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VARIACIÓN ADAPTATIVA EN EL ENCINO *Quercus rugosa* Née (Fagaceae) A LO LARGO DE UN GRADIENTE LATITUDINAL Y CLIMÁTICO

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Por medio de la presente me permito informar a usted, que el Subcomité de Ecología y Manejo Integral de Ecosistemas del Posgrado en Ciencias Biológicas, en su sesión ordinaria del día 29 de mayo de 2017, aprobó el jurado para la presentación del examen para obtener el grado de **DOCTOR EN CIENCIAS** al alumno **LLANDERAL MENDOZA JESÚS,** con número de cuenta **509021452**, con la tesis titulada, **"Variación adaptativa en el encino Quercus rugosa Née (Fagaceae) a lo largo de un gradiente latitudinal y climatico"**, bajo la dirección del **Dr. Antonio González Rodríguez** Tutor principal.

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ÍNDICE

RESUMEN …… **1**

ABSTRACT ……... **4**

CAPÍTULO I. INTRODUCCIÓN GENERAL …… **7**

CAPÍTULO II. CLIMATIC DETERMINANTS OF ACORN SIZE AND GERMINATION PERCENTAGE OF *Quercus rugosa* (Fagaceae) ALONG A LATITUDINAL GRADIENT IN MEXICO …….. **22**

CAPÍTULO III. FUNCTIONAL DIFFERENTIATION IN RESPONSE TO DROUGHT STRESS IN *Quercus rugosa* POPULATIONS ALONG A CLIMATIC GRADIENTE IN MEXICO …… **32**

CAPÍTULO IV. GEOGRAPHICAL PATTERNS OF GENOMIC VARIATION PROVIDE GUIDELINES FOR ASSISTED GENE FLOW IN MEXICAN POPULATIONS OF *Quercus rugosa* …… **65**

CAPÍTULO V. DISCUSIÓN Y CONCLUSIÓN FINAL …… **123**

LITERATURA CITADA ……... **132**

RESUMEN

Un tema central en la biología evolutiva es conocer la variación de rasgos funcionales a través de las poblaciones de una especie y detectar cuál es su valor adaptativo. Para diferentes especies de encinos se ha reportado que existe una gran variación en los atributos funcionales entre y dentro de las poblaciones, incluyendo el tamaño de las bellotas, la forma y el tamaño de las hojas, las funciones de intercambio de gases y la eficiencia del uso del agua, entre otras. La variación de estos rasgos funcionales se ha asociado a distintas estrategias para evitar o tolerar distintos tipos de estrés ambiental como es la disponibilidad de agua. Estudios con especies de encinos en Europa y el Norte de América muestran que la variación de estas estrategias se asocia a gradientes ambientales, mostrando evidencia de cómo la selección natural a nivel local juega un papel muy importante en la divergencia adaptativa a nivel intra-específico en este género. Sin embargo, pocos estudios se han realizado en especies de encinos mexicanos y mucho menos se ha reportado la variación y las respuestas adaptativas ante condiciones contrastantes de poblaciones de encinos a escalas geográficas amplias. De tal manera que en este estudio mostramos evidencia de cómo la variación de las condiciones geográficas y climáticas juega un papel muy importante en los procesos evolutivos de adaptación local en un encino blanco de amplia distribución, *Quercus rugosa*. Encontramos que la variación de la masa y el volumen de las bellotas de *Q. rugosa* a través del gradiente de distribución apoya la hipótesis de que estas variables se ven afectadas por una restricción ambiental en la precipitación de la temporada de crecimiento (gsp) y en los días en donde existe una temperatura mayor a 5ºC durante la temporada de crecimiento (gsdd5) en una gradiente en dirección sur-norte. De igual manera, se encontró una correlación positiva entre el tamaño de la bellota (volumen y masa) con el porcentaje de germinación de las semillas. Esto sugiere que esta disminución del tamaño, y por ende de la

reserva donada por la madre, puede ser deletérea para las plántulas y pudiera ser un factor que influye en la delimitación del área de distribución de esta especie.

En el segundo capítulo, se observó que las plántulas con mayor crecimiento al año de germinar eran las que provenían de las regiones con mayor precipitación anual y situadas al sur de México, mientras que las que provenían de las regiones más áridas eran de menor tamaño, con menor número de hojas y de menor tamaño. Al año y medio de haber comenzado el experimento las plántulas se dividieron en dos tratamientos, uno de humedad en donde se mantuvieron a capacidad de campo (TH – tratamiento de humedad) y otro en condiciones limitantes de humedad del suelo (TS – tratamiento seco). En este experimento se observó que las poblaciones de las regiones húmedas mantenían un mayor crecimiento que las de las regiones áridas en el tratamiento TH. En el tratamiento TS se siguió observando el mismo patrón, las poblaciones de las regiones áridas presentaron el menor tamaño, menor número de hojas y de menor tamaño. Además, las plántulas de estas regiones presentaron el mayor ajuste en la eficiencia del uso de agua (EUA) bajo las condiciones estresantes. Esto demuestra que las poblaciones divergen, y que las poblaciones de regiones áridas tienen adaptaciones que les confieren un mejor uso y conservación del agua. También se observó que las plántulas del sur tenían una gran respuesta plástica ante condiciones estresantes ya que reducían su crecimiento, la producción de hojas y disminuían el tamaño de estas bajo estas condiciones de estrés, reflejando un ajuste que reduce el área de pérdida de agua. También se encontró que las poblaciones del norte podían explotar más sus recursos, ya que presentaban las tasas fotosintéticas más altas, aún bajo condiciones limitantes de agua. No se encontró diferenciación poblacional en los potenciales hídricos de las hojas tomados antes del amanecer y al medio día, pero sí se encontró una variación entre tratamientos, mostrando su tolerancia para realizar sus funciones biológicas aún bajo condiciones estresantes.

Se encontró que las poblaciones estudiadas muestran valores bajos de diferenciación genética neutral (Fst = 0.09) lo que indica un alto flujo de genes, pero, a pesar de la baja diferenciación, se encontró una estructura génica que dividía a las poblaciones geográficamente en las del sur y del norte, o de regiones húmedas y regiones áridas, evidenciando que la variación de los atributos morfológicos y fisiológicos medidos es influenciada por selección de estos en respuesta a la adaptación local, además de estar afectada por un aislamiento por distancia. El análisis de escaneo genómico (GBS) en las poblaciones de la Faja Volcánica Transmexicana mostró valores bajos de diferenciación genética, pero una estructura que divide a las especies en un gradiente Este–Oeste, además de que se encontró evidencia de la huella genética producto de una adaptación local en respuesta a las condiciones de temperatura y precipitación de cada localidad.

En conclusión, la variación latitudinal observada en la reducción del tamaño de las bellotas esta determinada por una restricción climática que limita el desarrollo de las mismas y que, a su vez, esta disminución tiene un efecto deletero para el desarrollo de las plántulas, con implicaciones para la delimitación de los límites superiores septentrionales de las especies. Por otro lado, el conjunto de resultados obtenidos en cuanto a la variación de atributos morfológicos y fisiológicos sugieren que las plántulas de *Q. rugosa* tienen un considerable potencial para hacer frente a condiciones de baja humedad del suelo. Por otro lado nuestros resultados muestran que la selección natural ha conducido la adaptación local lo cual, a su vez, ha influido en una varianza genética entre poblaciones de esta especie que resulta en una importante implicación para la conservación de los bosques ante el cambio climático actual.

ABSTRACT

A central theme in evolutionary biology is to understand the variation of functional traits across populations of species and detect what is their adaptive value. For different species of oaks, it has been reported that there is a great variation in the functional attributes between and within populations, including the size of the acorns, the shape and the size of the leaves, the functions of gas exchange and water use efficiency, among others. The variation of these functional traits has been associated with different strategies to avoid or tolerate different types of environmental stress such as water availability. Studies with oak species in Europe and North America show that the variation of these strategies is associated with environmental gradients, showing evidence of how natural selection at local level plays a very important role in adaptive divergence in a intra-specific level in this genre. However, few studies have been conducted in Mexican species of oaks and much less have reported variation and adaptive responses to contrasting conditions of populations of oaks at broad geographic scales. This study shows evidence of how the variation of geographical and climatic conditions play a very important role in the evolutionary processes of local adaptation in a wide ranging white oak, *Quercus rugosa*. We found that the variation of the mass and volume of the acorns of *Q. rugosa* through the gradient of distribution supports the hypothesis that these variables are affected by a restriction in the precipitation of the growing season (GSP) and in the days where there is a higher temperature than 5ºC during the growing season (gsdd5) in a gradient in a south-north direction. Similarly, we found a positive correlation between the size of the acorn (volume and mass) with the percentage of germination of the seeds. This suggests that this decrease in size, may be deleterious for the seedlings and may be a factor in the delimitation of the area of distribution of this species.

In the second chapter, it was observed that seedlings with higher growth after a year of germination were those that came from the regions with the

highest annual rainfall and situated in the south of Mexico, while the ones that came from the most arid regions were smaller in size, with a lesser number of leaves and smaller in size. A year and a half after the beginning of the experiment the seedlings were divided into two treatments, one of moisture where it the soil was maintained at field capacity (TH - treatment of humidity) and the other in limiting conditions of soil moisture (TS - dry treatment). The populations from the humid regions showed higher growth than those of arid regions in the TH treatment. The plants in the TS treatment followed the same pattern, the populations of arid regions were smaller, with lower number of leaves and smaller in size. In addition, seedlings of these regions had the highest water use efficiency (WUE) under stressful conditions. This shows that the populations have diverged, and that the populations of arid regions have adaptations for a higher WUE. It was also observed that the seedlings of the south had a large plastic response to stressful conditions as they reduced their growth, the production of leaves and diminished their size under stress conditions, reflecting an adjustment which reduces the area of water loss. It was also found that the populations of the north could exploit more the resources, as they had higher photosynthetic rates, even under limiting conditions of water. Population differentiation was not found in the water potential of the leaves taken before dawn and in the middle of the day, but variation was found between treatments, showing tolerance to stressful conditions.

It was found that the populations studied showed low values of neutral genetic differentiation (Fst = 0.09) indicating a high gene flow, but, in spite of the low differentiation, we found a genetic structure that divided the populations geographically in the north and south, or humid and arid regions, demonstrating that the variation of the morphological and physiological attributes measured is influenced by selection of these in response to local adaptation, in addition to being affected by an isolation by distance.

The analysis of genomic scanning (GBS) in populations of the Transmexican Volcanic Belt showed low values of genetic differentiation, but a structure that divides the species in an east-west gradient. In addition to that, we found evidence of local adaptation in response to the conditions of temperature and precipitation of each locality.

In conclusion, the latitudinal variation observed in the reduction of the size of the acorns is determined by a climate restriction that limits their development and that, in turn, this decline has a deleterious effect for the development of the seedlings, with implications for the delimitation of the northern limits of the species. On the other hand, the set of results obtained with regard to the variation of morphological and physiological attributes suggest that seedlings of *Q. rugosa* have a considerable potential to adapt and adjust to conditions of low soil moisture. On the other hand, our results show that natural selection has led the local adaptation which, in turn, has influenced genetic variation between populations of this species, resulting in an important implication for the conservation of forests in the current climate change.

CAPÍTULO I

INTRODUCCIÓN GENERAL

Estrés hídrico en plantas

Tanto en condiciones naturales como de manejo experimental, las plantas están frecuentemente expuestas a algún tipo de estrés ambiental. El estrés se podría definir como cualquier alteración de la condición de crecimiento causada por factores que alteran el equilibrio en donde se estaba desarrollando la planta (Shao *et al*., 2008). El estrés puede ser provocado por factores bióticos o abióticos, los primeros producidos por ataques de insectos o microorganismos patógenos y dentro de los segundos encontramos aquellos generados por la falta de agua, la alta salinidad en el suelo, las temperaturas extremas, la hipoxia, el déficit de nutrientes minerales, la toxicidad por metales, contaminantes o radiación ultravioleta UV-B, por ejemplo (Smirnoff, 1998). El déficit hídrico probablemente es el factor estresante más importante que determina el crecimiento, productividad y distribución de las plantas (Anjum *et al*., 2011; Schwanz y Polle, 2001).

La sequía es un estrés que afecta el crecimiento de las plantas ya que disminuye el potencial hídrico del área geográfica de los sitios en donde estos organismos están creciendo. Se dice que es un estrés multidimensional que afecta a las plantas en todos sus niveles de organización (e. g. células, tejidos, órganos y al individuo completo) y la respuesta ante el estrés es de gran complejidad, dado que refleja una integración de los efectos del estrés y respuestas en todos los niveles de organización, en tiempo y espacio (Smirnoff, 1998). La resistencia a la sequía es una propiedad que caracteriza a ciertas especies o variedades de plantas que les permite adaptarse a áreas con poca disponibilidad de agua. El déficit hídrico puede presentarse por periodos largos de tiempo (días, semanas o meses) o sólo durante un corto tiempo (horas) (Chaves *et al*., 2003). En caso de que el estrés hídrico se desarrolle lentamente, algunas plantas pueden contrarrestar la deshidratación mediante el acortamiento de sus ciclos de vida o al optimizar sus recursos a largo plazo, a

través de respuestas de aclimatación (Figura 1). En el caso de una deshidratación rápida, las plantas reaccionan reduciendo al mínimo la pérdida de agua y/o mediante una protección metabólica, reduciendo los efectos perjudiciales de la pérdida de agua (Chaves *et al*., 2003).

Todo el conjunto de respuestas adaptativas de las plantas ante el estrés es el resultado de un proceso evolutivo que refleja una múltiple y compleja interacción de características fenológicas, morfológicas, fisiológicas y metabólicas que modulan el estado hídrico interno bajo condiciones edáficas y climáticas desfavorables (Taiz y Zeiger, 2002; Ceiller *et al*., 1998; Valliyodan y Nguyen, 2001; Wang *et al.,* 2003). En comunidades vegetales naturales, estas características son de gran importancia para la sobrevivencia de las plantas, y la manifestación de las distintas respuestas morfofisiológicas ante el déficit hídrico depende de la especie o variedad, como previamente he mencionado (Bray, 1997).

Figura 1.- Se muestran las respuestas de la planta ante un déficit hídrico que se desarrolla con rapidez (derecha) y cuando se desarrolla lentamente (izquierda) (Tomado y modificado de Chaves *et al*. 2003).

Estrategias fenológicas, morfológicas, fisiológicas y metabólicas ante el estrés

Tradicionalmente, la resistencia de las plantas ante el estrés hídrico provocado por la limitación de agua ha sido dividida en tres tipos de estrategias: escapar, evitar y tolerar (Levitt, 1972; Turner, 1986). Sin embargo, las plantas pueden combinar varios tipos de respuestas simultáneamente (Ludlow, 1989). Las plantas que escapan a la sequía tienen un alto grado de plasticidad y pueden completar sus ciclos de vida antes de que el déficit hídrico ocurra (Chaves *et al*., 2003). La estrategia de escape se basa en una reproducción exitosa antes de que aparezca un estrés severo. Esto es importante en zonas áridas, en donde las plantas anuales pueden combinar ciclos de vida cortos con altas tasas de crecimiento y de intercambio de gases, utilizando al máximo los recursos disponibles mientras haya humedad en el suelo (Maroco *et al*., 2000). De igual manera, para lograr el éxito reproductivo se debe tener una buena repartición de los recursos asimilados hacia los frutos en desarrollo, y esto está asociado con la habilidad de las plantas de almacenar reservas en algunos órganos (tallos y raíces) y después poder movilizarlos a la producción de frutos (Bruce *et al*., 2002; Chaves *et al*., 2003).

Las plantas también pueden soportar condiciones de sequía evitando la deshidratación de sus tejidos, mientras mantienen el potencial hídrico lo más alto posible, o tolerando bajos potenciales hídricos en estos. Evitar la deshidratación es común tanto en plantas anuales como perennes y está asociado a una variedad de rasgos adaptativos, entre los cuales se encuentra la reducción de la pérdida de agua y la maximización de su absorción. La pérdida de agua se puede reducir mediante el cierre de los estomas, o disminuyendo las áreas foliares limitando el crecimiento de las hojas o desprendiéndolas. La absorción de agua se maximiza al aumentar la superficie y profundidad de las raíces (Jackson *et al*., 2000). Además, la senescencia foliar que contribuye con el ahorro de agua puede verse como un programa de reciclaje dentro de la planta, permitiendo que los nutrientes almacenados en las hojas viejas se

muevan a los tallos o hacia hojas nuevas (Tyree *et al*., 2003; Chaves *et al*., 2003).

La tolerancia a los bajos potenciales hídricos implica un ajuste osmótico (Morgan, 1984), el desarrollo de paredes celulares rígidas y/o de células de menor tamaño (Wilson *et al*., 1980). Muchos arbustos de hojas perennes y árboles de zonas áridas o semi-áridas combinan la alta concentración de solutos en sus células (bajos potenciales osmóticos) con la esclerofilia y una baja capacidad fotosintética y conductancia estomática (Faria *et al*., 1998). Las hojas pequeñas están bien adaptadas a las altas intensidades de luz y a las altas temperaturas que existen en las regiones áridas ya que sus tamaños permiten la disipación del calor y una eficiencia en el control de la pérdida de agua dada por el cierre estomático (Jarvis y McNaughton, 1986).

Los árboles y la diferenciación adaptativa de rasgos funcionales

Algunas especies de árboles presentan amplias áreas de distribución a través de una gama considerable de condiciones ambientales y por lo tanto están sujetos a distintas presiones selectivas impuestas por los diversos ambientes ecológicos (Still *et al*., 2005). De esta manera, la selección a largo plazo ha conducido al desarrollo de adaptaciones morfológicas y fisiológicas locales, así generándose una diferenciación ecotípica de rasgos cuantitativos importantes (Kawecki y Ebert, 2004; Savolainen, 2007). Además, por lo general, las especies de árboles pueden hacer frente a la variabilidad ambiental exhibiendo una alta plasticidad fenotípica, es decir, la capacidad de expresar fenotipos alternativos en respuesta a la variación ambiental. Así que, si un rasgo dado es adaptativo para una especie o una población en particular, se espera que presente una diferenciación ecotípica causada por presiones de selección divergentes, una gran plasticidad fenotípica que haga frente a la heterogeneidad del ambiente, o ambas (Ramírez-Valiente *et al*., 2010a).

A corto plazo, la capacidad de las especies para hacer frente a las cambios en las condiciones ambientales se basa en la plasticidad fenotípica, así como en la variación genética intraespecífica subyacente a los rasgos funcionales adaptativos (Ward *et al*., 2000; Ward y Kelly, 2004; Marchin *et al*., 2008). A largo plazo, esta respuesta también dependerá tanto de la posibilidad de migrar a entornos favorables como de la adaptación de los rasgos funcionales a las nuevas condiciones ambientales (Geber y Dawson, 1993; Ward y Kelly, 2004; Parmesan, 2006). Aunque existe mucha información de las respuestas individuales de las plantas a cambios de los factores abióticos por la aclimatación y la plasticidad, se sabe menos acerca de la capacidad de las plantas para evolucionar, particularmente en el caso de especies de vida larga como son los árboles (Petit y Hampe, 2006; Valiente-Ramírez *et al*., 2010a).

El potencial para la evolución adaptativa de rasgos cuantitativos depende de la amplitud de la varianza genética aditiva, así como de las covarianzas genéticas entre rasgos (Falconer y Mackay, 1996; Roff, 1997). Por lo tanto, la capacidad de evolución de un rasgo puede ser estimada por dos parámetros genéticos: la heredabilidad y las correlaciones genéticas con otros rasgos (Valiente-Ramírez *et al*., 2010b). La heredabilidad determina el potencial de los cambios evolutivos y la velocidad a la que un rasgo puede responder a la selección (Falconer y Mackay, 1996). Las correlaciones genéticas entre rasgos cuantitativos y su adecuación también pueden determinar el potencial evolutivo de estos (Cheverud, 1984; Arnold, 1992).

Variación morfofisiológica y genética en árboles

Los árboles, que por lo general son organismos de vida larga, constantemente han hecho frente a los cambios naturales de las condiciones ambientales, tales como las variaciones climáticas que a veces han sido extremas, y su supervivencia está ligada con su capacidad de adaptarse a estas nuevas condiciones. Esta capacidad depende del grado de variabilidad de los genes

involucrados en el control de rasgos adaptativos y de la plasticidad de estos rasgos (Guevara *et al*., 2005). Por lo tanto, los estudios sobre la variación intra- e interpoblacional de caracteres ecológicamente relevantes relacionados a las respuestas plásticas o adaptativas ante la disponibilidad de agua, aunados a estudios de variación, estructura y diversidad genética, podrían ser particularmente informativos para conocer y estimar la probabilidad de sobrevivencia de algunas especies y poblaciones de árboles ante los escenarios de cambio climático global (González-Martínez *et al*., 2006). Recientemente, se han hecho avances importantes en el entendimiento de las estrategias y los rasgos cuantitativos relacionados con la disponibilidad de agua y la resistencia a la sequía de plántulas de especies arbóreas y la relación con su distribución (Tyree *et al*., 2003; Engelbrecht y Kursar, 2003; Slot y Poorter, 2007; Pineda-García, 2013).

Los encinos y su variación ecotípica

Estudios previos de algunos rasgos ligados a la poca disponibilidad de agua en encinos europeos tales como el tamaño de las bellotas y otros caracteres fenotípicos han sido ampliamente reportados y muestran una serie de variaciones de estos atributos en respuesta a los niveles de precipitación en poblaciones de especies que habitan gradientes ambientales (Aranda *et al*., 2007, Gouveia y Freitas, 2009, Ramírez-Valiente *et al*., 2009a, 2009b). Por ejemplo, se ha reportado que poblaciones de *Quercus suber* y *Quercus ilex* presentan una reducción de sus áreas foliares con la disminución de la precipitación, mientras que la masa específica foliar y el grosor de las hojas aumenta con la aridez (Castro-Diez, 1997). De igual manera, la selección natural y el potencial evolutivo de rasgos reproductivos y morfológicos han sido ampliamente investigados (Ramírez-Valiente *et al*., 2010b). Existen otros estudios enfocados a estudiar los rasgos fisiológicos relacionados con la tolerancia a la sequía (Geber y Griffen 2003; Ramirez-Valiente *et al*., 2010b) y sobre factores que afectan la sobrevivencia de las plántulas de encinos.

La genética cuantitativa, la genética del paisaje y la genómica del paisaje

Se han reportado numerosos experimentos de genética cuantitativa para determinar la base genética de algunos rasgos fenotípicos en especies de árboles, aunque la mayoría de estos estudios se han llevado a cabo para caracteres de interés en especies con valor económico, como son sobrevivencia, crecimiento, propiedades de la madera, tolerancia a la sequía o la congelación y resistencia a plagas o enfermedades (Rehfeldt *et al*., 1999). Se ha encontrado una variación geográfica con una fuerte base genética en algunos caracteres, que frecuentemente se manifiesta en forma de clinas altitudinales y latitudinales muy marcadas, presumiblemente resultado de la selección natural y la adaptación local (García-Gil *et al*., 2003).

Sin embargo, las limitaciones de espacio o de tiempo hacen que los métodos tradicionales de la genética cuantitativa sean muchas veces difíciles de aplicar en el caso de los árboles. Asimismo, estos métodos no permiten identificar la variación de genes específicos que están relacionados con la variación fenotípica y la variación ambiental (González-Martínez *et al*., 2006).

La genética del paisaje es un campo emergente que busca comprender cómo características específicas de un entorno interactúan con procesos evolutivos como el flujo de genes, la deriva génica y la selección para determinar la cantidad y la distribución espacial de la variación genética (Manel *et al*., 2010; Sork *et al*., 2010a). Dentro de los patrones espaciales más comunes que se han descrito en la literatura se encuentran las clinas (Sokal y Thomson, 1998), el aislamiento por distancia (Cassens *et al*., 2000), la discontinuidad en el flujo de genes (Piertney *et al*., 1998, Keyghobadi *et al*., 1999) y los patrones aleatorios (Piglucci y Barbujani, 1991). La identificación de estos patrones genéticos espaciales requiere de la obtención de datos tanto de individuos como de poblaciones cuya ubicación geográfica se conozca exactamente, de manera

que al utilizar herramientas genéticas y estadísticas se pueden utilizar las frecuencias alélicas para determinar los patrones genético-espaciales y correlacionarlos con el paisaje o con algunas características ambientales (Manel *et al*., 2003).

La genética del paisaje ofrece una gama de métodos espaciales para estudiar la influencia de los procesos ecológicos en la variación genética (Storfer *et al*., 2007; Sork y Waits, 2010b). Uno de estos métodos es la utilización de modelos de distribución de alelos que son útiles para reconocer regiones del genoma con relevancia adaptativa, ya que aún cuando se trata de marcadores neutros, estos pudieran estar ligados a genes sujetos a selección. Por lo tanto, la premisa básica de estos modelos es que la selección natural genera cambios graduales en las frecuencias de los alelos de los loci ligados a genes seleccionados a lo largo de gradientes ambientales o en paisajes heterogéneos (Manel y Segelbacher, 2009; Manel *et al*., 2010a).

Algunos estudios de genética forestal han demostrado que la heterogeneidad ambiental influye en la diferenciación genética entre las poblaciones de árboles, creando patrones geográficos de variación genética que son consistentes con rasgos fenotípicos (Savolainen *et al*., 2007; Sork *et al*., 2010a). De tal manera que la información genética y ambiental espacialmente explícita puede utilizarse para buscar los impactos de la selección (Manel *et al*., 2003; Storfer *et al*., 2007) y la asociación entre la variación genética y ambiental ha sido bien establecida como una evidencia de selección natural (Endler, 1986; Manel *et al*., 2010; Sork *et al*., 2010b). Al analizarse estas asociaciones se pueden detectar variables climáticas específicas que están configurando la estructura genética de las poblaciones (Foll y Gaggiotti, 2008), e incluso identificar genes específicos que pudieran estar bajo una selección natural pronunciada (Joost *et al*., 2006; Sork *et al*., 2010b). Por otro lado, la identificación de loci bajo selección también puede ayudarnos a entender las

bases genéticas de la adaptación local, la diferenciación adaptativa y la especiación (Manel *et al*., 2003).

Aunque la genética del paisaje ofrece una gran variedad de métodos para estudiar la influencia de los procesos ecológicos en la variación genética, los métodos tradicionales de amplificación, secuenciación e identificación de genes con relevancia adaptativa es limitada. En los últimos años, la disponibilidad de secuencias de todo el genoma mediante las técnicas de secuenciación masiva, ha permitido identificar un gran número de loci, incluyendo loci potencialmente adaptativos (Segelbacher *et al*., 2010), incluso en sistemas no modelo, creando así la oportunidad para la "genómica del paisaje", campo que puede examinar simultáneamente los efectos de la historia demográfica, la migración y la selección (Sork *et al*., 2013). El interés por las exploraciones genómicas, el estudio de la variabilidad genética a través de genomas completos o a través de un gran número de loci de ambientes distintos (Coop *et al*., 2010) está creciendo (Haasl y Payseur, 2016; Jensen *et al*. 2016), y bajo este contexto, los análisis de escaneos genómicos eliminan la necesidad de conocimiento previo de fenotipos o genes candidatos (Bonin *et al*., 2007; Talbot *et al*., 2016). La señal de selección que conduce a la adaptación local en una especie se puede inferir de algunas maneras de los escaneos del genoma, y una de estas formas es cuando un locus o conjunto de loci tienen una diferenciación significativamente mayor de lo esperado (Schoville *et al*., 2012, Bragg *et al* 2015, Rellstab *et al*., 2015). Estos loci con mayor diferenciación relativa se denominan loci “outlier”. Uno de los métodos más populares para detectar tales loci ha sido comparando los valores de diferenciación (Fst) de loci individuales con un valor Fst esperado estimado bajo modelos demográficos neutrales (Beaumont y Nichols, 1996). Si los valores de Fst de locus individuales son significativamente más altos que los valores estimados bajo modelos demográficos neutrales, esto es un indicio de que tales loci pueden estar localizados en un gen o físicamente ligados a un gen bajo selección, y consideramos que tales loci son potenciales “genes candidatos” (Talbot *et al*., 2016). Los genes candidatos son aquellos genes cuya variación

polimórfica está relacionada con la variación de rasgos metabólicos, fisiológicos o fenotípicos, entre otros (Pflieger *et al*., 2001).

