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Estructura genética, filogeografía e identificación de zonas híbridas en *Quercus magnoliifolia* y *Q. resinosa* (Fagaceae) en México.

T E S I S

QUE PARA OBTENER EL GRADO ACADÉMICO DE

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P R E S E N T A

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CONTENIDO

I. RESUMEN GENERAL

El presente trabajo es el primero en estudiar dos especies de encinos blancos mexicanos, *Q. magnoliifolia* y *Q. resinosa* a lo largo de toda su distribución, utilizando morfometría geométrica, modelado de nicho ecológico y marcadores moleculares nucleares y citoplasmáticos, con el objetivo de conocer la historia evolutiva histórica y contemporánea. La tesis está conformada de tres capítulos. En el primer capítulo encontramos que el efecto de la hibridación sobre la asimetría fluctuante (AF) foliar tiene una base genética. Los valores de AF más bajos fueron en los individuos parentales, después las retrocruzas y los valores más altos de AF fueron en los híbridos F1, apoyando la hipótesis de que la hibridación genera disrupción de los genes coadaptados (combinación favorable de genes, que hacen más aptos a los organismos para su desarrollo, funcionamiento y supervivencia), incrementando la inestabilidad del desarrollo. En el segundo capítulo encontramos que *Q. magnoliifolia* y *Q. resinosa* son dos especies morfológicamente diferentes, tienen nichos ecológicos divergentes y las zonas de simpatría ocurren en ambientes intermedios a las dos especies. Las diferencias de temperatura caracterizan el nicho ecológico de cada una de las especies. Dos zonas de simpatría geográfica y ambientalmente diferentes fueron predichas por los modelos. Los F_{ST} pareados a través de todos los loci muestran alta diferenciación intra-específica y baja diferenciación inter-específica. Se encontró aislamiento por distancia entre las dos especies y dentro de *Q. magnoliifolia*. Incluso, la diferenciación inter-específica fue baja entre poblaciones cercanas que entre poblaciones más distantes. La agrupación Bayesiana muestra dos grupos genéticos (K = 2), los cuales su correspondencia con la identificación morfológica es muy baja pero a nivel geográfico sí muestra estructura. La baja congruencia entre la morfología de la forma de la hoja y la asignación de genética sugiere que las especies permanecen morfológicamente y ecológicamente distintas,

debido posiblemente a la selección disruptiva, a pesar de los altos niveles de flujo génico inter-específico entre las poblaciones cercanas. La estructura geográfica de la introgresión entre *Q. magnoliifolia* y *Q. resinosa* soporta la hipótesis de flujo génico inter-específico por aislamiento por distancia. Análisis genómicos en loci bajo selección van hacer necesarios para identificar los genes asociados con la diferenciación inter-específica de estos encinos Mexicanos, debido a la extrema porosidad de su genoma. En el tercer capítulo, encontramos una gran diversidad de haplotípos de ADNcp en las poblaciones de *Q. magnoliifolia* (N_h = 56) y *Q. resinosa* (N_h = 34), con solo 13 haplotipos compartidos a lo largo de su distribución en México. La red de haplotipos de las dos especies muestran haplotipos ancestrales compartidos así como haplotipos derivados compartidos, lo cual sugiere intercambio citoplasmático recurrente entre las dos especies en diferentes periodos de tiempo. Los análisis demográficos muestran diferentes periodos de expansión para *Q. magnoliifolia* y *Q. resinosa*. Los modelos de distribución muestran que los cambios climáticos pasados durante el Último Glacial Máximo (UGM; ~20,000 años AP) y Último Interglacial Máximo (UIM; ~140,000 años AP) afectaron la distribución altitudinal de *Q. magnoliifolia* y *Q. resinosa*, con movimientos hacia altitudes más bajas durante el UMG y movimientos hacia altitudes más altas durante los periodos más cálidos del UIM con excepción de *Q. magnoliifolia*, la cual tuvo que moverse a altitudes aún más bajas durante el UIM, debido a que *Q. resinosa* ocupó las altitudes más altas durante este periodo. Estos movimientos altitudinales favorecieron una simpatría entre las especies. Los modelos de distribución también muestran contracción en el área de distribución de *Q. magnoliifolia* y *Q. resinosa*, durante el UGM y UIM, siendo el UGM el periodo de mayor contracción en la simpatría de las especies, caso contrario, durante el UIM se presentó la mayor área de simpatría entre las especies, debido principalmente al movimiento latitudinal de *Q.*

resinosa de la Planicie Central a la Sierra Madre del Sur y oeste de la Faja Volcanica Trans-Mexicana. Las áreas de distribución de la riqueza de haplotipos compartidos, muestran un incremento desde el UIM alcanzando su máxima área de distribución actualmente. El área de simpatría entre *Q. magnoliifolia* y *Q. resinosa* estuvo estrechamente relacionado con la riqueza de haplotipos compartidos.

II. ABSTRACT

This work is the first to study two Mexican white oak species, *Q. magnoliifolia* and *Q. resinosa* throughout its distribution using geometric morphometric, ecological niche modeling and nuclear and cytoplasmic molecular markers, in order to know the historical and contemporary evolutionary history. The thesis is comprised of three chapters. In the first chapter we found that the effect of hybridization on leaf fluctuating asymmetry (FA) has a genetic basis. FA values were lower in the parental individuals after backcrosses, and the highest values of AF were in the F1 hybrids, supporting the hypothesis that hybridization produces disruption of coadapted genes complex (i.e. favorable combination of genes that make organisms more fit for development, function and survival) increasing development instability. In the second chapter we find that *Q magnoliifolia* and *Q. resinosa* are two species morphologically different, have divergent ecological niches and sympatric zones occur at intermediates environments of the two species. Temperature differences characterize the ecological niche of each species. Two sympatric zones with different geographical location and environmental conditions were found. Pairwise F_{ST} across all loci showed high intraspecific differentiation and low interspecific differentiation. Isolation by distance between the two species and within *Q. magnoliifolia* was found. Moreover, interspecific differentiation was lower between nearby populations than between distant populations. A Bayesian genetic structure analysis identified two genetic groups whose correspondence with morphological species was limited but geographically structured. Such a low congruence between leaf shape morphology and genetic assignments suggests that the species remain morphologically and ecologically distinct, possibly to disruptive selection, despite very high levels of interspecific gene flow between nearby populations. The geographically structure of introgression between *Q. magnoliifolia* and *Q. resinosa* support the

hypothesis of inter-specific gene flow by isolation by distance. Much more genomic resolution will be needed to identify those genes associated with interspecific differentiation in these Mexican oaks, due to their extremely porous genomes. In the third chapter, we find a great diversity of cpDNA haplotypes in *Q. magnoliifolia* populations (N_h = 56) and *Q. resinosa* (N_h = 34), with only 13 shared haplotypes across its distribution in Mexico. The network of haplotypes of the two species exhibit shared ancestral haplotypes and shared derived haplotypes, suggesting recurrent cytoplasmic exchange between the two species at different periods of time. Demographic analysis shows different periods of expansion for *Q. magnoliifolia* and *Q. resinosa*. Distribution models showed altitudinal range shift for *Q. magnoliifolia* and *Q. resinosa* during the Last Glacial Maximum (LGM; ~20,000 years BP) and Last Interglacial Maximum (LIG; ~140,000 years BP), with downward altitudinal movement during the LGM and upward altitudinal movement during warm climate of LIG, with the exception of *Q. magnoliifolia*, which must to move downward due to *Q. resinosa* occupied higher altitudes, thus, these altitudinal movement favored the formation of sympatric areas. Also, the all distribution models showed contractions in the distribution area of the two species during the LGM, with a strong contraction during LIG. The sympatric areas has been lowest compare to current distribution areas of the two species, during the LGM the sympatric area contracted, whereas, during the LIG was presented the largest sympatric areas between the two species, mainly due to latitudinal movement of *Q. resinosa* from Central Plateau to Sierra Madre del Sur and western of Trans-Mexican Volcanic Belt. The distribution areas of the richness of shared haplotypes showed an increase from the LIG reaching its maximum range today. The sympatric area between *Q. magnoliifolia* and *Q. resinosa* was closely related to the richness of shared haplotypes

III. INTRODUCCIÓN GENERAL

La hibridación se lleva a cabo cuando las barreras reproductivas entre linajes o poblaciones genéticamente diferentes, distinguibles por uno o más caracteres heredables, son débiles, lo cual resulta en un individuo híbrido F1 (Barton and Hewitt 1985; Arnold 1997). La introgresión o hibridación introgresiva es la infiltración natural de genes de una especie a otra como resultado de un proceso de reproducción entre individuos híbridos fértiles (p. ej. F1, F2 o retrocruzas) con uno o ambos parentales (Anderson y Hubricht 1938; Rieseberg 1997).

La hibridación e introgresión entre especies de plantas es un proceso natural frecuente e importante en su evolución y mantenimiento de la diversidad genética (Stebbins 1959; Lewontin y Birch 1966). Estos permiten cambios genomicos rápidos como son rearreglos cromosómicos, expansión del genoma y expresión diferencial de genes (Baack y Rieseberg, 2007). La especiación es una de las consecuencias más conocidas y documentadas de la hibridación en plantas, la cual se lleva acabo cuando las barreras reproductivas entre los parentales e híbridos se fortalecen (Stebbins 1959; Grant 1981; Mallet 2005; Riesenberg 2006; Soltis y Soltis 2009). Las consecuencias de la hibridación e introgresión entre especies que se mantienen morfológicamente diferentes, a pesar de tener flujo génico inter-específico entre ellas, ha sido ampliamente documentado. Existe evidencia genética que el proceso de introgresión introduce genes de una especie en el genoma de la otra especie, así como nuevo material genético, lo cual puede resultar en la transferencia de caracteres adaptativos, sobre los cuales la selección puede actuar promoviendo la evolución adaptativa. Por lo anterior se ha considerado a la introgresión como un fuerte filtro selectivo entre las especies que hibridan (Anderson 1949; Martinsen et al. 2001; Martin et al. 2006).

La introgresión en caracteres adaptativos ha sido documentada entre dos especies de *Iris* que hibridan naturalmente. *Iris fulva* es una especie tolerante a las inundaciones e *I. brevicaulis* una especie adaptada a la sequía; se observó que cuando los híbridos retrocruzados con cada una de las especies fueron sometidos a un evento de inundación, los híbridos retrocruzados con *I. fulva* sobrevivieron en una frecuencia más alta que los híbridos retrocruzados con *I. brevicaulis*, por lo que, la supervivencia de los híbridos retrocruzados con *I. brevicaulis* fueron fuertemente influenciados por la presencia de alelos introgresados de *I. fulva* localizados a lo largo de todo el genoma, demostrando con ello el potencial de la introgresión en caracteres adaptativos entre especies (Martin et al. 2006).

Helianthus debilis y *H. annuus* son dos especies de girasoles que hibridan naturalmente en Norte América, en donde observaron que los caracteres de las retrocruzas hacia *H. debilis* permite un crecimiento rápido y reproducción antes del calor del verano y la sequía, por lo que, la selección natural actualmente favorece a los híbridos retrocruzados con *H. debilis*, demostrando con ello que la introgresión puede alterar aspectos fenotípicos de las especies en una manera adaptativa (Whitney et al. 2010). La introgresión ha sido documentada como un proceso de invasión y colonización de nuevos hábitats o hábitats ocupados por las especies progenitoras, a través de los genotipos introgresados y de la selección natural, la cual puede actuar en favor o en contra de estos (Potts y Reid 1988; Petit *et al*. 2003; Dodd y Afzal-Raffi 2004). La frecuencia de la hibridación y la subsecuente introgresión pueden estar relacionados con las condiciones ambientales que pueden favorecer o restringir el establecimiento de genotipos híbridos e introgresados (Fernandez-Manjarres *et al*.

2006), y/o la perturbación ambiental, ya sea natural o por el hombre (Stebbins 1959; Grant 1971).

La hibridación e introgresión tienen efectos positivos en las poblaciones naturales, ya que son fuente importante de variación genética que impacta en procesos ecológicos y evolutivos de les especies involucradas (Martinsen et al. 2001; Martin et al. 2006; Petit et al. 2003; Whitney et al. 2010), a pesar de que pueden ser mecanismos que pueden llevar a la extinción de especies por asimilación genética, siendo las especies raras las más amenazadas (Rhymer y Simberloff 1996).

El origen de la hibridación puede ser explicado por dos modelos: la hibridación por contacto primario y la hibridación por contacto secundario (Hewitt 2002). La hibridación por contacto primario ocurre cuando individuos de una misma especie se diferencia morfológicamente a lo largo de su distribución geográfica, debido a que la selección actúa en diferentes alelos hacia dos direcciones en un gradiente ambiental, produciendo una clina morfológica que no representa linajes evolutivos diferentes (Holman et al. 2003). La hibridación por contacto secundario ocurre cuando dos especies que divergieron alopátricamente se traslapan geográficamente en algún sitio a lo largo de sus áreas de distribución, produciendo una zona híbrida caracterizada por caracteres morfológicos y genéticos intermedios (González-Rodríguez et al. 2004).

El mantenimiento de las zonas híbridas depende del balance entre la dispersión que permite genotipos recombinantes y la selección que tamiza los genotipos parentales e híbridos, de acuerdo con su adecuación relativa (Barton y Hewitt 1985; Hewitt 2002). Hay dos tipos de selección que afectan la adecuación de los híbridos con respecto a los genotipos parentales: la selección endógena y la selección exógena (Hewitt 2002). La selección endógena o independiente del ambiente, actúa en contra de los genotipos

híbridos, debido a la disrupción genética de los genes coadaptados de los parentales (Dobzhansky 1936; Muller 1942; Palmer y Strobeck 1986). A este tipo de selección pertenece el modelo denominado “zona de tensión”, el cual produce una clina, producto del balance entre la dispersión y selección en contra de los genotipos híbridos, independiente del ambiente en el que se encuentren debido a la selección endógena (Barton y Hewitt 1985; Hewitt 2002).

La selección exógena o dependiente del ambiente, incorpora el efecto de los gradientes de selección en la estructuración de las zonas híbridas, debido a la heterogeneidad ambiental y el resultado se refleja en los genotipos sobrevivientes en un determinado tipo de ambiente (Arnold 1997). A este tipo de selección pertenecen los modelos de “mosaico” y de la “superioridad restringida del híbrido” (Arnold 1997). El modelo de “mosaico” está determinado por la selección extrínseca asociada a condiciones ambientales en parches, por lo que se observa un patrón de frecuencias genotípicas y fenotípicas en mosaico, las cuales se asume que surgen de la adaptación de los genotipos parentales, híbridos e introgresos a diferentes ambientes distribuidos en parches (Arnold 1997; Howard et al. 1997; González-Rodríguez et al. 2004; Tovar-Sánchez y Oyama 2004; Peñaloza-Ramírez et al. 2010). El modelo de “superioridad restringida del híbrido” se distingue del modelo de mosaico y de la zona de tensión por asumir que los híbridos son más aptos para sobrevivir que sus progenitores dentro de la zona híbrida, localizada en regiones de ecotonos o de transición entre los tipos de ambiente que favorecen a los parentales, porque los híbridos son menos aptos fuera de la zona, por lo que tienden a ocupar hábitats perturbados por causas naturales o antropógenas (Arnold 1997; Hewitt 2002).

Los encinos (género *Quercus*) modelos idóneos para estudiar hibridación e introgresión

Las especies del género *Quercus* (encinos o robles) son uno de los ejemplos más citados y documentados en la literatura en donde ocurren procesos de hibridación, introgresión y formación de zonas híbridas naturalmente debido a que las barreras reproductivas entre las especies son débiles y sus híbridos fértiles (Trelease 1924; Cooperrider 1957; Grant 1981; Rushton 1993; Bacilieri et al. 1996). En este sentido, se ha propuesto que los encinos son modelos evolutivos idóneos para el estudio de los procesos de hibridación, introgresión y formación de zonas híbridas (Futuyma 2005).

Las características de los encinos ha generado el desarrollo de conceptos de especie para su estudio (Burger 1975; Van Valen 1976; Nixon y Wheeler 1990) que son diferentes al concepto biológico o reproductivo de especies propuesto por Mayr (1942), el cual dice, “las especies son grupos de poblaciones reproductivas aisladas de otros grupos”. Burger (1975) propuso que las especies que intercambian material genético con otras especies deben estar debajo de un rango sub-específico dentro de una especie. Van Valen (1976) propuso el concepto ecológico de especie, el cual dice que una especie ocupa un mismo nicho o zona adaptativa, con todos los componentes del ambiente con el cual los organismo co-específicos interactúa. Nixon y Wheeler (1990) con su concepto diagnóstico de especie la definen como agregaciones de poblaciones de individuos diagnosticables por una combinación única de caracteres o atributos heredables.

A pesar de existir estos conceptos de especies, el estudio de la hibridación, introgresión e identificación de las zonas híbridas en encinos ha sido abordado principalmente con tres enfoques diferentes: 1) con el uso de caracteres taxonómicos

diagnósticos y morfológicos para la identificación de las especies parentales y sus híbridos, 2) usando marcadores genético-moleculares para delimitar las especies, describir la estructura genética y los patrones de diferenciación intra- e inter-específica, así como el análisis filogeográfico por medio de la variación del ADN de cloroplasto (ADNcp), 3) utilizado a los insectos (p. ej. minadores, agalleros, etc.) para identificar a las especies de encino y sus híbridos, en donde se han observado que la hibridación trae consigo una mayor diversidad en las comunidades de insectos endófagos (Ishida et al. 2003; Tovar-Sánchez y Oyama 2004b).

Estas aproximaciones han revelado que la historia evolutiva del género *Quercus* es compleja e interesante en parte debido a que el proceso de hibridación genera una gran diversidad morfológica, genética y ecológica.

Taxonomía e historia biogeográfica del género *Quercus*

El género *Quercus* (Fagaceae) se divide en dos subgéneros *Cyclobalanopsis* y *Quercus*; el subgénero *Quercus* se divide en tres secciones *Protobalanus*, *Lobatae* y *Quercus* (Nixon 1993). El género *Quercus* incluye árboles y arbustos, polinizados por viento, presentan entrecruzamiento obligado, periodos de vida muy largos y bellotas como frutos característicos (Nixon 1993). Existen dos centros de diversidad para el género *Quercus*, el primero se localiza en el sureste de Asia con 125 especies (Nixon 1993), y el segundo se localiza en México donde se encuentran aproximadamente 161 especies (Valencia 2004). Habitan principalmente en los bosques templados, regiones tropicales y subtropicales del Hemisferio Norte, así como en hábitats más secos del sureste de Asia y norte de África (Valencia 2004). En América se localiza desde el sur de Canadá

hasta Colombia, incluyendo Cuba. En el Viejo Mundo desde el norte de Europa hasta el norte de África a través de la región del Mediterráneo y en Asia a través de las partes montanas del sur hasta Kamchatka (Siberia al este de Rusia), Corea, Japón y Malasia (Nixon 1993; Valencia 2004). El subgénero *Cyclobalanopsis* es un importante componente de las tierras bajas del bosque tropical del Este de Asia y Malasia (Soapadmo 1972).

México es considerado el mayor centro de diversificación y endemismo específico para el subgénero *Quercus* en América (Rzedowski 1978; Nixon 1993; Valencia 2004). Cuenta con 161 especies del subgénero *Quercus* ubicadas en tres secciones: la sección *Lobatae* (encinos rojos) con 76 especies, de las cuales 61 son endémicas, la sección *Protobalanus* (encinos intermedios) con 4 especies y una de ellas es endémica, y la sección *Quercus* (encinos blancos) cuenta con 81 especies, de las cuales 47 son endémicas (Valencia 2004).

Mucha de la variación morfológica de las especies del subgénero *Quercus* es atribuida a la hibridación inter-específica (Valencia 2004). Sin embargo, el número de trabajos enfocados a identificar y analizar los procesos de hibridación en encinos mexicanos son aún pocos (Nixon 1993; Spellenberg 1995; Bacon y Spellenberg 1996; González-Rodríguez et al. 2004; Tovar-Sánchez y Oyama 2004; Albarrán-Lara et al. 2010; Peñaloza-Ramírez et al. 2010).

Diversificación del género *Quercus*

La edad mínima de las Fagáceas es del Paleoceno superior al Eoceno inferior, y su diversificación fue rápida en el Eoceno superior al Oligoceno inferior (30 millones de

años), en donde se ubican los géneros fósiles *Castaneoides, Trigonobalanoides, Quercoides y Fagoides* (Crepet y Nixon 1989; Walther 2000), los cuales se distribuyeron en Asia, Europa y Norte América (Axelrod 1983). El género *Quercus* se divide en dos grandes clados que corresponden a la separación geográfica entre los grupos del Nuevo y Viejo Mundo, sin embargo, dentro del clado del Nuevo Mundo, los encinos blancos sección *Quercus* están ampliamente distribuidos en Norte América-México, Europa y Asia (Fig. 1) (Manos y Stanford 2001).

