambio climático global (Ramírez *et al.* 2015; Yan *et al.* 2017).

Cabe resaltar que varios de los estudios de variación morfológicas se centran en especies que se distribuyen a lo largo de gradientes climáticos, altitudinales y latitudinales, debido a que estos estudios pueden brindar información importante para entender las respuestas ecológicas y evolutivas de las especies (Ogaya y Peñuelas; Fajardo *et al.* 2011). En el caso de los encinos, el género *Quercus* es considerado como un grupo taxonómico complicado, esto a consecuencia de la gran variabilidad morfológica (Valencia *et al*. 2004). Estudios previos han identificado una amplia variación fenotípica en especies con amplias áreas de distribución geográfica (Uribe Salas *et al*. 2008; Maya- García 2020). Sin embargo, la variación foliar en encinos se puede apreciar también a nivel paisaje (Lara De la Cruz *et al.* 2020). En el caso de este estudio, el rango de distribución de esta especie abarca un gradiente latitudinal de 1000 km, abarcando algunas partes de las principales regiones biogeográficas de México, como lo es la Sierra Madre Oriental, y zonas limítrofes de la Meseta Central y la FVTM, por esta razón, esta especie se considera un modelo idóneo para abordar la variación foliar a lo largo de gradientes ambientales.

Los resultados muestran que la latitud se correlacionó con la mayoría de los caracteres foliares evaluados. Estudios previos, consideran que la latitud es un factor que influye significativamente en la variación foliar en plantas (Tang y Ohsawa 1999; Uribe-Salas 2008; Pyakurel *et al.* 2014) al estar ligado indirectamente a la energía disponible para la fotosíntesis.

En el caso de *Q. mexicana*, la menor densidad de tricomas y apertura estomática se encontró hacia el sur. Contrario a esto, se encontró que el largo foliar, área foliar, largo y ancho del peciolo, área foliar especifica (AFE), número de nervaduras y grosor aumentan en latitudes norte (Figura 3 y Tabla 3, Capítulo II). Sin embargo, la temperatura y la precipitación son dos de los principales promotores de la variación de la variación foliar en plantas (Valladares *et al.* 2014; Wright *et al.* 2017) y son variables directamente relacionados a la función de las especies. En el caso de *Q. mexicana*, todos los caracteres morfológicos mostraron correlación con la precipitación y temperatura, excepto la densidad estomática. Las nervaduras secundarias, área foliar especifica, largo y ancho foliar se correlacionaron positivamente con la precipitación.

El grosor fue el carácter con el mayor número de diferencias significativas entre las poblaciones de *Q. mexicana*. El grosor foliar se correlacionó negativamente con la mayoría de las variables asociadas a la precipitación, y solo se relacionó de manera positiva con la estacionalidad de la temperatura, por lo que, este rasgo puede estar asociado a tolerancia a la sequía (Niinemets 2001). Las hojas más delgadas se encontraron hacia el sur en la Faja Volcánica Transmexicana y el sureste de la Sierra Madre Oriental; mientras que, las hojas más gruesas se encontraron en poblaciones al norte de la Sierra Madre Oriental, en lo que se conoce como la Sierra Plegada. Por otro lado, se ha documentado mayor densidad de venas foliares en ambientes más secos (Zhu *et al*. 2012) tal como sugieren los resultados de este estudio. Las hojas con un mayor número de venas secundarias se localizan también hacia el norte.

Las correlaciones muestran que las hojas con más venas y mayor AFE se asociaron positivamente con la temperatura, hay una explicación ecológica para este patrón, ambas variables morfológicas de alguna manera están asociadas con la fotosíntesis. En este sentido, un estudio mostró que la venación en las hojas es importante para el rendimiento de las plantas

(Sack *et al.* 2012 y 2013), su función es brindar apoyo, suministro de agua y transporte de carbohidratos. Son estructuras cruciales para mantener el estado hídrico de las hojas y la capacidad fotosintética (Walls 2011). Por otro lado, también se ha identificado que el AFE de una especie está correlacionado positivamente con la tasa fotosintética (Wright *et al.* 2004), está variable se asocia con la productividad de las plantas (Madani *et al.* 2017). Contrario a los resultados de este estudio, antecedentes han documentado una relación negativa entre el AFE y la temperatura (Lee *et al.* 2005), de la misma manera, se ha documentado una correlación positiva entre el AFE y áreas con mayor humedad (Gouveira y Freitas 2009).