La genómica del paisaje, una herramienta para estudiar la adaptación local de los árboles forestales

Los árboles forestales son una de las formas de vida dominantes sobre la Tierra y tienen una gran importancia tanto ecológica como económica. Pueden vivir durante largos periodos de tiempo en una sola localidad, a diferencia de algunas especies de animales que se pueden mover grandes distancias o de las plantas herbáceas, que se pueden dispersar anualmente (Petit y Hampe 2006; Sork *et al*., 2013). Frecuentemente tienen grandes poblaciones que habitan ambientes heterogéneos, lo que los conduce a presentar una gran variación genética tanto dentro como entre poblaciones. Tiene un alto flujo de genes que se da principalmente por el polen, el cual puede dispersar esta variación a través del paisaje, pero la selección local de algunos caracteres adaptativos clave puede ser fuerte a este nivel (Savolainen *et al*., 2007). Un tema clave para la investigación de la genética forestal es cuantificar la capacidad de estas especies para adaptarse a condiciones ambientales locales (Kremer *et al*., 2012), en donde la identificación de los factores que impulsan esta adaptación local es un desafío importante para la biología evolutiva y la ecología en general. Tradicionalmente, la evaluación de la adaptación local de árboles forestales se basó en mediciones fenotípicas en experimentos de jardín común mediante la medición de rasgos cuantitativos a través de poblaciones que provenían de regiones con climas diferentes (Savolainen *et al*., 2007; De Kort *et al*., 2014), asumiendo que al crecerlas bajo un mismo ambiente se puede controlar la plasticidad fenotípica, pero los efectos maternos y el ambiente del jardín común pueden inducir variación fenotípica en las plantas, segando así las inferencias sobre la divergencia adaptativa (De Kork *et al*., 2014). De tal manera que estos experimentos actualmente se pueden complementar con amplios análisis de

secuencias con el objetivo de encontrar señales de diferenciación adaptativa y que no se confunden con la plasticidad fenotípica (De Kork *et al*., 2014; Lepais y Bacles, 2014).

El género Quercus

Los encinos son un grupo de plantas ampliamente distribuido y representado por un conjunto de arbustos que van de los 10 a los 60 cm y por árboles de 3 a 40 m de altura. Los encinos tienen un gran valor ecológico (en el almacenamiento de carbono, en los ciclos del agua y del oxígeno en la biosfera), económico (elaboración de recipientes, pisos, mangos para herramientas) y cultural (Luna-José *et al*., 2003). Todos los encinos comparten una serie de características biológicas comunes como son los tallos leñosos, las hojas coriáceas y la presencia de bellotas. Su desarrollo es lento, lo que los hace organismos de vida larga.

Estos árboles se encuentran dentro de la familia Fagaceae que comprende de seis a nueve géneros y alrededor de 600 a 900 especies. Las fagáceas se encuentran en las regiones montañosas del hemisferio norte en Europa, el sureste de Asia y el noroeste de África y en América los podemos encontrar desde el sur de Canadá hasta Colombia (Rodríguez-Correa *et al*., 2015), siendo el género *Quercus* es el que presenta mayor distribución en todo el mundo (Nixon, 2002; Valencia, 2004). Los encinos crecen principalmente en bosques templados, aunque también se les puede hallar en matorrales, pastizales y de forma intercalada en algunas selvas secas (Arizaga *et al*., 2009).

Se han descrito dos centros de diversidad para los encinos, uno en Asia y el otro en América (Nixon, 2009), siendo México el área del continente Americano con el mayor número de especies. En el país se encuentran presentes tres secciones, las cuales son *Quercus* (encinos blancos), *Lobatae* (encinos rojos) y *Protobalanus* (encinos intermedios) (Arizaga *et al*., 2009). Algunos autores estiman que la riqueza de especies de encinos en México se

sitúa en más de 160 especies (Valencia 2004), de las cuales 109 son endémicas del país y 47 pertenecen a la sección *Quercus*, 61 a la sección *Lobatae* y cuatro a *Protobalanus*. Sin embargo, la riqueza específica total del grupo en México ha sido difícil de precisar, debido a la gran variación morfológica (aún dentro de un mismo organismo), la escasez, carencia e inaccesibilidad del material tipo, además de la sobre descripción de muchas de las especies (Valencia, 2004). Por otro lado, la amplia gama de ambientes a los que se ven sometidas las especies y poblaciones de encinos juega un papel fundamental modelando la variación tanto fenotípica como genética a través de su efecto en los procesos demográficos y mediante la selección natural, ya que el clima influye directamente sobre la expansión poblacional, la contracción y la migración, dando como resultado una gran variación asociada a gradientes climáticos que contribuyen en la especiación, la evolución adaptativa y la divergencia en el género.

Bajo este contexto, el presente estudio tiene como objetivo principal analizar la variación morfológica, fisiológica y genética de las poblaciones de un encino blanco (*Quercus rugosa*) de amplia distribución e importancia ecológica y económica en México, la cual está asociada a un gradiente latitudinal y climático.

Quercus rugosa Née, es un encino blanco que pertenece a la sección *Quercus* (Rzedowski, 1986). Su tamaño varía entre los 3 y los 25 metros de altura, tiene una corteza gris castaño y escamosa, las hojas son ovaladas de 4 a 17 cm de largo con un margen de 3 a 17 dientes, un haz verde oscuro y un envés amarillento. Su fruto es anual, solitario y en grupos de 2 a 3 bellotas ovoides y que presentan una coloración rosácea en su interior. Es una de las especies de mayor distribución que va del sur de los Estados Unidos a Guatemala atravesando todo México. Tiene una gran importancia ecológica y económica ya que es una de las especies dominantes en las regiones montañosas donde puede encontrarse en forma monoespecífica o cohabitando con otras especies de encinos o pinos (Rzedowski, 1978). La distribución geográfica latitudinal de esta especie en México va desde las zonas templadas

de la Sierra Tarahumara en el estado de Chihuahua, en el norte de México, hasta las regiones subtropicales en la sierra de los Altos de Chiapas al sur de México, encontrándose en un intervalo altitudinal que va de los 1700 m a los 3550 m. A través de esta área de distribución, *Q. rugosa* presenta un amplio intervalo de variación morfológica con respecto a la forma y tamaño de sus hojas. En un trabajo previo en el que se estudió la variación morfológica foliar de *Q. rugosa* Née, se observó que existe una marcada diferenciación en el fenotipo de las hojas (principalmente en el tamaño) a través del gradiente latitudinal mencionado (Uribe-Salas *et al*., 2008). Además, mediante la utilización de modelaje bioclimático, se encontró que esta diferencia en el tamaño de las hojas está estrechamente relacionada con variables climáticas, que fueron principalmente la precipitación media anual y el índice de aridez anual. De esta forma, la variación latitudinal en la precipitación media anual, que oscila de alrededor de los 1000 mm en las localidades de Chiapas, a los 366 mm en las localidades de Chihuahua, se ve reflejada en una marcada disminución en el tamaño de las hojas (Uribe-Salas *et al*., 2008).

Estos datos iniciales permiten proponer la hipótesis de que existe diferenciación en atributos morfofisiológicos de *Q. rugosa* en respuesta a la variación en la precipitación y el índice de aridez a lo largo del gradiente latitudinal. Nuestros datos muestran que donde las poblaciones que viven en las zonas con menor precipitación y con mayor índice de aridez (IA) son las mejor adaptadas para sobrevivir en condiciones de sequía. Finalmente, sería muy probable que esta variación adaptativa tenga una firma genética.

Una vez que comenzamos la colecta de las bellotas observamos un patrón de variación muy marcado en su tamaño, de manera que la primera etapa de este trabajo (Capítulo II) se dirigió a conocer que factores eran los que determinaban esta variación en el tamaño y como éste influye en la germinación de las bellotas. En la segunda etapa del estudio (Capítulo III) se realizó un experimento de estrés hídrico en un jardín común que nos permitió reconocer

la amplia gama de estrategias y divergencia de atributos poblacionales que desarrollaron las plántulas de *Q. rugosa*. Además, mediante la utilización de marcadores genéticos neutrales (microsatélites), se obtuvieron los valores de diversidad y estructura genética. En la parte final del estudio (Capítulo IV) se realizó un escaneo genómico que nos permitió obtener 6873 SNPs, y mediante herramientas bioinformáticas se encontró que 108 de estos SNPs se asocian a una clina ambiental de temperatura y precipitación, evidenciando la firma genética de una adaptación local de las poblaciones ante distintas condiciones climáticas y geográficas.

CAPÍTULO II

CLIMATIC DETERMINANTS OF ACORN SIZE AND GERMINATION PERCENTAGE OF *Quercus rugosa* (Fagaceae) ALONG A LATITUDINAL GRADIENT IN MEXICO

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Climatic determinants of acorn size and germination percentage of *Quercus rugosa* (Fagaceae) along a latitudinal gradient in Mexico

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Abstract

Background. Abiotic constraints, historical effects of the last glaciation, and differential dispersal, have been proposed as potential explanations to account for the latitudinal decrease in acorn size of wide-ranging oak species distributed in the U.S. and Canada.

Hypothesis. We specifically tested the abiotic constraints hypothesis on oak acorn size in a geographical area without the counfounding influence of glaciation and related dispersal history.

Data description. Acorns from seven populations of the white oak *Quercus rugosa* were collected, encompassing the distribution of the species in Mexico.

Study site and years of study. Mexico, 2009-2010.

Results. Acorn length, width, mass and volume differed significantly among populations and indicated a marked clinal latitudinal reduction in acorn size. A multiple regression model revealed that this reduction in size (measured as acorn volume) can be explained by two important bioclimatic variables (growing season precipitation and growing season degree-days above 5 °C), while spatial variables (latitude and longitude) are not significant. Furthermore, germination percentage was significantly correlated to acorn mass and volume.

Conclusions. The main determinants of the latitudinal decline in acorn size are climate factors constraining seed development. This decline is maladaptive for seedling establishment, with important implications for the delination of northern limits of species ranges.

Key words: acorn size, climate factors, geographical variation, latitudinal distribution, *Quercus*.

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Determinantes climáticos del tamaño de las bellotas y el porcentaje de germinación de *Quercus rugosa* (Fagaceae) a lo largo de un gradiente latitudinal en México

Resumen

Antecedentes. Las restricciones abióticas, el efecto histórico de la última glaciación y la dispersión diferencial son posibles explicaciones para la disminución latitudinal del tamaño de las bellotas en especies de encinos de amplia distribución en los EE.UU. y Canadá.

Hipótesis. Se probó específicamente la hipótesis de las restricciones abióticas sobre el tamaño de las bellotas de encinos en un área geográfica sin influencia de la historia de colonización postglacial.

Descripción de datos. Se colectaron bellotas procedentes de siete poblaciones del encino blanco *Quercus rugosa*, abarcando la distribución de la especie en México.

Sitio de estudio y fechas. México, 2009-2010.

Resultados. La longitud de las bellotas, el ancho, la masa y el volumen difirieron significativamente entre las poblaciones y mostraron una marcada reducción latitudinal clinal en el tamaño de la bellota. Un modelo de regresión múltiple reveló que esta reducción en el tamaño (medido como volumen de bellota) puede explicarse por dos variables bioclimáticas importantes (precipitación en la temporada de crecimiento y días-grado por encima de 5 °C de la temporada de crecimiento), mientras que las variables espaciales (latitud y longitud) no fueron significativas. Adicionalmente, el porcentaje de germinación se correlacionó significativamente con el volumen y la masa de las bellotas.

Conclusiones. Los principales determinantes de la disminución latitudinal del tamaño de las bellotas son los factores climáticos que limitan su desarrollo. Esta disminución afecta el establecimiento de las plántulas, lo cual tiene implicaciones importantes en el estudio de los factores que limitan la distribución de las especies.

Palabras clave: distribución latitudinal, factores climáticos, *Quercus*, tamaño de bellotas, variación geográfica.

Seed size and seed mass are key ecological traits that play a fundamental role in the life history of plants, influencing many aspects of the patterns of natural regeneration of species, including the number of seeds that can be produced with a given amount of resources, seed predation and dispersal, seedling survival rate, and fitness (Venable & Brown 1998, Leishman *et al*. 2000, Dalling & Hubbell 2002). Seed size variation has been widely studied in a large number of species, in different habitats, and at global and local scales (Moles & Westoby 2004, Moles *et al*. 2007). A general pattern that has been documented at the global scale is the reduction in seed size by two or three orders of magnitude with latitude, from the equator to 60 °N (Moles & Westoby 2003, Moles *et al*. 2007).

In genus *Quercus* L., acorn size has been correlated positively with the rates of germination, and seedling emergence, survival and growth, both within and among species (Tripathi & Khan 1990, Bonfil 1998, Jakobsson & Eriksson 2000, Moles & Westoby 2004). Larger acorns also have higher frost resistance and, in general, are more stress tolerant (Aizen & Woodcock 1992, Gómez 2004). However, larger acorns may suffer higher predation rates (Gómez 2004, Muñoz & Bonal 2008, Yi & Wang 2015, Zhang *et al*. 2015) and bird dispersers usually prefer smaller acorns (Moore & Swihart 2006), suggesting the existence of conflicting selection pressures on acorn size (Gómez 2004).

Two main patterns of geographical variation related to acorn size have been described for oaks in North America. The first is a positive association between acorn size and geographical range among species, probably because species with larger acorns have greater success in seedling establishment, especially under limiting climatic conditions (Aizen & Patterson 1990). The second is a within-species latitudinal clinal decrease in acorn size that could potentially be explained by three different hypotheses (Aizen & Woodcock 1992, Koenig *et al*. 2009). The first is the abiotic constraints hypothesis, which postulates that acorn size is limited by precipitation, temperature, primary productivity, or some other factor that decreases with increasing latitude (Moles *et al*. 2007), predicting a strong correlation between environmental factors and acorn size independently of latitude, in the direction of smaller acorns being produced at sites with more constrained conditions. According to the second hypothesis, called the vicariance hypothesis, acorn size is related to abiotic factors such as soils or nutrients associated to the historical effect of the last glaciation, and thus the latitudinal pattern can be explained by whether areas were glaciated or not. The third hypothesis states that differential dispersal by blue jays, which prefer smaller acorns, is responsible for the latitudinal cline. An examination of the three hypotheses in *Quercus macrocarpa* gave strong support to the first hypothesis and to the second in a lesser extent, but the third hypothesis was unsupported (Koenig *et al*. 2009).

Quercus rugosa Née is the white oak species with the broadest latitudinal distribution in Mexico, from the Sierra Tarahumara in Chihuahua south of the border with Arizona up to the subtropical mountains in the highlands of Chiapas in southeastern Mexico (Rzedowski 1986, Uribe-Salas *et al*. 2008). Since Mexico was not glaciated, and the distribution of temperate forest tree species was probably affected only moderately by climatic changes during the glacial cycles (Jaramillo-Correa *et al*. 2009, Gugger *et al*. 2011, Ornelas *et al*. 2013), it is unlikely that historical post-glacial colonization could have left an imprint on acorn size of Mexican oak species, as has been proposed for U.S. and Canadian species. However, Mexico is characterized by substantial climatic gradients that could strongly influence phenotypic traits of wide ranging tree species, offering an excellent opportunity to test the abiotic constraints hypothesis in the absence of the confounding influence of glacial history and related dispersal history. So far, no data are available on the geographical variation of acorn size in any Mexican oak species. Therefore, in this study, we evaluated if there is geographical variation in the acorn size of *Q. rugosa* along the latitudinal and climatic gradient of its distribution to test the abiotic constraints hypothesis. Also, considering the ultimate importance of acorn size on the population dynamics of tree species, we also determined the geographical variation in germination percentage of *Q. rugosa* acorns and its correlation with acorn size.

Materials and methods

Study species. *Quercus rugosa* (section *Quercus*) is an ecologically important tree that can reach 25 m in height. It is an evergreen or sometimes brevideciduous species. The leaves are usually

cupped, rarely flat, thick and leathery with a thick yellow-brown tomentum on the abaxial surface and up to 10 cm in length. The bark is brown and scaly. The acorn of *Q. rugosa* is ovoid, elongated and wider at the base than at the apex. It is produced individually or in groups of 2 - 3 with a cup of 10 - 15 mm in diameter with brown or blackish scales. The cotyledons are usually reddish or pinkish, which is a distinctive trait of the species. *Quercus rugosa* has a wide geographical distribution, with a latitudinal range in Mexico that extends from the temperate zones of the Sierra Tarahumara in the State of Chihuahua, to the subtropics in the highlands of Los Altos de Chiapas in southern Mexico, and Guatemala at altitudes ranging from 1,700 m to 3,550 m (Rzedowski 1986, Uribe-Salas *et al*. 2008). It is one of the dominant species over much of this range, and it can be found in monospecific stands or cohabiting with other species of oak or pine.

Acorn collection and measurement. During the last week of October and the first week of November of 2009 we collected a total of 2,810 acorns in seven populations representing the latitudinal gradient of the distribution of the species in Mexico, from Chiapas to Chihuahua (Figure 1, Table 1). Mature and undamaged acorns were collected directly off the tree or from the ground

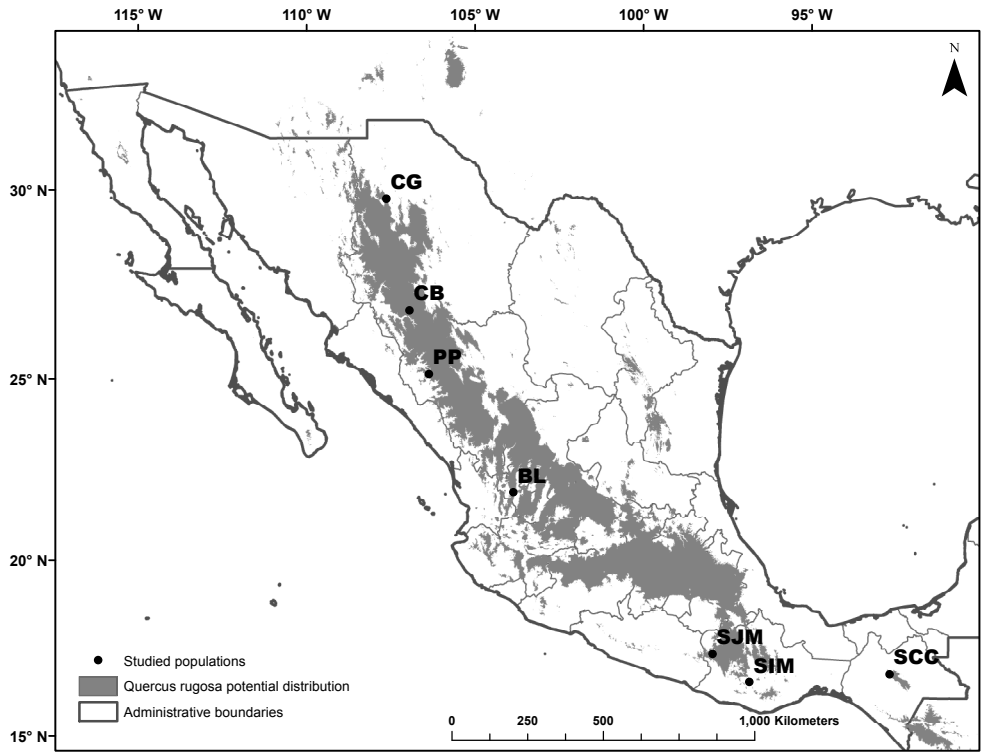

Figure 1. Map showing the seven *Quercus rugosa* populations collected in Mexico along the latitudinal/climate gradient. The numbering of populations is as in Table 1.

Table 1. Population name and population code, state, geographical, and climate data for the seven sampled sites listed south to north.

Population	Code	State	Latitude	Longitude	Altitude	GSP[a]	GSDD5[b]
1. San Cristóbal de las Casas	SCC	Chiapas	16.7639	-92.6935	2362	964	3338
2. Santa Inés del Monte	SIM	Oaxaca	16.9321	-96.8724	2527	760	3913
3. San Juan Mixtepec	SJM	Oaxaca	17.3454	-97.9510	2699	1059	2960
4. Bolaños	BL	Jalisco	21.8889	-103.8596	2300	771	3396
5. Papasquiaro	PP	Durango	25.1277	-106.3656	2573	849	1145
6. Caborachic	CB	Chihuahua	26.8343	-106.9455	2389	581	1359
7. Casas Grandes	CG	Chihuahua	29.7668	-107.6303	2409	409	1701

[a]GSP = growing season precipitation
[b]GSDD5 = growing season degree-days above 5 °C

below isolated trees from five different individuals per site. The number of seeds collected per mother tree varied between 16 and 180 and per site it varied between 240 (Casas Grandes) and 625 (San Cristóbal de las Casas) (Table 2). Groups of acorns from the same mother tree were packaged together in plastic burlaps, placed in a plastic cooler at environment temperature and transported to the laboratory where they were processed immediately. Transportation time was no longer than one week in all cases.

In a sample of 15 randomly chosen undamaged acorns from each of the five mother trees per population, we separated the cup from the nut and obtained the nut length (mm) and width (mm) with a digital caliper, and the fresh mass with an analytical balance. The volume (V) of each acorn was approximated assuming a prolate spheroid shape, using the formula $V = (4/3)\ \pi a^2 b$, where a is the width and b is the length (Aizen & Patterson 1990).

Table 2. Number of acorns collected per population, percentage of potentially viable acorns according to the water flotation test, and percentage of potentially viable acorns that germinated.

Population (code)	Number of acorns collected	Viable acorns (%)	Germination (%)
SCC	625	53.1	47.47
SIM	413	27.1	40.33
SJM	354	39.5	16.27
BL	298	81.9	39.58
PP	260	41.5	13.19
CB	620	54.0	21.04
CG	240	23.2	0.83

Viability and germination percentage. Acorns were sterilized by submerging them in water with 5 % chlorine bleach for ten minutes. At the same time, this served as a water flotation test of viability. In this way, for groups of acorns from each mother tree, we recorded the number and separated potentially viable seeds (the ones that sank) from inviable ones (the ones that floated). According with this test, the percentage of potential viability per population was between 27.1 and 53.1 % (Table 2). Then, all the potentially viable seeds were rinsed and humidified soaked in water for 24 h at 4 °C in a refrigerator. After that, acorns were individually sown in black plastic bags (25 cm long, 15 cm diameter, with drainage holes) filled with a substrate of 25 % agrolita, 25 % vermiculite and 50 % peat moss. Care was taken to introduce acorns into the substrate in approximately 2/3 of their length, with the apex pointing downwards. Bags were placed in a greenhouse with natural lighting in a randomized pattern and watered two times per week to keep a moisture level of approximately 30 % in the substrate. The position of the bags within the greenhouse was reshuffled every three or four weeks. The experiment was established in late November of 2009 and continued until early May 2010. Seeds were not moved or perturbed during this period and were considered germinated when the first leaves emerged. The germination percentage was recorded twice, in early March and in early May. After this date germination ceased and the results were analyzed, considering the total germination percentage.

Climatic variables. Climate data for each sampling site was downloded from the United States Department of Agriculture Forest Service web page (http://forest.moscowfsl.wsu.edu/climate/current/), a climate data set with derived variables designed for assessing plant-climate relationships (Rehfeldt 2006). We focused on two variables that capture the most important energy and water resource inputs for plants: growing season precipitation (gsp) and growing degree-days above 5 °C within the frost-free period (gsdd5) (a measure of heat accumulation) (Table 1).

Statistical Analyses. Differences among mother trees and among populations in acorn length, width, volume, and fresh mass were evaluated with nested analyses of variance. In these tests, both populations and mother trees within populations were considered to represent random samples from a larger pool. For each character, we calculated the proportion of the total varia-

tion (*i.e.* the variance components) due to differences at each of the two hierarchical levels. To compare germination percentage among populations, χ^2 tests were used. These tests were performed with the JMP 8 software (SAS Institute, Cary, North Carolina).

To test the abiotic constraints hypothesis, we performed multiple regressions of acorn volume on latitude, longitude, gsp, and gdd5 using R v3.0.0 languaje. All variables were centered prior to analysis and added variable (partial regression) plots were made to visualize the partial correlation of each climate variable after controlling for all other variables. Similar tests were done without including longitude or with only one climate variable at a time, and simple linear regressions were performed for each climate variable with each dependent variable to confirm their correlation. If the two derived climate variables were significant in the multiple regression models after accounting for the spatial variables (especially latitude), then we found support for the abiotic constraints hypothesis.