La sección *Quercus* (encinos blancos) es un grupo monofiletico con base en la morfología y evidencia molecular basada en ADN de cloroplasto y genoma nuclear (ITS) (Nixon 1993; Manos et al 1999). Evidencia molecular muestra que las especies de la sección *Quercus* se dividen en dos clados: uno que incluye las especies de Norte América y México, y otro que incluye a las especies de Europa y Asia (Manos y Stanford 2001). Los encinos blancos de la sección *Quercus* evolucionaron durante el oligoceno dentro de Norte América y migraron por el puente terrestre del estrecho de Bering durante el Oligoceno superior a Asia y Europa hace aproximadamente 17 millones de años (Axelrod 1983; Daghlian y Crepet 1983). Esta hipótesis está reforzada por el patrón general de la diversidad de especies presentes en Norte América y México con 125 especies y presentan los fósiles más antiguos, mientras que en Europa y Asia hay 20 especies (Axelrod 1983; Daghlian y Crepet 1983; Valencia 2004).

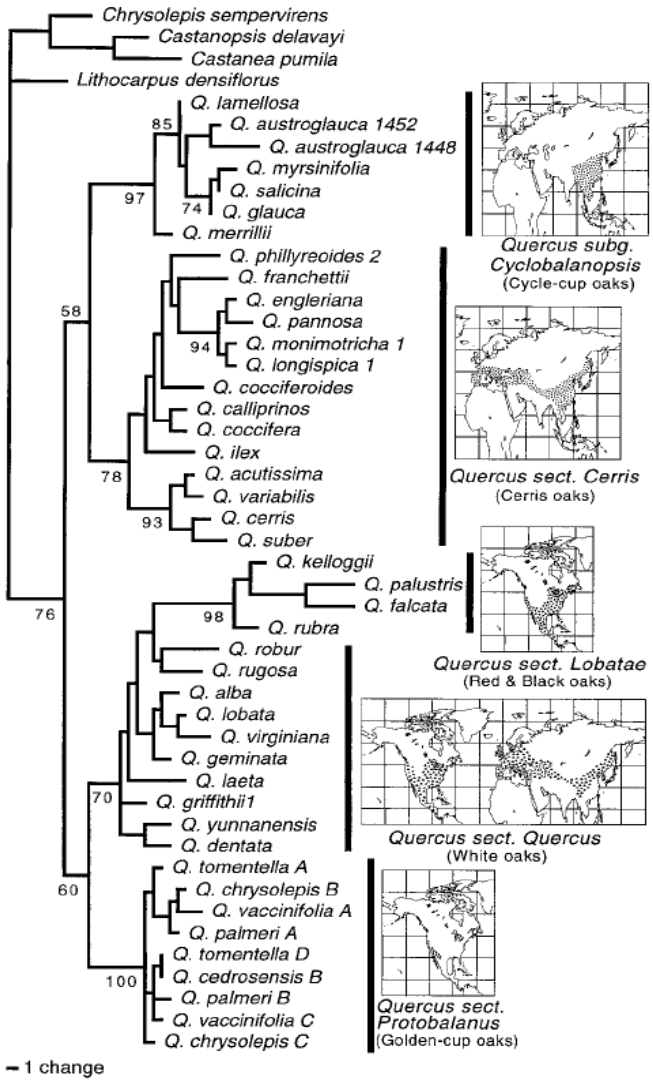

Figura 1. Árbol filogenético del género *Quercus*, representado por los subgéneros y secciones, así como su distribución geográfica de cada grupo. Tomado de Manos y Stanford (2001).

Los encinos blancos (sección *Quercus*) entraron a México durante el Oligoceno-Mioceno migrando a través del este de Estados Unidos hasta la Sierra Madre Oriental en el este de México y posteriormente dispersándose hacia el sur de México vía la Sierra Madre del Sur (Nixon 1993), sugiriendo un escenario de dispersión de encinos blancos

a través de estas dos sierras, cuando se encontraban en pleno proceso de formación de sus rangos montañosos (Oligoceno-Mioceno), lo cual pudo permitir la colonización de estas especies de encinos (Graham 1993). Fósiles de hojas de encinos blancos en los registros del Mioceno-Plioceno en el Paraje Solo Veracruz, muestran el paso de los encinos blancos por esta región (Suter 1984). Esta información coincide con los registros actuales de diversidad de encinos blancos, según los cuales existe una gran concentración de especies en las sierras de Nuevo León y Tamaulipas (Sierra Madre Oriental) y de la misma manera en la Sierra Madre del Sur (Nixon 1993). Los registros de polen y de hojas fósiles atribuibles a encinos blancos también nos permiten suponer una segunda dispersión de estos encinos a través de la Sierra Madre Occidental, como lo demuestra la evidencia de fósiles de bellotas de *Quercus* en los registros del Eoceno de Oregón (Manchester 1984), lo cual coincide con el levantamiento de la Sierra Madre Occidental para el Oligoceno temprano (28 millones de años), y Mioceno temprano (20 millones de años), en los cuales podemos observar ya algunas de las conformaciones montañosas a lo largo de toda la Sierra Madre Occidental (Rossotti *et al.* 2002).

La sección *Lobatae* (encinos rojos) se consideran el linaje más reciente del subgénero *Quercus*, con base en las características morfológicas y el análisis filogenético molecular (ver Fig. 1), se sugieren que los encinos Americanos pertenecientes a la sección *Protobalanus* son los ancestros de las especies americanas de la sección *Lobatae* (encinos rojos) y sección *Quercus* (encinos blancos) (Trelease 1924; Manos et al 1999; Manos y Stanford 2001). Estos autores sugirieron que los eventos de divergencia entre encinos blancos y rojos se llevaron a cabo durante el Oligoceno-Mioceno (20 millones de años). Según Axelrod (1983) durante el Oligoceno los encinos rojos (sección *Lobatae*) y blancos (sección *Quercus*) tuvieron una rápida evolución y

dispersión desde América del Norte, principalmente desde climas de condiciones más templadas y secas hacia climas con condiciones más cálidas y secas como las que ocurren en el suroeste de Estados Unidos, en México en la Sierra Madre Occidental, Oriental y Planicie Central de México, hasta Centroamérica. Posteriormente, el surgimiento de la Faja Volcánica Transmexicana, la cual comenzó su desarrollo durante el Terciario temprano desde el Mioceno, hasta su levantamiento principal y formación en el Cuaternario, hace 2.4 millones de años (Crepet y Nixon 1989; Rossotti *et al.* 2002), la cual, probablemente sirvió de puente de enlace y de dispersión de encinos entre las Sierras Madre Occidental y Oriental, y estas con la Sierra Madre del Sur, Planicie Central y sirvió para delimitar la abundancia de especies en el centro y sur de México, donde encontramos la mayor diversidad de especies de encinos reportadas para el país, con 60-75 especies (González, 1993; Nixon, 1993).

IV. ANTECEDENTES

Taxonomía y análisis morfológico en la identificación de encinos y sus híbridos

El análisis de las características macro- y micro- morfológicas como, la superficie abaxial de las hojas, los tricomas, las inflorescencias, las características del fruto, las características del tronco y la madera han servido para delimitar taxonómicamente a las especies de encinos (McVaugh 1974; Romero et al. 2002; Valencia 2004; Martínez-Cabrera et al. 2011) e identificar posibles eventos de hibridación (Trelease 1924; Coperrider 1957; Hardin 1975; Nixon 1993; Rushton 1993; Bacon y Spellenberg 1996), así como para describir nuevas especies producto de la hibridación (Spellenberg 1995; Tovar-Sánchez y Oyama 2004). El análisis de caracteres morfológicos de la hoja como

son largo, ancho, ancho máximo, largo del peciolo, número de aristas, numero de lóbulos, número de venas, tipo de tricomas, etc. (Cooperrider 1957; Bacon y Spellenberg 1996; Bruschi et al. 2005; Scareli-Santos et al. 2007), y/o utilizando la forma de la hoja por medio de morfometría geométrica (Jensen et al. 1993; Viscosi et al 2009; Albarrán-Lara et al. 2010; Peñaloza-Ramírez et al. 2010) han permitido identificar a las especies parentales y sus híbridos. Sin embargo, la detección de híbridos por medio de caracteres morfológicos no siempre es posible, porque los individuos con caracteres morfológicos intermedios tienden a tener baja frecuencia o a ser eliminados por tener baja adecuación o debido a efectos maternos (p. ej. los híbridos son más similares a la especie materna). La ausencia de individuos morfológicos intermedios ha sido observado entre pares de especies que tienen altos de nivel de introgresión a lo largo de toda su la distribución geográfica (Bacilieri et al. 1996; Bussotti y Grossoni 1997; Kremer et al. 2002; González-Rodríguez y Oyama 2005).

Estructura genética y diferenciación intra- e inter-específica en encinos

Los métodos moleculares pueden proveer un gran número de marcadores neutrales e independientes que son muy útiles en el análisis genético de las zonas híbridas porque pueden generan marcadores especie-específico (Rieseberg y Ellstrand 1993; Boecklen y Howard 1997). La amplificación de loci nucleares por medio de marcadores moleculares altamente variables, como son los microsatélites (SSR; simple sequence repeat), RAPDs (randomly amplified polymorphic DNA) y AFLPs (amplified fragment length polymorphism), ha sido ampliamente utilizado para inferir flujo génico inter-especifico contemporáneo entre especies de encinos, para discriminar entre especies cercanamente relacionadas y sus híbridos y en describir la estructura genética de las

zonas híbridas (Bacilieri et al. 1996; Bodénes et al. 1997; Howard et al. 1997; Bruschi et al. 2000; Muir et al. 2000; Balloux y Lugon-Moulin 2002; Craft et al. 2002; Ishida et al. 2003; González-Rodríguez et al. 2004; Tovar-Sánchez et al. 2004). Sin embargo, el uso de estos marcadores moleculares analizados con modelos basados en estadística bayesiana, por medio del programa STRUCTURE (Pritchard et al. 2000; Falush et al. 2003), han potenciado la detección de individuos híbridos de primera generación (F1) y retrocruzas hacia ambos parentales y así estimar el grado de introgresión y diferenciación inter- e intra- específico, dentro y fuera de las zonas de simpatría entre las especies de encino (Dodd y Afzal-Rafii 2004; Muir et al. 2005; Curtu et al. 2007; Burgarella et al. 2009; Lepais et al. 2009; Salvini et al. 2009; Albarrán-Lara et al. 2010; Neophytou et al. 2010; Peñaloza-Ramírez et al. 2010; Zeng et al. 2010). La mayoría de estos estudios muestran que existen altos niveles de flujo génico inter-específico e introgresión, dentro y fuera de las zonas de simpatría entre pares o más de tres especies involucradas en el proceso de hibridación, y que a pesar de ello, las especies mantienen sus características morfológicas y ecológicas.

La gran pregunta en el estudio de la hibridación e introgresión en especies de encino es ¿cómo las especies se mantienen diferentes, a pesar de los bajos niveles de diferenciación inter-específicos? Los bajos niveles de divergencia genética entre las especies de encino puede ser explicado por polimorfismos ancestrales compartidos debido a un evento de especiación reciente y/o por altos niveles de flujo génico inter-específico que les permite intercambiar genes por medio de la introgresión (Muir y Schötterer 2005; Lexer et al. 2006). El probar flujo génico por aislamiento por distancia puede ayudar a diferenciar entre polimorfismos ancestrales compartidos y flujo génico inter-específico porque el flujo génico inter-específico se espera que resulte en una

menor diferenciación entre poblaciones vecinas que entre las ubicadas distantemente (Lexer *et al*. 2006). En contraste, sí la baja divergencia inter-específica se debe unicamente a polimorfismos ancestrales compartidos, entonces las poblaciones vecinas seran igualmente similares que las ubicadas distantemente (Muir y Schötterer 2005). Los patrones de flujo génico intra- e inter-específicos puede ayudar a la delimitación de las especies (Petit y Excoffier 2009). Estos autores demostraron una correlación negativa entre los niveles de flujo génico intra- e inter-específicos y que altos niveles de flujo génico intra-específicos promueve la monofilia a nivel de especies, manteniendo la integridad y cohesión evolutiva. Es decir los altos niveles de flujo génico intra-específicos pueden prevenir la introgresión (Petit y Excoffier 2009). El mantenimiento morfológico y ecológico de las especies a pesar de los altos niveles de lujo génico inter-específicos e introgresión puede ser explicado por selección disruptiva o divergente (Wu 2001; Scotti-Saintagne et al. 2004; Minder & Widmer 2008).

La filogeografía en el estudio de la historia evolutiva de las especies

La filogeografía estudia los principios y procesos históricos que han moldeado la distribución geográfica de los linajes genealógicos de las poblaciones y la variación genética dentro y entre especies cercanamente relacionadas (Avise et al. 1987; Avise 2000; Knowles 2009). Los estudios filogeográficos proveen información sobre cómo los eventos geológicos, medioambientales, y los factores geográficos interactuaron con aspectos ecológicos de una especie y como todos estos factores moldearon su historia evolutiva (Knowles 2009). La filogeografia estadística considera la coalescencia y la mutación, proponiendo una gran variedad de enfoques para estimar los parámetros genéticos de la población y probar hipótesis de eventos históricos que sucedieron en el

pasado, como son, expansiones poblacionales, cuellos de botella, vicarianza, migración, patrones de flujo génico intra e inter-específicos y tiempo de divergencia (Knowles and Maddison 2002; Navascués et al. 2006; Carstens y Knowles 2007; Harter et al. 2004; Knowles 2009).

El ADN de cloroplasto (ADNcp), se encuentra en el citoplasma de las plantas y ha sido ampliamente utilizado en estudios evolutivos, porque es de herencia uni-parental y asexual, es decir, no recombina. Además, tiene una tasa baja de mutación, por lo que, provee información histórica preservada en sus secuencias después de muchas generaciones de reproducción sexual (Whittmore y Schaal 1991). En la mayoría de las angiospermas, el ADNcp es heredado de la madre y se transmite sólo a través de semillas, no a través del polen como algunas gimnospermas (Petit et al. 2005). El estudio de la variación del ADNcp por medio de marcadores moleculares analizados con filogeografia estadística, ha servido para estudiar los patrones de variación genética en un contexto geográfico, generando información sobre la demografía histórica como son expansiones poblacionales (Navascués et al. 2006), contracciones poblaciones (Bennett et al. 1991), rutas de colonización (Taberlet et al. 1998; Petit *et al.* 2002; 2003), colonización a gran distancia (Cuenca et al. 2003), la historia biogeográfica de las especies (Avise et al. 1987; Avise 2000; Knowles y Maddison 2002; Liu et al. 2009), así como evidencia de intercambio citoplasmático entre especies debido a eventos de hibridación e introgresión histórica y contemporánea (Matos y Schaal 2000; Cruzan 2004; Heurtz et al. 2006).

La distribución geográfica de las especies ha cambiado a lo largo del tiempo. Los registros palinológicos, fósiles y evidencia molecular muestran que los cambios climáticos cíclicos durante el Cuaternario afectaron la distribución geográfica y la

variación genética de las especies de plantas (Comes y Kadereit 1998; Hewitt 1996; 2000, 2004). Los cambios en la distribución geográfica de las especies de plantas a lo largo del tiempo, pueden inferirse directamente de los registros palinológicos o fósiles, sin embargo, para la mayoría de las especies estos registros son limitados o inexistentes (Carstens y Richards 2007). El modelaje de nicho ecológico ha sido ampliamente utilizado para reconstruir las áreas de distribución geográfica de las especies en el pasado (paleo-distribución) durante el Último Glacial Máximo (UGM; ~20,000 años AP) y/o Último Interglacial Máximo (UIM; ~140,000 años AP), lo cual ha ayudado a contrastar y complementar los patrones filogeográficos obtenidos con marcadores moleculares (Carstens y Richards 2007; Jakob et al. 2009).

En el caso de los encinos género *Quercus*, los estudios filogeográficos están basados en el análisis de la variación de ADNcp, el cual es heredado maternalmente por semillas (Dumolin et al. 1995). Los trabajos filogeográficos en encinos Europeos, muestran que la estructura genética del ADNcp es independiente de la especie, pero con una fuerte estructura filogeográfica, en donde los haplotípos relacionados tienen distribuciones geográficas similares, resultado que ha sido atribuido a hibridación introgresiva en los refugios glaciales y/o durante la recolonización posglacial (Petit *et al*. 1993; 1997; 2002; 2003; Dumolin-Lapegue et al. 1997; Olalde *et al*. 2002; Lumaret y Jabbour-Zahab 2009), sin embargo este resultado también puede ser explicado por polimorfismos ancestrales compartidos o selección balanceadora.

Los encinos de Norte América muestran un patrón similar, ya que la variación del ADNcp es independiente de la especie, pero concuerdan con la localización geográfica de las poblaciones simpátricas (Whittemore y Schaal 1991). En México, los patrones filogeográficos solo se han realizado en especies de encinos rojos como son los

complejos *Quercus affinis- Q. laurina* y *Q. crassifolia- Q. crassipes* los cuales muestran diferentes patrones de intercambio citoplasmático, como son variación haplotípica del ADNcp independiente de la especie y tener solamente cuatro haplotipos compartidos entre las dos especies, respectivamente, así como una débil estructura filogeográfica, altos niveles de variación genética y niveles relativamente bajos de diferenciación con respecto a los encinos europeos (González-Rodríguez et al. 2004; Tovar-Sánchez et al. 2008). Estos patrones contrastantes de intercambio citoplasmático sugieren que las especies respondieron de manera diferente durante los cambios climáticos del Pleistoceno. En México, se ha reportado que la temperatura descendió en promedio 6°C para el último glacial máximo (UGM), junto con un incremento en la precipitación en algunas áreas (Bradbury 1997; Metcalfe 2006), y un avance en los glaciares de 1300 m (Lachniet y Vázquez-Selem 2005; Lozano-García y Vázquez-Selem 2005). Estos cambios produjeron una migración hacia altitudes bajas y una amplia distribución de las comunidades de plantas montanas (p. ej. *Piceas* y *Pinus*) que han sido documentadas en los registro paleobotánicos (Van Denver 1990; Lozano-García et al. 2002; Metcalfe et al. 2002; Piperno et al. 2007; Caballero et al 2010). En México, estudios filogeográficos en *Pinus* muestran que los cambios durante el último glacial máximo, favorecieron la expansión de rango de las especies permitiéndoles flujo génico intra-específico, así como flujo génico inter-específico por la formación de zonas simpátricas debido a la expansión en los rangos de distribución (Matos y Schall 2000; Moreno-Letelier y Piñero 2009). Una importante zona de simpatría entre las especies de los complejos *Quercus affinis-Q. laurina* y *Q. crassifolia-Q. crassipes*, ha sido el Eje Neovolcánico Transverso, el cual pudo favorecer el intercambio citoplasmático entre estos complejos de especies (González-Rodríguez et al. 2004; Tovar-Sánchez et al. 2008). El Eje Neovolcánico

Transverso es considerado un “punto caliente” para la diversidad de encinos, un lugar en donde diferentes linajes maternos se fisionaron (Tovar-Sánchez et al. 2008).

Con base en los antecedentes mostrados anteriormente, el contrastar los patrones de herencia de los genes nucleares y citoplasmáticos puede ser usado para determinar y entender los patrones de flujo génico histórico y contemporáneo entre las especies de encinos (semillas y polen, respectivamente), así como conocer su historia evolutiva.

Sistema de estudio

En México un ejemplo de hibridación en encinos blancos (sección *Quercus*) es el caso de *Q. magnoliifolia* Née y *Q. resinosa* Liebm., las cuales pertenecen a series diferentes Circinatae y Macrophyllae, respectivamente (Trelease 1924).

Estas dos especies presentan caracteres morfológicos diagnósticos diferentes (Figs. 2 y 3) y son alopátricas en la mayor parte de su distribución. *Q. magnoliifolia* se distribuye en la Sierra Madre Occidental, el Eje Neovolcánico Transversal y la Sierra Madre del Sur, cubriendo un rango altitudinal que va desde los 400 a 2850 msnm. *Q. resinosa* se distribuye en la Planicie Central de México, parte suroeste de la Sierra Madre Occidental y Eje Neovolcánico Transversal en un rango altitudinal de los 1300 a 2800 msnm. Las dos especies se translapan en sus ciclos fenológicos, *Q. magnoliifolia* florece durante marzo y abril y *Q. resinosa* florece de marzo a mayo (McVaugh 1974; Valencia 1995; González-Villareal 1989). Diferentes zonas de simpatría entre estas dos especies han sido reportadas donde converge la Sierra Madre Occidental con el Eje Neovolcánico Transverso. En el estado de Jalisco individuos con características

intermedios han sido identificados como posibles híbridos (González-Villareal 1989; Susana Valencia com. pers.).