El ancho del peciolo mostró una correlación significativa con la mayoría de las variables asociadas a la precipitación. Cabe resaltar que, en distintas especies de encinos, el pecíolo es una de las características que consistentemente presenta patrones especiales de cambio a nivel de localidades, poblaciones e individuos, ya sea en longitud, forma o diámetro (Tovar- Sánchez *et al.* 2004; González-Rodríguez *et al.* 2005). El pecíolo, es una estructura importante para las hojas porque son una continuación de la estructura vascular del tallo. Los pecíolos largos favorecen la captación de luz para las hojas, al mantenerlas separadas entre sí (González-Rodríguez *et al.* 2005). Además, se ha documentado que los peciolos cortos son frecuentes bajo condiciones de estrés lumínico en *Q. acutissima* (Xu *et al.* 2008).

Con respecto a las variables micromorfológicos, entre todos los caracteres evaluados en este estudio, la densidad de tricomas fue el carácter foliar que mostró el mayor coeficiente de discriminación entre las poblaciones de *Q. mexicana,* las poblaciones al sur de la Sierra Madre Oriental y la FVTM presentan la mayor densidad de tricomas. Mientras que la menor densidad de tricomas la encontramos en poblaciones ubicadas al norte de la Sierra Madre Oriental en sitios más secos y temperaturas extremadamente altas. Estos resultados contrastan con el estudio en *Q.*

petraea, donde la densidad de tricomas aumenta como estrategia para evitar la desecación (Bruschi *et al.* 2003) Asimismo, los patrones en otras especies muestran que la mayor densidad de tricomas se encuentra en poblaciones ubicadas en zonas secas (Brushi *et al.* 2003; Benz and Martin 2006).

Los estomas son considerados caracteres con alta variación entre especies (Richardson *et al.* 2001; Pyakurel *et al.* 2014). Pero, curiosamente en este estudio, no encontramos diferencias entre las poblaciones en la densidad de los estomas y el tamaño de los estomas. Asimismo, los estomas no mostraron correlaciones significativas con la mayoría de las variables ambientales; sólo se encontró una correlación significativa con la evapotranspiración potencial, como se ha mostrado en otras especies de encinos (Nóbrega & Pereira 1992). Por otro lado, la apertura del poro estomático tiene una relación negativa con la temperatura. Las poblaciones con apertura estomática amplia se encontraron en las poblaciones de la FVTM y el sureste SMOr, donde hay mayor humedad. Esto es concordante con un estudio previo donde sugieren una disminución en la longitud de la apertura estomática, así como la apertura y tamaño de los estomas observados durante condiciones de sequía (Yan *et al*. 2017).

Los análisis de redundancia mostraron que la morfología se correlacionó significativamente con las variables ambientales. Los resultados muestran una amplia gama de variación foliar en caracteres funcionales en *Q. mexicana*, esta variación es originada a la alta diversidad ambiental en el rango de distribución de la especie. La variación de algunos caracteres foliares parece estar restringidas a condiciones climáticas o áreas geográficas específicas, mientras que otros rasgos varían a lo largo del rango de su distribución sin un patrón específico.

Por otro lado, estudios previos han probado los efectos del cambio climático, específicamente, se prevén condiciones más cálidas y secas en el área de distribución de esta

especie, existe evidencia de un aumento de 0.03 ° C en la temperatura media anual entre 1950 y 2009 y una disminución de 12 mm en la precipitación anual (Cuervo-Robayo *et al.* 2020) en la Zona de Transición Mexicana, que incluye la Sierra Madre Oriental y el Cinturón Volcánico Trans-Mexicano (Cuervo-Robayo *et al.* 2020). Considerando este escenario, y los resultados de este estudio es posible que las poblaciones de *Q. mexicana,* con afinidad a la sequía o temperaturas extremas respondan mejor a estos cambios climáticos. Sin embargo, las poblaciones con caracteres morfológicos afines a condiciones húmedas podrían ser afectadas negativamente.