Results

The nested analyses of variance showed highly significant ($P < 0.001$) differences among mother trees within populations and among populations for the four morphological acorn traits evaluated (Table 3). However, the proportion of the total variation explained by differences among mother trees wihin populations was in general much smaller (7.47-11.9 %, depending on the trait) than the proportion explained by differences among populations (75.11-79.53 %). The residual variance (9.13-14.39 %) was interpreted as being explained by differences among acorns within mother trees. The population mean values for the four variables evaluated clearly indicated that southern populations had larger acorns than northern populations (Figure 2). Simple linear regressions indicated significant, positive relationships of acorn volume with gsp

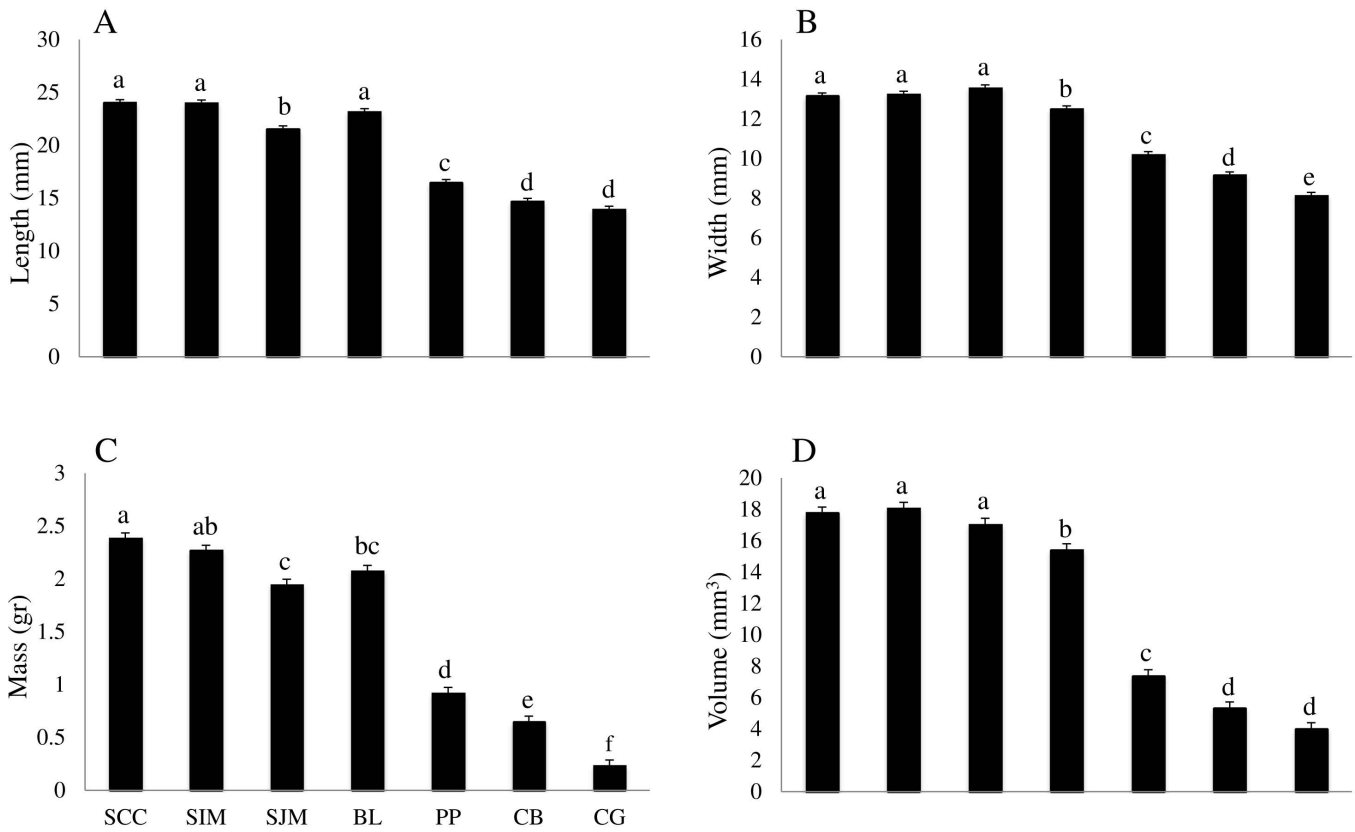

Figure 2. Mean and standard error for the four acorn morphological traits in the seven study populations: (a) length, (b) width, (c) mass, (d) volume. Different letters indicate significant differences according to an analysis of variance followed by Tukey-Kramer tests.

Table 3. Partitioning of variance by hierarchical level for four variables measured in the acorns of *Quercus rugosa*. Variance components and significance levels were determined with a nested ANOVA.

Variable	Level		
	Among populations	Among mother trees	Residual
Length	78.97***	11.90***	9.13
Width	79.53***	7.47***	12.99
Volume	75.57***	10.14***	14.29
Fresh mass	75.12***	10.49***	14.39

***$P < 0.0001$

($R^2 = 0.57$, $P = 0.049$) and with gdd5 ($R^2 = 0.86$, $P = 0.003$) and a strongly negative relationship with latitude ($R^2 = 0.94$, $P = 0.0003$).

According to the χ^2 tests, populations differed significantly in germination percentage ($\chi^2 = 239.6$; d.f. = 6; $P < 0.0001$) (Table 2). In general, germination percentage decreased latitudinally from 47.5 % in population San Cristobal de las Casas, Chiapas to 0.83 % in Casas Grandes, Chihuahua, although the relationship was only marginally significant ($R^2 = 0.54$; $P = 0.06$). Importantly, we found that germination percentage was significantly correlated with acorn volume ($R^2 = 0.60$; $P = 0.04$) and acorn fresh mass ($R^2 = 0.74$; $P = 0.013$), highlighting the importance of seed size on germination success.

In the multiple regression model of acorn volume with the two climate variables controlling for spatial variables, both climate variables were significant ($P < 0.034$), whereas spatial variables were not ($P > 0.23$) (Table 4, Fig. 3). This model has $R^2 > 0.99$. Multiple regression tests without longitude and with only one climate variable at a time gave similar results (not shown). These tests were not repeated for acorn fresh mass because of the high correlation (R^2=0.97; $P < 0.0001$) between this variable and volume.

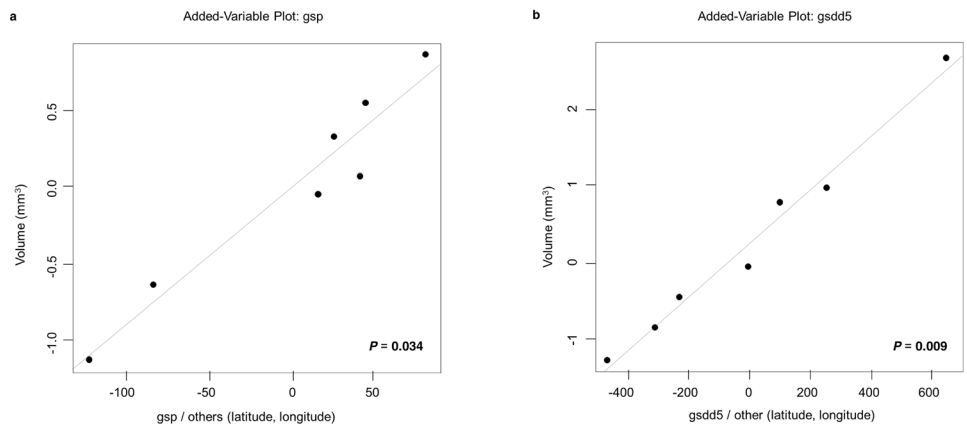

Figure 3. Added variable (partial regression) plots showing the partial correlation of (a) growing season precipitation (gsp) and (b) growing season degree-days above 5 °C (gsdd5) with acorn volume.

Discussion

Latitudinal variation in acorn size. *Quercus rugosa* showed a clinal pattern of south-north decrease in population mean acorn mass and volume across the distribution of the species in Mexico (Figure 2). According to the multiple regression analysis, growing degree-days > 5 °C and growing season precipitation are both significant when controlling for latitude and longitude, supporting the abiotic constraints hypothesis (Table 4, Figure 3). What is striking is that these relationships are highly significant even with a small sample size of seven sites. Although the relationship of latitude with acorn volume might become significant with increased sampling, this possibility would not change our interpretation. Most importantly, it is the significant correlations with climate that strongly support the abiotic hypothesis and specifically support the

Table 4. Results of the multiple regression analysis of acorn volume on spatial and climate variables of the seven acorn collection sites.

Acorn volume	Estimate	SE	*t*	*P**
Intercept	6.7E-10	0.12	0	1
Latitude	-0.26	0.15	-1.71	0.230
Longitude	-0.05	0.07	-0.73	0.541
GSDD5	3.5E-03	3.29E-04	10.76	**0.009**
GSP	8.8E-03	1.66E-03	5.31	**0.034**

*Bold values are significant

idea that primary water and energy inputs are important for acorn size and germination percentage in *Q. rugosa*.

Reduced seed size along latitudinal gradients in species exhibiting substantial northward postglacial migration have been interpreted as evidence of selection on small seed size because more dispersable seeds would be more likely to advance and establish northward (Cwynar & MacDonald 1987, Koenig *et al*. 2009). Our results from a Mexican oak whose history is not confounded by glacial migration history question that idea and instead support studies showing that the main determinants of within-species variation in acorn size are climate factors that constrain seed development.

Germination success. Several studies have shown a positive effect of acorn size on germination success, and seedling survival and growth at the intraspecific level (Bonfil 1998, Tripathi & Khan 1990, Tilki 2010, Urbieta *et al*. 2008, Sage *et al*. 2011). These effects are mostly due to the amount of resources stored in the seed, because larger seeds can survive for longer periods of time under unfavorable conditions, while smaller seeds will consume their food reserves in the process of respiration and physiological adjustment. After germination, seedlings that emerge from larger seeds will have more energy for initial growth and for recovery from herbivore attack (Bonfil 1998, Urbieta *et al*. 2008). However, most of these studies have involved a single or few populations, and in few cases the relationship between acorn size and germination success has been examined along large geographical or environmental gradients.

One important question that arises from the detection of a clinal pattern in acorn size is whether variation is a result of plastic responses to the limiting climatic factors or is genetically determined, as a result of selection for the production of viable offspring under the constraints of local climatic conditions (Aizen & Woodcock 1992). Our results showed that the latitudinal decrease in population mean acorn size was correlated with a concomitant reduction in germination percentage. In fact, the germination success in the northernmost population was extremely low, less than 1 %. However, it must be acknowledged that, in general, the germination percentages reported in this study are somewhat lower than the ones found in other studies with geographically restricted samples of the same species (40-92 %) (Bonfil & Soberón 1999, López-Barrera & Newton 2005, Huerta-Paniagua & Rodríguez-Trejo 2011). These differences could be due to methodological issues related to transportation and storage of the acorns but, most probably, to the criteria used to score germination. In other studies, seeds have been considered germinated when the radicle reaches a size equal to the length of the same seed (*e.g*. Huerta-Paniagua & Rodríguez-Trejo 2011), while in our case we waited until the emergence of the first leaves. However, even in this case, the extremely low germination percentage in the Casas Grandes populations seems odd and it could be explained if during the year of collection trees or acorns were subjected to an extreme stress factor (*i.e*. frost or drought). Therefore, further studies should also consider interannual variation in seed size and viability.

Nevertheless, we consider that our data in general reflect the fact that seed production and development faces more severe constraints at the northernmost part of the range of *Q. rugosa*. Overall, our results do not seem to support the size-optimization-through-selection hypothesis to explain acorn size variation in *Quercus rugosa*. Also, according to our personal observations,

populations of this oak species become progressively smaller and its distribution more fragmented towards the north, suggesting limited recruitment success at higher latitudes. As was also stated by Aizen & Woodcock (1992), this evidence indicates that the latitudinal decline in acorn size is maladaptive for seedling establishment, with important implications for the delineation of northern limits of species ranges.

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CAPÍTULO III

FUNCTIONAL DIFFERENTIATION IN RESPONSE TO DROUGHT STRESS IN *Quercus rugosa* POPULATIONS ALONG A CLIMATIC GRADIENTS IN MEXICO

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INTRODUCTION

The spatial heterogeneity of climatic factors is considered one of the main selective forces in nature (Jump *et al*., 2006; Ramírez-Valiente *et al*., 2010a) that may lead to the differentiation of functional traits among plant populations as a consequence of local adaptation (Savolainen *et al*., 2007; Marchin *et al*., 2008; Ramírez-Valiente *et al*., 2015a). Population genetic diversity and structure, as well as the distribution ranges of species are also related in part to processes of local adaptation (Ramírez-Valiente *et al*., 2009; 2010b; 2014a; Berlin *et al*., 2014), which promotes genetic differentiation even with gene flow (Allen *et al*., 2006; Ramírez-Valiente *et al*., 2015a). Additionally, neutral evolution and demographic history also contribute to population differentiation (Leinonen *et al*., 2006; Ramírez-Valiente *et al*., 2014b).

Numerous studies have reported functional adjustments among tree populations as a response to specific climatic factors, particularly in foliar traits and growth rate (Ramírez-Valiente *et al*., 2015b), crown architecture (Ramírez-Valiente *et al*., 2014a), physiological and metabolic functions such as water use efficiency and the accumulation of photoprotective pigments (Ramírez-Valiente *et al*., 2015a). Such correlations help to understand which are the key environmental factors that influence morphological and physiological variation in trees (Traiser *et al.,* 2005; Uribe-Salas *et al*., 2008).

In several oak species (e. g. *Quercus ilex, Q. petraea*, *Quercus suber*, *Q. virginiana, Q. oleoides*) a positive correlation between foliar area and precipitation has been observed in natural populations, and between foliar area and soil moisture in the case of greenhouse experiments, while leaf thickness and specific mass increase with aridity (Aranda *et al*., 2007; Gouveia and Freitas 2009; Ramírez-Valiente *et al*., 2009a, 2009b). In oak species with large distribution ranges such as *Q. alba, Q. macrocarpa, Q. rubra* and *Q. velutina*, it also has been observed that acorn size and mass show a latitudinal decrease

(Aizen and Woodcock, 1992), in association with climatic gradients (Koenig *et al*., 2009). These examples illustrate the quantitative traits that can show plastic or adaptive variation, allowing oak species to develop under a wide range of environmental conditions and resource availability.

Quercus rugosa Née is one of the white oak species (section Quercus) with larger distribution ranges in Mexico. This species is found from the temperate areas in the Sierra Tarahumara in Chihuahua state in northern Mexico to the subtropical mountains in the Sierra de Los Altos de Chiapas in southern Mexico, in an altitudinal range from 1700 to 3550 m. Throughout this distribution area, *Q. rugosa* shows a wide variation in leaf size and shape. In a previous study that quantified this foliar variation it was found that there is a clinal reduction in leaf size in a south-north direction, in association with two climatic variables, mean annual precipitation and an annual aridity index (Uribe-Salas *et al*., 2008). This environmental variation is considerable; for example, mean annual precipitation for *Q. rugosa* populations varies from 1200 mm in the localities in Chiapas to 500 mm in the localities in Chihuahua (Uribe-Salas *et al*., 2008).

This previous result allows hypothesizing that there is also differentiation among *Q. rugosa* populations in other important functional traits, as a result of local adaptation through divergent selection along the environmental gradient experienced by the species in its distribution area in Mexico. Therefore, in this study we asked the following questions: 1) Is there phenotypic differentiation in morphological and physiological traits expressed in *Q. rugosa* seedlings in a common garden? 2) What are the morphological and physiological responses of seedlings from different populations to chronic drought stress? 3) Are phenotypic differentiation and plastic responses to drought stress related to the climatic gradient along the latitudinal distribution of the species in Mexico, and 4) Are the phenotypic patterns related to neutral genetic structure?

Material and methods

Acorn collection and germination

During the last week of October and the first week of November of 2009, acorns were collected from six *Q. rugosa* populations along a latitudinal and climatic gradient (Table 1 and Fig. S1; see Llanderal-Mendoza *et al*. 2017 for more details). In each population, acorns were collected from at least five mother plants, directly off the tree or below isolated individuals. The methods for acorn germination are described in Llanderal-Mendoza *et al*. (2017). Following germination, seedlings were grown under homogeneous conditions for one year in a green house. During this period, the seedlings were watered every three days and their position within the green house was randomly reshuffled every three weeks.

Phenotypic variation among populations after one year

After one year (2010) seedlings from each population were measured. We registered the total height of each seedling from the base of the plant to the apical meristem, as well as the length and width of the three fully developed newest leaves. For the leaves, we also calculated a shape index by dividing the length by the width.

Drought stress experiment

In December 2010, the same number of seedlings between 15 and 75 from each population were assigned to one of two treatments: well-watered plants were maintained at about 30% of soil moisture (representing a water potential of about 0 MPa), while the drought-stressed plants were maintained at about 5% of soil moisture (-1.5 MPa). Moisture levels were monitored and adjusted twice per

week with the aid of a soil moisture meter (ML3 ThetaKit, Delta-T Devices). The treatments were maintained over the course of one year, with the position of each individual seedling being randomly reshuffled within the green house every month. After the one year period, we performed the morphological and physiological measurements described below.

Morphological and physiological measurements

In at less 12 seedlings per population and treatment, we quantified the height of each seedling (as defined above), the stem diameter at the base of the plant, the number of leaves and, in the last three fully developed leaves, length and maximal width were measured. Leaf shape was evaluated by dividing length by maximal width. In the same three leaves, thickness was determined with a digital caliper and chlorophyll concentration was estimated with a SPAD 502DL Plus instrument (Minolta). In three leaves from each of five randomly selected individuals per population, we cut a 1 cm^2 square and obtained its fresh weight. After that, the squares were dried to constant weight at 70 °C and the leaf dry mass content was calculated as the dry weight divided by the fresh weight.

Physiological functions of CO_2 assimilation (*Amax*), transpiration (*E*) and stomatal conductance (*G*) were measured between 0800 and 1200 hours with a LI-6400XT portable infrared gas analyzer (IRGA, LICOR Biosciences). Measurements were performed on two mature leaves per seedling and ten randomly selected plants per population and treatment at ambient CO_2 concentration (400 µmol mol^{-1}), photon flux density of 1600 µmol m^{-2} s^{-1} and a leaf temperature of 25 °C.

Dark-acclimated chlorophyll fluorescence (Fv/Fm) was measured to determine the maximum photosynthetic quantum yield and non-photochemical quenching (NPQ) in one leaf from six individuals per population and treatment using pulse-amplitude modulation (PAM) fluorometry with the 6400-40 leaf

chamber flurometer attached to the LI-6400XT system. Measurements were taken after 2100 hr to allow dark adaptation of the photosystem using actinic light intensity of 1400 μmol m^{-2} s^{-1} and 400 μmol m^{-2} s^{-1}.

To evaluate plant internal water status under the two watering treatments, predawn (ψ_{PD}) and midday (ψ_{MD}) leaf water potential was measured in 88 leaves from 88 individuals using a Scholander pressure chamber.

Microsatellite amplification and genetic differentiation

Genomic DNA was extracted from 100 mg of leaf tissue from 136 individuals randomly selected per population using the Qiagen DNeasy plant mini kit (QIAGEN). Eight nuclear microsatellites (QpZAG36, QpZAG110, QrZAG39, quru-GA OC19, quru-GA OC11, quru-GA OI01, quru-GA OM07 and quru-GA OM05), previously designed for *Quercus petraea* (Steinkellener *et al*., 1997), *Q. robur* (Kampfer *et al*., 1998) and *Q rubra* (Aldrich *et al*., 2002) were selected on the basis of the quality of amplification in preliminary trials. Polymerase chain reactions were performed in two separate multiplex reactions, the first one containing primers QpZAG36, QrZAG39, QpZAG110 and quru-GA OC19 and the second containing primers quru-GA OC11, quru-GA OI01, quru-GA OM07 and quru-GA OM05). Final volume of the reactions was 5 μL, containing 1.5 μL of template DNA with a concentration of 20 ng/μL, 2.5 μL 2x Multiplex PCR Master Mix, and 1 μL of the primer mix with a concentration of 2 μM each one. Thermal cycling conditions were 35 cycles with a denaturation step at 94ºC for 1:30 min, alignment for 1:30 min at 50 °C for the first primer group and at 48 °C for the second primer group and extension at 72 ºC for 1:30 min. A final extension step for 10 min at 72 °C was included. PCR products were run in an automatic capillary sequencer Avant 3100 with the GenScan-500 LIZ (Applied Biosystems) size marker. Final sizing analysis of the fragments was performed with the software Peak Scanner 1.0 (Applied Biosystems).

To assess the pattern of genetic structure, microsatellite data were subjected to a Bayesian clustering analysis in the program STRUCTURE (Pritchard *et al*., 2000; Falush *et al*., 2003; Hubisz *et al*., 2009). Runs were performed using the admixture model without prior population information. The program was set to run with values for the number of assumed genetic clusters (*k*) from one to six, with ten independent runs for each *k*. The length of the burn-in period was 50,000, followed by 10^5 Markov chain Monte Carlo repetitions. The value of k best fitting the data was identified with the method of Evanno *et al*. (2005) to compute Δ*k*, an ad hoc quantity that is a good predictor of the real number of genetic clusters.

The amount of genetic differentiation among populations was determined with an Analysis of Molecular Variance (AMOVA) in ARLEQUIN 3.5 (Excoffier et al. 2005), grouping the populations according to the STRUCTURE analysis (see Results). For AMOVA, F_{ST} (infinite alleles mutation model) was used as the measure of differentiation and 10,000 permutations were performed to estimate significance levels (Excoffier *et al*. 2005). A matrix of pairwise F_{ST} values among populations and their significance was also calculated in ARLEQUIN 2.5.

Geographic and climate variables

Geographic variables latitude, longitude and altitude were taken at each collection site with a GPS instrument. The 19 bioclimatic variables were downloaded from the WorldClim site (http://www.worldclim.org/) while 20 additional variables were obtained from United States Department of Agriculture Forest Service web page (http://forest.moscowfsl.wsu.edu/climate/current/). Redundancy among variables was assessed through a matrix of pairwise correlations, eliminating one from each pair of variables with a correlation equal of higher than 0.8. A set of nine variables was obtained in this way, including mean annual precipitation (map), growing season precipitation (gsp), degree-days > 5 °C accumulating within the frost-free period (gsdd5), annual dryness

index (adi), summer precipitation balance (spb), maximum temperature of the warmest month (mtwm), mean temperature of the coldest quartet (mtcq), precipitation seasonality (ps) and precipitation of the driest quartet (pdq) (Table 1).

Data analysis

First year measurements were subjected to analyses of variance (ANOVA) for each trait, with population as the explanatory factor. Results from the drought stress experiment were compared among populations within the two treatments with one-way ANOVAs for each trait, and also a global two-way ANOVA analysis was performed, with population, treatment and their interaction as the explanatory factors.

To visualize the pattern of multivariate phenotypic variation between the two treatments and the six populations, a principal components analysis was performed (PCA). The association between variation in each phenotypic trait and geographic (latitude) and climatic variables (the nine variables specified above) was assessed through simple pairwise correlation tests. Multivariate redundancy analyses (RDA) with variance partitioning were performed to separate the effect of geography, climate and their combination on overall phenotypic variation. RDA has proven more powerful in detecting complex species–environment relationships and spatial structures in multivariate data than Mantel tests or regression on distance matrices (Riordan *et al*., 2016). Separate RDA analyses were performed for plants in the well-watered and the drought stress treatments. All analyses were performed in JMP ver. 8 (SAS Institute) except RDAs which were performed in R using the vegan package (Oksanen *et al*., 2011).

RESULTS

Phenotypic differentiation under homogeneous conditions

After one year of growth in the green house under optimal watering conditions populations displayed significant variation in all traits measured (Table 2). In general, a pattern of south-north differentiation was observed, with southern plants being larger in size, with more leaves which were also larger and had a more elongated shape. Variation in all traits was negatively and significantly correlated with latitude, and almost all traits were positively correlated with the values of MAP, gsdd5 and mtcq of the collection localities (Table S1).

Drought stress experiment

After one year of growth under the two watering treatments all measured morphological and physiological traits displayed significant differences (Tables 3 and 4). Differences between the two watering regimes were observed in all cases except for leaf shape, while the population effect was also significant except for leaf thickness, dry matter content and ψ_{MD}. Finally, a significant interaction between population and treatment was observed for seedling size, stem diameter, leaf thickness, leaf area, stomatal density (Table 3) and all physiological traits except ψ_{MD} (Table 4).

In general, plants grown under water stress were smaller by all measures and had lower chlorophyll content and less stomata (Table 3). Also, the differentiation pattern among populations was similar to the one observed at the first measurement: plants from the northern sites are smaller than plants from the southern populations. However, differences between the two treatments were in general more considerable for the southern populations and less pronounced in the northern populations (Table 3). For the physiological functions, it was interesting to observe that in the drought stress treatment, northern populations retained a higher *Amax* than southern populations and had a considerably higher *WUE* (Table 4).

As in the first-year measurements, both morphological and physiological traits showed significant correlations with geographical and climatic variables of the collection localities (Tables S2 and S3). The climatic variable that showed the highest number of correlations with morphological traits in both the well-watered and drought stress treatments was gsdd5, followed by spb and ps (Table S2). In the case of the physiological traits, most correlations with climatic variables were observed only for plants under the drought-stress treatment. A_{max} and *WUE* were positively correlated with latitude (Table S3), while A_{max}, *G*, *E* and *WUE* were negatively correlated with gsdd5. Both ψ_{PD} and ψ_{MD} were negatively correlated with spb.

RDA analyses, conducted to separate the effects of geography and climate in phenotypic variation, separately for the plants in the two watering treatments, indicated that gsp, spb, gsdd5 and mtcq explained 68.3% of the phenotypic variation in the well-watered treatment, while geography explained 17% and the combination of geography and climate explained 14.8% (Figure 1). In the case of the drought-stress treatment, 37.5% of the phenotypic variation was explained by spb, gsp, gsdd5 and ADI, while geography alone explained 10.2 % of the variation and the combination between geography and climate explained 52.2% (Figure 2).

Genetic structure

The analysis in STRUCTURE indicated that the most probable value of *k* is 3 (Fig. S2). According to this result the three southern populations (San Cristobal, Santa Inés and Mixtepec) showed a large proportion of their genetic ancestry in the first genetic group, while populations Bolaños and Papasquiaro showed predominantly the second genetic group and in population Caborachic the third genetic group predominated. However, the AMOVA analysis revealed that this genetic structuring is not very strong. With populations grouped according to the STRUCTURE results, the amount of the genetic variation among the groups was

4.89% (P = 0.01), while the amount of genetic variation among populations within groups was 3.11% (P < 0.0001) and the variation within populations was 92%. The overall differentiation value was F_{ST} = 0.08 (P<0.0001). The pairwise F_{ST} values (Table S4) ranged from 0.008 (between populations Santa Inés and Mixtepec) to 0.11 (between populations Papasquiaro and Caborachic). All differentiation values were significant except for populations Santa Inés and Mixtepec.

DISCUSSION

Numerous studies have been published showing that forest tree species, like oaks, express a wide variation in functional traits as a result of local adaptation in response to differing climatic conditions, mostly related to temperature and precipitation levels (Acherar and Rambal, 1992; Long and Jones 1996; Fotelli *et al*., 2000; Aranda *et al*., 2007; Ramírez-Valiente *et al*., 2014; 2015; Aguilar-Romero *et al*., 2017;). However, fewer studies have simultaneously assessed the association of trait variation with the underlying genetic structure of the populations. In this study, we showed that differences in functional traits related to the growth of *Q. rugosa* seedlings persisted for a year under homogenous conditions in samples collected along a climatic gradient from southern to northern Mexico and also under the well-watered treatment for a second year. We also showed that plants subjected to a chronic drought stress expressed plastic responses that differed among populations. Furthermore, the overall phenotypic differentiation patterns were concordant with the neutral genetic structure of the populations.

In general, the divergence pattern observed between northern and southern populations is expected for plants adapted to contrasting temperature and precipitation regimes (Ramírez-Valiente *et al*., 2010b). Size was in general smaller (reflecting a slower growth rate) in plants from the northern populations, but these populations showed a higher capacity to maintain physiological

functions and higher *WUE* in drought stress conditions. These plants had smaller but thicker leaves, an adaptation that is common in arid environments that allows the avoidance of excessive water loss and the maintenance of photosynthetic activity for longer periods under water deficit (Dudley 1996a; 1996b; Faria *et al*., 1996; Corcuera *et al*., 2002). A higher maximum photosynthetic rate (*Amax*) could be a trait associated positively with more sclerophyllous leaves, compensating the reduction in leaf size by increasing the efficiency of the photosynthetic apparatus. Additionally, *Amax* correlated positively with *G*, *E* and *WUE*, further indicating the advantages of sclerophyllous leaves in more arid climates. Similar changes have been observed in Mediterranean evergreen oaks (Leiva and Fernandez-Alés 1998; Bussoti *et al*., 2002).