Con base en la revisión de herbario tomando como fuente principal el Herbario MEXU-UNAM, obtuvimos la variación morfológica presentada por *Q. magnoliifolia* y *Q. resinosa* a lo largo de su distribución en México para poderlas identificar en campo, las localidades únicas para cada especie y los estados en los cuales están distribuidas. Ejemplares de herbario colectados en el Volcán de Tequila, Jalisco mostraron inconsistencia en determinación de la especie, incluso González-Villareal (1989) reporta individuos posiblemente híbridos en esta zona. Las primeras colectas se realizaron en diferentes localidades del estado de Jalisco, siendo el Volcán de Tequila uno de los sitios de colecta. Los ejemplares colectados fueron identificados siguiendo la clave dicotómica de McVaugh (1974), bajo la enseñanza y asesoría del Dr. Sergio Zamudio. Los ejemplares colectados en el Volcán de Tequila, Jalisco, efectivamente muestran individuos con caracteres diagnósticos de *Q. magnoliifolia* y *Q. resinosa*, así como individuos con características intermedias, lo que nos hizo suponer que esta localidad era una putativa zona híbrida, además muchos de los de individuos putativamente híbridos mostraban una gran asimetría en sus hojas, lo cual, nos hizo plantearnos las siguientes preguntas, ¿A qué se debe esta gran asimetría? ¿Es producto del ambiente o al efecto de la hibridación?

La tesis se divide en tres capítulos. En el primer capítulo se presenta un análisis morfológico y genético, con el cual probamos que la asimetría fluctuante foliar incrementa con la hibridación e introgresión entre *Q. magnoliifolia* y *Q. resinosa* a lo largo de un gradiente altitudinal en México. Los objetivos particulares de este capítulo fueron: (1) identificar morfológica y genéticamente los individuos puros de cada

especie, los híbridos F1 y retrocruzas, (2) determinar los niveles de asimetría fluctuante por individuo y promediando los individuos pertenecientes a cada grupos genéticos, (3) probar si los niveles de asimetría fluctuante tienen una base genética o se deben al gradiente altitudinal en el Volcán de Tequila, por medio de correlaciones y (4) determinar la estructura genética y geográfica de esta putativa zona híbrida (clina o mosaico).

El segundo capítulo examinamos la diferenciación morfológica, ecológica y genética de *Q. magnoliifolia* y *Q. resinosa* a través de toda su distribución geográfica en México utilizando morfometría geométrica, modelado de nicho ecológico y ocho loci de microsatélites nucleares usados para determinar la estructura genética y los niveles de introgresión entre estas dos especies. El modelado de nicho ecológico se utilizo para determinar la divergencia de nicho entre estas dos especies e identificar las características geográficas y ecologicas de las areas de simpatría. Con el fin de diferenciar entre flujo génico y polimorfismos ancestrales compartidos, probamos la hipótesis de aislamiento por distancia comparando los valores de F_{ST} inter-específico a diferentes escalas geográficas. Los objetivos particulares de este capítulo fueron: (1) determinar el nivel de diferenciación morfológica entre *Q. magnoliifolia* y *Q. resinosa* a diferentes escalas espaciales, (2) determinar la distribución geográfica y diferenciación ecológcia entre *Q. magnoliifolia*, *Q. resinosa* y las areas de simpatría utilizando modelado de nicho, y (3) caracterizar su estructura genética utilizando marcadores moleculares.

En el tercer capítulo estudiamos la variación del ADN de cloroplasto (ADNcp) de *Q. magnoliifolia* y *Q. resinosa* a través de su rango de distribución en México, para determinar la estructura filogeográfica, inferir eventos demográficos tales como

expansiones y contracciones poblacionales, migración y colonización, y para reconstruir la distribución de las especies en el presente y el pasado, durante el último glacial máximo (Last Glacial Maximum; LGM; ~20,000 años AP) y el último integlacial máximo (Last Interglatial Maximum; LIG; ~140,000 años AP; Otto-Bliesner et al. 2006) por modelaje utilizando MAXENT y GARP, con el fin de entender los procesos que favorecieron el intercambio citoplasmático entre estas dos especies. Los objetivos específicos fueron: (1) determinar la diversidad genética del ADN de cloroplasto y la estructura geográfica de los haplotípos de las poblaciones de *Q. magnoliifolia* y *Q. resinosa*, (2) determinar las relaciones genealógicas entre los haplotípos, (3) determinar los eventos demográficos históricos de las poblaciones de las dos especies y (4) reconstruir la distribución presente y pasada de las especies utilizando MAXENT y GARP para dilucidar cambios los rangos de distribución de las especies durante los últimos cambios climáticos que pudieron haber favorecido el intercambio citoplasmático inter-específico.

Descripción taxonómica de las especies de estudio

Quercus magnoliifolia Née presenta varias sinonímias: *Q. macrophylla* Née (1801), *Q. circinata* Née (1801), *Q. lutea* Née (1801), *Q. nudinervis* Liebm. (1854), *Q. magnoliifolia macrophylla* (Née) A. DC (1864), *Q. haematophlebia* Trel. (1924), *Q. tepicana* Trel. (1924), *Q. erubescens* Trel. (1934), *Q. rubescens* Trel. (1934) y *Q. platyphylla* Warb. (1939). La mayoría de estas sinonimias corresponden a las especies que Trelease (1934) propone como parte de la serie *Circinatae*. Estudios taxonómicos realizados por la especialista en encinos Susana Valencia Avalos, propone que la serie

Circinatae está formada solo por *Q. magnoliifolia* y *Q. liebmanii* Oersted (1869) (Valencia 1995). *Q. liebmanii* Oerst. Solo se distribuye en la Sierra Madre del Sur.

La descripción taxonómica de *Q. magnoliifolia* se basa en la descripción hecha por Valencia (1995) y González-Villareal (1986): **Árbol** frondoso hasta 25m de alto o arbustos, **copa** irregular con ramificaciones desde los 1.5 m; **tronco** torcido de 26-60 cm de diámetro a veces hasta de 1 m, **corteza** rugosa café-ceniza a pardo amarillenta en el interior, se desprende en escamas grandes; **ramillas** estriadas longitudinalmente pero no muy marcadas, glabras, ligeramente exfoliante con numerosas lenticelas pálidas largas y conspicuas; **yemas** ovoides a anchamente ovoides de color ámbar, con frecuencia obtusas, casi glabras, margen ciliado; **hojas maduras** con **pecíolos** glabros y largos; **lámina** coriáceas, obovadas, anchamente obovadas o rara vez elípticas de 10.4-39 cm de largo son 1.5-2.5 veces más largas que anchas; **ápice** obtuso o subagudo, **base** cuneada, aguada, redonda o auriculada, subcordada o truncada; **margen** o **borde** lobado-dentado por arriba de la base, **haz** glabro, venas lisas o impresas, ocasionalmente puberulento o bien con algunos pelos glandulares vermiformes coloración ámbar cercanos a vena media y con pelos estrellados regularmente distribuidos; **envés** densa o laxamente cubierto por pelos glandulares vermiformes amarillo-ámbar y por pelos estrellados, epidermis ampulosa y papilosa, **hojas jóvenes** con el **haz** verde, **envés** tomentoso, glandular principalmente cerca de las nervaduras; **flores masculinas** sésiles regularmente distribuidos sobre el raquis, **flores femeninas** solitarias o en grupos pequeños, dispersas sobre un pedúnculo glabrescente de 5-10 cm de largo; **frutos** anuales, **cúpula** hemisférica a obcónica-aplanada, escamas triangulares cortamente seríceas y canescentes o hialinas; **bellotas** ovoides, glabras o en ocasiones conservan tomento canescente hacia el ápice. Habita principalmente en transiciones de

selva baja caducifolia con bosques de pino-encino, en laderas de cerro y barrancas, terrenos planos, suelos someros, arenosos y pedregosos o profundos y con rocas ígneas (Fig. 4).

Quercus resinosa Liebm. presenta solo una sinonímia: *Q. macrophylla sensu* Trel. (1924). Pertenece a la serie *Macrophyllae*; la cual incluye a *Q. purulhana* Trel.

La descripción taxonómica de *Q. resinosa* se basa en la descripción hecha por McVaugh (1974) y González-Villareal (1986): **Árboles** de 7-15 m de altura, **copa** ancha y baja, **tronco** corto de 30-70 cm de diámetro, **corteza** gruesa gris escamosa, **ramillas** muy cortas y gruesas, densamente cubiertas por un tomento amarillento, con pelos estrellados glandulares puberulentos, simples rojizos o enegrecidos, **yemas** muy anchamente ovoides a depresamente ovoides de color pardo claro a café rojizo, densa o medianamente piloso-pubescentes, **hojas maduras** con **peciolos** grueso, cubiertos por pelos estrellados y glandular puberulentos; **lámina** coriáceas, ampliamente obovadas, rara vez elípticas, de 8-22(-50) cm de largo por 3.5-20(-30) cm de ancho, **ápice** obtuso, redondeado, rara vez agudo, ocasionalmente apiculado; **base** obtusa a subcordada, **margen o borde** cartilaginosos ligeramente revoluto, **haz** duro, con una fina textura aterciopelada formada por pelos estrellados regularmente esparcidos en toda la superficie, **envés** densamente cubierta por pelos estrellados, glandulares vermiformes y pubérulo suave y delgado con epidermis ligeramente ampulosa-papilosa, **hojas jóvenes** muy tomentosas en ambas superficies, **haz** teñido de rojo debido a la cubierta de pelos glandulares vermiformes, **envés** densamente pálido tomentoso, con abundantes pelos glandulares solamente sobre las nervaduras; **flores masculinas** sésiles con una bracteola en la base de la flor, regularmente dispuestas sobre el raquis, **flores femeninas** de 1-5(-10), agrupadas o dispersas a lo largo de un pedúnculo tomentoso, **frutos** anuales,

solitarios o en par, sésiles sobre un pedúnculo tomentoso, **cúpulas** tomentosas, hemisféricas o profundas, escamas triangulares, ascendentes, **bellotas** ovadas, glabras, excepto el ápice que es ligeramente tomentoso. Habita principalmente en matorrales semiáridos, xerófilos junto con otras especies de encino, y bosque de pino-encino en laderas graníticas, riolíticas, andesítica y muy pedregosas, con suelos pedregosos, rocosos, arenosos-cuarzosos, someros, bien drenados, de colores café-claro, rojo (Fig. 5).

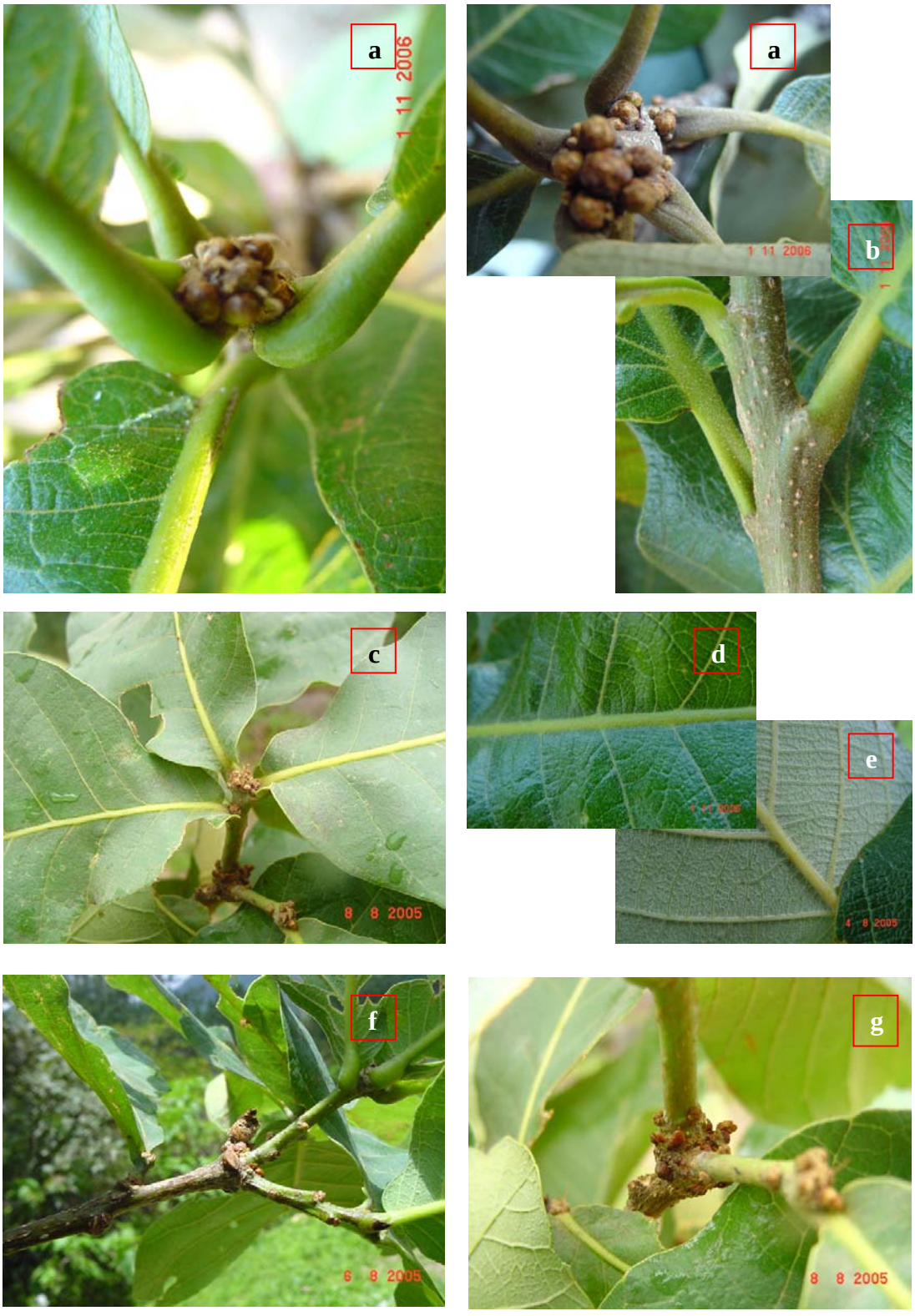

Figura 2. Caracteres diagnósticos de *Q. magnoliifolia*: a) yemas ovoides, b) lenticelas amarillas pálidas, c) hojas pecioladas glabrecentes, d) haz lustroso, e) envés pusbescente, f) y g) ramillas glabrescentes.

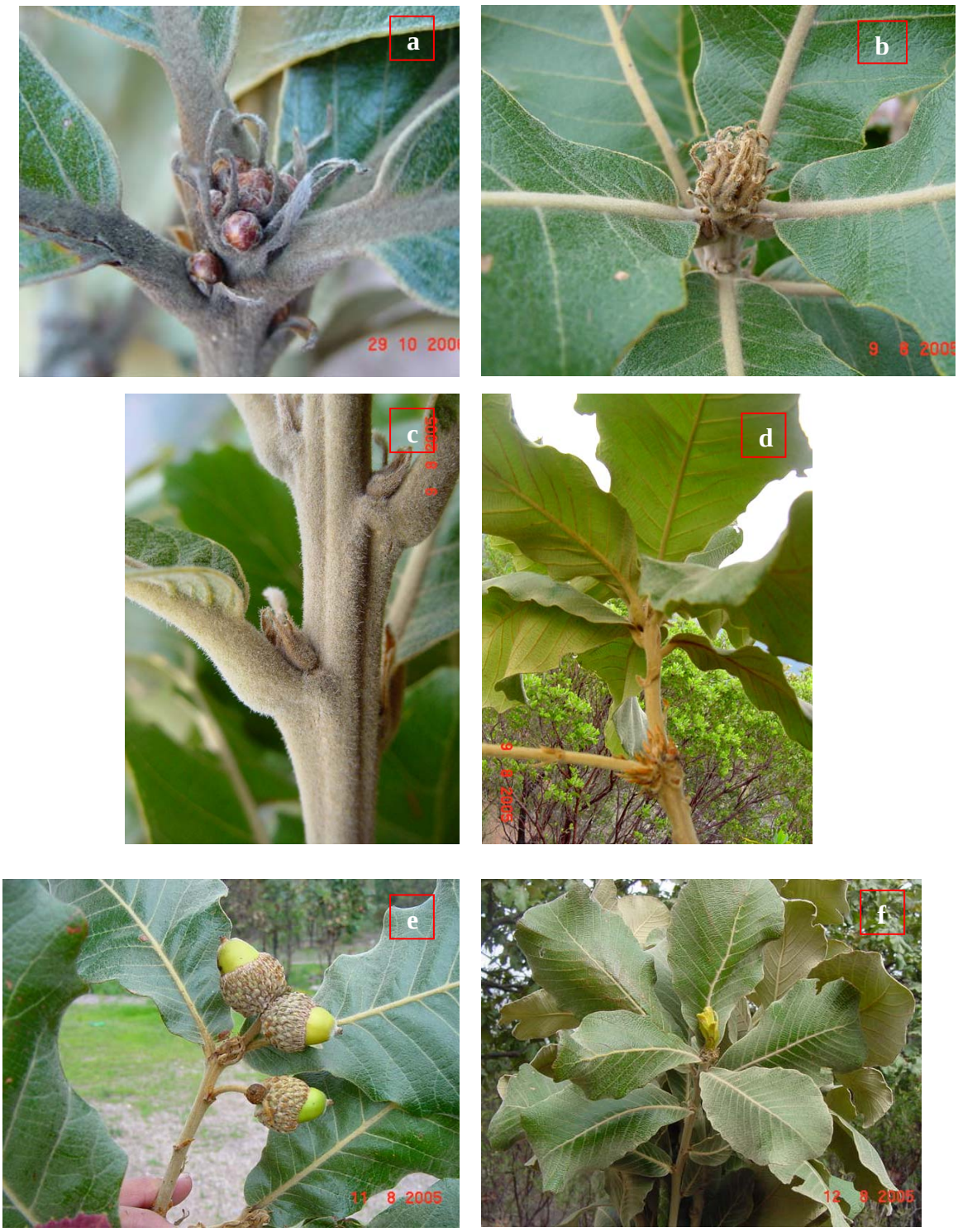

Figura 3. Caracteres diagnósticos de *Q. resinosa*: a) yemas anchamente ovoides con estípulas, b) estípulas largas puberulentas, c) y d) ramillas gruesas cubiertas por un denso tomento pubescente, e) bellotas,. f) hojas sésiles densamente pubescentes.

Figura 4. Fotos de árboles de *Q. magnoliifolia*, está especies que habita en las transiciones entre la selva baja caducifolia y los bosques de pino-encino, principalmente.

Figura 5. Fotos de árboles de *Q. resinosa*, está especies que habita principalmente en matorrales xerófilos.

VI.

Ana Luisa Albarrán-Lara, Luis Mendoza-Cuenca, Susana Valencia-Avalos, Antonio González-Rodríguez and Ken Oyama

Leaf fluctuating asymmetry increases with hybridization and introgression between *Quercus magnoliifolia* and *Quercus resinosa* (Fagaceae) through an altitudinal gradient in Mexico

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LEAF FLUCTUATING ASYMMETRY INCREASES WITH HYBRIDIZATION AND INTROGRESSION BETWEEN *QUERCUS MAGNOLIIFOLIA* AND *QUERCUS RESINOSA* (FAGACEAE) THROUGH AN ALTITUDINAL GRADIENT IN MEXICO

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We tested the effects of hybridization and introgression on the levels of leaf fluctuating asymmetry (FA) in a hybrid zone between *Quercus magnoliifolia* and *Quercus resinosa* at the Tequila volcano, Jalisco state, Mexico, in which the species are distributed along an altitudinal gradient ranging from 1400 to 2100 m. Bayesian clustering analysis was performed with STRUCTURE on data for eight nuclear microsatellite loci to assign individuals from reference populations and from the hybrid zone to pure or hybrid genotypic classes. To test the performance of the assignment procedure and to determine optimal thresholds for genetic assignment pure, hybrid and backcrossed genotypes were simulated (from the allelic frequencies found in real pure populations of the two species) and reanalyzed with STRUCTURE. Leaf FA and morphological identification of parental and hybrid individuals were obtained using geometric morphometric techniques. We found genetic and morphological evidence of a hybrid zone by secondary contact at the Tequila volcano. The genotypes and phenotypes were significantly correlated with altitude along the mountain, but no correlation between them was found. FA was higher in F_1 hybrids and backcrossed genotypes than in pure species. Levels of FA were more correlated with the proportion of genetic ancestry of each individual than with leaf morphology or altitude, supporting the hypothesis that hybridization is associated with development instability potentially caused by disruption of coadapted gene complexes characteristic of each species.