La mayoría de los caracteres evaluados en este estudio tiene una función estructural y fisiológica (Tabla 2). En este estudio, la mayoría de los caracteres morfológicos evaluados mostraron variación con respecto a algún factor abiótico. Por lo tanto, es evidente que la diferenciación morfológica intraespecífica de *Q. mexicana* pueda ser el resultado de la heterogeneidad ambiental de la región donde se distribuye. En términos generales, estos resultados son consistente con previos estudios en plantas, donde argumenta que la variación foliar está asociada con factores abiótico (Abrams 1990; Uribe Salas *et al.* 2008; Riordan *et al.* 2016; Albarrán-Lara *et al.* 2019). La explicación a este patrón es debido a que las especies con alta variación foliar en caracteres ligados a la supervivencia presentan ventajas en ambientes inestables, heterogéneos o de transición, estos cambios pueden facilitar la exploración de nuevos nichos, dando como resultado el aumento de la tolerancia ambiental (Levin *et al.* 2009; Pyakurel *et al.* 2014).

Cuadro 2. Se muestra el listado de algunos caracteres morfológicos evaluados en este estudio y los patrones encontrados en previos estudios.

Variable foliar	Patrones en otras especies	Función
Tamaño foliar	Hojas pequeñas se asocian con condiciones climáticas severas como el frío (Gates 1980), altas T° (Smith 1977) y sitios secos (McDonald 2003).	El tamaño influye en una serie de procesos fisiológicos importantes, como la fotosíntesis, la transpiración y la termorregulación, y varían con una serie de factores medioambientales (Yates *et al.* 2010).
Grosor	Hojas de mayor grosor en sitios secos (Warren *et al.* 2005)	Hojas esclerófilas y gruesas proporcionan refuerzos estructurales para soportar el marchitamiento en ambientes secos y evitar un exceso de pérdida de agua (Warren *et al.* 2005)
Área foliar específica	La reducción del AFE en sitios secos y con altas T^O (Aranda *et al.* 2014).	Se asocia con la productividad de las plantas (Mandani *et al.* 2017), con la tasa fotosintética (Wright *et al.* 2004).
Pecíolo	Pecíolos cortos son frecuentes bajo condiciones de estrés lumínico en *Q. acutissima* (Xu *et al.* 2008).	Favorecen la captación de luz para las hojas, al mantenerlas separadas entre sí (González *et al.* 2005).
Venas secundarias	Mayor número de venas en sitios con mayor exposición solar (Sack y Flore 2006) y suelos secos (Dunbar-Co *et al*. 2009)	Suministro de agua, y exportación de carbohidratos. Mantiene el estado hídrico y la capacidad fotosintética (Walls 2011).
Tricomas	Mayor densidad en zonas secas (Benz y Martin 2006) o bajas T^O (Agrawal *et al.* 2004). La densidad aumenta como estrategia para evitar desecación (Brushi *et al.* 2003).	Regulación hídrica, barrera protectora contra la alta radiación (Levizou 2004) y regulador de la difusión de gases (Brewer *et al.* 1994). Protege a la planta contra insectos (Schilmiller *et al.* 2008)
Estomas	Aumenta en sitios áridos (Yan *et al.* 2017), cierra sus estomas ante eventos de estrés hídrico (Florido *et al*. 2014).	Regulación del ciclo del H_2O y carbono (Yang *et al.* 2017).
Apertura estomática	Disminución en condiciones de sequía (Yan *et al.* 2017).	Facilita el cierre más rápido en respuesta a ambientes más secos (Lawson and Blatt 2014).

Con respecto al capítulo II, los resultados indicaron altos valores de diversidad genética en todas las poblaciones de *Q. mexicana*, en microsatélites de núcleo y cloroplasto. Cabe señalar que los principales procesos evolutivos que influyen en la variación genética de las poblaciones son la expansión del área de distribución, el flujo de genes, la dispersión de semillas, o la migración a larga distancia (Hamrick *et al.* 1992; Chiang *et al.* 2006). Asimismo, estudios previos en otras especies de plantas han relacionado positivamente la diversidad genética con la estabilidad del hábitat (Gugger *et al.* 2013; Loera *et al.* 2017). También, se ha documentado que la diversidad genética es importante para la adaptación de las especies a las diversas condiciones, por lo tanto, puede influir de manera positiva en la adecuación y la persistencia de las

poblaciones (Jump *et al.* 2009). La diversidad genética es considerada la materia prima sobre la que actúa la selección natural (Amos y Harwood 1998), se considera que tiene importantes implicaciones en la evolución y la conservación de las especies (Ellegraen and Galtier 2016) debido a que contribuye a la capacidad de una especie para responder a los cambios en su entorno (Jump *et al*. 2009). Los encinos mexicanos presentan altos niveles de diversidad genética dentro y entre poblaciones (Ramos-Ortiz *et al.* 2016; Oyama *et al.* 2018; Peñaloza-Ramírez *et al.* 2020), lo que concuerda con lo encontrado en este estudio. En el caso de *Q. mexicana* se puede inferir que la persistencia histórica, la distribución estable a largo plazo, la migración local, los tamaños efectivos poblacionales durante un largo período y el flujo de genes entre poblaciones mantienen altos niveles de diversidad genética.