Both univariate correlations and the RDA analyses indicated that the trait variation is associated to the variation in climatic variables across the distribution range of *Q. rugosa* in Mexico. The variables that appeared to have the strongest correlation with phenotypic variation were in general gsdd5, spb, gsp mtcq and ADI. These results indicate that temperature or precipitation are not independently driving phenotypic patterns in this oak species. Instead, it seems like their particular combinations along the environmental gradient are defining different strategies for optimal growth and survival under different limiting conditions. Overall, our results strongly suggest that the clinal pattern of phenotypic differentiation in the functional traits of *Q. rugosa* populations is the result of local adaptation that has evolved in response to divergent selection pressures across the environmental gradient. Interestingly, the phenotypic variation in leaf size and shape that we observed in our common garden experiment exactly matches the pattern previously observed in field-collected leaves from adult individuals (Uribe-Salas *et al*., 2008), further supporting the conclusion that these differences are genetically controlled and expressed in both seedlings and adults.

However, one important aspect that should be considered is the possible impact of conflicting selection pressures or effects at different stages of the life cycle, leading to alternative optimization solutions in terms of the life history traits. As was demonstrated in the previous chapter (Llanderal-Mendoza *et al*., 2017), acorn mass and germination percentage in *Q. rugosa* also follow the latitudinal trend towards a reduction at more northern populations, in association with more limiting growing conditions at these sites. Still, it was concluded that this acorn mass reduction is probably not adaptive. In general, at the intraspecifc level, smaller acorns show lower germination success, and result in seedlings with lower survival and growth (Bonfil 1998; Tripathi and Khan 1990; Tilki 2010; Urbieta *et al*. 2008; Sage *et al*. 2011). We did not record survival in this study but it was in general similar among all populations. However, it is remarkable that the smaller acorns from northern populations, as expected, produced smaller and slow-growing seedlings, but these were much better adapted to withstand drought stress than seedlings from southern populations.

Even though local adaptation along an environmental gradient may be a good explanation for clinal patterns in functional traits, other historical processes such as secondary contact between previously diverged populations or isolation by distance may create such patterns. Therefore, in testing the local adaptation hypothesis it is important to attempt to disentangle the correlation between trait variation and geography on one side and climate on the other side (e. g. Riordan *et al*. 2016). RDA is a multivariate procedure which allows the separation of both effects as much as possible. In general, our results indicated a stronger contribution of climate variables alone than geography alone on overall phenotypic variation. However, a generally large proportion of the variation remained explained by both geography and climate combined.

Finally, the microsatellite analysis permitted a broad evaluation of genetic structure and gene flow among the evaluated populations. The results indicated that differentiation among populations is low (F_{ST} = 0.08) implying historically

high gene flow levels among *Q. rugosa* populations despite the considerable geographical distance, as was also observed along the Trans-Mexican Volcanic Belt populations using a genomic approach (F_{ST} = 0.055; Martins et al. in prep, chapter 3 this thesis). The Bayesian assignment procedure (i. e. STRUCTURE) found evidence of three main genetic groups that followed the latitudinal order of the populations, that is, the three southern populations (San Cristóbal, Santa Inés and Mixtepec) had a larger proportion of the first genetic group, the second genetic group predominated in Bolaños and Papasquiaro and the third genetic group was mostly found in population Caborachic. However, the assignment also indicated a considerable degree of admixture among the groups and the AMOVA analysis showed that the differentiation between these three population groups is low (4.89%). The pairwise F_{ST} analysis showed that differentiation values between population pairs varied between 0.008 (Santa Inés and Mixtepec) and 0.11 (Papasquiaro and Caborachic). All pairwise differentiation values were significant except between Santa Inés and Mixtepec. Together, these results indicate that the genetic structure of *Q. rugosa* populations resembles more an isolation by distance pattern than a clear-cut structuring in population groups. Nevertheless, it seems like the remarkable phenotypic differentiation documented here has occurred on the face of very high historical gene flow levels. A similar conclusion was obtained in chapter 3 (Martins *et al*. in prep.) in which only a few SNPs showed F_{ST} values higher than the average background differentiation and some of those also showed significant associations with climate variables, providing further strong support to divergent selection and local adaptation processes on the evolution of *Q. rugosa* populations.

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Table 1.Geographical coordinates and climatic variables for the sampling localities. Alt, altitude (m); map, mean annual precipitation (mm); gsp, growing season precipitation (mm); gsdd5, degree-days > 5 ºC accumulating within the frost-free period; adi, Annual dryness index; spb, summer precipitation balance (jul+aug+sep)/(apr+may+jun); mtwm, maximum temperature of the warmest month (ºC); mtcq, mean temperature of the coldest quarter (ºC); ps, precipitation seasonality; pdq, precipitation of the driest quartet (mm).

Code	Nearest locality	Lat N	Long W	Alt	map	gsp	gsdd5	adi	spb	mtwm	mtcq	ps	pdq
SC	San Cristóbal	16º45’	92º41’	2382	1203	964	3338	2.942	347	24.3	14.1	102.03	58
SIM	Santa Inés del Monte	16º55’	96º52’	2237	1458	760	3913	2.703	388	29.5	16.7	132.6	14
SJM	San Juan Mixtepec	17º20’	97º57’	2699	1120	1059	2960	2.830	432	27.3	14.8	130.4	57
Bol	Bolaños	21º53’	103º51’	2294	689	771	3396	5.694	450	28.7	13.0	118.4	6
Pap	Santiago Papasquiaro	25º07’	106º56’	2573	615	849	1145	3.317	510	26.8	11.4	91.2	46
Cab	Caborachic	26º50’	106º56’	2389	508	581	1359	4.626	362	29.6	9.9	92.6	34

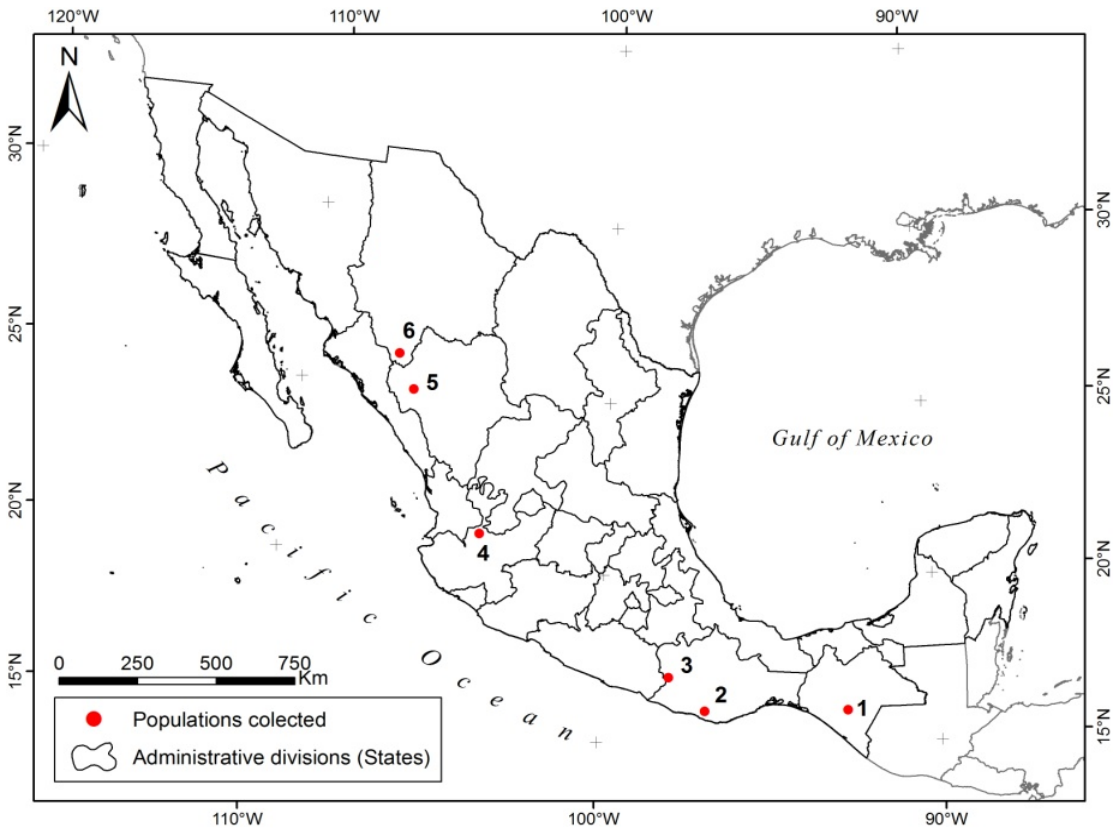

Figure S1. Map of Mexico showing the geographical location of the six sampling localities.

Table 2. Analysis of variance for five morphological traits in six populations of *Q. rugosa* after one year of growth in a common garden under optimal watering conditions. Different letters indicate significant differences after a post-hoc Tukey test. Ss: seedling size, Nl: number of leaves, Ll leaf length, Lw: leaf width, Ls: leaf shape index.

Population (Code)	Ss (cm)	Nl	Ll (cm)	Lw (cm)	Ls (Ll/Lw)
SC	11.71 ± 0.63 a	8.03 ± 0.40 a	6.47 ± 0.27 a	3.41 ± 0.15 a	1.93 ± 0.03 a
SIM	12.99 ± 0.59 a	7.77 ± 0.38 a	6.00 ± 0.25 a	3.35 ± 0.14 a	1.80 ± 0.03 ab
SJM	11.28 ± 0.82 a	7.15 ± 0.52 ab	5.97 ± 0.35 a	3.46 ± 0.20 a	1.73 ± 0.04 bcd
Bol	10.84 ± 0.69 a	7.40 ± 0.44 a	4.50 ± 0.30 b	2.53 ± 0.17 b	1.77 ± 0.03 bc
Pap	4.74 ± 0.69 b	5.40 ± 0.43 bc	3.06 ± 0.30 c	1.87 ± 0.17 b	1.62 ± 0.03 cd
Cab	4.57 ± 0.59 b	5.05 ± 0.38 c	3.55 ± 0.25 bc	2.31 ± 0.14 b	1.57 ± 0.03 d
P -value	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**	**< 0.0001**

Table S1. Pearson correlation coefficients between population means of five morphological traits with the latitude and nine climatic variables of the collection sites. Only significant values (P < 0.05) are shown.

Morphological variables	**Climatic variables**									
	Lat	Map	gsp	gsdd5	adi	spb	mtwm	mtcq	ps	pdq
Ss	-0.910	0.855		0.983				0.920	0.830	
Nl	-0.919	0.811		0.961				0.858		
Ll	-0.966	0.904		0.852				0.849		
Lw	-0.937	0.891						0.831		
Ls	-0.852			0.838						

Table 3. Population means and standard errors of 11 morphological traits measured in *Q. rugosa* seedlings under two watering treatments after one year. The p-values of within treatment ANOVA analyses for the comparison of populations are shown. Different letter indicate significant differences among populations after a Tukey test. The last three rows show the P-values for a two-way ANOVA with population, treatment and the interaction of population and treatment as the explanatory factors. WW, well-watered treatment; DS, drought-stress treatment; Ss; seedling size; Nl, number of leaves; Ll, leaf length; Lw, leaf width; Ls, leaf shape; Sd, stem diameter; Lt, leaf thickness; Tla, total leaf area; DMC, dry matter content; Chl, chlorophyll content; Stom, stomata.

Treat	Pop code	Ss (cm)	Nl	Ll (cm)	Lw (cm)	Ls	Sd (mm)	Lt (mm)	Tla (cm2)	DMC (%)	Chl (spad units)	Stom
WW	SC	33.6±2.2b	32.5±2.9a	11.9±0.4a	7.0±0.2a	1.7±0.04a	18.5±1.9ab	0.55±0.02ab	73.3±5.9a	34.4±1.5ab	48.14±1.3ab	78.2±2.8bc
	SIM	41.32±2.1ab	32.6±2.9a	10.8±0.4ab	6.5±0.2ab	1.6±0.04bc	20.1±2.0ab	0.49±0.02b	52.1±7.1ab	36.23±1.5a	48.71±1.2a	93.6±2.8a
	SJM	42.7±3.0ab	28±4.1ab	9.2±0.6bc	6.1±0.3ab	1.5±0.05ab	23.9±2.2a	0.54±0.02b	43.2±7.7b	33.01±1.5ab	48.18±1.7ab	63.6±2.8d
	Bol	45.3±2.3a	41.3±3.1a	9.7±0.4bc	5.8±0.2b	1.7±0.04ab	17.3±2.0abc	0.56±0.02ab	46.6±7.1ab	34.53±1.5ab	46.2±1.3ab	68.6±2.4cd
	Pap	19.5±3.8c	15±5.1b	7.8±0.7c	5.2±0.4b	1.5±0.07abc	9.43±2.0c	0.66±0.02ab	20.1±8.4b	28.62±1.5b	42.1±1.7b	75.2±2.8bcd
	Cab	22.4±1.5c	21.3±2.0b	8.2±0.3c	5.9±0.1b	1.4±0.03c	14.7±1.9bc	0.59±0.02a	45.2±6.6b	31.54±1.5ab	49.8±1.1a	81.2±2.6b
	P-value	**< 0 .0001**	**< 0 .0001**	**< 0.0001**	**0.0054**	**< 0 .0001**	**0.0011**	**0.0027**	**0.0004**	**0.0254**	**0.0188**	**< 0.0001**
DS	SCC	28.08±1.0b	21.9±1.4ab	10.3±0.2a	5.9±0.1a	1.7±0.02a	9.43±1.1a	0.45±0.02a	36.2±3.4a	26.56±1.7b	44.45±1.0a	64.0±3.1ab
	SIM	35.09±1.1a	27.5±1.6ab	8.5±0.3b	5.0±0.2bc	1.7±0.03a	10.5±1.1a	0.43±0.02a	28.6±3.6ab	35.88±1.7a	45.06±0.9a	75.6±3.1a
	SJM	24.6±2.8bc	25.0±4.1ab	8.4±0.7ab	5.2±0.4abc	1.6±0.08ab	12.0±1.1a	0.44±0.02a	25.6±4.1ab	31.80±1.7ab	43.98±1.3a	36.8±3.1d
	Bol	31.01±1.5ab	25.2±2.1ab	8.0±0.4b	4.5±0.2c	1.7±0.04a	9.90±1.1a	0.42±0.02a	29.9±3.6ab	34.25±1.7a	42.99±1.2a	56.4±3.1bc
	Pap	17.5±2.8c	11.8±4.1b	7.7±0.7b	5.0±0.4abc	1.5±0.08b	8.93±1.1a	0.41±0.02a	22.9±4.5ab	29.32±1.7ab	41.63±1.4a	49.6±3.1cd
	Cab	18.9±1.1c	19.1±1.6b	7.6±0.3b	5.5±0.2ab	1.4±0.03b	11.9±1.1a	0.39±0.02a	19.9±3.6b	29.45±1.7ab	45.20±1.0a	43.1±2.9cd
	P-value	**< 0.0001**	**0.0011**	**< 0 .0001**	**0.0002**	**< 0 .0001**	0.2863	0.4037	**0.0443**	**0.0085**	0.3360	**< 0.0001**
	P-value pop	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	**0.0002**	0.1214	**<0.0001**	**0.0005**	**0.0041**	**< 0 .0001**
	P-value treatment	**<0.0001**	**0.0004**	**0.0002**	**<0.0001**	0.2045	**<0.0001**	**<0.0001**	**<0.0001**	0.0590	**<0.0001**	**< 0.0001**
	P-value interaction	**0.0044**	0.0527	0.1298	0.1085	0.7120	**0.0094**	**0.0028**	**0.0401**	0.1428	0.8279	**0.0003**

Table 4. Population means and standard errors of six physiological traits measured in *Q. rugosa* seedlings under two watering treatments after one year. The p-values of within treatment ANOVA analyses for the comparison of populations are shown. Different letters indicate significant differences among populations after a Tukey test. The last three rows show the P-values for a two-way ANOVA with population, treatment and the interaction of population and treatment as the explanatory factors. WW, well-watered treatment; DS, drought-stress treatment; *Amax*, maximum photosynthetic rate; *G*, stomatal conductance; *E*, transpiration rate; *WUE*, water use efficieny; Ψ_{PD}, predawn leaf water potential; Ψ_{MD}, midday leaf water potential.

Treatment	Pop Code	*Amax* (mmol $m^{-2}s^{-1}$)	*G* (mmol $m^{-2}s^{-1}$)	*E* (mmol $m^{-2}s^{-1}$)	*WUE* (*Amax*/*E*)	Ψ_{PD}	Ψ_{MD}
WW	SC	6.31 ± 0.64b	0.091 ± 0.01b	1.68 ± 0.23b	3.75 ± 0.42a	0.270 ± 0.02a	0.310 ± 0.02a
	SIM	7.52 ± 0.68b	0.131 ± 0.01ab	2.14 ± 0.24b	3.79 ± 0.45a	0.301 ± 0.02a	0.315 ± 0.02a
	SJM	7.52 ± 0.78b	0.139 ± 0.02ab	2.32 ± 0.28b	3.50 ± 0.52a	0.265 ± 0.02a	0.279 ± 0.02a
	Bol	7.44 ± 0.68b	0.095 ± 0.01b	1.74 ± 0.24b	4.62 ± 0.45a	0.293 ± 0.02a	0.321 ± 0.02a
	Pap	7.08 ± 0.78b	0.097 ± 0.02b	1.72 ± 0.28ab	4.84 ± 0.52a	0.216 ± 0.02a	0.233 ± 0.03a
	Cab	10.82 ± 0.60a	0.198 ± 0.05a	3.14 ± 0.21a	3.51 ± 0.40a	0.268 ± 0.02a	0.302 ± 0.02a
	P-value	**0.0002**	**0.0002**	**0.0003**	0.2153	0.3288	0.3132
DS	SC	0.55 ± 0.89c	0.032 ± 0.01a	0.59 ± 0.22a	1.06 ± 0.62c	3.187 ± 0.31a	2.899 ± 0.25a
	SIM	0.95 ± 1.00bc	0.027 ± 0.01a	0.52 ± 0.25a	1.88 ± 0.70bc	2.055 ± 0.33a	2.600 ± 0.27a
	SJM	2.50 ± 1.00abc	0.048 ± 0.01a	0.85 ± 0.25a	2.72 ± 0.70abc	2.354 ± 0.39a	2.636 ± 0.29a
	Bol	2.00 ± 1.00abc	0.049 ± 0.01a	0.93 ± 0.25a	2.46 ± 0.70abc	2.261 ± 0.32a	1.996 ± 0.25a
	Pap	5.64 ± 0.89a	0.087 ± 0.01a	1.51 ± 0.25a	3.94 ± 0.62ab	1.651 ± 0.45a	1.892 ± 0.36a
	Cab	4.49 ± 0.66ab	0.055 ± 0.01a	1.01 ± 0.17a	4.53 ± 0.46a	2.845 ± 0.32a	2.481 ± 0.25a
	P-value	**0.0018**	0.1247	0.0737	**0.0021**	0.070	0.1280
	P-value population	**<0.0001**	**0.0023**	**0.0021**	**0.0030**	**0.0485**	0.1866
	P-value treatment	**<0.0001**	**<0.0001**	**<0.0001**	**0.0002**	**<0.0001**	**<0.0001**
	P-value interaction	**0.0302**	**0.0036**	**0.0041**	**0.0035**	0.0525	0.2312

Table S2. Pearson correlation coefficients between population means of eleven morphological traits with the latitude and nine climatic variables of the collection sites. Only significant values (P < 0.05) are shown. See table 3 for the abbreviation of morphological traits and table 1 for the abbreviation of climatic variables.

	Morphological variables	Climatic variables									
Treatment		Lat	Map	gsp	gsdd5	adi	spb	mtwm	mtcq	ps	pdq
WW	Ss				0.898					0.9011	
	Nl										
	Ll	-0.828			0.845						
	Lw						-0.821				
	Ls										
	Sd									0.835	
	Lt				-0.881				-0.821	-0.840	
	Tla						-0.855				
	DMC	-0.432	0.420		0.547		-0.329		0.440	0.424	
	Chl						-0.884				
	Stom										
DS	Ss				0.963				0.826		
	Nl				0.895					0.868	
	Ll										
	Lw										
	Ls				0.915						
	Sd										
	Lt		0.851								
	Tla										
	DMC							0.454	-0.820	0.481	
	Chl						-0.919				
	Stom										

Table S3. Pearson correlation coefficients between population means of six physiological traits with the latitude and nine climatic variables of the collection sites. Only significant values (P < 0.05) are shown. See table 3 for the abbreviation of morphological traits and table 1 for the abbreviation of climatic variables.

	Physiological variables	Climatic variables									
Treat		Lat	Map	gsp	gsdd5	ADI	smrp	mtwm	mtcq	ps	pdq
WW	*Amax*										
	E										
	G										
	WUE										
	Ψ PD				0.814						
	Ψ MD										
DS	*Amax*	0.872			-0.953						
	E				-0.860						
	G				-0.860						
	WUE	0.898			-0.886						
	Ψ PD						-0.857				
	Ψ MD						-0.831				

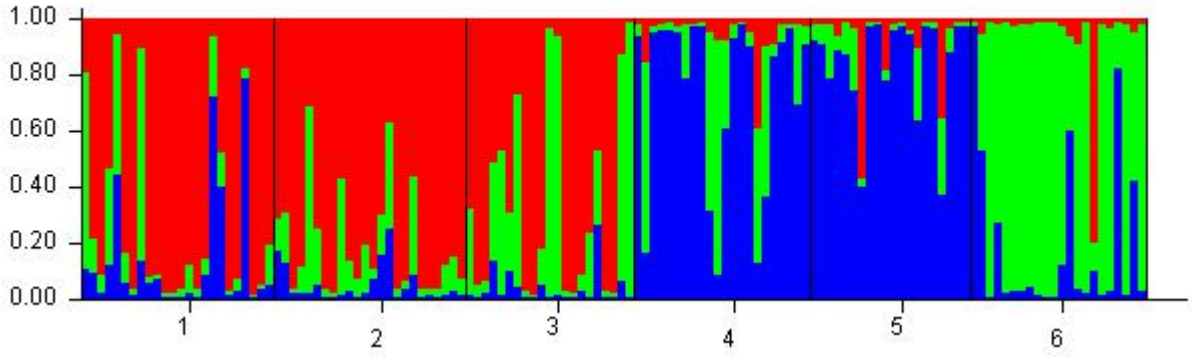

Figure S2. Genetic assignment of individuals and populations according to the Bayesian method implemented in STRUCTURE. Each thin vertical line represents an individual and the proportion of each color represents the proportion of ancestry inferred for the two genetic groups. Populations are separated by black lines.

Table S4. Matrix of pairwise F_{ST} values among populations.

	SC	SIM	SJM	Bol	Pap	Cab
SC	0.00000					
SIM	0.02553	0.00000				
SJM	**0.04505**	**0.00783**	0.00000			
Bol	**0.06994**	**0.05949**	**0.06758**	0.00000		
Pap	**0.08923**	**0.07437**	**0.09488**	**0.04826**	0.00000	
Cab	**0.09432**	**0.07450**	**0.07167**	**0.09778**	**0.10635**	0.00000

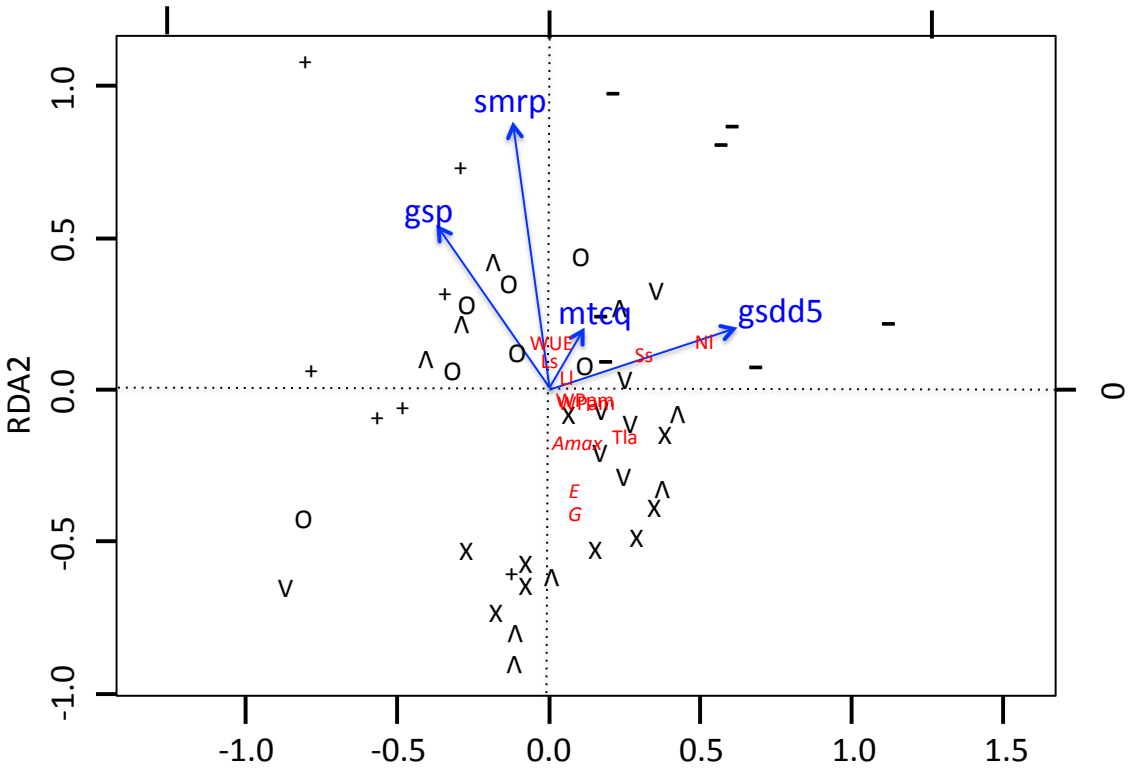

Figure 1. RDA between the morphophysiological and climatic variables conditioned by Latitude and Longitude in WW treatment.

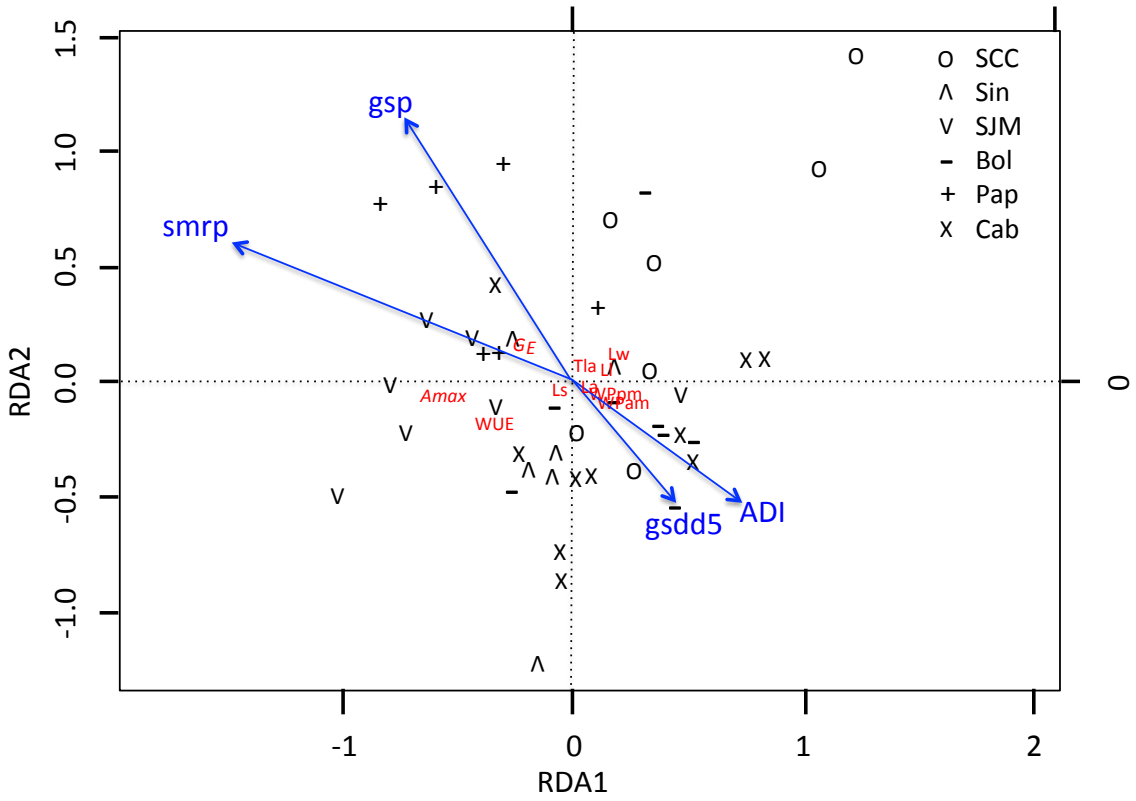

Figure 2. RDA between the morphophysiological and climatic variables conditioned by Latitude and Longitude in DS treatment.