Keywords: genetic assignment, hybridization, introgression, leaf fluctuating asymmetry, *Quercus*.

Introduction

Developmental instability arises from genetic or environmental stressors that disturb the normal developmental pathways of different continuous characters, producing developmental noise, which is commonly measured as fluctuating asymmetry (FA) in phenotypic traits (Van Valen 1962; Palmer and Strobeck 1986; Leamy and Klingenberg 2005). FA is the variance in subtle differences between the left and the right sides in bilaterally symmetrical organisms or parts of them, and it provides a measure of how well an individual can buffer its development against internal genetic and external environmental stress during ontogeny (Van Valen 1962; Palmer 1996).

Hybridization is one of the biological factors underlying the changes in levels of FA in plants (Siikamäki and Lammi 1998; Wilsey et al. 1998). Two closely related hypotheses have been proposed to explain the levels of FA in hybrids. The first one is that FA is reduced in hybrids because of the increase in genetic heterozygosity (Soulé 1979). The second hypothesis is that FA increases by disruption of coadapted gene complexes in hybrids (Soulé 1967; Levin 1970; Graham and Felley 1985; Wilsey et al. 1998; Hochwender and Fritz 1999; Siikamäki 1999). It seems that increments of FA in hybrids depend on how closely related the parents of the hybrids are; intraspecific hybrids (closely related parents) present lower levels of FA than do hybrids of interspecific species (distantly related parents; Markow 1995).

Hybridization is a frequent and important natural process involved in plant evolution and speciation, with at least 25% of plant species engaged in hybridization and potential introgression events with other species (Stebbins 1959; Grant 1981). Hybrids and hybrid zones entail the breeding between genetically distinct groups or taxa, resulting in offspring of mixed ancestry (Barton and Hewitt 1985; Arnold 1997). Hybrid zone structure is influenced by dispersal ability of parental and hybrid individuals and by both environment-independent ("endogenous") and environment-dependent ("exogenous") selection (Barton and Hewitt 1985; Arnold 1997; Howard et al. 2003). Endogenous selection against hybrids results from genomic incompatibilities due to the disruption of parental coadapted gene complexes and/or the interaction between genes that have diverged functionally between the species, giving rise

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to sterility, inviability, developmental instability, or, in general, fitness reduction in hybrids with respect to parental individuals (Dobzhansky 1936; Muller 1942; Palmer and Strobeck 1986; Palmer 1996; Arnold 1997; Siikamäki 1999). Exogenous selection implies that differential survivorship of hybrid genotypes is dependent on particular environmental conditions (Endler 1977; Barton and Hewitt 1985; Arnold 1997; Howard et al. 2003), leading to ecological underdominance or ecological character displacement producing low fitness of the hybrids in the two main habitats of the parental species (Schluter 1996; Hatfield 1997).

Hybridization and hybrid zones are unusually common among species of the genus *Quercus* and have been widely studied using morphological (e.g., Trelease 1924; Nixon 1993; Spellenberg 1995; Bruschi et al. 2000; Kremer et al. 2002; González-Rodríguez and Oyama 2005) and molecular (e.g., Howard et al. 1997; Craft et al. 2002; Dodd and Afzal-Rafii 2004; González-Rodríguez et al. 2004; Tovar-Sánchez and Oyama 2004; Curtu et al. 2007; Valbuena-Carabaña et al. 2007; Burgarella et al. 2009; Lepais et al. 2009) markers. However, the impact of hybridization among pure and hybrid genotypes on developmental instability measured as FA has not been studied in oaks. This analysis could provide evidence of genetic or environmental stress in hybrid populations and contribute to the evaluation of the fitness of pure-bred and hybrid genotypes under different environmental conditions.

In Mexico, two species of white oak (section *Quercus*), *Quercus magnoliifolia* Née and *Quercus resinosa* Liebm., are allopatric and morphologically distinct species in most of their geographic distribution (McVaugh 1974; González-Villareal 1986; Valencia-Avalos 1995). However, both species are distributed at several localities in Jalisco, where the Sierra Madre Occidental and the Central Plateau of Mexico joins with the western part of the Trans-Mexican volcanic belt. Sympatric populations of *Q. magnoliifolia* and *Q. resinosa* have been reported at the Tequila volcano, Jalisco, where the species are distributed through an altitudinal gradient ranging from 1400 to 2100 m (González-Villareal 1986). At this site, *Q. magnoliifolia* individuals predominate at low altitudes (from 1400 to 1500 m) in the transition between tropical deciduous forest and pine-oak forest, and *Q. resinosa* individuals occur from 1900 to 2100 m, with individuals with intermediate leaf morphological traits present in abundance between 1600 and 1800 m. This pattern has been interpreted as a hybrid zone with interspecific hybridization between *Q. magnoliifolia* and *Q. resinosa* (González-Villareal 1986). Along this altitudinal gradient, we have also observed leaves with different levels of deformations in leaf shape in both oak species at different altitudes. Thus, we chose this hybrid zone to test the effects of hybridization and introgression between *Q. magnoliifolia* and *Q. resinosa* on the levels of FA. First, we assessed the genetic ancestry of all the individuals of both oak species along the altitudinal gradient using nuclear microsatellites followed by Bayesian clustering analyses using STRUCTURE (Pritchard et al. 2000). Optimal thresholds for genetic assignment of pure, hybrid, and backcrossed individuals were tested using simulations (Vähä and Primmer 2006). To this analysis we added trees of isolated populations of both oak species located outside of the hybrid zone as references of "pure" individuals. Then we proceeded to morphologically characterize and determine the levels of leaf FA of all parental and hybrid individuals using geometric morphometric techniques. These approaches allowed us to identify genotypes and test the hypotheses that levels of developmental instability as by-products of endogenous selection increase with hybridization. We also assessed whether the spatial segregation of phenotypes and genotypes vary along the altitudinal gradient at the Tequila volcano.

Materials and Methods

Study Species

Quercus magnoliifolia is a 25-m-tall tree with very broad to narrow obovate leaves with glabrescent petioles. The size of the leaves is ~35 cm (Rzedowski 1978). Staminate flowers are produced in March and April (Mc Vaugh 1974; González-Villareal 1986; Valencia-Avalos 1995). Its altitudinal distribution ranges from 400 to 2850 m, through the Sierra Madre Occidental, the Trans-Mexican volcanic belt, and the Sierra Madre del Sur, often in almost pure stands forming open woodlands, sometimes with pines or other oaks or in the transition of tropical deciduous forests with pine-oak forests (McVaugh 1974; Rzedowski 1978). *Quercus resinosa* is a tree that is 7–10 m in height, with broad, obovated, rounded leaves with short, densely tomentose petioles. The size of the leaves of *Q. resinosa* (~50 cm) is considered to be the largest within the oak group (Rzedowski 1978). Staminate flowers are produced from March to May (McVaugh 1974; González-Villareal 1986; Valencia-Avalos 1995). It has a comparatively more restricted altitudinal range (1300–2800 m) and is distributed in the Central Plateau of Mexico and the north of the Trans-Mexican volcanic belt. It occurs in semiarid grasslands with scattered trees, often in almost pure stands but sometimes with other oak or pine species (McVaugh 1974).

Study Site

The Tequila volcano (20°50′N; 103°51′W) reaches 2980 m in altitude and is a part of the Trans-Mexican volcanic belt. It is considered to be an active volcano (Ferrusquía-Villafranca 1993), and it is covered by pine-oak forest at high altitudes and tropical deciduous forest at low altitudes (González-Villareal 1986).

Population Samples

A total of 176 trees separated by at least 20 m were randomly selected at seven sites located along an altitudinal transect from 1400 to 2100 m at the Tequila volcano (table 1). From each individual, 10 mature and complete leaves were sampled, pressed, and dried for morphometric analysis, and five young fresh leaves were stored at −80°C in the laboratory for genetic analysis. A total of 40 trees were sampled from the four reference populations of both species. Reference populations of *Q. magnoliifolia* were sampled in Compostela (Sierra Madre Occidental) and Juxtlahuaca (Sierra Madre del Sur), while reference populations of *Q. resinosa*

Table 1

Populations Sampled along an Altitudinal Gradient through the Hybrid Zone between *Quercus magnoliifolia* and *Quercus resinosa* at the Tequila Volcano and Reference Populations of Each Species, Latitude, Longitude, Altitude, Sample Size, and Genetic Assignment for Each Sampled Population

Species, population	Latitude (N)	Longitude (W)	Altitude (m)	Sample (*N*)	Genetic assignment				
					Q. resinosa	*Q. magnoliifolia*	F_1 hybrid	*resinosa*-like	*magnoliifolia*-like
TEQ:									
1	20.80	103.87	2100	21	2	1	5	2	11
2	20.81	103.85	1900	14	–	1	3	4	6
3	20.82	103.85	1800	29	1	1	3	8	16
4	20.83	103.85	1700	28	–	5	4	6	13
5	20.84	103.84	1600	28	2	7	1	4	14
6	20.85	104.84	1500	43	–	4	9	7	23
7	20.86	103.83	1400	13	–	4	–	1	8
Quercus magnoliifolia:									
Juxtlahuaca	17.48	98.02	2200	10	–	3	–	–	7
Compostela	21.22	104.80	1300	10	–	8	–	–	2
Quercus resinosa:									
Mina	21.68	100.05	1500	10	5	–	–	5	–
El plateado	21.92	103.03	2150	10	7	–	–	3	–
Total				216	17	34	25	40	100

Note. TEQ = Tequila volcano population.

were sampled in El Plateado and Minas (Central Plateau of Mexico; table 1).

Morphological Characterization of Individuals

Five of the 10 preserved leaves from each tree were randomly chosen, and they were photographed from the abaxial part together with a ruler as a size reference (fig. 1) using a digital camera (Sony 7× optical zoom, 8.0 megapixels). In total, 1080 leaves (880 and 200 leaves from the Tequila volcano transect and the reference populations, respectively) were included in the analysis.

Morphometric analysis was based on unambiguous and repeatable anatomical marks along the leaf margin. The coordinates (x, y) of a total of 29 such anatomical marks were registered for each leaf image using the program TpsDig (Rohlf 2005). Three of these anatomical marks (i.e., apex, lamina base, and petiole extreme) correspond to homologous traits or "landmarks" (sensu Bookstein 1991), and 26 correspond to semilandmarks, which correspond to morphological points that incorporate information about leaf contour in a morphometric analysis (Zelditch et al. 2004). Landmarks 1 (lamina base) and 15 (apex) were used to constructed a "fan" (radial guidelines with equal angular spacing on images) with 80 radial guidelines covering the whole leaf contour, which was used to digitalize the 26 semilandmarks (fig. 1). Two additional marks were placed on the ruler for size reference (fig. 1). The MakeFan6 program from the "Integrated Morphometrics Package" IMP series (http://www.canisius.edu/~sheets/morphsoft.html) was used for this procedure.

A Procrustes superimposition analysis for the configuration of landmarks and semilandmarks was performed using the CoordGen6 program in the IMP series (http://www.canisius.edu/~sheets/morphsoft.html). The first step of superimposing configurations of landmarks in two-dimensional shapes (x_1 y_1, x_2 y_2 ...) is a generalized least squares Procrustes superimposition that minimizes differences between landmark configurations by translation, scaling, and rotation to remove all information unrelated to shape and to obtain shape variables (Procrustes distances; Rohlf 1990). After the superimposition, resulting Procrustes coordinates were averaged across all five leaves by individual. Three independent traditional length measurements were also generated from the landmark and semilandmark data sets: (1) lamina length (distance from lamina base to apex; landmarks 1–15), (2) petiole length (distance from lamina base to petiole extreme; landmarks 1–29), and (3) lamina maximal width (semilandmarks 9–21; see fig. 1), using the TmorphGen6 program in IMP (http://www.canisius.edu/~sheets/morphsoft.html).

Shape variables (Procrustes distances) of each individual were used to perform a principal components analysis (PCA) with the PAST software, version 1.79 (Hammer et al. 2001), to obtain synthetic variables of leaf shape variation. The resulting first two principal components, which jointly explained 99.9% of the variation in leaf shape, together with the three length measurements (see above), were used as input data for a canonical discriminant function analysis (Tabachnik and Fidell 1989) in order to assess the morphological differentiation between the two species and to extract a canonical discriminatory function for the classification of individuals. This analysis was performed with SPSS 11.0, following Ferrán (2001).

Measurement of Leaf Fluctuating Asymmetry

There are three types of deviation from perfect bilateral symmetry: fluctuating asymmetry (FA), directional asymmetry (DA), and antisymmetry (AS; Van Valen 1962). FA measures the variance in left-right (L-R) differences, which are distributed around 0, whereas in the case of DA, the L-R differences are distributed about a mean that is significantly ei-

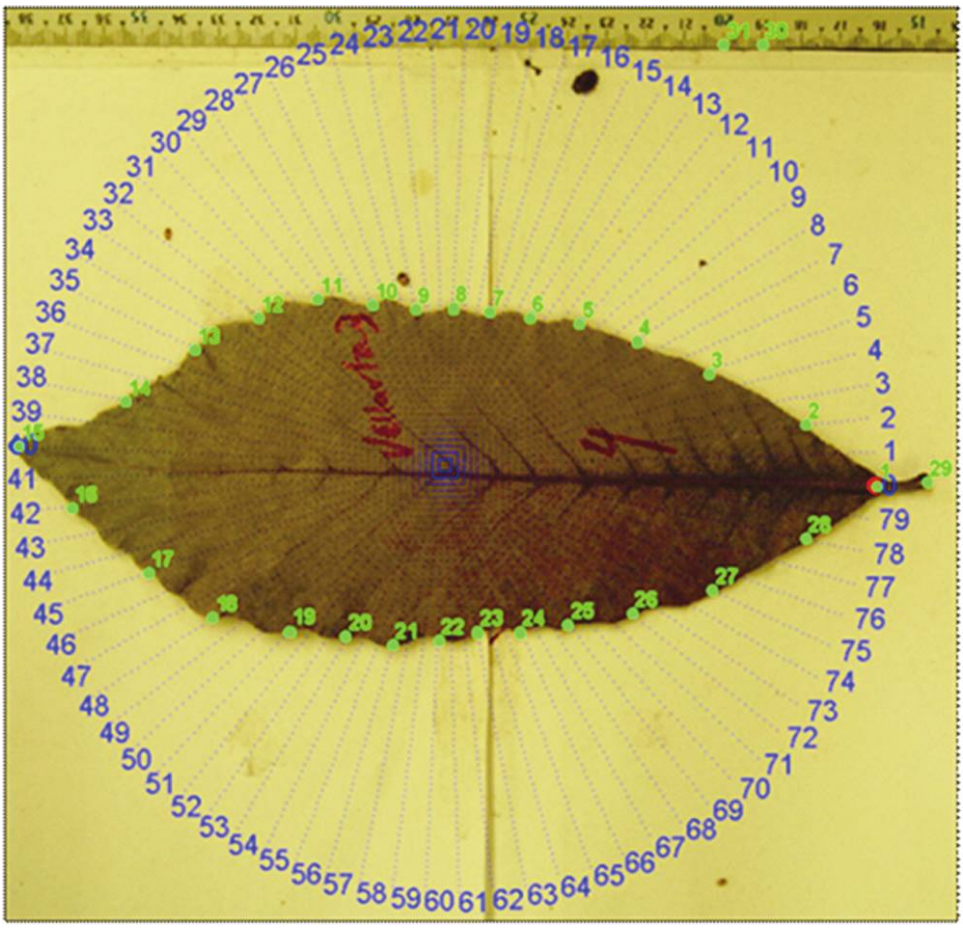

Fig. 1 Digital image of a leaf of *Quercus magnoliifolia* showing the fan with the 80 radial guidelines and the 29 semilandmarks. Fluctuating asymmetry was analyzed using points 2-28, 3-27, 4-26, 5-25, 6-24, and 7-23 as pairing semilandmarks and points 15, 1, and 29 as landmarks along the axis of symmetry. Two additional landmarks (30 and 31) were added on a reference ruler as a scale.

ther greater than or less than 0 (i.e., it occurs when one side of a character is consistently larger than the other). AS is the lack of symmetry in normally developing traits, and it is distinguished by a platykurtic (broad-peaked) or bimodal distribution of *L*-*R* differences about a mean of 0 (Van Valen 1962; Palmer and Strobeck 1986, 1992). FA corresponds to a random variation and can be used to measure developmental instability, whereas DA and AS are considered to be inappropriate as descriptors of developmental stability because both are developmentally controlled and are probably adaptive as asymmetries (Van Valen 1962; Palmer and Strobeck 1992).

Individual levels of leaf FA were obtained using the "Symmetry and Asymmetry in Geometric Data" (SAGE) program, version 1.0 (Marquez 2006). This software analyzed the *x*- and *y*-coordinates of the 29 landmarks of the five leaves per individual, using a configuration protocol that divides both sides of the leaf by considering the primary vein as the symmetry axis (Mardia et al. 2000; Klingenberg et al. 2002). Our configuration protocol considered 13 paired landmarks to estimate FA level (e.g., 2–28, 3–27; fig. 1). Procrustes superimposition analysis is then performed with the original and mirrored configurations simultaneously. The least squares Procrustes consensus of our set of landmark configurations and their relabeled mirror images is a perfectly symmetrical shape, while FA is the deviation from perfect bilateral symmetry (Klingenberg and McIntyre 1998; Klingenberg et al. 2002). The squared average of Procrustes distances for all specimens is the individual contribution to the FA component of leaf variation within a sample (Zelditch et al. 2004).

Shape asymmetry data were analyzed using a Procrustes factorial ANOVA with 1000 permutations using SAGE software (Marquez 2006). Sides (directional asymmetry; DA), individual × side interaction (FA), and their respective error were included as effects.

PCAs of the covariance matrix associated with the component of FA variation were performed for each genotypic class (i.e., pure, hybrid, and backcrossed; see below) to carry out an interpolation based on a thin-plate spline to visualize shape changes as landmark displacement in a deformation grill (SAGE, ver. 1.0; Marquez 2006).

Genetic Analysis

DNA isolation was performed using a cetyltrimethyl ammonium bromide (CTAB) protocol with an additional phenol-chloroform cleaning step (Lefort and Douglas 1999). Isolated

Table 2

Standardized Canonical Discriminant Function Coefficient of Each Morphological Trait Analyzed

Morphometric variable	Coefficient
Leaf shape:	
PCA 1	.816
PCA 2	1.369
Lamina length (LL)	−1.154
Petiole length (PL)	.311
Lamina width (LW)	1.173

DNA was stored in deionized water at −20°C. DNA concentration was obtained with a BioPhotometer (Eppendorf). All isolated DNA samples were diluted to a final concentration of 20 ng μL^{-1}. Eight nuclear microsatellite loci were amplified in multiplex polymerase chain reactions (PCRs). Two groups of primer pairs were arranged according to allele sizes and fluorescent labels. The first group was formed by primer pairs QpZAG36, QpZAG110 (Steinkellner et al. 1997), QrZAG39 (Kampfer et al. 1998), and quru-GA-0C19 (Aldrich et al. 2002). The second group of primers included quru-GA-0C11, quru-GA-0M07, quru-GA-0I01, and quru-GA-1C08 (Aldrich et al. 2002). The selection of these primers was based on the quality of preliminary amplification trials. PCR was performed using the QIAGEN Multiplex PCR kit (QIAGEN) in 5-μL reactions as follows: 1X Multiplex PCR Master Mix, 2 μM each primer, deionized water, and 20 ng DNA (Cortés-Paloméc et al. 2008). The thermal cycling program was run on an Applied Biosystems thermocycler. The program consisted of one cycle at 95°C for 15 min and then 35 cycles, at 95°C for 30 s each, annealing at 50°C for 1.5 min, and extension at 72°C for 1 min. A final extension at 60°C for 30 min was included. Multiplex PCR products were diluted 1 : 1 in deionized water and run in an ABI-PRISM 3100-Avant sequencer with the GeneScan-500 LIZ size standard included (Applied Biosystems). Fragment analysis and final sizing was performed using Peak Scanner software, version 1.0 (Applied Biosystems).