Por otro lado, los resultados también mostraron la existencia de estructura filogeográfica en las poblaciones de *Q. mexicana*, relacionado a la presencia (o ausencia) de barreras que establecen diferentes niveles de aislamiento geográfico entre las poblaciones y que, repercuten en el flujo génico entre poblaciones (Avise 1987; Avise 2000). Con respecto a los resultados en este estudio, el flujo de genes, las barreras físicas y el aislamiento por distancia parecen haber determinado un papel fundamental en promover la estructura filogeográfica en *Q. mexicana*, lo cual ha sido documentado en otros estudios (Soto *et al.* 2007; Sosa *et al.* 2009; Zhang *et al.* 2015; Ochoa-Zavala *et al.* 2020). Estudios previos han documentado los patrones filogeográficos de especies de encinos en las regiones tropicales y subtropicales donde se encuentra una alta riqueza de especies (Cavender-Bares *et al.* 2011; Rodríguez- Gómez *et al.* 2018). Particularmente, los estudios filogeográficos en los encinos en México han reportado fuerte estructura filogeográfica, en todos los casos a excepción de *Q. rugosa* (Cuadro 1). En *Q. mexicana* los análisis de varianza molecular mostraron estructura genética significativa en

microsatélites del núcleo y del cloroplasto. Estos resultados coinciden con datos reportados en otras especies de encinos mexicanos (Rodríguez- Gómez *et al.* 2018; Oyama *et al.* 2018), donde han resaltado el papel de las barreras geográficas como el principal promotor de dicha estructura.

Cuadro 1. Antecedentes filogeográficos en *Quercus* en México. Se muestra la referencia, tipo de marcador, estructura filogeográfica, diversidad genética, número de haplotipos, especie y región donde se distribuye la especie de estudio (FVT: Faja Volcánica Transmexicana; SMOr: Sierra Madre Oriental; SMS: Sierra Madre del Sur; SMOc: Sierra Madre Occidental; SMC: Sierra Madre de Chiapas; MC: Meseta Central).

Especie	Región	Pob/Ind	Haplotipos	Hs/ H_T	Estructura filogeográfica	Marcador	Referencia
Q. affinis	FVT-SMOr	20/109	6	0.299	NST=0.566 GST=0.499	AFLP	González-Rodríguez *et al.* 2004
Q. laurina	FVT-SMS	19/294	6	0.299	NST=0.566 GST=0.499	AFLP	González-Rodríguez *et al. 2004*
Q. affinis- Q.laurina	SMS-FVT-SMOr	7/105	42	0.845/0.970	NST=0.27 GST=0.038	6 Cp SRR	Ramos Ortiz 2015
Q. rugosa	SMOc-SMO-SMS-FVT	25/237	80	0.765/0.987	RST= 0.296 GST= 0.225	6 Cp SRR	Uribe Salas, 2009
Q. Hypoleuicoides	SMOc	14/120	24	0.671/0.932	RST=0.643 GST=0.280	8 Cp SRR	Peñaloza Ramírez *et al*, 2011
Q. scytophylla	SMOc-FVT- SMS	21/174	57	0.708/0.972	RST=0.708 GST=0.271	8 Cp SRR	Peñaloza Ramírez *et al*, 2011
Q. sideroxyla	SMOc, FVT	16/138	41	0.695/0.981	RST=0.809 GST=0.291	8 Cp SRR	Peñaloza Ramírez *et al*, 2011
Q. castanea	SMOc-FVT-SMS-MC	36/341	90	0.730/0.989	RST=0.711 GST=0.261	7 Cp SRR	Peñaloza Ramírez *et al*, 2011
Q. magnolifolia	SMOc-FVT-SMS	38/242	56	0.67 SEP/0.98	RST=0.734 GST=0.364	6 Cp SRR	Albarrán Lara *et al.*, 2011
Q. resinosa	MC-FVT- SMOc	23/155	34	0.62/0.92	RST=0.830 GST=0.360	6 Cp SRR	Albarrán Lara *et al.*, 2011