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CAPÍTULO IV

Geographical patterns of genomic variation provide guidelines for assisted gene flow in Mexican populations of *Quercus rugosa*

ABSTRACT

Forest trees species show geographic patterns of phenotypic and genetic variation, which largely reflect the signature of local adaptation. A major concern is whether tree populations that have evolved in response to past climate conditions will be able to respond to rapid climate change. Landscape genomics methods, such as F_{ST} and environmental association outlier tests, coupled with a model that incorporates geography and climate, Gradient Forests (GF), provide the tools to study the spatial patterns of adaptive molecular variation in trees. Here, we deploy those three methods to guide the definition of forest restoration strategies for the widely distributed high-elevation oak species *Quercus rugosa*, in Mexico. Using 5354 single-nucleotide polymorphisms (SNPs) genotyped from 105 individuals across 17 populations in the Trans-Mexican Volcanic Belt, we first identified SNPs associated with local adaptation, using the two outlier tests mentioned above. We found 78 outlier F_{ST} SNPs and 88 SNPs that are significantly associated with climate. We then used GF to identify the most important drivers of genomic variation in the landscape. Genetic structure is primarily driven by precipitation seasonality, precipitation of the wettest quarter and geographical distance. We then modelled and mapped the turnover in allele frequencies across the landscape, using GF, and identified the regions where *Q. rugosa* populations are expected to be more sensitive to climate change. The genetic variation shows a pattern of population differentiation concordant with precipitation gradients. Based on genetic and climatic similarities, we organized the distribution area of *Q. rugosa* in Mexico in three seed zones. Populations in one seed zone are expected to be more sensitive to climate change due to the higher expected velocity of climate change in that region. Our findings provided specific recommendations for assisted gene flow and forest restoration in Mexico. We suggest that effective conservation measures should consider the landscape approach of adaptive genomic differentiation.

KEYWORDS: Genotyping by sequencing, landscape genomics, *Quercus*, natural selection, Trans-Mexican Volcanic Belt, assisted gene flow, climate change, restoration

INTRODUCTION

Forest tree species show geographic patterns of phenotypic and genetic variation that are largely shaped by local adaptation (Langlet 1971; Morgenstern 1996; Savolainen, Pyhäjärvi, and Knürr 2007). Forest trees have long received attention because they have great economic value and because of their ecological importance as drivers of terrestrial biodiversity and their role in sequestering carbon (Neale and Kremer 2011; Alberto *et al*., 2013; Cavender-Bares 2016). More recently, there is a general concern on whether tree species with long life span will be able to adapt to rapid climate change (Aitken *et al*., 2008; Sork *et al*., 2013). Thus, it is important to manage both plantation and natural populations with knowledge of the genetic basis of tree performance (Savolainen 2011; Christmas, Breed and Lowe 2015). Provenance studies that compare population divergence in a range of traits such as growth, drought tolerance, cold-hardiness, and phenology, by planting seeds of different origin in one or more common gardens, provide compelling evidence of local adaption that needs to be incorporated into forest management practices (Bower and Aitken 2008; Sork *et al*., 2013; Aitken and Bemmels 2016). However, when such long-term studies are not feasible, the analysis of geographic patterns of genetic variation provides an alternative approach to studying the genetic basis of local adaptation (Manel, Joost, *et al*. 2010; Savolainen, Lascoux, and Merila 2013; Sork *et al*. 2013). Sometimes called landscape genetics or genomics, this approach aims to analyze spatial patterns of genetic variation to identify evidence of local adaptation (Holderegger, Kamm, and Gugerli 2006; Joost *et al*., 2007; Joost *et al*., 2013; Sork *et al*., 2013; Bragg *et al*., 2015).

The knowledge of the spatial patterns of adaptive variation in trees may be used to guide forest management decisions, because it has the potential to predict the genetic response of trees to rapid climate change (Aitken *et al.,* 2008; Schoville *et al*., 2012; Sork *et al*., 2013; Aitken and Bemmels 2016). Spatially explicit predictive models would help to identify priority regions for conservation, define seed zones and guide the choice of seed sources for reforestation based on assisted gene flow (AGF), the movement of individuals or propagules across the species range to facilitate faster adaptation to future predicted climates" (Aitken and Bemmels 2016). But, translating information on adaptive genomic variation into sound management decisions is still challenging (Schoville et al. 2012; Fitzpatrick and Keller 2015) as it requires the development of accurate predictive models that consider the interaction between adaptive genetic variation and multiple environmental gradients (Aitken *et al*., 2008; Schoville et al. 2012; Fitzpatrick and Keller 2015). Initial efforts of predictive models using genetic data relied on a classical species distribution modelling (SDM) framework (Sork *et al*., 2010; Fournier-Level *et al*., 2011; Jay *et al*., 2012). Fitzpatrick and Keller (2015) argued that SDMs have the disadvantage of not accounting for the multidimensionality of genomic variation across the landscape. They have demonstrated that community-level modelling frameworks (Ferrier and Guisan 2006), such as Gradient Forests (GF - Ellis, Smith, and Pitcher 2012) and Generalized Dissimilarity Models (GDM - Ferrier *et al*., 2007) are powerful strategies to model and map the turnover in allele frequencies along environmental gradients because these regression-based models use nonlinear functions of environmental gradients.

The overall objective of this study is to analyze patterns of adaptive genetic variation to illustrate how to develop management guidelines for the widely distributed high-elevation oak species, *Quercus rugosa* Née (Fagaceae). Given research on other oak species that reported evidence of selection on genes associated with phenology, drought resistance, and other traits (Deans and Harvey 1996; Alberto *et al*., 2011; Koehler, Center, and Cavender-Bares 2012; Homolka *et al*., 2013; Ramirez-Valiente, Koehler, and Cavender-Bares

2015; Sork *et al*., 2016), we designed this study to test the hypothesis that spatially divergent selection is driving differentiation among *Q. rugosa* populations in an environmentally heterogeneous region of Mexico, especially at specific loci under selection by climate. We then modeled the spatial patterns of adaptive variation across the distribution range of *Q. rugosa* in Mexico in order to identify the most critical regions under climate change and propose management guidelines.

Our first specific objective is to quantify differentiation of SNPs across populations to identify candidate genes under selection. This approach is based on the premise that loci under divergent selection show larger variation in allele frequencies among populations on the landscape than neutral genomic regions (Lewontin and Krakauer 1973). Therefore, SNPs showing larger population differentiation (F_{ST}) than neutral expectations may be indicative of local adaptation. These loci with significantly high F_{ST}, however, do not point to which environmental factors might be the cause of selection (Schoville et al. 2012). Thus, our second objective is to identify candidate SNPs that are linearly associated with climate variation across the landscape. Using environmental association analyses (Vasemägi and Primmer 2005), we test for significant linear relationships between allele frequencies and an environmental gradient to detect candidate genes under selection while controlling for population structure (Joost *et al*., 2007; Coop *et al.,* 2010; Frichot *et al*., 2013). In our study, we used Latent Factor Mixed Models (Frichot et al. 2013) to find candidate genes associated with temperature and precipitation variables. Our third objective is to identify candidate genes associated with the outlier loci by searching the genomic database available for *Quercus lobata* to identify gene models based on predicted functional annotation of our candidate SNPs. Finally, our fourth objective is to quantify the association between climate and genome-wide genetic variants using the candidate SNPs by modeling and mapping the turnover in allele frequencies across current and future predicted environmental gradients using the Gradient Forests (GF) community-level modelling framework. The GF modelling is a flexible model that uses a machine-learning regression

tree approach to directly model the compositional turnover in genomic variation and efficiently handles complex relationships between environmental predictors (Ellis, Smith, and Pitcher 2012; Fitzpatrick and Keller 2015). Findings from the specific objectives, especially the GF maps, will provide the basis for recommendations on management of these oak populations under conditions of future climate change.

MATERIALS AND METHODS

Study species and sampling

Quercus rugosa is a white oak species (section *Quercus*) with a wide geographical distribution, from Honduras and Guatemala in Central America to Arizona, New Mexico and western Texas in the United States. In Mexico, it can be found from the subtropics in the highlands of Los Altos de Chiapas to the temperate zones of the Sierra Tarahumara in the State of Chihuahua, at altitudes ranging from 1700 m to 3550 m (Rzedowski 2006; Uribe-Salas *et al*., 2008). It is one of the dominant species over much of this range, often found in monospecific stands or with other species of oak or pine. The species is most abundant along the Trans-Mexican Volcanic Belt (TMVB), with a distribution from the western areas in the states of Jalisco and Nayarit to the eastern region in the state of Veracruz, at altitudes between 2300 and 3200 m (Rzedowski 1986). The TMVB is a region with a complex geologic and climatic history. The highlands of the TMVB cross Mexico in an east-west orientation at latitude ~19°N (Metcalfe 2006). It is an area of diverse topography and geological composition that result in a wide range of elevations and climate conditions (Metcalfe 2006; Gómez-Tuena, Orozco-Esquivel, and Ferrari 2007). The highlands forests of the TMVB are dominated by oak and pine species (Metcalfe 2006).

Leaves of 10 randomly selected individuals were collected in each of 17 natural populations of *Q. rugosa* from 11 states in Mexico (Table S1). Most of the sampling sites are located along the TMVB, but a population from Chiapas

(Tenejapan) in southeastern Mexico was also included (Fig. 1). The latitudinal and longitudinal breadth of the sampling is from about 16.7 to 21.2 °N and from 92.9 to 103.2 °W. At each site, sampled trees are separated by at least 50 m to avoid sampling closely related individuals. The samples were labeled, transported to the laboratory, and stored at −80°C until processed.

Laboratory procedures

Total genomic DNA was extracted from the leaves using the DNeasy Plant Mini Kit (Qiagen) according to the manufacturer's instructions. For samples that produced final products with coloration due to unremoved secondary compounds, we repeated the extractions applying a prewash protocol (Li *et al*. 2007; Gaddis *et al*. 2014). Total genomic DNA was prepared for sequencing using an efficient restriction-enzyme-based approach, genotyping by sequencing (GBS) (Elshire et al. 2011). Briefly, DNA is digested with a restriction enzyme, common and unique barcoded adapters with overhangs complementary to the cut site are ligated to each sample, samples are pooled in equimolar ratios, and the pooled library is PCR-amplified and sent for Illumina sequencing. We largely followed the original protocol, including the same adapter concentration and restriction enzyme (*Ape*KI). All steps were performed manually rather than robotically with a few changes to optimize the protocol for *Quercus* spp. (Gugger *et al.* in prep.). Using 48 samples per preparation, adapters were added during the ligation step. AMPure XP bead-based size selection/purification steps were added after the ligation step and repeated after the PCR step to ensure a consistent distribution of fragment sizes between 200 and 500 bp (including adapters) among all preps. We reduced the number of PCR cycles to 16 from 18. Final libraries were checked for the proper size distribution on an Agilent BioAnalyzer with the High Sensitivity DNA assay and quantified using a Qubit fluorometer. Samples were sent to the UCLA Broad Stem Cell Research Center for single-end, 100-bp sequencing on an Illumina HiSeq2000 v3. GBS libraries can have a large fraction of fragments without the proper combination of adapters and thus will not form clusters and sequence. Therefore, we arranged

with the sequencing staff to "pretend" the sample was half as concentrated (nM) as it appeared based on initial quantification assays. The sequences used in this study were generated from three lanes of Illumina sequencing, some of which were multiplexed with other *Quercus* spp. for another project.

Genomic data processing

Illumina reads in FASTQ format were quality filtered and demultiplexed using the 'process_radtags' command in STACKS 1.28 (Catchen et al. 2011; Catchen et al. 2013) to remove adapter sequence with up to two mismatches (adapter_mm), recover barcodes with up to one mismatch to the expected barcodes (r), remove any read with an uncalled base (c), discard low quality reads as defined by default settings (q), and trim all reads to 92 bp (t). Using BWA 0.7.12 (Li and Durbin 2010), the filtered reads were aligned to the v. 0.5 draft *Quercus lobata* reference genome (NCBI accession # ID 308314, also available at http//valleyoak.ucla.edu). We used GATK 3.3 (DePristo et al. 2011) to identify SNPs in each aligned sample using a minimum confidence threshold (Phred-scaled) of 30. We then used "VariantFiltration" and "SelectVariants" tools in GATK to exclude low quality variants. We applied the following filters: QD < 20.0, MQ < 40.0, MQRankSum < -12.5, and ReadPosRankSum < -8.0. We used VCFTOOLS 0.1.12b (Danecek et al. 2011) to filter the SNPs to include only diallelic sites, present in at least 95% of individuals, with minimum mean coverage depth of 5, and minor allele frequency (MAF) ≥ 0.10. Statistics of coverage depth per loci and per samples were also performed in VCFTOOLS. SNPs were pruned in PLINK (Purcell et al. 2007) using the "indep" parameter. We used a variance inflation factor (VIF) threshold of 2, window size in SNPs of 5, and the number of SNPs to shift the window at each step of 5.

Climatic variables

We downloaded 19 climatic variables from the Digital Climatic Atlas from Mexico (http://uniatmos.atmosfera.unam.mx, 926 m resolution, period: 1902-2011) and extracted values for 17 *Q. rugosa* point locations. This procedure was performed

in R 3.2.0 (R CoreTeam 2015) using the 'dismo' 1.0-12 package (Hijmans et al. 2015). We excluded variables that are highly correlated (|*r*| > 0.70) resulting in the following set of climate variables: temperature seasonality (BIO4), minimum temperature of coldest month (BIO6), precipitation seasonality (BIO15), precipitation of wettest quarter (BIO16) (Table S1). Some of these variables are also correlated with either latitude or longitude (Table S2).

Population structure and isolation by distance

To understand the overall genetic structure of the study populations, we first characterized population structure using the Bayesian clustering method implemented in STRUCTURE (Pritchard, Stephens, and Donnelly 2000). We tested *K*-values ranging from 1 to 17 and ran three independent repetitions at each *K*. We used the admixture model; the length of burn-in period was 10,000; and the number of MCMC repetitions after the burn-in was 100,000. We used the Δ*K* method of Evanno, Regnaut, and Goudet (2005) implemented in STRUCTURE HARVESTER (Earl and vonHoldt 2011) to decide the number of *K* that best describes our data set.

To explore whether restricted gene flow and isolation by distance influence the genetic structure of our populations, we first estimated pairwise population differentiation using F_{ST} (Weir and Cockerham 1984) and then regressed $F_{ST}/(1 - F_{ST})$ between population pairs to the log of pairwise spatial distances between populations as proposed by Rousset (1997). These analyses were performed in GENEPOP 4.3 (Rousset 2008). A Mantel test was performed in R using 'ape' library (Paradis, Claude, and Strimmer 2004) and 9999 permutations. We also calculated gene diversity H_E and F_{IS} per population in GENEPOP.

Population divergence of individual loci

To identify genomic regions under spatially divergent selection, we used the Bayesian method implemented in BayeScan 2.1 (Foll and Gaggiotti 2008) that has been recognized the most efficient population differentiation method (Narum

and Hess 2011; De Mita et al. 2013; Lotterhos and Whitlock 2014). We tested 5354 SNPs using default values. In summary, prior odds for the neutral model was set to 10 and the following parameter values: 5000 of outputted iterations, thinning interval size of 10, 20 pilot runs, pilot runs of 5000 iterations, burn-in length of 50000 iterations. To decrease the chance of false positives due to multiple testing, we adopted the false discovery rate (FDR) criterion (Benjamini and Hochberg 1995). *Q*-values were calculated in R 3.2.0 (R CoreTeam 2015) using 'qvalue' package (Storey 2015). We considered outliers to be SNPs with $q < 0.05$ ($-\log_{10}q > 1.3$).

Environmental association analysis of individual loci

As a second way of identifying SNPs under natural selection for local adaptation, we tested for associations between SNPs and climatic gradients using a latent factor mixed model implemented in LFMM 1.3 (Frichot et al. 2013). This method estimates allele-environment correlations while correcting for background population structure using latent factors. The number of latent factors (*K*) is included in the model as a fixed effect to both control for demographic history, as well as environmental gradients not included in the study (De Villemereuil et al. 2014). Although most environmental association (EA) analysis methods are prone to false negatives when demography and environment are correlated, LFMM is less prone to both false negatives and false positives (Frichot et al. 2013; Lotterhos and Whitlock 2015) than competing methods, such as BAYENV2 (Gunther and Coop 2013), because it does not rely on a specific demographic model when accounting for population structure (De Villemereuil et al. 2014; Lotterhos and Whitlock 2015).

As *K* influences test outcomes, we used the two methods recommended by Frichot et al. (2013) to decide the range of *K*-values to be explored in the genotype–environment association analyses. First, we ran a principal component analysis (PCA) followed by Tracy-Widom test (Patterson, Price, and Reich 2006) implemented in LFMM to select the number of significant eigenvalues as one

estimate of *K*. Second, we used the *K*-value from STRUCTURE results (see Methods above) as an alternative estimate. Using LFMM, we calculated the |*z*|-score, which is the strength of genetic-environment association, for each SNP and each of six climatic variables. Based on the results of Tracy-Widom test (*K* = 5) and Bayesian clustering method (*K* = 6, see Results, Fig.S1), we ran LFMM with *K* = 5 and *K* = 6. For each *K*, we did 5 independent LFMM runs using 10,000 iterations and burn-in of 5,000. To increase the power of LFMM statistics, we calculated median |*z*|-scores for each locus among five runs and considered a FDR of 5% to be significant (Frichot and François 2015). Adjusted *p*-values (*q*) were calculated using the genomic inflation factor (λ) procedure described in Devlin and Roeder (1999). To confirm that the confounding effects of population structure were under control, we relied on visual observation of histograms of adjusted *p*-values as recommended in LFMM manual (Frichot and François 2015). Correct distributions are expected to be flat with a peak close to zero. We performed these analyses in R using scripts available in the LFMM manual. We considered candidates to be SNPs that are significant (FDR < 0.05) for all investigated values of *K* (5 and 6).

Testing population differentiation of climate-associated SNPs

We compared the results of F_{ST}-outlier and environmental association analyses to evaluate whether climate is driving population differentiation. First, we tested if the F_{ST} of climate-associated SNPs are significantly higher than the background F_{ST}, using the non-parametric Wilcoxon rank sum test. Second, we tested whether F_{ST} values of climate-associated SNPs are enriched in the upper tail of the overall F_{ST} distribution, using one-sided Fisher's exact test.

Genomic contexts of candidate SNPs

SnpEff (Cingolani et al. 2012) and BEDTools v2.25.0 (Quinlan and Hall 2010) were used to identify positions of candidate SNPs with respect to predicted gene models on the *Quercus lobata* genome (http://valleyoak.ucla.edu). The gene models were predicted by mapping contigs of the *Quercus lobata* transcriptome

(Cokus, Gugger, and Sork 2015) to the genome using GMAP (Wu and Watanabe 2005) and Sim4db (Walenz and Florea 2011). Table S3 lists the genes for which candidate SNPs fall within, plus the closest upstream and downstream genes and their distances from the SNP. For genes with candidate SNPs within, Table S3 also lists predicted functional annotation for the genes, transferred from the carefully curated annotation of the *Quercus lobata* transcriptome to identify gene annotations and orthologs with *Arabidopsis thaliana* TAIR10 gene models (Swarbreck et al. 2008).

Genomic landscape of current and future environmental adaptation

We used Bayescan and LFMM results to generate five SNP sets. The five SNPs set are: (1) the complete SNP set (5354 SNPs), (2) climate associated SNPs (88 SNPs), (3) SNPs associated with temperature (76 SNPs), (4) SNPs associated with precipitation (12 SNPs), and (5) F_{ST} outlier that were also associated with climate in LFMM (4 SNPs). We then modeled climatic and spatial drivers of genomic variation for each SNP set using Gradient Forests (GF; Ellis, Smith, and Pitcher 2012). We followed procedures described in Fitzpatrick and Keller (2015). The SNP data was converted into minor allele frequencies and SNPs polymorphic in fewer than four of the 17 populations were removed to ensure robust regression. For each model, we used the four climatic variables chosen for LFMM analyses as environmental predictors. The effect of spatial processes and unmeasured environmental variation were included in the models using Moran's eigenvector map (MEM) variables as spatial predictors. We used the first half of the MEM eigenfunctions with significant positive eigenvalues as predictors of broad-scale spatial structure and unaccounted environmental variation as proposed in previous studies (Manel, Poncet, et al. 2010; Sork et al. 2013). We calculated MEM variables in R using 'spacemakeR' 0.0-5 package (Dray 2013). We used the same threshold parameters described in Fitzpatrick and Keller (2015) to fit GF models, 2000 regression trees per SNP and a variable correlation threshold of 0.5. We also used default values for the proportion of samples used for training (~0.63) and testing (~0.37) each tree. The relative

importance of each predictor variable was assessed through weighted R^2 values of each GF model. We used GF models to predict changes in allele frequencies along each environmental gradient within the geographic range of *Q. rugosa* in Mexico. For this purpose, the environmental variables of 10,000 random location points were transformed into genetic importance values. The GF analyses were performed in R, using 'gradient forests' 0.1-17 package (Ellis, Smith, and Pitcher 2012).

We then used Principal Component Analysis (PCA) to reduce the transformed environmental variables into three factors. The PCA was centered but not scaled to preserve the differences between genetic importance values among the environmental variables. For each of the five GF models, the first three PCs were assigned to a RGB color palette and visualized in geographic space. In our maps, color similarity corresponded to the similarity of expected patterns of genetic composition. We then performed a Procrustes superimposition on the PCAs to compare mapped genetic composition for the complete SNP set and the four candidate SNP sets. The Procrustes residuals represent the absolute distance in genetic composition between SNP sets for each point location. The Procrustes residuals were 0-1 rescaled and mapped. PCAs and Procrustes superimpositions were performed in R, using 'vegan' v.2.3.1 library (Oksanen et al. 2015).

To estimate the vulnerability to climate change we transformed future climate scenarios for 2080 into genetic importance values using the previous GF functions calculated for current climate. For each data point, we averaged future climate data corresponding to the Representative Concentration Pathway 6.0 (RCP 6) scenario of greenhouse gas concentration trajectories (Fujino et al. 2006; Hijioka et al. 2008) of three coupled atmosphere-ocean climate models: i. BCC-CSM1.1(m) (Wu 2012, Xin et al. 2012, Xin et al. 2013), ii. CSIRO-Mk3.6.0 (Rotstayn et al. 2012) and iii. MIROC5 (Watanabe et al. 2010). We then calculated the Euclidian distance between current and future genetic

compositions to identify geographic regions where gene-environmental relationships will be most disrupted due to climate change (named as 'genetic offset' in Fitzpatrick and Keller, 2015). We mapped the genetic offsets for each SNP set to identify regions predicted to experience greater impacts under future environments in the lack of adaptive evolution or migration (Fitzpatrick and Keller 2015).

RESULTS

The final data set included 5354 SNPs and 105 samples distributed in 17 populations (with a mean number of samples per population of six) (Table S1). On average, samples have only 1.68% of missing data and 91.4% of the samples has less than 5% of missing data (the sample with the greatest number of missing loci had 16.8%). The mean depth of coverage per locus per sample is 21.6 and 88% of our 5354 loci have a mean depth larger than 10 (Fig. S2). Out of 105 samples, 73.3% have a mean depth greater than 10.

Genetic diversity, population structure and isolation by distance

Mean population differentiation is F_{ST} = 0.055 and mean inbreeding coefficient is F_{IS} = 0.050. Average pairwise F_{ST} per population ranges from 0.037 to 0.095 (Table S1). Average gene diversity is H_E = 0.364, SD = 0.012. The Mpio. Bolaños population, which is northwestern located shows the lowest gene diversity (H_E = 0.327) and the highest mean pairwise F_{ST} (0.095) (Table S1). Populations exhibit a pattern of isolation by distance (R^2= 0.238, Mantel test z = 21.548, p = 0.009, Fig. S3). Bayesian analysis identified K = 6 gene pools (Fig. S1). The distribution of gene clusters in the landscape follows an east-west gradient (Fig. 1).

Population divergence of individual loci

BayeScan identified 78 SNPs (1.46% of 5354 SNPs) with elevated F_{ST} consistent with divergent selection. Mean F_{ST} of these outlier SNPs is 0.192 (SD = 0.036)

and the range is from 0.147 to 0.318. We did not detect low outlier F_{ST} values indicative of balancing or purifying selection.

Environmental association analysis of individual loci

Histograms of adjusted *p*-values are uniformly distributed and thus indicate that the two values of *K* tested in LFMM adequately control for the confounding effects of population structure (Fig. S4, S5). We found 88 SNPs (1.64% of 5354 SNPs) that are significantly associated with climate variables for all values of *K* and 4 SNPs that are associated with two or three climatic variables. We considered only the climate variable with the strongest association (i.e. highest |*z*|-score) for these SNPs in case that additional environmental associations are due to the correlation among climate variables (De Kort et al. 2015). Out of the 88 outlier SNPs, 76 are associated with temperature variables and 44 of those are associated with temperature seasonality (mean |*z*| = 3.91) and 32 with minimum temperature of the coldest month (mean |*z*| = 4.15). Only 12 SNPs are associated with precipitation, but mean |*z*|-scores are usually higher than in temperature variables. Six of these SNPs are associated with precipitation seasonality (mean |*z*| = 5.61) and six with precipitation of the wettest quarter (|*z*| = 4.51).

Testing population differentiation of climate-associated SNPs

BayeScan and LFMM identified altogether 162 candidate SNPs, and four SNPs were identified with both methods. Three of these SNPs were associated with temperature seasonality and one with precipitation seasonality. Climate-associated SNPs (n = 88) have mean F_{ST} = 0.070 (range: 0.039 – 0.217, SD = 0.029), slightly higher than the background overall population differentiation (F_{ST} = 0.055), and this difference is significant according to a Wilcoxon rank sum test (W = 302480, p = 4.211e-07). However, most of the climate-associated SNPs are not located at the 5% upper tail of the F_{ST} distribution (one-sided Fisher's exact test p = 0.999), revealing that the elevated F_{ST}-values of climate-associated SNPs are mainly due to seven SNPs with F_{ST} > 0.10 (Fig. 4).