Bayesian Assignment Analysis

The Bayesian statistical methods have been used to detect hybridization and hybrid individuals in nature (Pritchard et al. 2000; Anderson and Thompson 2002). The efficiency and accuracy of these Bayesian methods to determine objective and optimal thresholds for genetic assignment of pure, hybrid, and backcrossed individuals has been assessed using simulation of artificial genotypes of known ancestry (Vähä and Primmer 2006; Schwartz and Beheregaray 2008; Burgarella et al. 2009; Lepais et al. 2009).

Genetic ancestry of individuals was determined using the Bayesian clustering method implemented in STRUCTURE, version 2.1 (Pritchard et al. 2000). All 216 individuals from the hybrid zone and the reference populations were analyzed jointly, without prior population information, under the admixture model with uncorrelated allele frequencies. We ran K values (number of potential genetic clusters) from 1 to 5 with 10 independent runs for each K. The length of the burn-in was 500,000 steps followed by 10^6 iterations. The K value with the highest posterior probability was identified in this way, and also by using the ΔK statistics, which quantifies the second-order rate of change of the likelihood function with respect to K (Evanno et al. 2005).

After this analysis individuals were assigned to one of five possible genotypic categories according to their inferred admixture coefficient (q value), as follows: $q \geq 0.90$ represents individuals belonging to a single genetic group (i.e., the

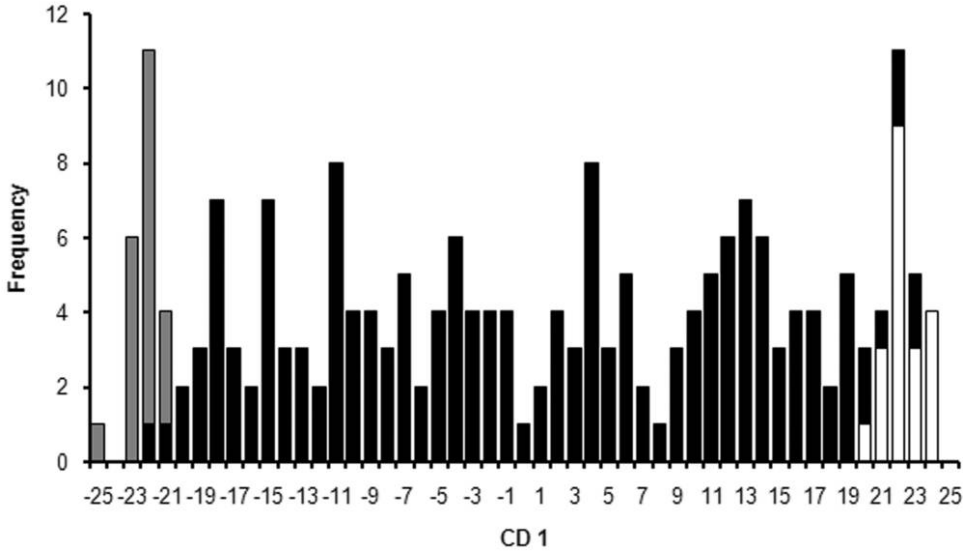

Fig. 2 Frequency histogram of the first canonical discriminant function scores (CD1) of leaf morphological characters in representative populations of *Quercus resinosa*, *Quercus magnoliifolia*, and the hybrid zone. Gray columns represent parental individuals of *Q. magnoliifolia* (mean CD1 = −22.32, SD = 0.95), filled columns represent individuals from the Tequila volcano hybrid zone (mean CD1 = 0.5, SD = 12.32), and white columns represent parental individuals of *Q. resinosa* (mean CD1 = 22.32, SD = 1.05).

two parental species); $0.41 < q < 0.59$ indicates F_1 hybrid individuals; and $0.89 > q > 0.6$ are the two possible backcrosses. To verify the efficiency of the assignment procedure in the recognition of the different genotypic classes potentially represented in our samples, parental, hybrid, and backcrossed genotypes were generated through simulation with the HYBRILAB 1.0 program (Nielsen et al. 2006), using the allelic frequencies estimated from individuals that had a high probability of belonging to a single genetic cluster, that is, with admixture coefficients (q values) greater than 0.90 (Vähä and Primmer 2006). In total, 500 pure genotypes for each species, 25 F_1 hybrids, 40 backcrosses to *Q. resinosa*, and 100 backcrosses to *Q. magnoliifolia* were simulated. The number of hybrid genotypes simulated is approximately equal to that which was observed in the real samples. Afterward, the simulated genotypes were analyzed with STRUCTURE, using the same parameters as before, to test the efficiency (the proportion of individuals correctly identified), accuracy (the proportion of true hybrid and pure individuals assigned in a correct category), and overall performance (efficiency multiplied by accuracy) of these Bayesian methods.

Statistical Analysis

FA levels were compared among the five genotypic classes (parental individuals of the two species, F_1 hybrids, "*resinosa*-like" backcrosses, and "*magnoliifolia*-like" backcrosses) with ANOVA and a posteriori Tukey-Kramer HSD tests using the JMP 6.0 software (SAS Institute).

To evaluate the possible combined effects of genetic ancestry, leaf morphology (as measured by the scores of the canonical discriminant function; CD1), and altitude on individual FA levels within the hybrid zone, a stepwise regression analysis was performed with JMP 6.0 (SAS Institute). Also, we determined how leaf morphology and genetic ancestry are correlated with each other and their pattern of variation along the altitudinal gradient. These analyses were performed using only the genetic, morphological, and altitudinal data of the hybrid zone at the Tequila volcano.

Results

Morphological Data

The first canonical discriminant function derived from morphometric data analysis explained 100% of leaf shape and size variation between *Quercus resinosa* and *Quercus magnoliifolia*, meaning that discrimination between reference individuals of the two species was absolute and highly significant (Wilks's lambda = 0.0019; $\chi^2 = 222.37$; df = 5; $P < 0.0001$). Leaf shape (PCA 2) was the morphological trait that contributed the most to the discrimination, according to the values of standardized canonical discriminant function coefficients (table 2). Histograms of the discriminant function scores for individuals from the reference populations of *Q. magnoliifolia* and *Q. resinosa* and for the hybrid zone (Tequila) samples are shown in figure 2. Score values of the Tequila individuals form a continuous series covering the whole interval that separates reference populations of the two species.

Bayesian Assignment Data

The results of the STRUCTURE analysis showed that the estimated log probability of data decreased sharply from $K = 1$ ($\ln P(D) = -8913.2$) to $K = 2$ ($\ln P(D) = -8707.0$) and then decreased slightly from $K = 2$ to $K = 3$ ($\ln P(D) = -8607.0$; fig. 3*a*). According to Pritchard and Wen (2004), this pattern should be interpreted as favoring $K = 2$ as the most probable number of clusters. The values of ΔK also indicated that $K = 2$ is the most likely number of genetic groups (fig. 3*b*). The genetic assignments of individuals with $K = 3$ are similar to those with $K = 2$, with the addition of a third genetic group that predominates in hybrids but without showing a clear structure (fig. 4). Considering two main clusters, the assignment of individuals detected a total of 17 pure *Q. resinosa* trees (7.8%; five from Tequila and 12 from the reference populations), 34 pure *Q. magnoliifolia* trees (15.7%; 23 from Tequila and 11 from the reference populations), 25 F_1 trees from Tequila (11.5%), 40 backcrosses to *Q. resinosa resinosa*-like (18.5%; 32 from Tequila and eight from the reference populations), and 100 backcrosses to *Q. magnoliifolia magnoliifolia*-like (46.4%; 91 from Tequila and nine from the reference populations). Simulations indicated a high efficiency in STRUCTURE in the assignment of pure species individuals (100%) but a relatively modest efficiency for hybrid classes (72%–79%; table 3). Seven simulated F_1 individuals (28%) were misclassified as backcrosses, six were *resinosa*-like, and

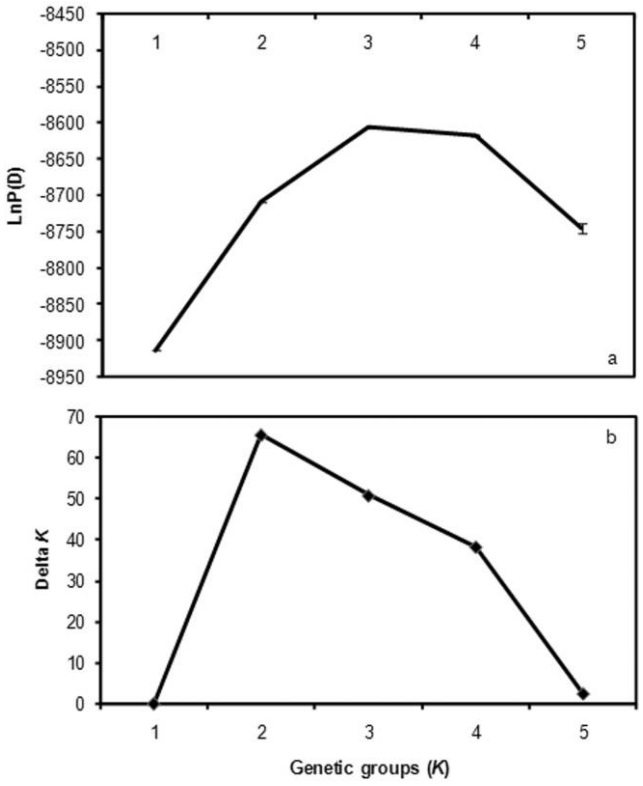

Fig. 3 Estimated genetic groups (K) from STRUCTURE clustering analysis: *a*, mean and standard deviation of log probability of data over 10 independent runs for each K and *b*, plot of statistics ΔK with respect to genetic clusters K (from 1 to 5).

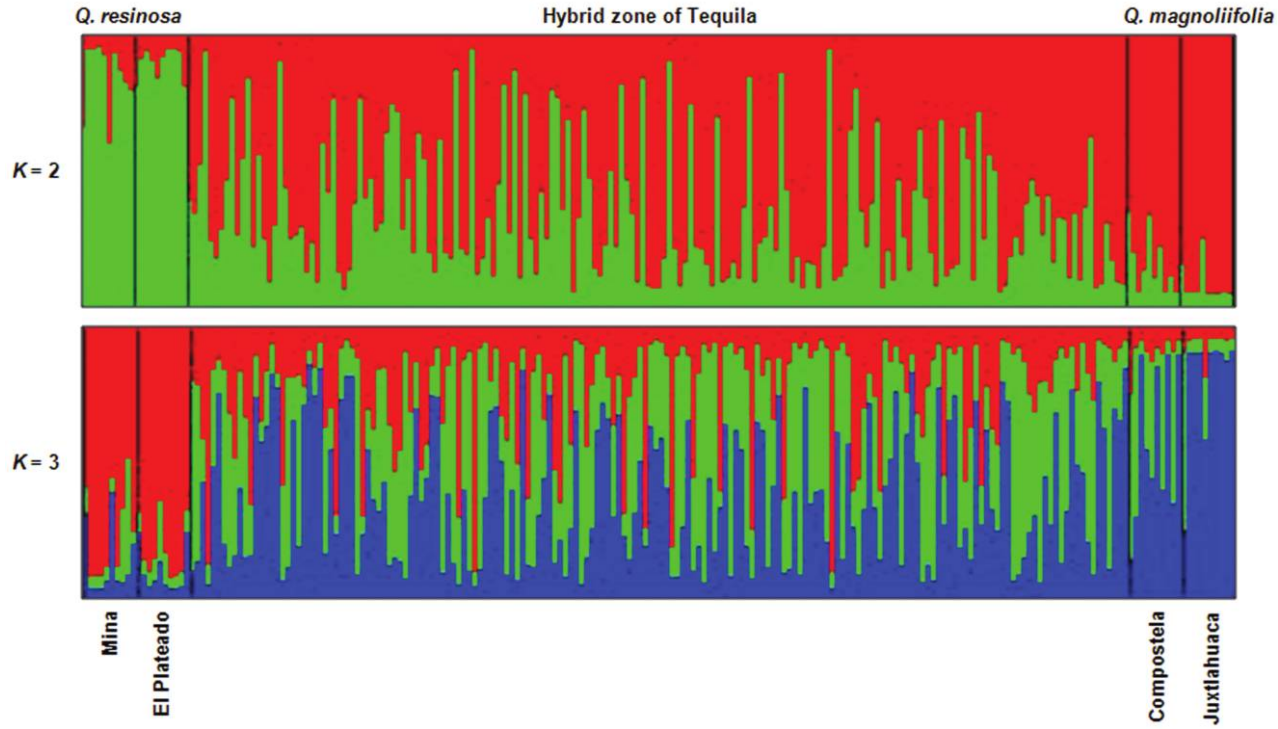

Fig. 4 Structure clustering analysis results for $K = 2$ and $K = 3$. Individual genetic ancestry is represented by vertical lines partitioned into K different colors that represent the admixture genetic ancestry in the corresponding genetic cluster. Species reference populations and the population of the Tequila hybrid zone are separated by black lines, as indicated at the top; at the the bottom are the names of those populations. Individuals from the Tequila population were ordered by altitude.

one was *magnoliifolia*-like, while 20% of simulated *resinosa*-like individuals and 12% of *magnoliifolia*-like individuals were misclassified as pure species (table 3). Accuracy was high for both simulated pure species and backcrosses (*Q. magnoliifolia* [97.7%], *Q. resinosa* [98.4%], *resinosa*-like trees [80.5%], and *magnoliifolia*-like trees [98.8%]), but it was relatively low for F_1 hybrids (62%), mainly because 38% of the simulated backcrosses were misclassified in the F_1 hybrid category, affecting the accuracy. The overall performance of the assignment method was high for pure species: it was 97.7% for *Q. magnoliifolia* and 99% for *Q. resinosa*. The overall performance for hybrid classes was modest (between 78% and 44.7%).

Leaf Fluctuating Asymmetry Data

Procrustes ANOVA indicated that asymmetry variation in the samples is due to FA (the individual × sides interaction term is significant in all cases) and not to directional asym-

Table 3

Results of Simulated Pure F_1 Hybrids and Backcrosses, Genotypes (Columns), Number of Simulated Individuals (Rows), Correct Assignment Individuals Highlighted in Bold, Percentage of Efficiency, Accuracy, and Overall Performance of the Assignment Method

Simulated/assigned	*Quercus resinosa*	*Quercus magnoliifolia*	F_1	*resinosa*-like	*magnoliifolia*-like	Total
Q. resinosa	**500**					500
Q. magnoliifolia		**500**				500
F_1			**18**	6	1	25
resinosa-like	8		3	**29**		40
magnoliifolia-like		12	8	1	**79**	100
Total	508	512	29	36	80	1165
Efficiency (%)	100	100	72	72.5	79	
Accuracy (%)	98.4	97.7	62	80.5	98.8	
Performance (%)	98.4	97.7	44.7	58.4	78	

Note. F_1 = hybrid of first generation; *resinosa*-like = backcrosses of *Q. resinosa*; *magnoliifolia*-like = backcrosses of *Q. magnoliifolia*.

metry (the side terms are not significant; table 4). The PCA axis 1 of leaf FA for each genotype class is shown as deformation grids (fig. 5). This axis explains 84% of the total variation in FA for *Q. resinosa*, 78% of that in *Q. magnoliifolia*, 89% of that in F_1 hybrids, 83.53% of that in *resinosa*-like trees, and 77.73% of that in *magnoliifolia*-like trees.

The ANOVA comparing the five genotype classes indicated significant differences in FA levels ($F = 2.83$; $df = 4$; $P = 0.025$; fig. 6). The higher level of leaf FA was found in the F_1 hybrid class (mean = 0.000924, SE = 0.00016), followed by *resinosa*-like (mean = 0.000817, SE = 0.00013), *Q. resinosa* (mean = 0.000625, SE = 0.00019), *magnoliifolia*-like (mean = 0.000575, SE = $7.87 \times 10_{-5}$), and *Q. magnoliifolia* (mean = 0.000319, SE = 0.00013) individuals. The Tukey-Kramer tests indicated that F_1 hybrids and *Q. magnoliifolia* individuals were significantly different (fig. 6).

The results of the stepwise regression showed that individual FA levels within the Tequila population were significantly correlated with values of the admixture coefficient ($R^2 = 0.0344$; $P = 0.01$), but not with CD1 ($P = 0.67$) values and altitude ($P = 0.51$). The admixture coefficient of each tree was significantly correlated with the altitudinal gradient ($R^2 = 0.0433$; $P = 0.007$). Leaf morphological variation (CD1 scores) was strongly correlated with altitude ($R^2 = 0.891$; $P = 0.0001$; fig. 7). However, CD1 scores and admixture coefficients were not significantly correlated ($R^{2=}11.53$; $P = 0.4$).

Discussion

Quercus resinosa and *Quercus magnoliifolia* are two distinct morphological species in most of their distribution areas, but they have intermediate leaf morphologies at Tequila, Jalisco, Mexico. The hypothesis of hybridization between *Q. magnoliifolia* and *Q. resinosa* at the Tequila volcano was strongly supported by the analysis of leaf morphology and the genetic assignment of individuals by the Bayesian clustering method implemented in STRUCTURE. The analysis of simulated genotypes allowed us to optimize the thresholds to discriminate between the different genotypic classes, minimizing the assignment error rate (Vähä and Primmer 2006; Schwartz and Beheregaray 2008; Burgarella et al. 2009; Lepais et al. 2009). According to these results, the performance of the assignment procedure was adequate, since simulated pure F_1 hybrids and backcrossed individuals of *Q. magnoliifolia* and *Q. resinosa* were identified by STRUCTURE with acceptable efficiency and accuracy.

Table 4

Procrustes ANOVA Results for Each Genotype Classes

Effect	*F*	df	MS	*P*
Quercus resinosa:				
Sides	1.207	27	.0010	.27
Individuals × sides	2.168	432	.0008	.001*
Measurement error		3672	.0004	
Quercus magnoliifolia:				
Sides	.215	27	8.45×10^{-5}	.87
Individuals × sides	1.342	891	.0003	.01*
Measurement error		7344	.0002	
F_1 hybrid:				
Sides	.802	27	.0006	.384
Individuals × sides	1.833	567	.0007	.0009*
Measurement error		4752	.0004	
Q. resinosa–like:				
Sides	.218	27	.0001	.80
Individuals × sides	1.786	972	.0006	.0009*
Measurement error		7992	.0003	
Q. magnoliifolia–like:				
Sides	.971	27	.0004	.358
Individuals × sides	1.219	2565	.0004	.01*
Measurement error		20,736	.0003	

Note. Sides = side-directional asymmetry; individual × sides interaction = fluctuating asymmetry; measurement error effect analyzed within each genotype classes. Significance was tested with 1000 permutations.

* $P < 0.01$.

The genotypic composition of individuals was weakly correlated with altitude along the Tequila volcano. Seemingly, *Q. resinosa* genotypes predominated at higher altitudes, from 1600 to 2100 m, and *Q. magnoliifolia* genotypes, although present all along the altitudinal gradient (from 1400 to 2100 m), increased in frequency at low altitudes, between 1600 and 1700 m. However, these differences are not significant according to a χ^2 contingency table analysis (not shown). The hybrids and backcrosses were frequent throughout the whole gradient (see table 1). A possible explanation for this lack of correlation between the individual genotypes and their altitudinal distribution is that *Q. resinosa* and *Q. magnoliifolia* have very wide altitudinal ranges, and the gradient at the Tequila volcano only represents a fraction of these ranges. *Quercus resinosa* has an altitudinal distribution range from 1300 to 2800 m, whereas *Q. magnoliifolia* has a range from 400 to 2850 m. These results suggest that exogenous selection related to altitude probably does not have a strong effect on genotype distribution at the Tequila volcano. On the other hand, the genetic assignment analysis showed that there are more backcrosses and F_1 hybrids than pure genotypes within the hybrid zone, which suggests that hybrids could be more fit than parental genotypes at this site.