Las características del paisaje y la historia de vida afectan el flujo de genes, e influyen en la estructura genética de las especies (Hevroy *et al*. 2018). Sin embargo, en *Q. mexicana* la ubicación de las barreras no coincide precisamente con las barreras reportadas en estudios filogeográficos previos (Anducho-Reyes *et al.* 2008; Bryson *et al.* 2011, 2012; Ruiz- Sánchez *et*

al. 2012, 2013). Los resultados del cloroplasto identificaron barreras a lo largo de la distribución de *Q. mexicana*, resaltando que una de las barreras indica una separación de las poblaciones del norte de la SMOr, que corresponden a la Sierra Plegada, del resto. En contraste, en los microsatélites núcleo las discontinuidades se identificaron al sur de la distribución de la especie en poblaciones cercanas a la FVTM.

El flujo génico, es un proceso responsable que determina la variación y la estructura genética, e influye en el potencial evolutivo de las especies (Nakanishi *et al.* 2004). El flujo genético puede ocurrir a través del movimiento del polen y de la dispersión de semillas (Sujii *et al*. 2021). En especies de plantas, relativamente inmóviles, la dispersión del polen es probablemente el componente más importante del movimiento de genes (Nakanishi *et al.* 2004), y suele ser extenso en plantas que son polinizadas por el viento como en el caso de los encinos (Kremer *et al.* 2012; Ashley *et al.* 2015). Las discrepancias de las barreras geográficas entre los datos del núcleo y el cloroplasto se pueden explicar por el flujo de genes, es decir, la variedad de mecanismo de dispersión observados a través de polen y/o semillas que produce un patrón diverso de intercambio genético (Shaal *et al.* 1998). El uso de marcadores de herencia citoplasmática y nucleares fue determinar los patrones de flujo génico histórico y contemporáneo (semillas y polen), y de esta manera conocer su historia evolutiva (Cavender- Bares *et al.* 2011; Ohtani *et al.* 2013).

En cuanto a los resultados de la demografía histórica y los modelos de nicho ecológico, estos indicaron una expansión demográfica en varias poblaciones de *Q. mexicana*. Los ENM mostraron que la distribución actual de *Q. mexicana* es más extensa que en el pasado, específicamente, en el escenario del Último Máximo Glacial (UMG ~ 21 ka AP), la amplitud del nicho se redujo. En este escenario, la mayor reducción se mostró en el norte de su distribución,

es decir, lo que se conoce como la Sierra Plegada. Un punto importante es que los modelos de nicho sugieren la prevalencia y conectividad de las poblaciones de *Q. mexicana* a lo largo de los períodos glaciales. En este sentido, estudios previos han resaltado el efecto que tuvieron las glaciaciones en la estructura genética y filogeográfica en especies de encino en América (Cavender Bares *et al.* 2011; Gugger *et al.* 2013 Rodríguez- Gómez *et al.* 2018). Específicamente, en el Pleistoceno, se ha documentado que las migraciones de las especies de encinos mexicanos fueron altitudinales (González- Rodríguez *et al.* 2004; Gugger *et al.* 2013) y no latitudinales. De esta forma, es factible que las poblaciones de encinos persistieran durante varios episodios de cambio climático a lo largo del Pleistoceno, y hayan experimentado contacto secundario con poblaciones de otras especies originando zonas de hibridación de especies (González- Rodríguez *et al.* 2004). Se ha documentado que las glaciaciones del Pleistoceno fueron menos severas en los encinares en México, por lo que, las especies de encinos fueron más persistentes localmente, con algunos eventos de expansión y contracción de rango, pero sin una extensiva colonización post-glacial (Grivet *et al.* 2006).

Las conclusiones principales de este capítulo fueron que las poblaciones de Q. *mexicana* muestra estructura filogeográfica, alta diversidad genética, estructura genética y rangos de expansión histórica entre sus poblaciones. Factores como el flujo de genes, las barreras geográficas, el aislamiento por distancia, la prevalencia y conectividad de las poblaciones de *Q. mexicana* a lo largo de los períodos glaciales podría haber tenido un papel importante en la configuración poblacional de esta especie a lo largo de su distribución en la Sierra Madre Oriental y zonas adyacentes.

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