Genomic contexts of candidate SNPs

The genomic contexts of the 162 candidate SNPs were determined based on *Quercus lobata* gene models using the SnpEff variant annotator. We predicted 64 SNPs to fall within 61 genes and 45% of these 64 SNPs were intron variants. Ninety-eight SNPs were located in intergenic regions, including the four SNPs that were identified by both LFMM and BayeScan (Table S3). Out of 61 genes, 53 have annotations in *Q. lobata* transcriptome, 27 from the outlier F_{ST} analysis and 26 associated with climate (Table S3). We identified proteins involved in a broad range of biological processes, as transcription (transcription factors and regulatory proteins), signal transductions (protein kinases, proteins involved in ubiquitination, proteases), and ion transport (Table S3). Furthermore, four of these proteins are directly involved in response to abiotic and biotic stimuli, such as response to water deprivation (Tetratricopeptide repeat (TPR) like superfamily protein - Yuan and Liu 2012), salt and osmotic tolerance (phosphopantothenoylcysteine decarboxylase, HAL3A gene - Kupke, Hernandez-Acosta, and Culianez-Macia 2003), oxidative and osmotic stress (Mitogen-activated protein kinase 3, MPK3 gene - Wang et al. 2007; Kim et al. 2011), and drought tolerance (Kang et al. 2010) and lead resistance (Lee et al. 2005) (pleiotropic drug resistance 12, PDR12 gene).

Genomic landscape of current and future environmental adaptation

Out of the five SNP sets, the best model performances were achieved with the LFMM precipitation candidates (mean R^2 = 18.50%) and the four F_{ST} outlier SNPs associated with climate (mean R^2 = 19.96%) (Table 1). Considering the SNP set that included all the 5354 SNPs, almost 20% of the SNPs had positive R^2 values and those SNPs (10% upper tail of R^2 distribution) with the greatest R^2 were not included in other data sets (i.e. they are not candidates of climate association). The percentage of SNPs with positive R^2 increased in the other four smaller data sets (Table 1). The two precipitation variables and MEM spatial variables were the most important predictors in the five GF models (Fig. 5),

indicating a strong spatial influence on the distribution of genetic diversity. The strong role of MEM variables may also suggest that they have captured important unmeasured environmental predictors. As the two precipitation variables and the MEM spatial variables were the most important predictors in all the five GF models, the predicted turnover in allele frequencies across the landscape were similar and followed an east-west direction (Fig. 6 and S6). The four SNP sets of climate associated SNPs showed a rapid turnover in allele frequencies on the eastern portion of *Q. rugosa* distribution, which was not evident in the all SNPs data set. Indeed, the difference between the pattern of genetic distribution predicted for this SNP set and the patterns of each of the climate associated SNP sets, evaluated trough the mapping of Procrustes residuals (Fig 6c, S7), was small and restricted to some small areas in the eastern range of *Q. rugosa* distribution. GF future predictions also indicated that north-eastern populations are expected to present the greatest genetic offsets under climate change (Fig. 7, S8). Populations in these regions are predicted to be less adapted to future climate if there is no adaptive evolution or migration.

DISCUSSION

Our study of genomic variation in the TMVB populations of *Q. rugosa* reveals numerous loci with evidence of being locally adapted and identifies the most likely climatic drivers of genetic structure. We demonstrate that the combination of landscape genomic approaches, through the identification of locally adapted SNPs, and community modelling approach is an efficient strategy to identify regions across *Q. rugosa* distribution where gene-environment relationships will be most disrupted due to climate change. By modelling and mapping the adaptive variation along environmental gradients using GF we propose restoration strategies for *Q. rugosa* in most of its distribution range in Mexico.

Population diversity and structure

The STRUCTURE analysis indicates that *Q. rugosa* individuals can be assigned to six genetic clusters that showed a strong east-west gradient. This pattern was detected in other species occurring in the TMVB (Bryson Jr and Riddle 2012; Parra-Olea et al. 2012; Velo-Anton et al. 2013; Ruiz-Sanchez and Specht 2014) and may reflect a phylogeographic signature of its orogenic history (Mastretta-Yanes et al. 2015). However, the population differentiation was weak (F_{ST} = 0.055) and genetic diversity was high (H_E = 0.364), indicating that gene flow has been sufficient to overcome history of uplift, and interglacial cycles in the TMVB region. The overall genetic structure as a footprint of the uplift of TMVB emerges as a testable biogeographical hypothesis for future studies.

Population divergence of individual loci

BayeScan indicated that 78 SNPs showed signs of divergent selection, providing evidence that these populations are locally adapted despite evidence of extensive historical gene flow. Outlier F_{ST} values ranged from 0.147 to 0.318, which are 2.7- to 6-fold higher than the background F_{ST} (0.055). Even though the method is based on the premise that it is able to distinguish between the genetic signatures left by neutral and selective processes, we might consider possible bias before advocating that all these F_{ST} outlier SNPs are truly evidence of divergent selection. Some theoretical studies have shown that departures from the island model of population structure may cause false positives in population differentiation methods (Narum and Hess 2011; De Villemereuil et al. 2014; Lotterhos and Whitlock 2014). *Quercus rugosa* exhibited a pattern of isolation by distance, but the level of differentiation was far weaker than other TMVB tree species studied to date (Ruiz-Sanchez, Specht, and Ladiges 2013; Moreno-Letelier, Mastretta-Yanes, and Barraclough 2014; Ruiz-Sanchez and Ornelas 2014). Thus, the departure from the island model seems to be weak. Simulation studies also have shown that BayeScan has the best performance under departure from the island model than other population differentiation methods (Narum and Hess 2011; De Mita et al. 2013). We then advocate that F_{ST} outlier

analyses gave strong evidence of spatially divergent selection in these *Q. rugosa* populations.

Environmental association analysis of individual loci

The environmental association analysis identified 88 SNPs as significant outliers. Almost all of these outliers were correlated with the two temperature variables, suggesting that temperature gradient is an important driver of adaptive variation in *Q. rugosa* at the geographic scale encompassed in this study. Studies of other temperate and subtropical tree species have also identified a greater proportion of SNPs associated with temperature than with precipitation (Cox et al. 2011; De Kort et al. 2014; De Kort et al. 2015; Huang et al. 2015; Jaramillo-Correa et al. 2015). Other studies have documented a strong and significant historical influence of temperature variables in shaping geographic distribution of high altitude co-occurring species (Velo-Anton et al. 2013; Ruiz-Sanchez and Specht 2014). At the same time, we found 12 SNPs associated with precipitation variables, but the strength of association of these few SNPs was greater than SNPs associated with temperature. Thus, precipitation may be a stronger selection pressure on certain genes while temperature-driven selection pressure may influence more genes.

Precipitation seems to be a key factor shaping geographic patterns of adaptive variation (see GF discussion below), but our EA tests may be under-detecting its impact. For example, when an environmental variable co-varies with a gradient in population structure, the model may result in false negatives (Eckert, Bower, et al. 2010). Indeed, precipitation seasonality with fewer significant SNPs in LFMM were highly correlated with longitude (r = 0.86, Table S2). Precipitation seasonality also was highly correlated with population structure (r = 0.76). This covariance may have caused a lower number of significant precipitation-associated SNPs in our environmental association tests. In general, EA models may under-detect environmental variables that co-vary with neutral demographic structure (De Villemereuil et al. 2014; Lotterhos and Whitlock

2015). We then conclude that both precipitation and temperature are likely important drivers of selection in *Q. rugosa* TMVB populations.

Combining population differentiation and environmental association

We did find only four SNPs in common among the set of outliers from environmental association and those from population differentiation tests. Moreover, the SNPs that were significantly associated with climate did not show high divergence among populations, nor did the F_{ST} outliers have high climate associations (only one outlier F_{ST} SNP was associated with climate in LFMM). One explanation for this incongruence is that climate variables causing population divergence are undetected because they co-vary with demographic history. Indeed, the strength of association (|*z*|-scores) in LFMM was moderate and even lower in associations with temperature. Thus, the climate variables we detect through environmental associations may not be the important drivers of spatial divergence at the BayeScan outlier loci. Secondly, other environmental gradients or biological interactions may have imposed stronger selective pressures than climate and caused the observed population divergence. The lack of concordance between outlier F_{ST} and environmental association tests also highlights important differences between these methods, as discussed by Eckert, van Heerwaarden, et al. (2010) and Hancock et al. (2010). F_{ST} outlier tests are known to be very efficient in identifying strong instances of divergent selection (Narum and Hess 2011) acting on new mutations, but has less power to detect a weak selection acting on standing variation (Narum and Hess 2011; De Villemereuil et al. 2014) and may not detect genes that are under selection only in part of the populations (Narum and Hess 2011). EA tests, on the other hand, have more power to detect weak selection (De Mita et al. 2013) and are better able to detect candidate genes showing subtle variation in allele frequencies across populations (Jones *et al.,* 2013). The few overlap between the two sets of outlier SNPs has been found in other tree species, such as *Pinus taeda* (Eckert, van Heerwaarden *et al*. 2010), *Populus balsamifera* (Keller *et al*., 2012) and *Populus trichocarpa* (Evans et al. 2014), and those studies argue that different

provide distinct views of selection, especially because they may be detecting differentiation operating at different temporal scales. Thus, the lack of overlap in outliers indicates that different selective pressures have influenced these candidate SNPs. BayeScan likely identified loci with strong spatial divergence, whereas LFMM identified candidates of selection by climate with subtle variation in allele frequencies across the landscape.

Discovery of candidate genes

Forty percent (n = 64) of our 162 candidate SNPs are in genic regions and 53 are annotated in *Q. lobata* transcriptome. We also identified 31 candidate genes with previously identified orthologs in *Arabidopsis thaliana*. These genes are involved in a variety of physiological processes, including regulation of transcription and translation, transport of ions, metabolic processes, and response to abiotic stimuli. Although the number of genes we find here is too few for enrichment tests, other studies find enrichment for these categories. For example, Evans *et al*., (2014) reported an enrichment of gene annotations involved in response to stimuli, regulation of transcription, and metabolic processes in *Populus trichocarpa*. Eckert, Bower, *et al*., (2010) and Eckert, van Heerwaarden, *et al*., (2010) also found that many candidate genes identified through population differentiation or EA methods encode proteins associated with abiotic and biotic stress responses. The 53 candidate genes found here are targets for future investigation of their roles in phenotypic responses to environment.

Genomic landscape of current and future environmental adaptation

The five GF models indicate that precipitation gradients represent the main environmental driver of the turnover in allele frequencies in *Q. rugosa* in Mexico. Geography and unaccounted environmental gradients were also important predictors, as revealed by the greater importance of MEM variables in comparison with temperature gradients. Consequently, for all the five SNP sets, the predicted turnover in allele frequencies across the landscape followed the same east-west direction of the overall genetic structure (Fig. 1) and the

precipitation seasonality gradient. Indeed, the SNPs showing the strongest associations in the all SNPs model were not climate-associated candidates, revealing that the pattern of neutral differentiation coincides with adaptive variation.

Although the difference between models is subtle, which reveals that the all-SNPs model is a good predictor of tree performance, only for the climate associated SNPs there is a rapid turnover in allele frequencies in some small areas in the eastern portion of *Q. rugosa* distribution (areas in red in Fig. 6c and Fig. S7). In these areas, even a small change in precipitation seasonality and/or precipitation of the wettest quarter results in greater variation in allele frequencies. Based on the GF model built with precipitation candidates (Fig. 6b) we recommend the organization of Mexican distribution of *Q. rugosa* in three seed zones (zone 1: larger area in green tones, zone 2: smaller area in red and pink colors, and zone 3: smallest area in blue and purple. Our future projections indicated that populations in the north-eastern portion of the *Q. rugosa* distribution in Mexico (seed zone 2) are predicted to exhibit a significant disruption in the gene-environment relationship (warmer colors in Fig. 7 and Fig. S8). Considering long term persistence under a scenario of climate change, trees in zone 2 are expected to be less adapted to future climate if there is no adaptive evolution or migration. Indeed, in our precipitation data, the difference between current and future climates is expected to be greater in eastern regions than in western regions (data not shown). In eastern regions, populations are adapted to smaller precipitation seasonality and smaller precipitation of the wettest quarter than in the west. In our climate change scenarios, eastern regions would suffer from a greater increase in precipitation seasonality but also a greater decrease in precipitation of wettest quarter, while the velocity of climate change is comparatively slow in western regions. Under this scenario, we recommend that restoration plans in seed zone 2 should consider the benefits of the assisted gene flow concept (AGF; Aitken and Whitlock 2013), where seed sources would include preadapted genotypes to future precipitation conditions.

Seed transfers from western regions (seed zone 1, for example), whose genotypes are preadapted to greater precipitation seasonality, would be an adequate match. But, as precipitation seasonality and precipitation of the wettest quarter are changing in opposite directions, these genotypes could not be preadapted to the drier seed zone 2. To avoid a failure of future restoration or plantations, and following recommendations in Aitken and Bemmels (2016), we suggest a composite seed sourcing, that would mix local seeds, preadapted to a smaller precipitation of wettest quarter, and translocated seeds from seed zone 1, preadapted to a broader precipitation seasonality.

CONCLUSIONS

We found strong evidence of spatially divergent selection and local adaptation in natural populations of *Quercus rugosa* in TMVB. We, then, demonstrated that the combination of landscape genomics and GF modelling is an efficient strategy to identify and map the most critical regions under climate change. Our findings provided guidelines to the definition of three seed zones and specific recommendations for assisted gene flow in most of the *Q. rugosa* distribution range in Mexico.

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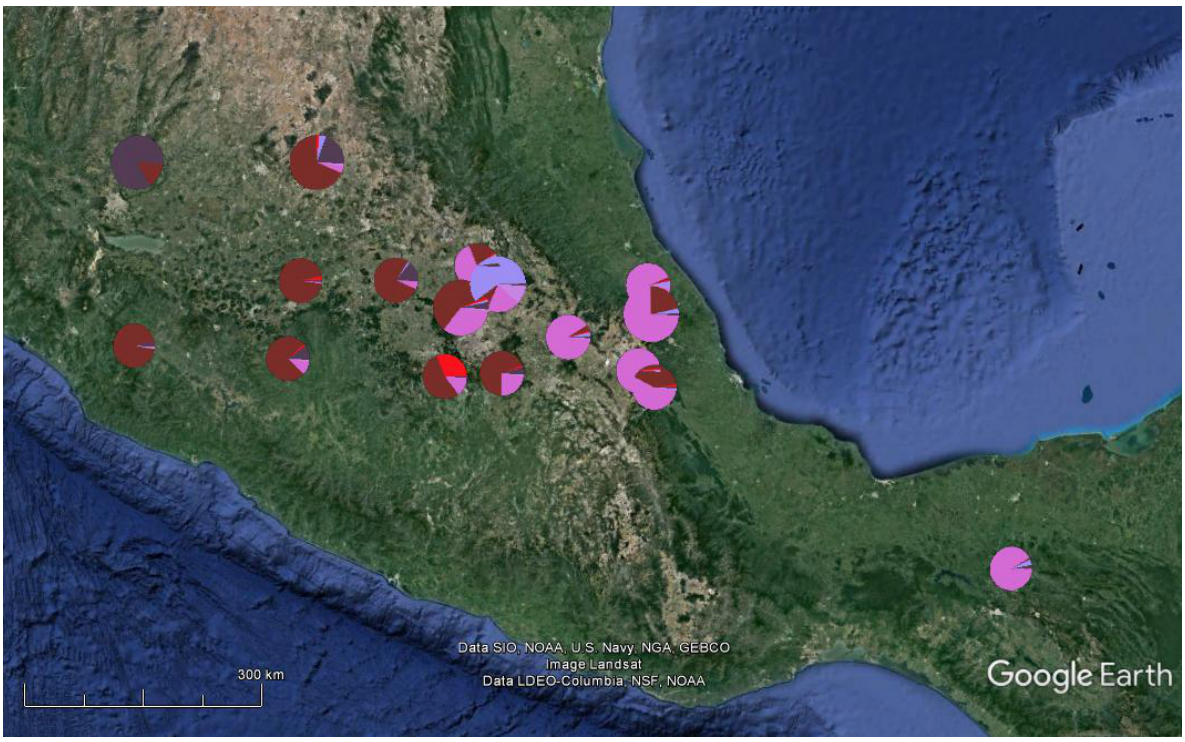


Fig. 1 Geographic distribution of population memberships (*K*=6), based on Bayesian clustering method in STRUCTURE. Black dots represent the actual location of the populations.

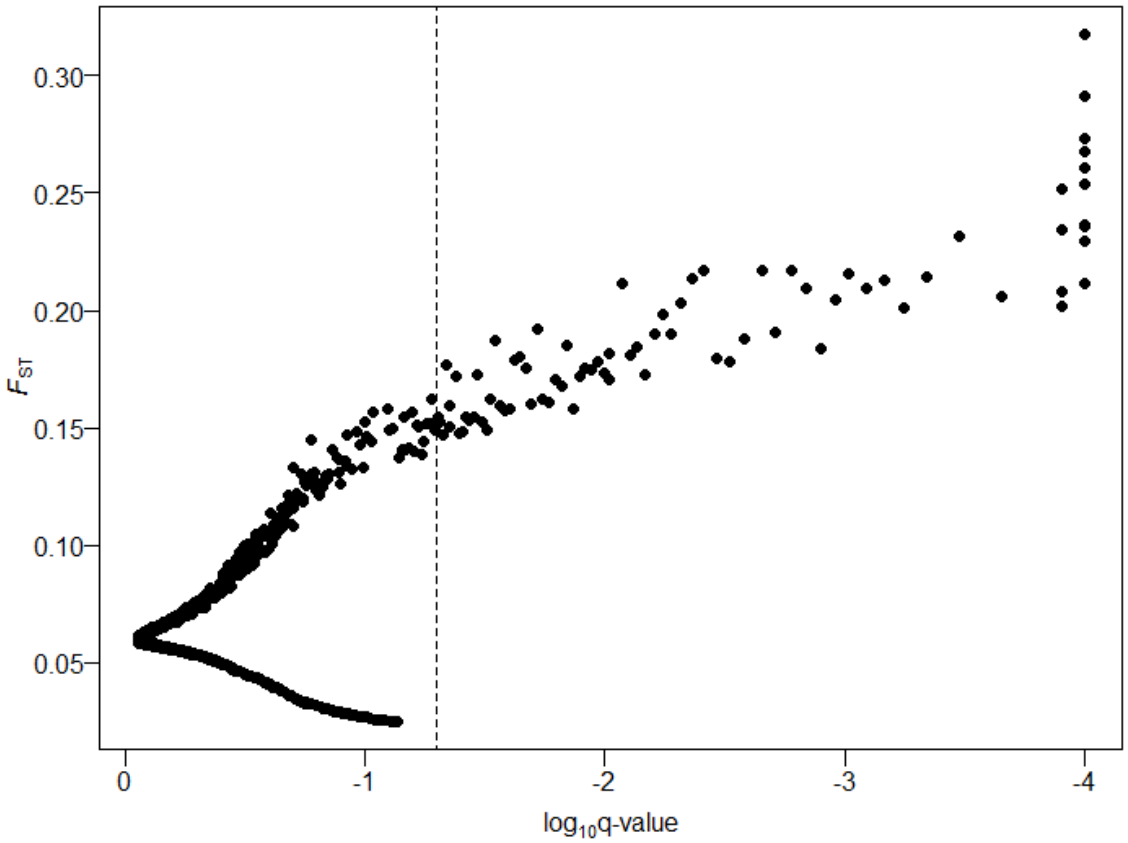


Fig. 2 Results for the outlier F_{ST} test. SNPs exceeding $\log_{10}q < -1.3$ are outliers. Values of $\log_{10}q = -4$ had $q = 0$ and were truncated at -4.

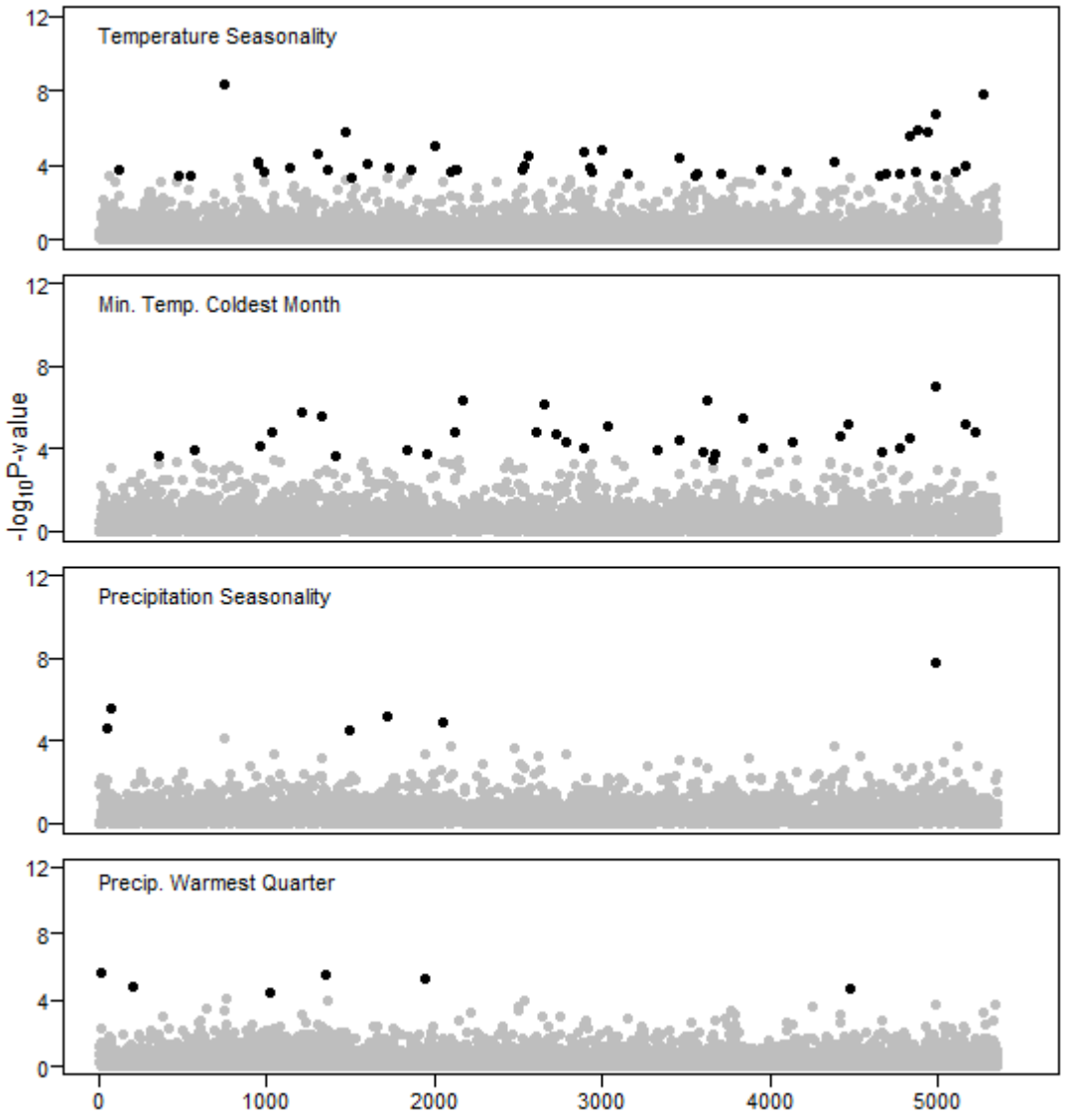


Fig. 3 SNPs associated with temperature and precipitation variables in LFMM. Black dots are SNPs significantly associated with climate in K =5 and 6 (adjusted-p < 0.05).

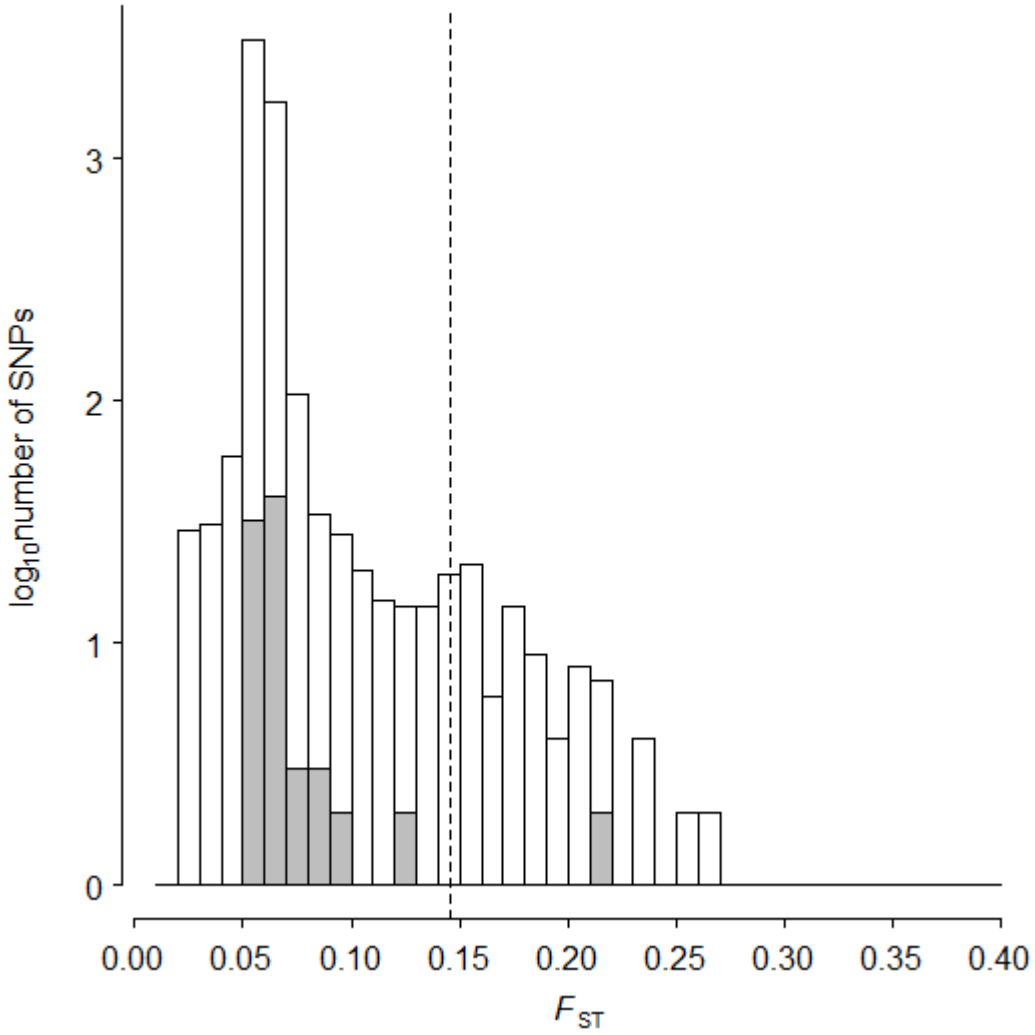


Fig. 4 Distribution of F_{ST} values across 5354 SNPs. Grey bars indicate 88 SNPs significantly associated with climate in LFMM. Dashed line is the lower limit of significant F_{ST} estimates.