The use of geometric morphometric techniques allowed us to clearly discriminate the morphological leaf characters of shape and size between parental species and their hybrids. A very strong correlation was found between foliar morphological variation (CD1) and altitude. This variation was markedly clinal throughout the hybrid zone (a gradual change from the typical morphology of one pure parental phenotype to the other). In contrast to what was observed with genotypic variation, in this case it is possible that environmental conditions (exogenous selection) could be favoring the establishment of pure parental phenotypes at both ends of the gradient (*Q. magnoliifolia* predominating at lower altitudes and *Q. resinosa* predominating at higher altitudes), whereas intermediate phenotypes are favored at middle elevations. Morphological clines along altitudinal or geographical gradients have been observed in other studies of oak species in sympatry (e.g., Jensen et al. 1993; Spellenberg 1995, Tovar-Sánchez and Oyama 2004), and they are usually interpreted as supporting the hypothesis that hybrid zones with extensive interspecific gene flow are by-products of secondary contact of species. However, this pattern is not always found. For example, in the well-studied European oak complex (*Quercus*

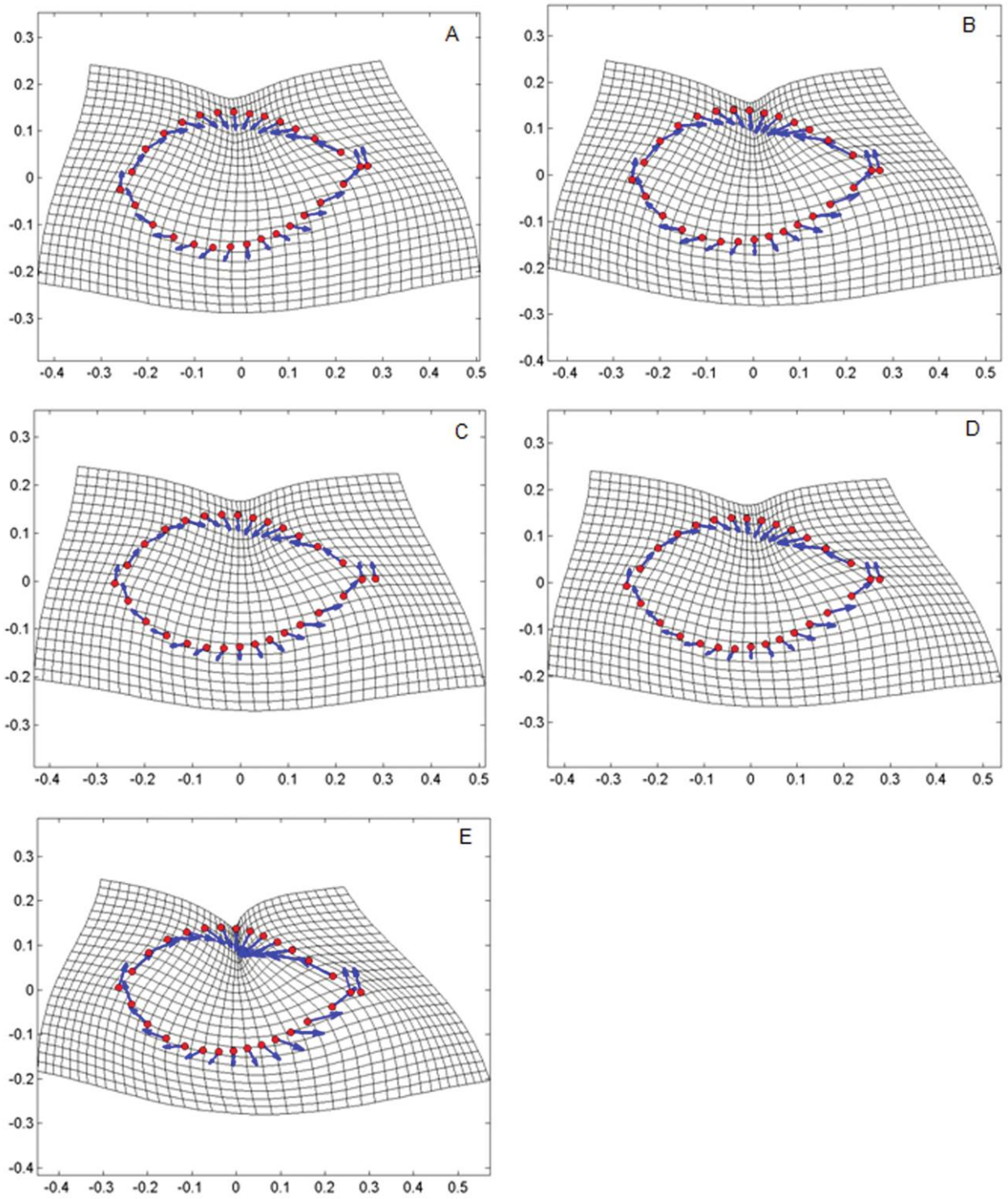

Fig. 5 Deformation grid of leaf shape fluctuating asymmetry (FA) for two parental species and putative hybrids: (*A*) *Quercus resinosa*, (*B*) *resinosa*-like individuals, (C) *Quercus magnoliifolia*, (*D*) *magnoliifolia*-like individuals, and (*E*) hybrids.

robur–Quercus petraea), morphological variation has a bimodal distribution, with few clearly intermediate trees, despite extensive introgression inferred with genetic markers (Kremer et al. 2002).

No correlation was found between genotypic and phenotypic variation within the *Q. magnoliifolia–Q. resinosa* hybrid zone along the Tequila volcano. A possible explanation is that extensive hybridization could break down the genes

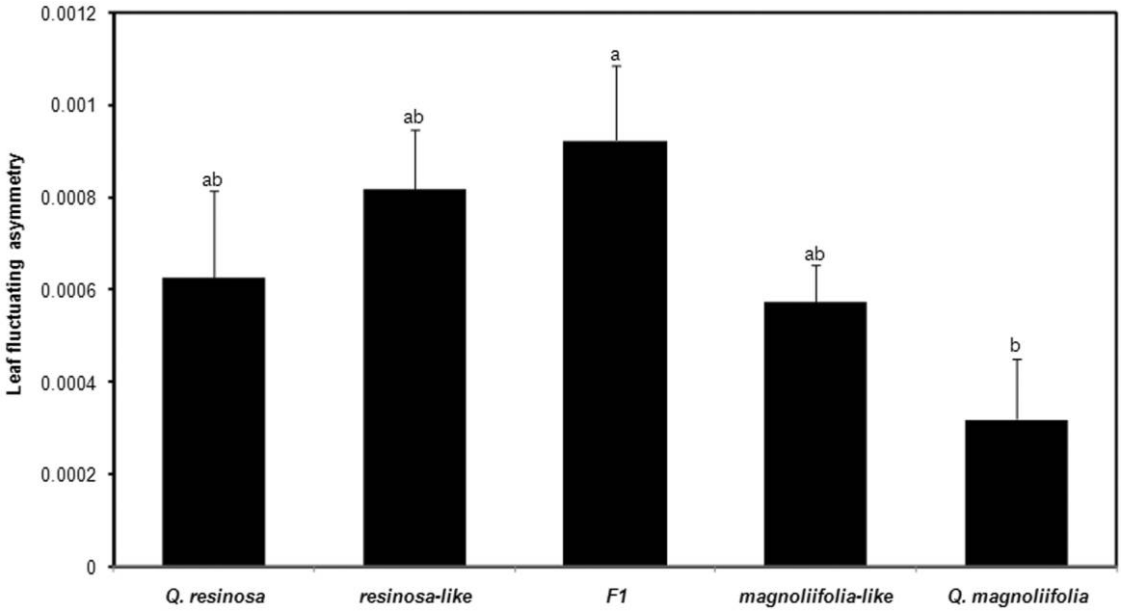

Fig. 6 Leaf-fluctuating asymmetry (FA) variation between pure genotypes of *Quercus resinosa* (mean = 0.000625, SE = 0.00019), *Quercus magnoliifolia* (mean = 0.000319, SE = 0.00013), F_1 hybrids (mean = 0.000924, SE = 0.00016), backcrosses of *resinosa*-like individuals (mean = 0.000817, SE = 0.00013), and backcrosses of *magnoliifolia*-like individuals (mean = 0.000575, SE = $7.87 \times 10 - 5$). Data shown are means and standard errors. Different letters indicate significant differences between genotype classes.

associated with morphological characters. Another possibility is that microsatellite loci are completely unliked with QTL for leaf morphology. A similar lack of correlation between morphological and molecular variation was found in the *Quercus affinis–Quercus laurina* complex, in which only partial congruence between genetic assignment and morphology was found (González-Rodríguez et al. 2005), as well as in the case of a few hybrid individuals of *Quercus suber* and *Quercus ilex* that were morphologically assigned to one species but genetically assigned to the other species (Burgarella et al. 2009). Contrasting findings have been reported in other studies, such as one of *Q. robur*, *Q. petraea*, *Quercus pubescens*, and *Quercus frainetto* in west-central Romania, in which a strong association between genetic assignment and morphology was found, but with very low levels of hybridization (Curtu et al. 2007), or another study of *Q. robur*, *Q. petraea*, *Q. pubescens*, and *Quercus pyrenaica* in France that showed a clear concordance between genetic cluster and morphological features of the populations, but with different levels of hybridization among the species through the different sites (Lepais et al. 2009).

Few studies exist on the effects of interspecific hybridization on plant character asymmetry (Levin 1970; Wilsey et al. 1998; Hochwender and Fritz 1999; Siikamäki 1999). Hybridization could either reduce or increase the levels of developmental instability, as a result of increased heterozygosity or the disruption of coadapted gene complexes of each species in hybrids (Soulé 1967, 1979; Graham 1992; Klingenberg 2003), respectively. Also, there is evidence indicating that the effects of hybridization on the levels of FA depend on how closely related the parental taxa are (Markow 1995; Hochwender and Fritz 1999; Siikamäki 1999; Alibert and Auffray 2003). In this study, we measured the effect of hybridization and introgression on the levels of FA in *Q. magnoliifolia* and *Q. resinosa*. As far as we know, our study is the first to compare the level of FA among parental and hybrid genotypes of oak species despite the fact that hybridization among *Quercus* species is an emblematic example in evolutionary biology (Futuyma 2005). The results of the Procrustes ANOVA indicated random variation (FA) between the left and the right sides of the leaves in all genotypic clas-

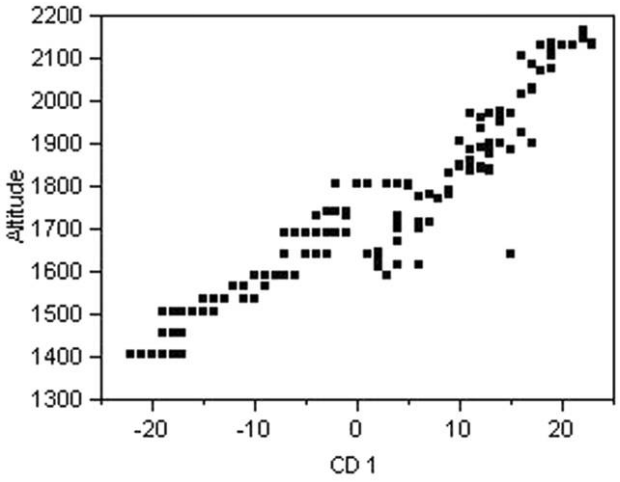

Fig. 7 Correlations analysis between altitude and individual scores of the first canonical discriminant function of morphological analysis in the Tequila hybrid zone ($R^2 = 0.891$; $P = 0.0001$).

ses, rather than nonrandom differences (DA) among sides. Also, asymmetry values followed a normal distribution (not shown), which is contrary to what would be expected in the case of antisymmetry that would produce a binominal distribution. Levels of FA were higher in F_1 hybrids and backcrosses compared with in pure *Q. magnoliifolia* and *Q. resinosa* individuals, suggesting that in this case, hybridization might be increasing FA as a result of the disruption of the coadapted gene complexes of the parental species. Similar patterns of high levels of FA in hybrid systems have been observed in interspecific hybrids among *Betula pubescens*, *Betula nana*, and *Betula pendula* growing in common gardens at different elevation sites (Wilsey et al. 1998). In the hybrid zone between *Liatris aspera*, *Liatris cylindracea*, and *Liatris spicata*, hybrids showed higher levels of FA than did parental species (Levin 1970). Petals of hybrids between *Lychnis viscaria* and *Lychnis alpina* have higher levels of FA than do petals of parental species when they are grown in a common garden (Siikamäki 1999). In a *Salix* hybrid system, F_1 and F_2 hybrid plants showed higher levels of FA than did parental species, with F_2 showing greater FA (Hochwender and Fritz 1999). Nevertheless, some studies failed to find differences in FA between parental individuals and hybrids, such as in the case of intraspecific hybrid line products of distant populations of *Dalechampia scandens* (Pélabon et al. 2004, 2005) or in the hybrid zone between the two subspecies *Artemisia tridentata* subspecies *tridentata* and subspecies *vaseyana* (Freeman et al. 1995). In these cases, coadapted gene complexes could be very similar and, thus, hybridization would result in little or no disruption of these complexes (Graham and Felley 1985; Markow 1995; Hochwender and Fritz 1999). Also, if the hybrid zone is old, gene complexes may have reevolved (Felley 1980; Graham and Felley 1985).

The nature and extent of the genetic basis of FA are not fully understood (Leamy and Klingenberg 2005). Common garden experiments show that genetic stress through hybridization increases the levels of FA in hybrids more than does environmental stress (i.e., water stress, pathogen attack, competition, and altitude; Wilsey et al. 1998; Siikamäki 1999; Hochwender and Fritz 1999). Our study suggests that the effect of hybridization on FA has a genetic basis. We found that leaf FA was correlated more with the proportion of genetic ancestry of each individual than with leaf morphology or altitude. This supports the hypothesis that FA is related more to hybridization than to environmental conditions. According to our data, backcrosses have lower values of FA compared with that of hybrids, which also supports the genetic hypothesis.

We conclude that *Q. magnoliifolia* and *Q. resinosa* are two different evolutionary lineages that overlapped on the Tequila volcano, forming a hybrid zone, and, in this case, hybridization produced an increase in the levels of DI, probably as a result of the disruption of coadapted gene complexes characteristic of each species. Measures of levels of FA as indicators of DI in natural populations are an indirect method to determine how genetic composition and environmental stress interact under hybridization events. The genetic basis and the effects on fitness are still unknown, and future studies are needed.

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VI.

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Limited genetic differentiation between two distinct morphological and ecological giant-leaved Mexican oaks

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Title: **Limited genetic differentiation between two distinct morphological and ecological giant-leaved Mexican oaks**

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Running title: Limited genetic differentiation between two oak species.

- *Background and Aims* Patterns of intra- and interspecific gene flow can help to resolve the problem to define species boundaries. We examined morphological, ecological and genetic differentiation between two hybridizing Mexican white oak species, *Quercus magnoliifolia* and *Q. resinosa*, throughout their distribution range.

- *Methods* We analyzed leaf shape by geometric morphometrics, ecological differentiation between the two species by ecological niche modeling and genetic structure using eight highly informative nuclear microsatellites (SSRs) loci. Inter- and intra-specific pairwise genetic differentiation (F_{ST}) between populations was estimated to evaluate the extent of gene flow at different geographic scales.

- *Key Results* The principal component analysis of leaf shape revealed two clear groups corresponding well to field-based species determinations. *Q. magnoliifolia* and *Q. resinosa* have divergent niches and sympatric zones occur at intermediates environments of the two species as shown by the first principal component of the environmental features. Temperature differences characterize the ecological niche of each species. Two sympatric zones with different geographical location and environmental conditions were found. Pairwise F_{ST} across all loci showed high intra-specific differentiation and low inter-specific differentiation. Isolation by distance between the two species and within *Q. magnoliifolia* was found. Moreover, inter-specific differentiation was lower between nearby populations than between distant populations. A Bayesian genetic structure analysis identified two genetic groups whose correspondence with morphological species was limited but geographically structured.

- *Conclusions* Such a low congruence between leaf shape morphology and genetic assignments suggests that the species remain morphologically and ecologically

distinct, possibly to disruptive selection, despite very high levels of inter-specific gene flow between nearby populations. The geographically structure of introgression between *Q. magnoliifolia* and *Q. resinosa* support the hypothesis of inter-specific gene flow by isolation by distance. Much more genomic resolution will be needed to identify those genes associated with inter-specific differentiation in these Mexican oaks, due to their extremely porous genomes.

***Key words*:** Limited genetic differentiation, ecological niche modeling, leaf shape analysis, nuclear microsatellites, isolation by distance, interspecific gene flow, introgression, sympatric zone, *Quercus magnoliifolia*, *Quercus resinosa*, Mexico.

Introduction

Hybridization is a mechanism contributing to maintain genetic diversity in natural populations (Stebbins, 1959; Lewontin and Birch, 1966). It can lead to rapid genomic changes such as chromosomal rearrangements, genome expansion, and differential gene expression (Baack and Rieseberg, 2007). Introgression or introgressive hybridization is the natural infiltration of genes from one species into another as a result of interspecific hybridization followed by successive backcrosses with one or both parental species (Rieseberg, 1997). Introgressive hybridization is potentially important in the incorporation of genetic variability in natural populations necessary for natural selection to produce new adaptations resulting in fitter genotypes capable to colonize new habitats or sites already occupied by a related species (Stebbins, 1959; Lewontin and Birch, 1966; Potts and Reid, 1988; Petit *et al*., 2003; Dodd and Afzal-Rafii, 2004; Baack and Rieseberg, 2007). The distribution of introgressed genes in the current geographic range of a species is a consequence of many generations of gene flow between hybridizing groups and the particular environmental conditions that could

promote (or restrict) gene exchanges and the establishment of hybrids (Fernández-Manjarres *et al*., 2006).

Oak species (genus *Quercus*) have been of interest to evolutionary biologist since Darwinian times for their ability to overcome sexual barriers thanks to the fertility of their hybrids promoting gene flow between diverging taxa. They have therefore been used as models to study hybridization, the formation of hybrid zones and introgression (Grant, 1981; Rushton, 1993; Futuyma, 2005), but also has been a source of debate regarding species concepts (Burger, 1975; Van Valen, 1977). Oak species could maintain their morphological identity despite interspecific gene flow and introgression (Whittemore and Schaal, 1991; Bacilieri *et al*., 1996; Howard *et al*., 1997; Kremer *et al*., 2002; Dodd and Afzal-Rafii, 2004; González-Rodríguez and Oyama, 2005) but low levels of molecular differentiation between oaks pairs have been reported as a consequence of high levels of interspecific gene flow (Bodénès *et al*., 1997; Howard *et al*., 1997; Bruschi *et al*., 2000; Kashani and Dodd, 2002; Aldrich *et al*., 2003; Ishida *et al*., 2003; González-Rodríguez *et al*., 2005; Craft and Ashley, 2006; Salvini *et al*. 2009; Peñaloza-Ramírez *et al*., 2010; Zeng *et al*., 2010; Curtu *et al*., 2011). Patterns of interspecific gene flow and introgression between oak species have been strongly associated with their sympatry (Whittemore and Schaal, 1991; González-Rodríguez *et al*. 2005; Neophytou *et al*., 2010).

Extensive gene flow or shared genetic ancestry has been proposed as alternative or complementary hypotheses to explain the low genetic divergence between oak species (Muir and Schötterer, 2005; Lexer *et al*., 2006). The study of genetic differentiation among species pairs distributed in different geographical regions could help to differentiate between gene flow and shared ancestry because interspecific gene flow is expected to result in less differentiation between neighbouring populations than

among distantly located ones (Lexer *et al*., 2006). In contrast, if low interspecific genetic divergence is caused solely by the retention of ancestral alleles, geographical neighbors are expected to be also similar than distantly located ones (Muir and Schötterer, 2005).

The integration of ecological niche models and molecular data has been used to delimitate species (Raxworthy *et al*., 2007), to improve historical inferences (Jakob *et al*., 2009) and to know the role of niche similarity in the patterns of inter-especific gene flow (Arteaga *et al*., 2011).

Q. magnoliifolia and *Q. resinosa* are two Mexican white oak species that are remarkable by their huge leaves that can reach over 30 cm and are among the largest in the genus *Quercus* (Rzedowski, 1978) (Fig. 1). The resulting tree architecture with large leaves borne on thick twigs might be an adaptation to the fire prone environments where these species occur (Peña-Ramírez and Bonfil, 2003; Rodríguez-Trejo, 2008). Sympatric populations of these two oak species occur at the south of Sierra Madre Occidental and Trans-Mexican Volcanic Belt, whereas allopatric populations of *Q. magnoliifolia* occur in the north of Sierra Madre Occidental and the Sierra Madre del Sur and allopatric populations of *Q. resinosa* occur in the Central Plateau (Fig. 2). Morphological and molecular analysis at local scale showed a hybrid zone by secondary contact between these two species (Albarrán-Lara *et al.*, 2010). In the present study, we examine the morphological, ecological and genetic differentiation between *Q. magnoliifolia* and *Q. resinosa* throughout their geographical distribution range using leaf shape geometric morphometry, ecological niche modeling and eight highly informative nuclear microsatellites (SSRs) loci to determine the genetic structure and degree of introgression between species. The ecological niche modeling was using to determine the divergence of niche between these two species and identified the

137 geographical and ecological characteristic of sympatric areas. In order to differentiate
138 between gene flow and shared ancestral polymorphism, we test the hypothesis of
139 isolation by distance by comparing interspecific F_{ST} values at different geographic
140 scales. The objectives of this study were to: i) determine the levels of morphological
141 differentiation between *Q. magnoliifolia* and *Q. resinosa* at different spatial scales, ii)
142 determine the geographical distribution and ecological differentiation between *Q.*
143 *magnoliifolia, Q. resinosa* and sympatric areas using niche models, and iii) characterize
144 their genetic structure using molecular markers.

145

146 **Materials and Methods**

147 *Study species*

148 *Quercus magnoliifolia* Née and *Q. resinosa* Liebm. were classified as members of
149 different taxonomic series: *Circinatae* and *Macrophyllae*, respectively (Trelease 1924).
150 Like many other oaks, including other Mexican white oaks, they have been reported to
151 hybridize frequently (Albarrán-Lara *et al.*, 2010). *Q. magnoliifolia* has very broad to
152 narrow obovate, seldom elliptic leaves with long glabrescent petioles and twigs that are
153 longitudinally striate with numerous long pale lenticels, whereas *Q. resinosa* has broad
154 obovate and rounded leaves with sessile petioles and twigs covered by a dense yellow
155 tomentum with glandular trichomes (McVaugh, 1974; Valencia, 2004). The production
156 of flowers is in March and April for *Q. magnoliifolia* and in April and May for *Q.*
157 *resinosa*. *Q. magnoliifolia* has a wide geographic distribution with an altitudinal
158 distribution range from 400 to 2850 m and *Q. resinosa* has a more restricted geographic
159 distribution with an altitudinal range from 1300 to 2800 m. These two species are
160 dominant in the communities where they grow, often forming pure stands in different

161 habitats; *Q. magnoliifolia* is almost always present in transition zones between tropical
162 deciduous and pine-oak forests, whereas, *Q. resinosa* occurs in semi-arid grasslands and
163 pine-oak forests (McVaugh, 1974; Rzedowski, 1978).

164 *Population samples*

165 A total of 392 trees in 60 populations of *Q. magnoliifolia* and *Q. resinosa* were
166 collected across their distribution range in Mexico (Fig. 2). For each population, 10
167 trees separated by at least 20 m were randomly selected. From each tree, several leaves
168 and branches were collected, pressed and dried for taxonomical identification and
169 morphometric analysis, and fresh leaves were stored at -80°C in the laboratory for
170 genetic analysis. Plant specimens were deposited in the Herbarium of Facultad de
171 Ciencias, UNAM.

172 *Leaf shape morphometric analysis*

173 We analyzed the leaf shape of *Q. magnoliifolia* and *Q. resinosa* individuals using
174 geometric morphometry analysis. For each of the 392 white oak trees, five leaves were
175 randomly chosen and photographed from the abaxial part together with a ruler as size
176 reference using a digital camera (Sony 7x optical zoom, 8.0 mega pixels). A total of
177 1960 leaves were included in the analysis. Along the leaf margin, a total of 29
178 anatomical marks were registered for each leaf image with the program TpsDig (Rohlf,
179 2005); three of these anatomical marks (i.e. apex, lamina base and petiole extreme)
180 corresponded to homologous traits or "landmarks" (Bookstein, 1991), and the
181 remaining 26 marks to semi-landmarks that corresponded to morphological points that
182 incorporate information about leaf contour in a morphometric analysis (Zelditch *et al*.,
183 2004). To digitize the 26 semi-landmarks, we constructed a "fan" (radial guidelines with
184 equal angular spacing on images) with 80 radial guidelines covering the whole leaf

contour, based on landmarks "1" (lamina base) and "15" (apex) to construct a "fan" using MakeFan6 program from the "Integrated Morphometrics Package" IMP series (http://www.canisius.edu/~sheets/morphsoft.html). Two additional marks were placed on the ruler as size reference.

The analysis of landmarks and semi-landmarks configuration by population was performed by Procrustes superimposition using the CoordGen6 program in IMP. This analysis allows to calculate leaf shape variables without size effect. The resulting shape variables (procrustes distance) were averaged across all five leaves by tree. For each tree 58 procrustes distance plus their centroid size were obtained. A principal component analysis (PCA) was performed using the procrustes distances of the 392 trees to determine the leaf shape morphological differentiation among individuals of each species with the PAST software, ver. 1.79 (Hammer *et al*., 2001).

Potential geographic distribution of species using ecological niche modeling

We compiled herbaria data for *Q. magnoliifolia* and *Q. resinosa* from the National Herbarium of Mexico (MEXU, UNAM), online available herbarium information especially from Global Biodiversity Information Facility (Gbif Accesed) and our own field work data. Occurrence information of species was used in the form of unique latitude–longitude combinations; duplicate records for the same species collected at the same site were removed. Thus, we obtained 462 unique records for *Q. magnoliifolia* and 136 for *Q. resinosa* that cover the localities of their entire distribution ranges.

Current environmental scenarios were represented by a series of 19 variables summarizing aspects of climate (appendix 1), drawn from the WorldClim database (Hijmans *et al*., 2005).

Ecological predictive models

209 To reduce methodological biases and obtain more robust models of ecological niches of
210 each species and sympatric area we used the Genetic Algorithm for Rule-set Production
211 (GARP; Stockwell and Noble, 1992; Stockwell and Peters, 1999) implemented in
212 DESKTOPGARP v. 1.1.6 (http:// www.nhm.ku.edu/desktopgarp/), and the maximum
213 entropy machine learning algorithm in MAXENT 3.3.1 (Phillips *et al*., 2004, 2006).
214 For GARP, we developed 100 replicate models of the ecological niche of each
215 species in order to capture all variation and optimize model performance. Then the 10
216 best models were selected following Anderson *et al*., (2003) and were summed in a GIS
217 to provide a best estimate of the potential geographic distribution of each species. This
218 procedure was repeated independently 10 times. For MAXENT 3.3.1, we used the
219 default convergence threshold and 1000 maximum number of iterations for each of 10
220 replicates independently. MAXENT provides as output a continuous probability value
221 ranging from 0 to 1 in log format. Each map obtained in each replicate with MAXENT
222 was "binarized" taking as threshold the 10 percentile training presence, and was
223 summed to get a single map of maximum entropy. Finally, the consensus models
224 obtained with GARP and MAXENT for each species were obtained in a single map
225 using the converge area between the two algorithms. To obtain the sympatric area we
226 intersect the final consensus model of each species.
227 To evaluate the congruence and accuracy of the models we measured the values
228 of omission and commission. Omission is the number of records no predicted by the
229 model and commission is the predicted area where the species are not recorded or did
230 not occur. To evaluate the commission values a subset of 100 and 55 records where *Q.*
231 *magnoliifolia* and *Q. resinosa* not occurs were generated, then we compared the number
232 of absences generated for this procedure versus area predicted by the models. The
233 evaluations were made for each one of the 10 independent repetition models obtained

234 with MAXENT and GARP as well as the final consensus models of the 10 repetitions
235 obtained with MAXENT and GARP.
236 To corroborate that the predicted area by the models are not to chance, a chi-
237 square analysis comparing the area predicted by the model with real records versus
238 predicted area by the models with random records were performance. Thus, 462 random
239 records inside the distribution area of *Q. magnoliifolia* and 136 random records inside
240 the distribution area of *Q. resinosa* were generated.

241 *Ecological differentiation*

242 In order to examine the divergence of niches between *Q. magnoliifolia*, *Q. resinosa* and
243 sympatric areas, we performed a Principal Component Analysis (PCA). To performance
244 the PCA we extracted the information of the 19 current climatic variables of the 574
245 records of both species and 114 records occurred inside the sympatric areas.

246 *DNA isolation and microsatellites genotyping*

247 DNA isolation was performed using a cetyltrimethyl ammonium bromide (CTAB)
248 protocol with an additional phenol-chloroform cleaning step (Lefort and Douglas,
249 1999). All isolated DNAs were diluted with deionized water to a final concentration of
250 20 ng/μL and storage at -20°C. Eight nuclear microsatellite loci were amplified in two
251 multiplex PCR reactions using an Applied Biosystems thermocycler (Albarrán-Lara *et*
252 *al*., 2010). The first multiplex included the following four loci: QpZAG36, QpZAG110
253 (Steinkellner *et al*., 1997), QrZAG39 (Kampfer *et al*., 1998) and quru-GA-0C19
254 (Aldrich *et al*., 2002). The second multiplex included also four loci: quru-GA-0C11,
255 quru-GA-0M07, quru-GA-0I01 and quru-GA-1C08 (Aldrich *et al*., 2002). Multiplex
256 PCR products were diluted 1:1 in deionized water and run in an ABI-PRISM 3100-
257 Avant sequencer with the GeneScan-500 LIZ size standard included (Applied

Biosystems). Fragment sizing analysis was performed using Peak Scanner software v1.0 (Applied Biosystems). We verified and corrected the individual genotype assignment of the eight nuclear microsatellite loci at least three times to corroborate our genotyping. We tested homozygote excess due to null alleles (non-amplified alleles), short allele dominance (large allele dropout) and scoring of stutter peaks errors during the polymerase chain reaction (PCR) for each population using Micro-Checker program (Oosterhout *et al*., 2004).

Genetic diversity

Allelic richness (A) and private allelic richness (P_A) were estimated by rarefaction due to differences in sample size between the two species using ADZE 1.0 (Szpiech *et al*., 2008). Rarefaction size was 155 corresponding to overall sample size of *Q. resinosa*. Expected heterozygosity (H_E), observed heterozygosity (H_O) and inbreeding coefficient (f_{is}) were calculated by locus for each species (as defined by leaf shape morphological analysis) using GENETIX 4.03 (Belkhir *et al*., 2004) with 10,000 permutations for significance testing.

Interspecific and intraspecific differentiation

For each locus, we evaluated differentiation within and between species (as defined by leaf shape morphological analysis) by calculating F_{ST} using ARLEQUIN version 3.0 (Excoffier *et al*., 2005). Significance of F_{ST} was tested using 10,000 permutations.

To differentiate between gene flow and shared ancestral polymorphism we compared F_{ST} values of neighboring populations of species pairs from different geographic location. Due to low sample size per population, we pooled nearby populations of species pair of three different regions: *Q. magnoliifolia* [pop 2, 4, 5, 6, 7 and 8] and *Q. resinosa* [populations 45, 47, 48, 50 and 52] located at Sierra Madre

Occidental and western of Trans-Mexican Volcanic Belt; populations of *Q. magnoliifolia* [11, 14, 15, 16, 17, 18 and 20] and *Q. resinosa* [53, 54 and 56] located in the center of the Trans-Mexican Volcanic Belt; and populations of *Q. magnoliifolia* [13, 22 and 23] located at north of Sierra Madre del Sur and populations of *Q. resinosa* [58 and 59] located at the south of the Central Plateau. To corroborated our result we test isolation by distance comparing the pairwise F_{ST} genetic distance matrix with pairwise geographical distance for the two species and for each species using ARLEQUIN version 3.0 (Excoffier *et al*., 2005) with 10,000 permutations for statistical significant of F_{ST}.

In order to identify groups of genetically alike individuals, we run a Bayesian clustering methods implemented in the software STRUCTURE version 2.3.1 (Pritchard *et al*., 2000). All 382 trees of both species were analyzed jointly, without prior taxonomic information of populations, under the admixture model and assuming correlated allele frequencies. We ran *K* values from 1 to 10 with 10 independent runs for each *K*. The length of the burn-in was 500,000 steps followed by 10^6 iterations. To identify the correct number of genetic groups *K*, we used the highest posterior probability of each *K* ran as well as the ΔK statistics, which quantifies the second order rate of change of the likelihood function with respect to *K* (Evanno *et al*., 2005). To run STRUCTURE, we ordered the populations of each species as shown in Table 1. To visualize the pattern of introgression between the two species along their distribution, the admixture proportion by population was plotted in a map.

To know if the pattern of admixture is associated with the geographical location of populations independently of species, we build a neighbour-joining tree with a F_{ST} distance matrix using SplitsTree version 4.11.3 (Huson and Bryant, 2006) and edited in Dendroscope version 2.4 (Huson *et al*., 2007). Then, to determine the admixture level of

each cluster populations, we pooled the all individuals belonging to these cluster and their admixture proportion was obtained with the STRUCTURE results. Finally, to explore the association between admixture levels and predicted sympatric areas, we quantified the number of nearby and inside populations in the sympatric area.

Results

Leaf shape morphometric analysis

Principal components analysis of the Procrustes analysis of leaf shape of 392 white oak trees yielded two main components explained all the observed variation with 74% for PCA 1 and 26% for PCA 2. The PCA 1 showed two clear groups that belong to *Q. magnoliifolia* (PCA 1 = -15 to -212; mean = -100) and *Q. resinosa* (PCA 1 = 6 to 331; mean = 135), indicating a clear morphological differentiation between them (Fig. 3). Landmark 1 (shape of petiole) for *Q. magnoliifolia* and Landmark 15 (shape of apex) for *Q. resinosa* were the leaf shape traits that contributed most to species differentiation. The results of morphological assignment based on scores of PCA 1 corresponded, in almost all cases, to our provisional taxonomic identification, except for ten individuals identified as *Q. magnoliifolia* but grouped with *Q. resinosa* in the PCA analysis (Table 1). We removed these ten individuals from subsequent genetic analyses. Nine of the ten individuals were located in different populations in the Sierra Madre del Sur and only one was located at Trans-Mexican Volcanic Belt (Table 1).

Predicted geographic distribution of species using ecological niche modeling

Ecological models

329 The final consensus model for *Q. magnoliifolia* and *Q. resinosa* are given in Fig. 4.
330 These models are quite accurate representations of the current geographical distribution
331 of both species. In almost all cases, the chi square was statistically significant ($\chi^2_{0.05}$ (9)
332 = 18.3) indicated that all predicted models were not due to chance. The predicted model
333 of sympatry showed two overlapping areas. The sympatric zone with greater
334 geographical extension is located at south of Sierra Madre Occidental and western of
335 Trans-Mexican Volcanic Belt, whereas, the other sympatric zone is located in the
336 central part of Trans-Mexican Volcanic Belt and is comparatively smaller in area (Fig.
337 4).

338 *Ecological differentiation*

339 Principal components analysis of the environmental features yielded three main
340 components accounting for 81.2% of the total variance observed with 47% for PCA 1
341 and 19.3% for PCA 2. The PC1 grouped at least three distinct groups, an exclusive
342 group of *Q. magnoliifolia* (PCA 1 = -1 to -0.2); the second group include records of *Q.*
343 *magnoliifolia* and a sympatric zone with very few *Q. resinosa* records (PCA 1 = -0.19 to
344 0.59) and the third group representative of *Q. resinosa* including fewer records of *Q.*
345 *magnoliifolia* and a sympatric zone (Fig. 5). The most important environmental features
346 that explain the PCA 1 are the temperature in the coldest and driest quarters (BIO 11
347 and BIO 9), and the minimum temperature of the coldest month (BIO 6). Descriptive
348 statistics with a 95% confidence interval for the mean in environmental features
349 correlated with PC1, showed that the ecological niches of *Q. magnoliifolia* and *Q.*
350 *resinosa* were different and the existence of sympatric zones between the two species
351 (Fig. 6). Descriptive statistics with a 95% confidence interval for the mean in the
352 altitude distribution range obtained from herbaria data showed that *Q. magnoliifolia*

should have its optimum between 700-1950 m in altitude and *Q. resinosa* between 1500-2250 m and sympatric zone occurs between 1400-1700 m.

Genetic diversity

Allelic richness (*A*) was high for all loci, with 11-26 alleles per locus, whereas the private allelic richness (P_A) by locus ranged from 3 to 13. For both parameters, *Q. magnoliifolia* was more variable across all loci than *Q. resinosa* (Table 2). The mean expected heterozygosity (H_E) by locus ranged from 0.69 to 0.95, the mean observed heterozygosity (H_O) by locus ranged from 0.58 to 0.95, and for both H_E and H_O the mean across all loci was higher in *Q. resinosa* than in *Q. magnoliifolia*. The inbreeding coefficient (f_{is}) by locus range from -0.05 to 0.23, with only two loci (QrZAG39 and quru-GA-0C11) showing an excess of heterozygous genotypes (negative f_{is} values) in *Q. resinosa* (Table 2).

Interspecific and intraspecific differentiation

Low but significant genetic differentiation (F_{ST}) between species and much higher differentiation among populations within species (more than 10 times higher) was found at each locus (Table 2). The comparison of F_{ST} values for neighbouring populations of species pairs showed low genetic differentiation (F_{ST}) between nearby populations of *Q. magnoliifolia* and *Q. resinosa* located at Sierra Madre Occidental and western part of Trans-Mexican Volcanic Belt, and between nearby populations of *Q. magnoliifolia* and *Q. resinosa* located at center of Trans-Mexican Volcanic Belt. In contrast, interspecific differentiation was much higher between distant populations (Table 3). Also, low genetic differentiation was found between nearby populations of *Q. magnoliifolia* located at center of Trans-Mexican Volcanic Belt and north of Sierra Madre del Sur, and between nearby populations of *Q. resinosa* located at center of Trans-Mexican

377 Volcanic Belt and south of Central Plateau (Table 4). Mantel test indicated that
378 geographical distance had a significant correlation with genetic differentiation between
379 *Q. magnoliifolia* and *Q. resinosa* ($r = 0.12$; $P = 0.01$) and within *Q. magnoliifolia* ($r =$
380 0.29; $P = 0.001$) but isolation by distance was not found within *Q. resinosa* ($r = -0.05$; P
381 $= 0.7$).

382 The results of the Bayesian clustering analysis of the 382 white oak trees
383 implemented in STRUCTURE showed that the 'log probability of data' decreased
384 sharply from $K = 1$ [LnP(D) = -15830] to $K = 2$ [LnP(D) = -14623] while at $K = 3$
385 [LnP(D) = -14230] it remained unchanged (Fig. 7). The highest posterior probability
386 and ΔK value indicated that $K = 2$ is the correct number of genetic groups (Fig. 7). The
387 genetic structure for $K = 2$ was represented by two different colors red and green, the
388 populations of *Q. magnoliifolia* (as defined by leaf shape morphological analysis) is the
389 group 1 and the populations of *Q. resinosa* (as defined by leaf shape morphological
390 analysis) is the group 2 (Fig. 8). The two genetic groups ($K = 2$) did not correspond to a
391 species delimitation because each species includes both the red and the green genotypes,
392 roughly in equal proportion. In the group 1, of the 227 individuals morphologically
393 identified as *Q. magnoliifolia*, 63% had the red genotype and 11.5% had the green
394 genotype with a proportion of $q \geq 0.90$ to belong a single cluster. In the group 2, of the
395 155 individuals morphologically identified as *Q. resinosa*, 49.7% had the red genotype
396 and 30% had the green genotype with a proportion of $q < 0.90$ to belong a single cluster.
397 Thus, there is no way to assign the two genetic groups to species. The admixture
398 proportion by population showed widespread introgression in both sympatric and
399 allopatric populations of *Q. magnoliifolia* and *Q. resinosa* (Fig. 9).

400 Analyses of genetic distances between populations confirmed that the admixture
401 proportion is geographically structured (Fig. 10). The populations of *Q. magnoliifolia*

402 and *Q. resinosa* located at Sierra Madre Occidental, western of Trans-Mexican Volcanic
403 Belt and five populations located at Central Plateau group together in the same common
404 node and 78% of individuals had the red genotype and 3% had the green genotype with
405 a $q \geq 0.90$ to belong a single cluster. The populations of *Q. magnoliifolia* and *Q.*
406 *resinosa* located at Central Plateau and central part of Trans-Mexican Volcanic Belt
407 group together and 56.4% had the green genotype and 19% of individuals had the red
408 genotype with a $q \geq 0.90$ to belong a single cluster. The populations of *Q. magnoliifolia*
409 located at Sierra Madre del Sur group together and 66% of individuals had the red
410 genotype and 1.5% had the green genotype ($q \geq 0.90$). Seven sampled populations occur
411 inside and seven populations occur nearby the predicted sympatric areas (Fig. 4).
412

413 **Discussion**

414 The persistence of morphological and ecophysiological integrity of oak species despite
415 inter-specific gene flow and introgression has been explained by divergent selection at a
416 limited number of loci in the genome, with a large proportion of the nuclear genome
417 remaining permeable to interspecific gene flow and introgression (Bodénès *et al*., 1997;
418 Wu, 2001; Petit *et al*., 2003; Scotti-Saintagne *et al.*, 2004; Minder and Widmer, 2008).
419 We found no strong evidence for interspecific differentiation at SSR loci between *Q.*
420 *magnoliifolia* and *Q. resinosa* across the distribution range; yet, each species maintains
421 its morphological and ecological traits might to divergent selection (Figs. 3, 4, 5 and 6).
422 *Quercus petraea* and *Q. robur* represent another example of a pair of oak species that
423 maintain morphological and ecophysiological integrity despite extensive interspecific
424 gene flow (Kremer *et al*., 2002). Genomic and ecological studies also indicate that these
425 species maintain distinction through disruptive selection (Bodénès *et al*., 1997; Petit *et*
426 *al*., 2003; Scotti-Saintagne *et al.*, 2004). Similarly, other studies have documented

morphological and/or ecological differentiation with low genetic differentiation (Bruschi *et al*., 2000; Kashani and Dodd, 2002; Aldrich *et al*., 2002; Ishida *et al*., 2003; Craft and Ashley, 2006; Wood and Nakazato, 2009; Cooper *et al*., 2011), concluding that the use of few neutral molecular markers to assess species divergence will often be limited, especially when closely related species hybridize or have diverged recently.