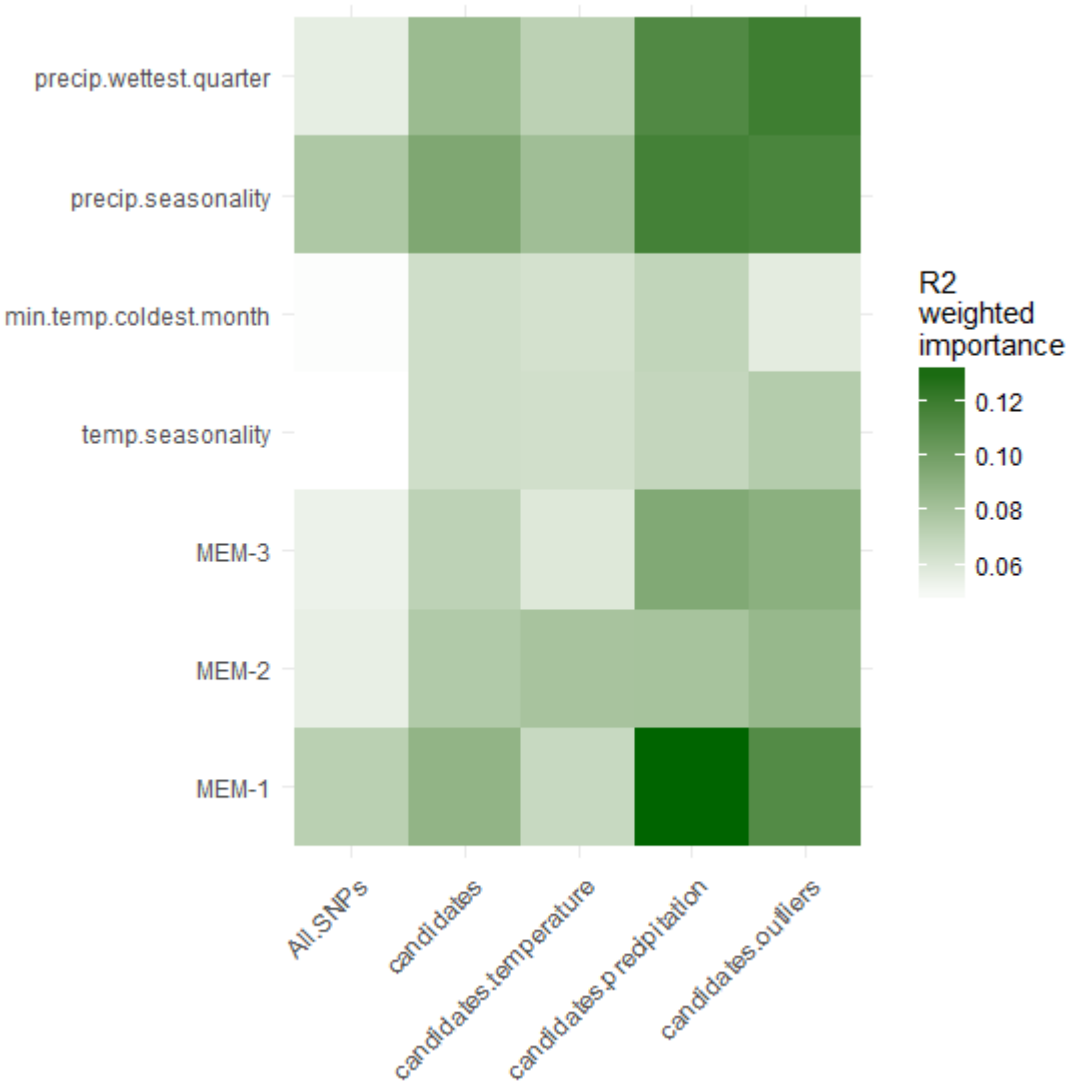


Fig. 5 The relative importance of climatic and spatial predictors used in GF for the five SNP sets. Darker shading indicates greater relative importance, measured as R^2 of each GF model. Candidates SNPs were those significantly associated with climate variables in LFMM. This SNP set was further separated in SNPs associated with temperature and SNPs associated with precipitation. Candidates outliers are SNPs that are both associated with climate and F_{ST} outlier.

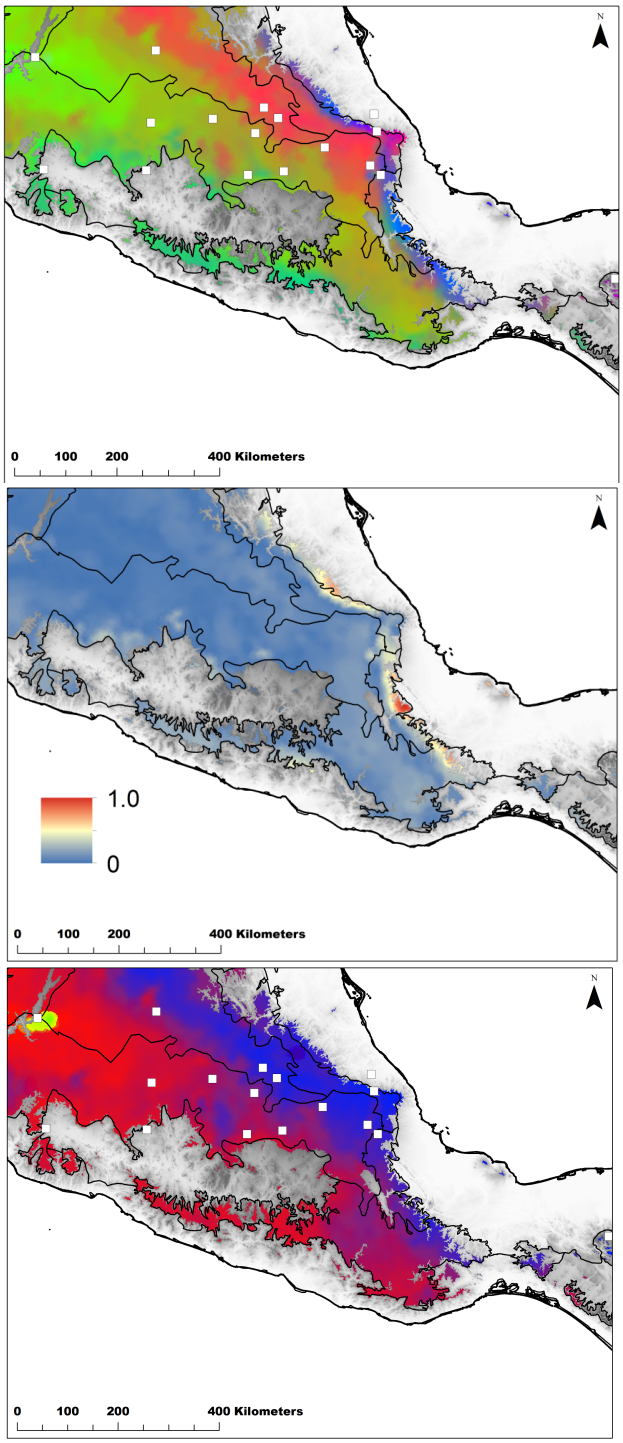


Fig. 6. Predicted spatial turnover in allele frequencies from GF for all SNPs (a) and for SNPs associated with precipitation (b). Regions with similar colors are expected to harbor populations with similar genomic compositions. The difference between GF models (c) mapped in (a) and (b) is based on Procrustes residuals, transformed to a 0-1 scale. White squares in (a) and (b) indicate the locations of populations used to fit GF models.

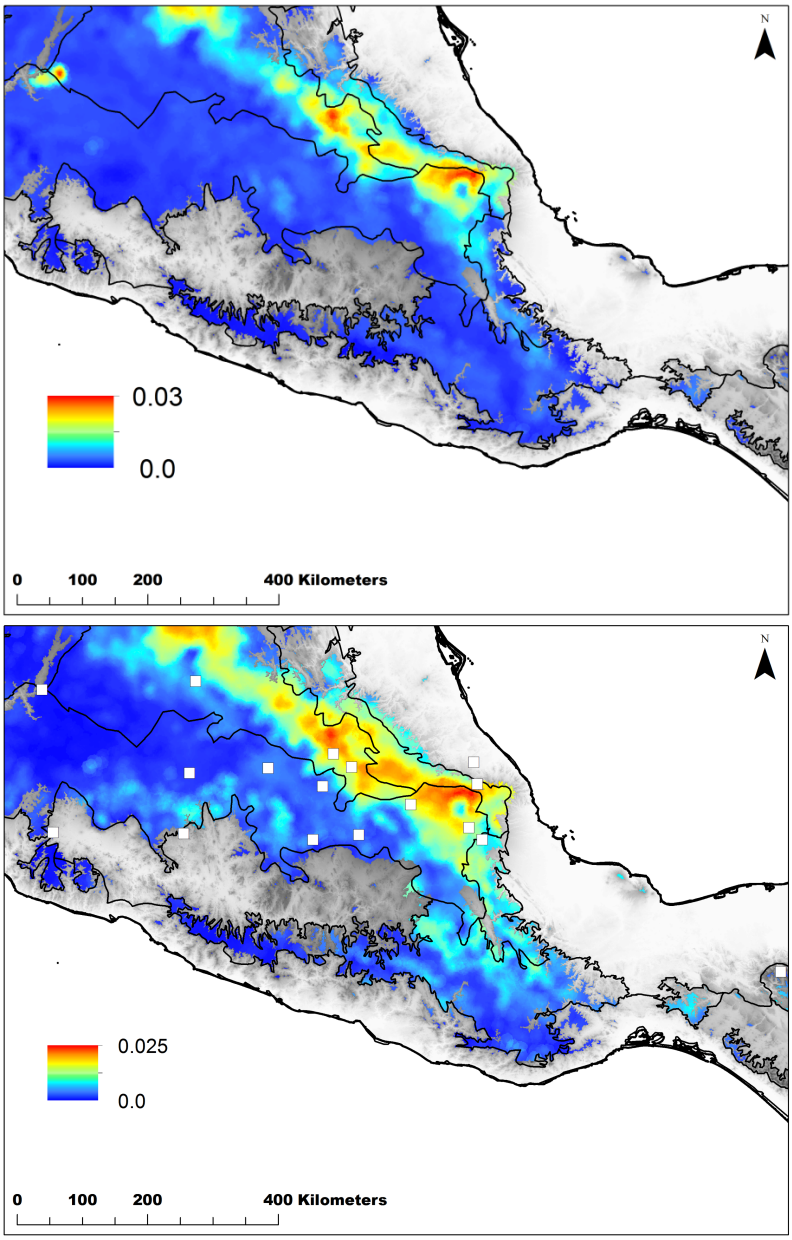


Fig. 7. Mean predicted genetic offset for SNPs associated with precipitation (a) and for F_{ST} Outlier SNPs associated with climate (b) for GF from three scenarios of 2080 climate change. Map unities are Euclidian distances between current and future genetic spaces for each model. Regions with greater Euclidian distances represent large predicted genetic offset.

Table 1. Summary of the five SNP sets used to fit GF models and parameters of model performance.

SNP sets	SNPs polymorphic in more than 4 populations	SNPs with $R^2 > 0$ (%)	Mean R^2 [range]
All	5354	1006 (18.8)	16.14 [0.03-74.62]
LFMM candidates	88	26 (29.5)	15.98 [0.39-53.92]
Temperature candidates	76	17 (22.3)	14.68 [0.19-32.48]
Precipitation candidates	12	8 (66.7)	18.50 [3.19-52.89]
F_{ST} outlier associated with climate in LFMM	4	4 (100)	19.96 [10.69-34.80]

Supporting Information

Fig. S1 Magnitude of Δ*K* as a function of *K*, with *K*-values ranging from 1 to 17.

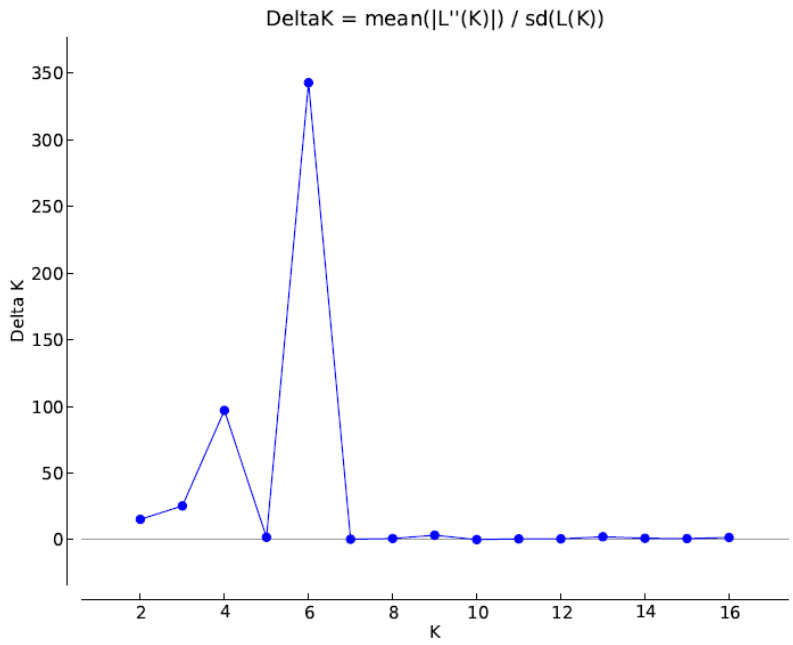


Figure S1

Fig. S2 Distribution of mean coverage depth per locus. Only mean depth above 5x was included.

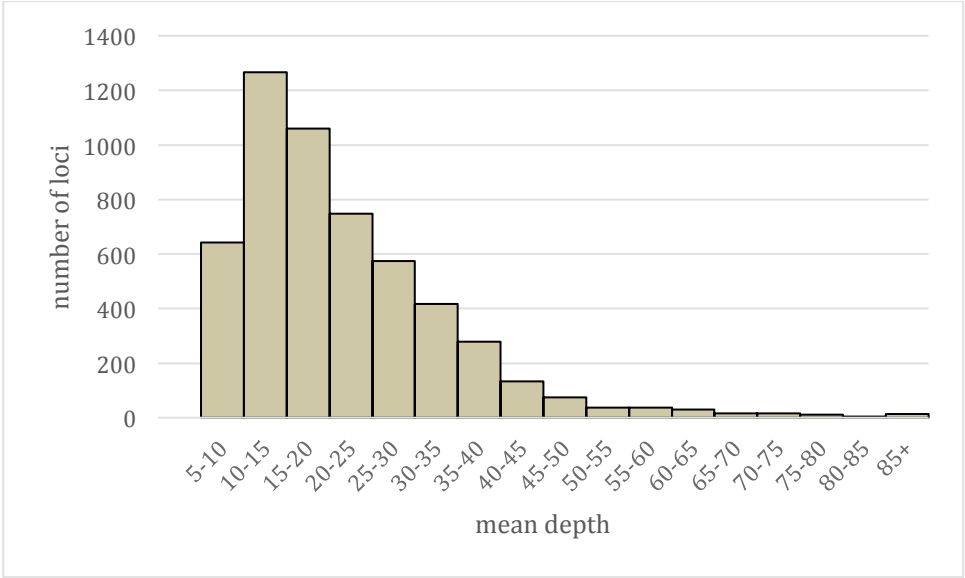


Figure S2

Fig. S3 Evidence of isolation by distance.

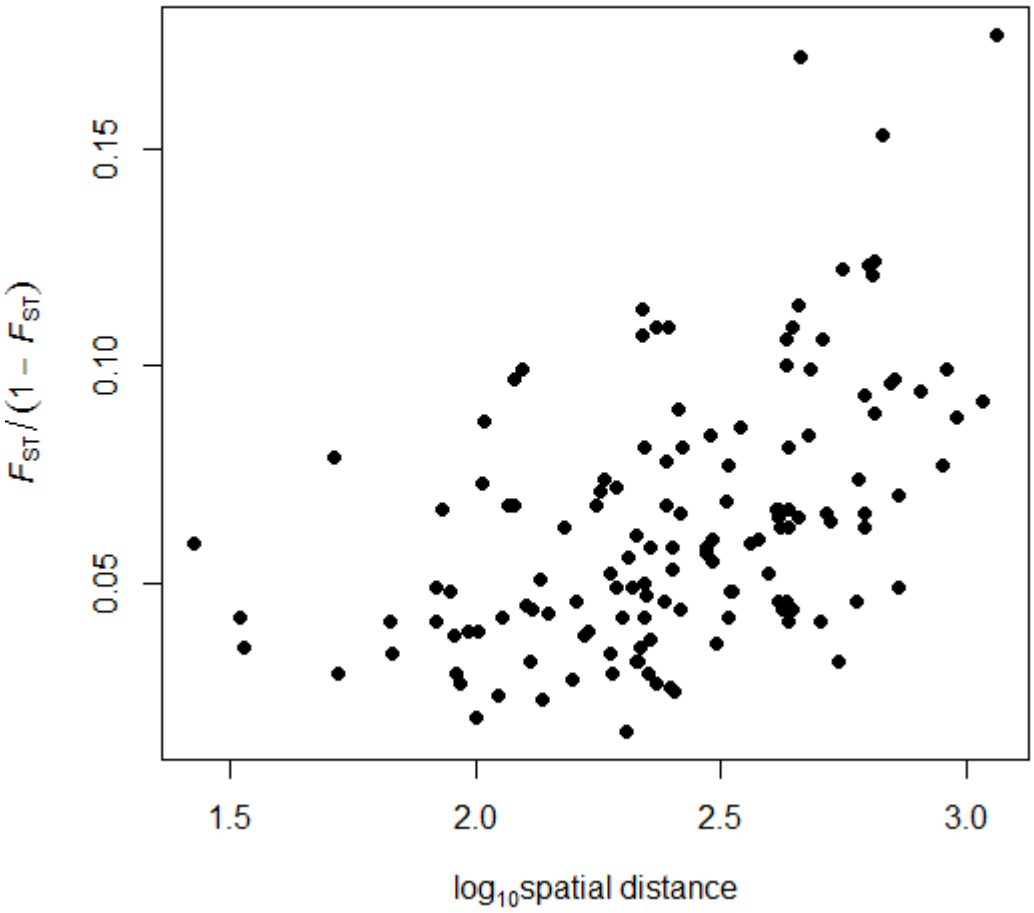


Figure S3

Fig. S4 Histograms of adjusted *p*-values for *K*=5 and *K*=6 and temperature variables (bio4: temperature seasonality, bio6: minimum temperature of coldest month, bio10: mean temperature of warmest quarter).

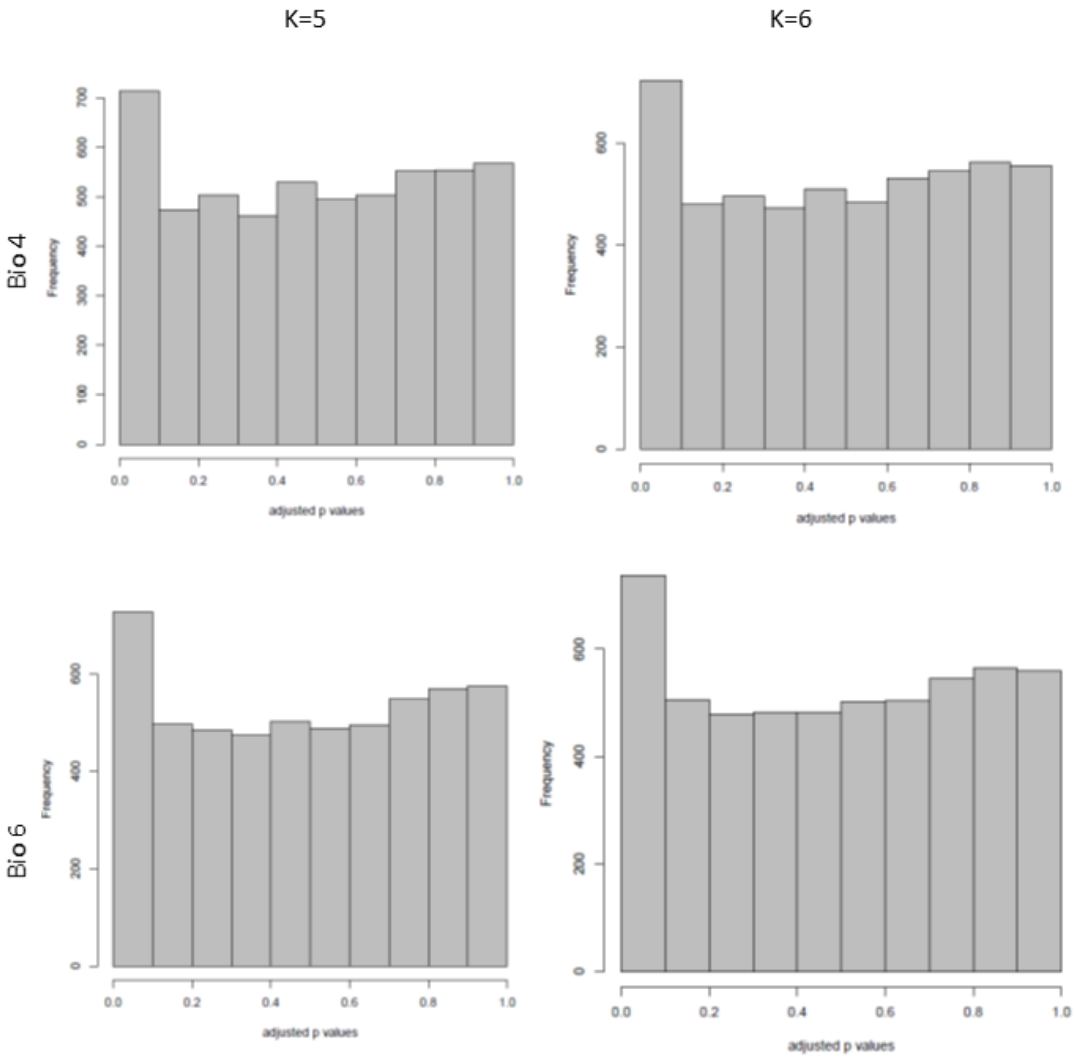


Figure S4

Fig. S5 Histograms of adjusted *p*-values for *K*=5 and *K*=6 and precipitation variables (bio15: precipitation seasonality, bio16: precipitation of wettest quarter).

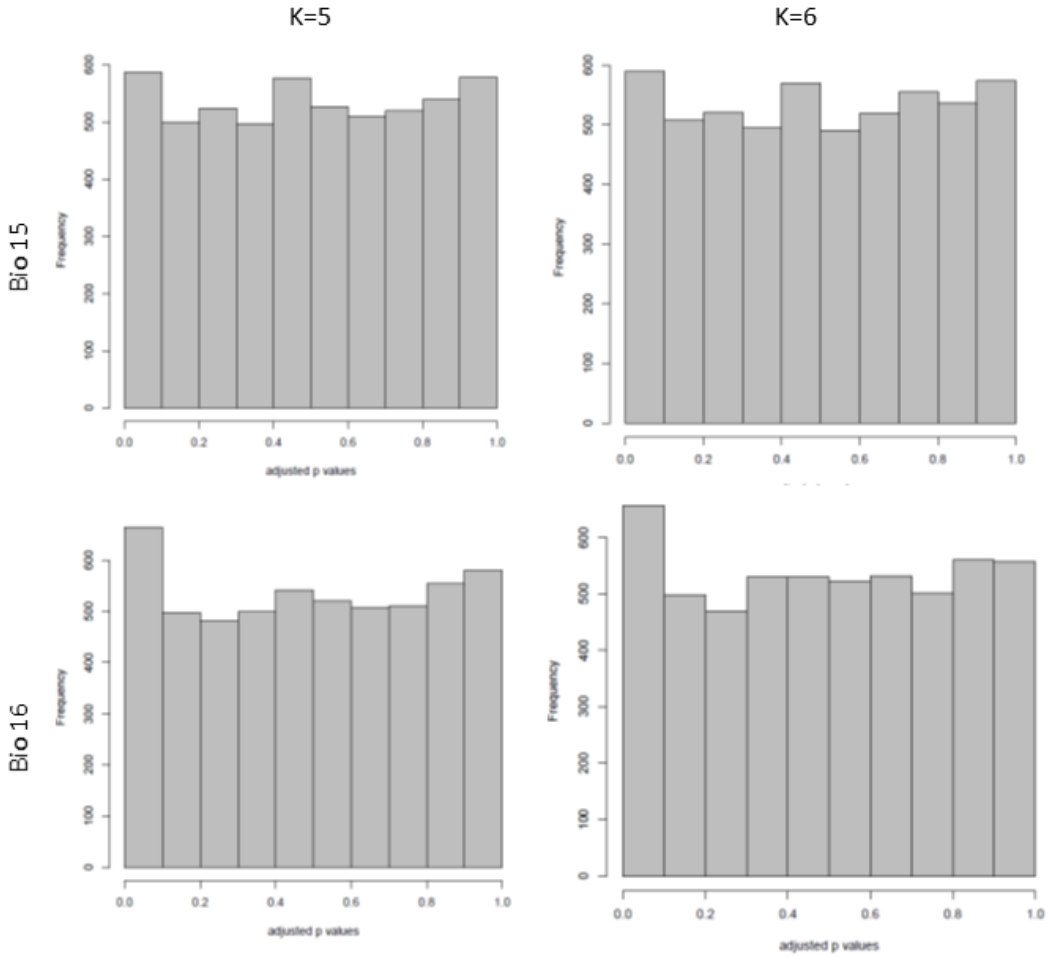
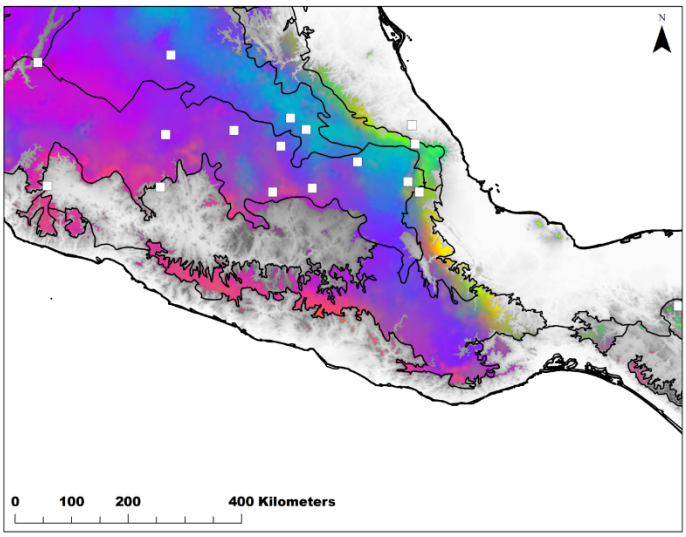
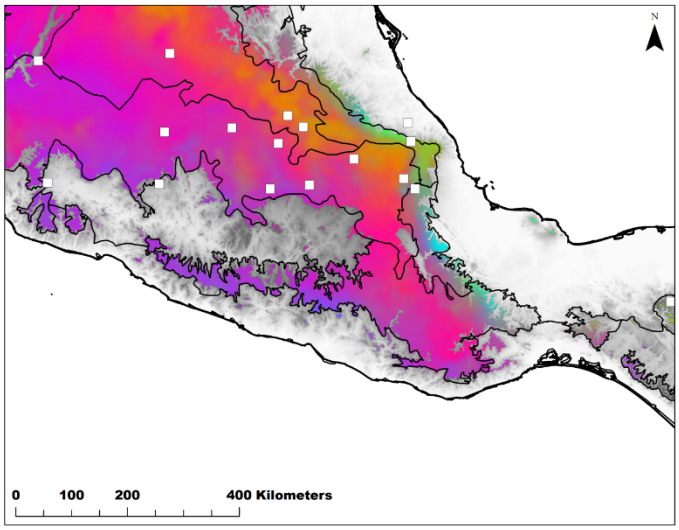


Figure S5

Fig. S6 Predicted spatial turnover in allele frequencies from GF for LFMM candidates (a), SNPs associated with temperature (b) F_{ST} outlier SNPs associated with climate (c). Regions with similar colors are expected to harbor populations with similar genomic compositions. White squares indicate the locations of populations used to fit GF models.





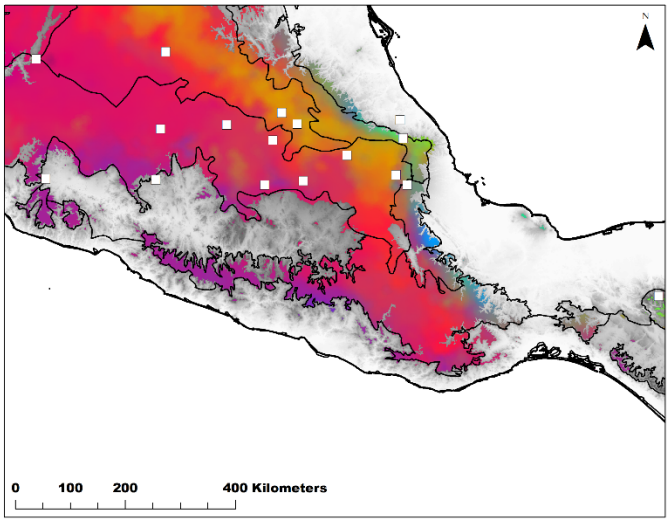
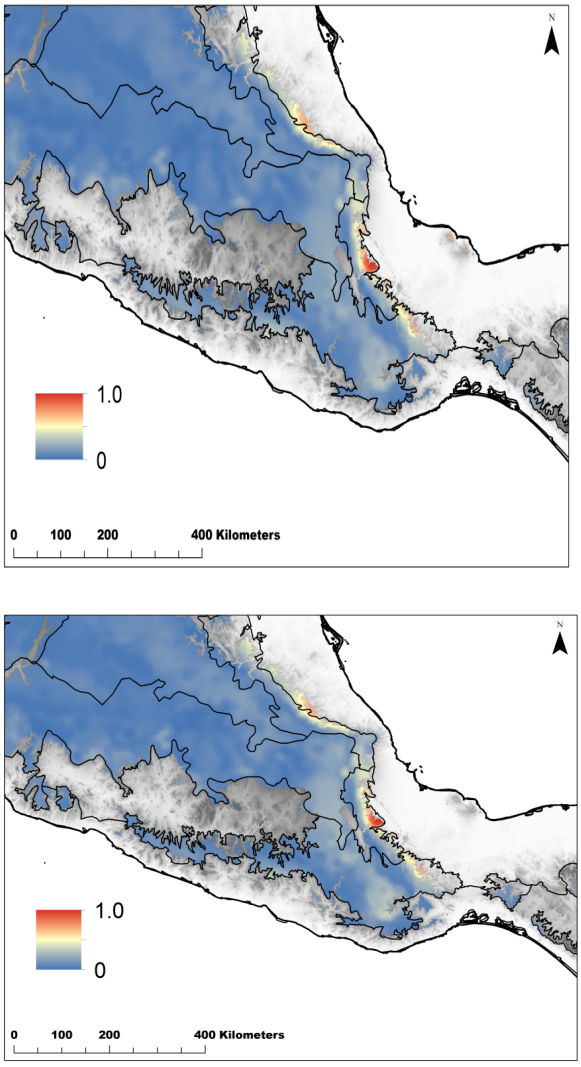

N
0
100
200
400 Kilometers

Fig. S7 The difference between the GF model fitted to the complete SNP set and the models fitted with LFMM candidates (a), SNPs associated with temperature (b) F_{ST} outlier SNPs associated with climate (c). Differences are based on Procrustes residuals, transformed to a 0-1 scale. White squares indicate the locations of populations used to fit GF models.



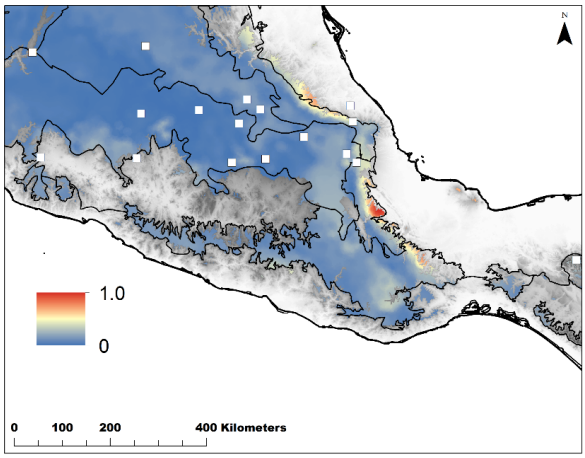
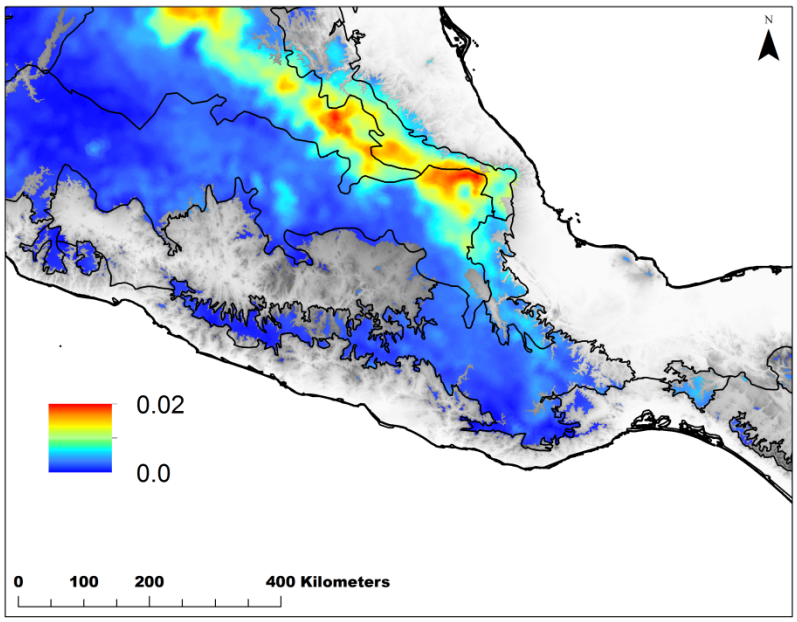

N
1.0
0
0 100 200 400 Kilometers

Fig. S8 Mean predicted genetic offset for LFMM candidates (a) and for SNPs associated with temperature (b) for GF from three scenarios of 2080 climate change. Map unities are Euclidian distances between current and future genetic spaces for each model. Regions with greater Euclidian distances represent large predicted genetic offset.



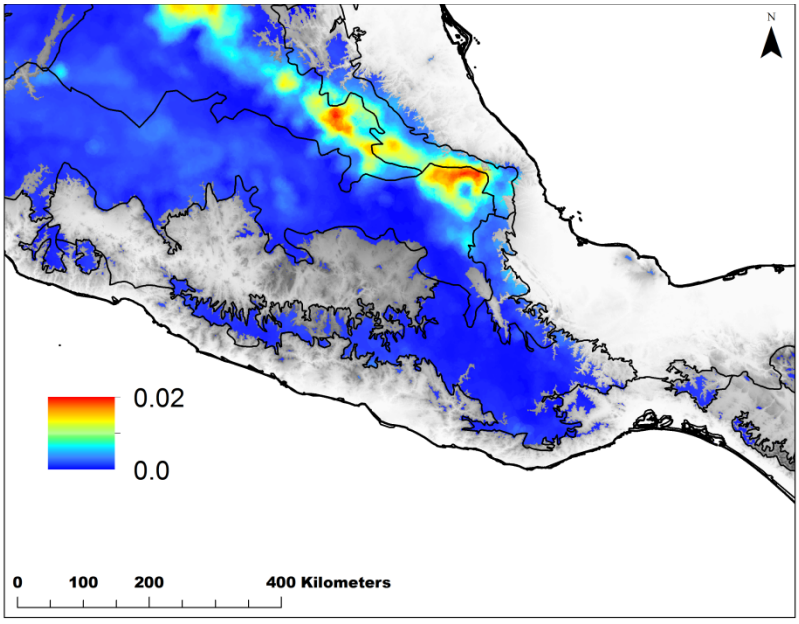


Table S1 Geographic locations and climatic data of 17 sampling sites in Mexico, mean pairwise F_{ST} per population, gene diversity (H_E) and inbreeding coefficient (F_{IS}). Temperature seasonality (BIO4), minimum temperature of coldest month (BIO6), precipitation seasonality (BIO15), precipitation of wettest quarter (BIO16).

Population name	Code	State	Sample size	Latitude	Longitude	Bio4	Bio6	Bio15	Bio16	Mean F_{ST}	H_E	F_{IS}
Amealco	AM	Querétaro	6	20.0	-100.0	164.21	2.05	96.64	479.68	0.050	0.362	0.009
Mpio. Bolaños	BOL	Jalisco	5	21.1	-103.2	272.60	5.11	113.59	550.37	0.095	0.327	-0.017
Cacahualco	CA	Veracruz	7	19.2	-97.3	107.03	-2.40	81.34	952.20	0.048	0.370	0.036
Cerro de la Malinche	CR	Tlaxcala	5	19.1	-97.1	173.39	6.79	73.41	911.37	0.069	0.355	-0.022
Los Dinamos	DIN	DF	8	19.1	-99.4	132.44	1.40	94.06	736.61	0.056	0.358	0.009
Erongarícuaro	ERO	Michoacán	6	20.0	-101.1	217.65	4.83	100.75	411.81	0.055	0.357	0.097
Alfahayucan (Hidalgo2)	H-2	Hidalgo	9	20.1	-98.9	174.55	2.19	73.45	286.22	0.076	0.364	0.034
Mazamitla	MA	Jalisco	5	19.2	-103.0	159.54	14.07	105.13	530.25	0.056	0.359	0.188
Mineral del Monte	MM	Hidalgo	6	20.2	-99.1	205.27	3.84	72.30	199.51	0.037	0.383	0.140
Santa Catarina	SC	Edo. Méx	6	19.8	-99.3	192.64	2.19	88.60	464.21	0.047	0.371	0.014
San Nicolás de los Ranchos	SNR	Puebla	5	19.1	-98.8	161.39	2.41	91.24	502.79	0.047	0.367	-0.094
Santa Rosa	SR	Guanajuato	5	21.2	-101.0	253.27	3.55	87.92	278.39	0.051	0.370	0.021
Tatatila (veracruz1)	TAT	Veracruz	8	20.1	-97.2	298.28	13.92	48.41	592.53	0.046	0.378	0.233
Tenejapan	TE	Chiapas	3	17.2	-93.0	167.59	10.79	41.94	815.89	0.069	0.358	0.016
Terranate	TER	Tlaxcala	8	19.8	-97.2	251.68	10.00	54.48	1054.78	0.051	0.369	0.019
Tlalpujahua	TLA	Michoacán	5	19.1	-101.2	175.92	13.48	109.15	709.23	0.041	0.370	-0.050
Zacatepec	ZA	Puebla	8	19.5	-98.1	160.09	0.87	78.61	351.88	0.049	0.374	0.155

Table S2 Correlation of spatial and climate variables associated with 17 localities *of Quercus rugosa* (see Figure 1).

	Precip., seasonality	Precip., wettest quarter	Temp. seasonality	Min. temp., coldest month	Latitude × longitude	Longitude	Longitude^2	Latitude^2
Precipitation, wettest quarter	-0.262	1	-	-	-	-	-	-
Temperature seasonality	0.272	0.105	1	-	-	-	-	-
Minimum temperature, coldest month	-0.236	0.251	-0.553	1	-	-	-	-
Latitude × longitude	0.178	-0.096	0.098	-0.017	1	-	-	-
Longitude	-0.859	0.429	0.140	-0.022	-0.293	1	-	-
Longitude^2	0.001	0.183	-0.154	0.440	-0.786	0.000	1	-
Latitude	0.200	-0.476	-0.628	-0.107	0.208	-0.548	-0.276	1
Latitude^2	-0.106	-0.049	-0.263	0.148	-0.920	0.087	0.780	0.000

CAPÍTULO V

DISCUSIÓN Y CONCLUSIÓN FINAL

Variación en el tamaño de las bellotas

Uno de los caracteres clave para la sobrevivencia y dispersión de las plantas es el tamaño de sus semillas. Este rasgo además de reflejar una serie de características biológicas (e. g. representa la cantidad de inversión materna en una descendencia) está relacionado a distintas variables ambientales que determinan el establecimiento de las especies. Moles (2007) reporta la existencia de un patrón global de disminución del tamaño de las semillas que va de 2 a 3 órdenes de magnitud entre el Ecuador y los 60^{o} de latitud norte, asociado fuertemente a la divergencia de formas de crecimiento (gramíneas, hierbas, arbustos, árboles), mientras que autores como Aizen y Woodcock (2009) y Koenig (2009) relacionan que el patrón de disminución en el tamaño de las bellotas de especies de encinos del norte América (e.g. *Q. macrocarpa*, *Q. alba*, *Q. rubra* y *Q. velutina*) esta dado por la restricción ambiental de las temporadas de crecimiento.

Para el género *Quercus*, el tamaño de las bellotas ha sido correlacionado positivamente con el porcentaje de germinación, la emergencia, sobrevivencia y crecimiento de las plántulas, así como con el área total de la hoja. También se ha observado que las bellotas mas grandes pueden establecerse en lugares con condiciones ambientales distintas a las de su proveniencia e incluso pueden tolerar mejor condiciones estresantes como la sequía (Aizen y Patterson, 1990). De manera interesante, otros estudios muestran una relación positiva entre el tamaño de las bellotas y el crecimiento de las plántulas en otras especies de *Quercus* como son *Q. prinus* y *Q. rubra* (McComb 1934; Kolb y Steiner 1989).

La gran variación reportada en el tamaño de las bellotas que se presenta en el género *Quercus* tanto a nivel inter como intraespecífico ha llevado a plantear distintas hipótesis dirigidas a conocer cuales son los factores involucrados en la determinación del tamaño de las semillas. En nuestro primer capítulo exploramos la hipótesis de la restricción abiótica con el objetivo de

conocer si la variación en el tamaño y volumen de las bellotas de *Q. rugosa* estaba influenciada por distintas variables climáticas relacionadas con la precipitación y la temperatura.

Hay otras dos hipótesis propuestas para explicar la clina latitudinal en los tamaños de las bellotas que ya fueron descartadas pero que vale la pena mencionar. La hipótesis de la vicarianza que trababa de explicar los patrones de variación a través de factores abióticos relacionados a las regiones geográficas que sufrieron glaciación y aquellas que no y la hipótesis de que la dispersión diferencial de las bellotas esta conferida por los agentes dispersores, principalmente aves y debido a que prefieren mover semillas pequeñas (Kening *et al*., 2009).

Nuestros datos, al igual que los reportados por Aizen y Woodcok (1992) y Koening *et al*. (2009) para especies del género *Quercus* (*Q. alba*, *Q. macrocarpa*, *Q. rubra*, *Q. velutina, Q. macrocarpa*) muestran una fuerte correlación negativa de los rasgos cuantificados (Largo, Ancho, Volumen y Masa) con la latitud (Tabla 2 y Figura 2, Capítulo II). También muestran una fuerte correlación positiva con la precipitación de la temporada de crecimiento (gsp) y con los días grado mayores a 5^0C de la temporada de crecimiento (GSDD5). Estos datos soportan la hipótesis de que la restricción ambiental dada por la disminución en la precipitación y la duración de las temporadas de crecimiento tienen un efecto en el desarrollo de las bellotas. A su vez, esta restricción ambiental tiene un efecto negativo (o restrictivo) en la germinación de las plántulas. Además, se ha reportado una relación positiva entre el peso y tamaño de las bellotas con la altura total, el peso total y el área foliar total de plántulas al término de la primera temporada de crecimiento, lo que indica que toda la energía almacenada en los cotiledones es consumida en ese primer año y es la que aporta el primer impulso para el crecimiento (Long y Jones, 1996). Por otro lado, hay estudios que muestran que las plántulas son más vulnerables a la sequía durante los primeros años posteriores a su establecimiento y que el

tamaño de las bellotas es más importante en esta etapa para el crecimiento (Ramírez-Calcerrada *et al*., 2011). Finalmente, se han reportado diferencias poblacionales en el grado de tolerancia a la sequía de distintas poblaciones de *Q. suber* asociadas al lugar de procedencia de las bellotas, en donde aquellas que vienen de sitios secos tienen una mayor sobrevivencia bajo estas condiciones (Ramírez-Valiente *et al*., 2009a).

Variación en atributos morfológicos y fisiológicos

Existen otros caracteres funcionales (atributos morfológicos, fisiológicos, fenológicos) de gran importancia que representan las estrategias ecológicas que determinan cómo las plantas responden al ambiente. Estas estrategias están además, relacionadas con la diferenciación morfológica temprana de las plántulas (Matsuda *et al*., 1989). De manera que, en el segundo capítulo de la tesis, se examinaron los patrones de variación morfológica y fisiológica de las plántulas de *Q. rugosa* que germinaron de las bellotas colectadas y que fueron crecidas bajo dos condiciones de riego (TH - Tratamiento de Humedad y TS - Tratamiento de sequía). Lo que encontramos fue que las plántulas al año de germinar y de haber crecido bajo las mismas condiciones (antes de hacer los tratamientos) presentaban una variación morfológica significativa en la altura del tallo, el número de hojas y el largo y ancho de la hojas, en donde las plántulas que provenían de regiones con menor precipitación media anual y menor temporada de crecimiento eran las mas pequeñas (Tabla 2; Capítulo III). Esto podría ser un efecto materno, ya que las plántulas del sur de México, que fueron las más grandes, provenían de bellotas con mayor volumen. Sin embargo, ya que este mismo patrón de variación clinal en estos rasgos se siguió observando durante los dos años posteriores, además de presentar una alta correlación positiva con gsdd5, sugerimos que efectivamente la variación observada es el producto de una respuesta adaptativa ante el ambiente. Previamente se ha demostrado que especies de encinos (e. g. *Q. laevis* y *Q. margaretta*) que habitan regiones xerófilas presentan tasas de crecimiento menores, menor

número de hojas y de menor tamaño, así como menores áreas foliares con respecto a especies de zonas húmedas (e. g. *Q. lyrata, Q. rubra*), reflejando una respuesta evolutiva ante el estrés hídrico (Long y Jones, 1996; Matsuda *et al*., 1989; Fotelli *et al*., 2000). Por otro lado, se sabe que en ambientes secos las plantas pueden aumentar su desempeño al disminuir la pérdida de agua al reducir sus áreas foliares, aumentando la eficiencia del uso del agua, o ambas (Gouveia, 2009). Nuestros resultados sugieren que entre las poblaciones de *Q. rugosa* existe diferenciación intraespecífica funcional de forma tal que las plántulas de *Q. rugosa* que provienen de las zonas más secas (norte de México) tiene una estrategia para reducir la pérdida de agua al disminuir el tamaño y número de hojas, además de presentar una menor tasa de crecimiento.

Por otro lado, se encontraron diferencias significativas a nivel poblacional al comparar la respuesta de los atributos morfológicos en plántulas crecidas bajo dos condiciones de humedad (TH –TS), observándose una variación en el crecimiento (cm), el número de hojas y el largo y ancho de éstas, así como en el área foliar total. Se vió que, las poblaciones que provenían de las regiones con menor precipitación anual (Papasquiaro y Caborachic) presentaban el menor crecimiento (cm), el menor número de hojas y de menor longitud, siguiéndose este patrón tanto en el TH como en el TS. Al comparar los atributos entre los tratamientos observamos que la limitación de agua en el suelo de las plántulas del TS indujo una disminución significativa de estos rasgos (Tabla 3; Capítulo III), mostrando que efectivamente el tratamiento tuvo un efecto y evidenciando la gran capacidad plástica en el ajuste fenotípico de las plántulas de *Q. rugosa* ante la limitación de agua. Más aún, encontramos evidencia de cómo la selección local ha tenido un fuerte impacto en la fijación de algunos atributos que favorecen el establecimiento de estas plántulas ante condiciones de baja humedad de suelo, ya que las poblaciones que provenían de las regiones más áridas (Papasquiaro y Caborachic), a pesar de haber crecido en condiciones húmedas, siempre mantuvieron un menor tamaño, menor número de hojas y de menor tamaño, demostrando la significancia adaptativa de la reducción foliar

bajo condiciones secas. En conclusión, estos datos muestran tanto la gran respuesta plástica de las plántulas de *Q. rugosa*, ante la limitación de agua en el suelo, como evidencia contundente de una variación morfológica y fisiológica en las poblaciones de provenían de las regiones áridas producto de una adaptación local.

Variación en el estado hídrico interno y en el intercambio de gases

Entonces, si la disminución del área foliar está relacionada con la reducción de pérdida de agua, nosotros esperaríamos encontrar variación en atributos fisiológicos relacionados con la optimización de este recurso, ya que se ha reportado una relación significativa entre el estrés hídrico y el desarrollo de mecanismos de tolerancia ante éste, tales como el ajuste osmótico, la disminución de los potenciales hídricos de las hojas y del intercambio de gases (Abrams, 1990; Aranda *et al.,* 2004). En nuestro estudio encontramos que en las plántulas del TH los valores del potencial hídrico de las hojas tanto antes del amanecer como al medio día fueron relativamente altos (-0.21 a -0.32 MPa) y consistentes con los encontrados en otras especies de encinos (*Q. austrina, Q. chapmanii, Q. margaretta, Q. michauxii*), indicando que las plántulas tenían acceso al agua. De igual manera, los potenciales hídricos en el TS presentaron valores similares a los reportados en otras especies de encinos tanto en las mediciones antes de amanecer (-1.8 a -2.9 MPa en *Q. rubra* y *Q. alba*; Comin et al., 1987) como del medio día (-2.33 a -3.34 MPa en *Q. macrolepis, Q. germinata*; Cavenders-Bares y Holbrook 2001). Esto muestra que es una estrategia de tolerancia generalizada de los encinos para hacer frente a la sequía y de acuerdo con algunos autores esto sugiere una adaptación ancestral conservada (Aguilar-Romero *et al*., 2017). De esta forma, la tolerancia ante el déficit hídrico les permite a las plántulas de los encinos sobrevivir manteniendo sus funciones fotosintéticas bajo condiciones de desecación e incluso se ha reportado que las especies perennes de encinos pueden tolerar y recuperarse de embolismos producto del déficit hídrico (Cavender-Bares y Holbrook, 2001).

Los parámetros de intercambio de gases mostraron diferencias significativas a nivel poblacional en el TH para la tasa fotosintética máxima (*Amax*), la conductancia estomática (*G*) y la transpiración (*E*), y al comparar estos valores con los encontrados en el tratamiento TS se observó una disminución altamente significativa de estos valores, mostrando que la variación de estos atributos durante el estrés hídrico es una respuesta que contribuye a la regulación de la pérdida del agua en las hojas evitando la pérdida de turgencia en las células. Por otro lado, se observó que las poblaciones de las zonas más secas presentaban los valores más altos en sus tasas fotosintéticas con respecto a las plántulas que provenían de las regiones con mayor precipitación (*Amax* Papasquiaro = 5.64 ± 0.89, *Amax* Chiapas = 0.55 ± 0.89), y lo más interesante fue que en este tratamiento se observó que en las poblaciones del norte (Caborachic y Papasquiaro) tienen un mejor ajuste en la eficiencia del uso del agua durante periodos con poca disponibilidad de agua.

En conjunto, en este estudio encontramos evidencia de cómo las plántulas de *Q. rugosa* hacen frente a la baja disponibilidad de agua desarrollando una combinación de estrategias que van desde el ajuste fenotípico que muestra la alta capacidad plástica de estas plántulas, así como respuestas de tolerancia y adaptativas, concluyendo que las plántulas de *Q. rugosa* tienen una gran capacidad para mantener sus actividades biológicas durante periodos de sequía, no obstante, mostramos evidencia que las plántulas originarias de las poblaciones del norte están mejor adaptadas a las condiciones de sequía que aquellas que provienen de regiones humedad dado su gran ajuste en la eficiencia del uso del agua.

Diferenciación genética

En nuestro estudio encontramos que los valores de diferenciación genética de 8 microsatélites nucleares (Fst) fueron parecidos a los reportados en otras especies de encinos (Americanos y Europeos) y dentro de los valores estimados

para especies de árboles forestales, los cuales son menores a 0.1 (Savolainen *et al*., 2007). Estos datos muestran el alto flujo de genes histórico entre las poblaciones de *Q. rugosa*, el cual es el responsable de mantener la diversidad genética al mover alelos de una población a otra (Sork *et al*., 2010). Sin embargo, también encontramos que a pesar de la baja diferenciación genética las poblaciones muestran un gradiente Norte - Sur que separa las poblaciones en dos grupos geográficos (de acuerdo al análisis en Structure), en donde el grupo del sur de México estaba conformado por las poblaciones de Chiapas, Santa Inés y San José Mixtepec y el grupo del Norte con Bolaños, Papasquiaro y Caborachic. Esta diferenciación genética muy posiblemente está influenciada por el grado de heterogeneidad ambiental, ya que el gradiente latitudinal de México (Sur – Norte) está asociado a variables climáticas relacionadas a la precipitación (PMA) y al índice de aridez (IA), además de que encontramos que esta estructura clinal también está influenciada por el aislamiento por distancia ya que se encontró una correlación positiva entre los pares de valores de Fst y la distancia geográfica.

De igual manera, al estudiar 17 poblaciones de *Q. rugosa* distribuidas a lo largo de la Faja Volcánica Trasnmexicana encontramos valores de diferenciación (Fst) por pares de poblaciones que iban de 0.037 a 0.096, los cuales son parecidos a los encontrados en las plántulas (0.093). En este caso, se encontró un gradiente de distribución genética Este – Oeste influenciado por un flujo de genes restringido que está contribuyendo a la divergencia de las poblaciones, ya que se encontraron 96 SNPs que mostraban señal de selección divergente. Además, mediante un análisis de asociación ambiental se encontraron 108 SNPs, de los cuales 8 se correlacionaron con la precipitación y la mayoría de los restantes a 3 variables relacionadas a la temperatura, lo que sugiere que tanto la precipitación como la temperatura ejercen una presión selectiva sobre este gradiente longitudinal en la Faja Volcánica.

En conclusión, nuestro estudio muestra evidencia de la presencia de una selección espacial divergente en asociación con gradientes climáticos a los cuales se ven sometidas las distintas poblaciones de *Q. rugosa* a lo largo de su área de distribución. Se hace evidente porque *Quercus rugosa* es una de las especies con mayor distribución en México al mostrar su gran capacidad plástica para responder a condiciones contrastantes de humedad del suelo ajustando sus fenotipos ante la disponibilidad de este recurso, así como mostrando una marcada diferenciación de estrategias y atributos que muestran una adaptación local dada por la heterogeneidad ambiental. El encontrar variación genética (SNPs) asociada a variables climáticas relacionadas con la precipitación fortalecen la hipótesis de que las poblaciones que viven en regiones más secas están mejor adaptadas que las de regiones húmedas a condiciones ambientales desfavorables de humedad.

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