The genetic differentiation among populations within *Q. magnoliifolia* and *Q. resinosa* was >10 times greater than differentiation between species for all loci. The low genetic differentiation between oak species can be explained by high rates of gene flow between species or shared ancestral polymorphism (Muir and Schötterer, 2005; Lexer *et al*., 2006). We tested the hypothesis of isolation by distance and we found that the differentiation between neighbouring species pairs in the same geographical region is lower than among distantly conspecific populations in different geographical regions. This evidence showed that the local lower SSR differentiation between *Q. magnoliifolia* and *Q. resinosa* is due to interspecific gene flow and the high intraspecific differentiation is due to isolation by distance. *Q. magnoliifolia* and *Q. resinosa* present the lowest values of genetic differentiation reported for previous studies of hybridization and introgression in oaks to date using SSR loci (Bruschi *et al*., 2000; Gugerli *et al*., 2008; Salvini *et al*., 2009; Neophytou *et al*., 2010; Peñaloza-Ramírez *et al*., 2010; Zeng *et al*., 2010; Curtu *et al*., 2011) with the exception of *Q. macrocarpa* and *Q. bicolor* (Craft and Ashley, 2006) and *Q. rubra*, *Q. shumardii* and *Q. palustris* (Aldrich *et al*., 2003). However, in oaks the hypothesis of weak interspecific barriers is the most parsimonious to explain the limited interspecific divergence observed at most loci between species (Lexer *et al*., 2006).

The genetic structure of *Q. magnoliifolia* and *Q. resinosa* resulted in two genetic groups ($K = 2$) that did not reflect the morphological species identification because each

452 species includes both genotypes (red and green) roughly in equal proportion, indicating
453 that most alleles were shared between species (Figs. 8 and 9). However, this admixture
454 proportion is geographically structured (Fig. 10). This pattern of introgression between
455 *Q. magnoliifolia* and *Q. resinosa* geographically structured support the hypothesis of
456 inter-specific gene flow due to a pattern of isolation by distance. Further analysis to test
457 several SSR loci under selection is necessary to find the genomic differentiation
458 between these two species due to coding regions expressed higher differentiation than
459 noncoding regions (Scotti-Saintagne *et al*., 2004), and also loci experiencing high rates
460 of intraspecific gene flow should be used for species delimitation due to high rates of
461 gene flow within species is beneficial for the maintenance of species and their cohesive
462 evolution (Petit and Excoffier, 2009).
463 *Q. magnoliifolia* and *Q. resinosa* have divergent niches and sympatric zones
464 occur between the environmental characteristics of the two species (Figs. 5 and 6).
465 These findings suggest that niche divergence can evolve comparatively quickly unlike
466 the genetic divergence. The sympatric zone from south of the Sierra Madre Occidental
467 and western part of Trans-Mexican Volcanic Belt has different environmental
468 characteristics from the sympatric zone of central part of Trans-Mexican Volcanic Belt
469 (Figs. 4 and 5). This pattern of geographical and environmental differences of the two
470 sympatric zones could be explained and supporting our geographical structure of
471 admixture between the two species. *Q. magnoliifolia* niche overlap partially inside the
472 *Q. resinosa* niche favor ecological sympatry and interspecific gene flow. GARP model
473 of *Q. magnoliifolia* predicted the niche of *Q. resinosa*, but *Q. resinosa* not predict the
474 niche of *Q. magnoliifolia* (Fig. S1). This data suggest that *Q. magnoliifolia* and *Q.*
475 *resinosa* are two different species closed related based on concept of niche conservatism
476 (Peterson *et al*., 1999; Wiens and Graham, 2005). The ecological overlapping between

477 species can facilitate dispersal and gene flow between divergence lineages (Arteaga *et*
478 *al*., 2011). The environmental features that characterize the ecological niche of each
479 species showed that *Q. magnoliifolia* and *Q. resinosa* had differences in temperature
480 (Fig. 6). The ecological niche of *Q. magnoliifolia* is congruence with Rzedowski (1978)
481 which describe to *Q. magnoliifolia* as ecological transition species between tropical dry
482 forests to pine-oak forest, being not tolerant to low temperatures and has not marked
483 seasonality. The ecological niche of *Q. resinosa* is congruence with McVaugh (1974)
484 which describe it habitat as xeric shrublands and in pine-oak forest almost always
485 forming a monospecific forest or sometimes with other oak species, so these ecological
486 characteristics could explain their tolerance to lower temperatures and strong
487 seasonality.
488 We conclude that *Q. magnoliifolia* and *Q. resinosa* are two different
489 morphological and ecological species despite the high levels of gene flow due to
490 divergent selection. The pattern of isolation by distance between the two species and
491 within *Q. magnoliifolia* can be explained the local lower SSR differentiation between *Q.*
492 *magnoliifolia* and *Q. resinosa* at different geographical scales and the high intraspecific
493 differentiation. The geographical and environmental differences of the two sympatric
494 zones could be explained and supporting our geographical structure of admixture
495 between the two species and the asymmetrical niche of *Q. magnoliifolia* favor the
496 ecological overlapping with *Q. resinosa* and the interspecific gene flow and
497 introgression. Much more genomic resolution will be needed to identify those genes
498 associated with interspecific differentiation in these Mexican oaks, due to their
499 extremely porous genomes.

500

501

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Table 1. Sampled populations number and name, number of individuals (*N*) assignment to *Q. magnoliifolia* (Qm) and *Q. resinosa (Qr)* obtained from leaf shape analyses, biogeographic region, altitude, latitude and longitude by population. SMOc = Sierra Madre Occidental, TMVB = Trans-Mexican Volcanic Belt, SMS = Sierra Madre del Sur, CP = Central Plateau.

	N					
No./Population	*Qm*	*Qr*	Biogeographic region	Altitude	Latitude	Longitude
1. Canelas	10		SMOc	2561	25.117	-106.5
2. Santa Lucía	10		SMOc	1547	23.45	-105.85
3. Cacalutan	5		TMVB	1048	21.083	-104.23
4. Compostela	10		TMVB	1174	21.217	-104.8
5. Ocotillo	6		TMVB	1228	21.267	-104.65
6. Puerto Vallarta	10		TMVB	473	20.433	-105.28
7. Guadalupe	5		TMVB	1810	19.867	-103.459
8. El Llano	8		TMVB	406	19.75	-104.77
9. Casimiro Castillo	4	**1**	TMVB	1366	19.7	-104.38
10. Cuzalapa	6		TMVB	584	19.45	-104.52
11. Manantlán	7		TMVB	1687	19.6	-104.22
12. Nogal	5		TMVB	523	19.317	-104.13

14. Puerto del Gato	5		TMVB	1905	19.483	-100.37
15. Morelia	5		TMVB	2120	19.659	-101.168
16. Guayabos	6		TMVB	1430	19.233	-101.33
17. Benito Juárez	5		TMVB	1902	19.35	-100.4
18. Valle de Bravo	8		TMVB	1878	19.25	-100.13
19. Temascaltepec	5		TMVB	1724	19.05	-100.07
20. Ixcateopan	6		TMVB	1974	18.55	-99.7
13. Coalcomán	6		SMS	1643	18.733	-103.27
21. Filo de Caballo	3	**3**	SMS	1954	17.783	-99.7
22. Platanillos	6		SMS	1354	17.467	-100.58
23. Chila de las Flores	4	**1**	SMS	1975	17.95	-97.88
24. Magueyal	11		SMS	1975	17.183	-97.78
25. Sta. Inés del Monte	6		SMS	2072	16.95	-96.85
26. San Bernardo	5		SMS	1721	16.85	-96.92
27. Ojo de Agua	5		SMS	1167	16.417	-97.08
28. Mitla	7		SMS	1910	16.933	-96.3
29. Papalutla	6		SMS	1761	17.733	-97.9
30. Juxtlahuaca	10		SMS	1987	17.483	-98.02
31. Pinos	6		SMS	2007	17.233	-97.72

32. Cerezal	5		SMS	2118	17.267	-96.53
33. San Miguel del Río	5		SMS	1822	17.317	-96.55
34. Sola de Vega	5		SMS	1816	16.583	-96.93
35. Matatlán	5		SMS	1946	16.833	-96.35
36. Cerro Metate	3	**2**	SMS	2131	16.25	-96.53
37. Coatlán	3	**3**	SMS	2104	16.283	-96.68
38. La Congoja		5	CP	2296	22.183	-102.52
39. Calvillo		10	CP	2087	21.917	-102.58
40. Arroyo Seco		6	CP	1988	21.717	-102.63
41. Cerro de los Gallos		5	CP	2139	21.667	-102.22
42. Estancia del Cubo		6	CP	2049	21.383	-101.12
43. Dolores		6	CP	2144	21.167	-101.12
44. Minas		10	CP	1469	21.683	-100.05
57. Santa Catarina		6	CP	2137	20.9	-101.05
58. Guanajuato		7	CP	1983	20.883	-101.15
59. Agua Zarca		5	CP	2174	20.767	-101.02
45. Mezquital		10	SMOc	2067	23.45	-104.35
46. Valaparaíso		6	SMOc	2082	22.667	-103.77
47. Mexquitic		8	SMOc	1881	22.517	-103.77

48. Sierra de Bolaños		8	SMOc	2513	21.883	-103.87
49. San Lorenzo		6	SMOc	1938	21.967	-103.2
50. El Plateado		10	SMOc	1937	21.917	-103.03
51. Puertecito		6	SMOc	2528	21.683	-103.17
52. Teul de González		6	SMOc	1946	21.4	-103.52
53. Talpa		7	TMVB	1316	20.4	-104.88
54. Cuquio		6	TMVB	2053	21.017	-103
55. Avigel		5	TMVB	2099	20.867	-102.8
56. Tepatitlán		5	TMVB	1932	20.867	-102.77
60. Tumbiscatío		6	TMVB	2072	19.592	-101.11
Total	227	165				

In bold the 10 individuals removed of genetic analyses

Table 2. Genetic diversity estimates overall populations of *Q. magnoliifolia* and *Q. resinosa* and intraspecific and interspecific genetic differentiation (F_{ST} values) at each analyzed locus

	N		A_R		P_A		H_E		H_O		f_{is}		F_{ST} Intraspecific		F_{ST} Interspecific
Locus / Species	*Qm*	*Qr*	*Qm*	*Qr*	*Qm*	*Qr*	*Qm*	*Qr*	*Qm*	*Qr*	*Qm*	*Qr*	*Qm*	*Qr*	*Qm vs. Qr*
QrZAG39	227	155	26	23	10	6	0.93	0.93	0.88	0.95	0.05	-0.02	0.051***	0.042**	0.004**
QpZAG110	227	155	21	17	10	6	0.82	0.87	0.65	0.68	0.2	0.22	0.127***	0.144***	0.015**
quru-GA-0C19	227	155	11	13	3	5	0.69	0.85	0.58	0.78	0.16	0.09	0.135***	0.126***	0.041***
QpZAG36	227	155	21	18	10	7	0.87	0.90	0.74	0.71	0.16	0.21	0.163***	0.126***	0.029***
quru-GA-0C11	227	155	21	14	11	5	0.90	0.88	0.87	0.93	0.03	-0.05	0.104***	0.094***	0.011***
quru-GA-0I01	227	155	26	18	13	5	0.93	0.91	0.83	0.83	0.12	0.09	0.116***	0.153***	0.009**
quru-GA-0M07	227	155	25	18	10	3	0.95	0.92	0.83	0.76	0.13	0.18	0.087***	0.121***	0.007**
quru-GA-1C08	227	155	24	25	9	10	0.92	0.92	0.72	0.71	0.22	0.23	0.159***	0.121***	0.006*
Overall	227	155	175	146	75	46	0.88	0.90	0.76	0.79	0.13	0.12	0.116***	0.130***	0.011**

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

Table 3. F_{ST} values between neighbouring populations of species pairs from different geographic location. *Qm*_SMOc + wTMVB = *Q. magnoliifolia* located at Sierra Madre Occidental and western of Trans-Mexican Volcanic Belt, *Qr*_SMOc = *Q. resinosa* located at Sierra Madre Occidental, *Qm*_cTMVB and *Qr*_cTMVB = *Q. magnoliifolia* and *Q. resinosa* located at center of Trans-Mexican Volcanic Belt, *Qm*_nSMS = *Q. magnoliifolia* located at north of Sierra Madre del Sur and *Qr*_sCP = *Q. resinosa* located at south of Central Plateau

	*Qm*_SMOc + wTMVB	*Qr*_SMOc	*Qm*_cTMVB	*Qr*_cTMVB	*Qm*_nSMS
*Qr*_SMOc	0.012*	-			
*Qm*_cTMVB	0.044***	0.037***	-		
*Qr*_cTMVB	0.099***	0.071***	0.035*	-	
*Qm*_nSMS	0.088***	0.075***	0.013ns	0.033*	-
*Qr*_sCP	0.138***	0.105***	0.045**	0.036*	0.033*

*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; n.s. = non-significant $P > 0.05$.

Figures

Fig. 1 Photograph of *Q. magnoliifolia* leaves (left) collected in Canelas population and *Q. resinosa* leaves (right) collected in Mezquital population both at Durango state in the Sierra Madre Occidental.

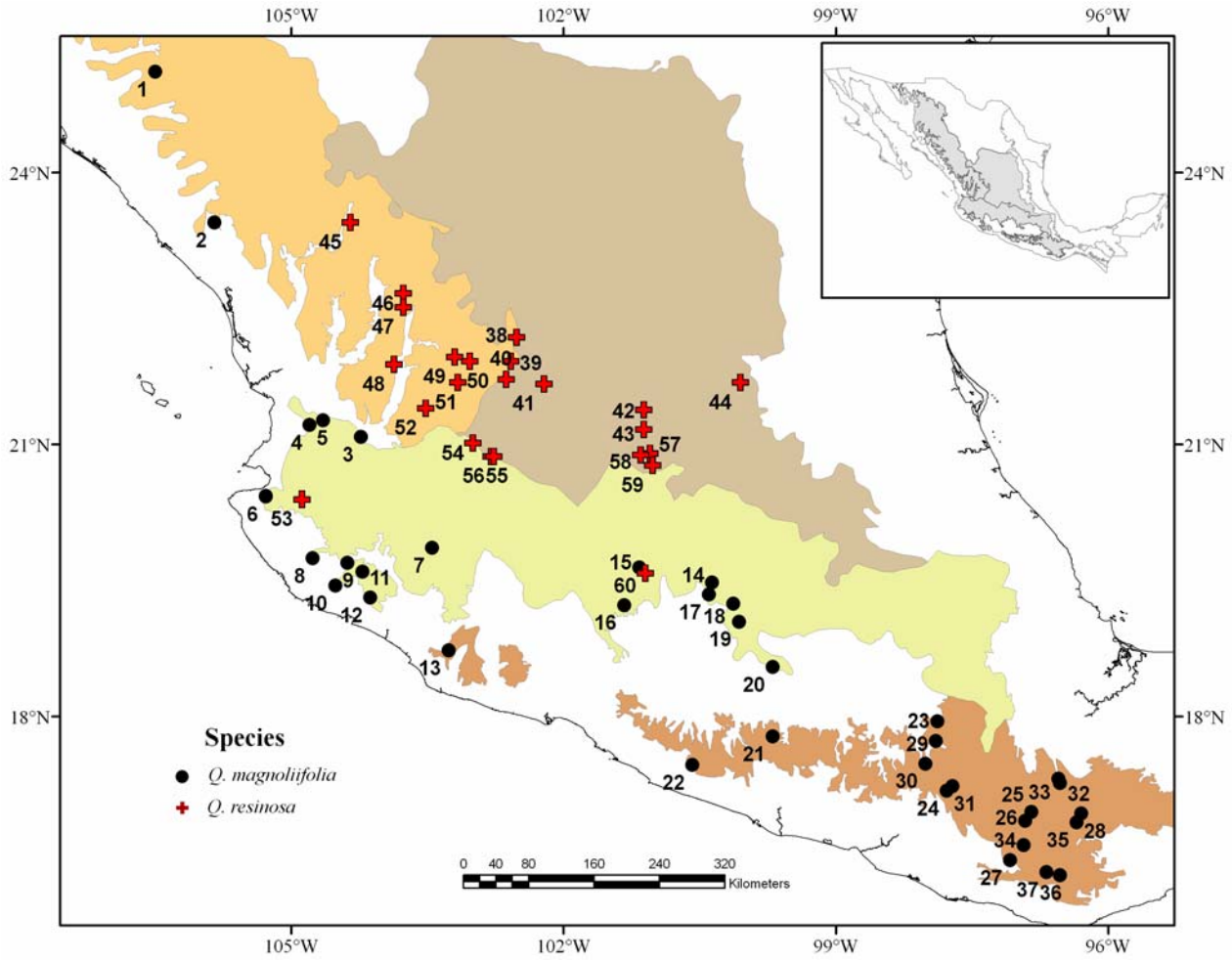

Fig. 2 Geographical distribution of *Q. magnoliifolia* and *Q. resinosa* in Mexico and populations sampled. The biogeographic regions are represented by different colors: Sierra Madre Occidental in orange, Trans-Mexican Volcanic Belt in lime green, Sierra Madre del Sur in brown, Central Plateau in sand color.

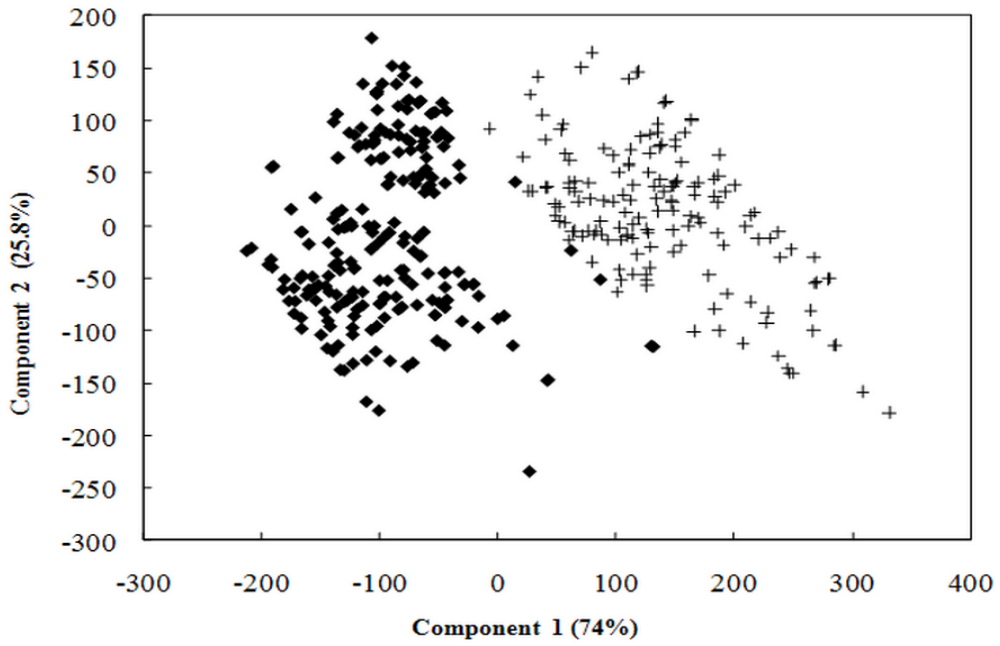

Fig. 3 Principal component analysis of the leaf shape analysis. Crosses represent individuals identified as *Q. magnoliifolia* (PCA 1 = -15 to -212; mean = -100) and diamonds individuals identified as *Q. resinosa* (PCA 1 = 6 to 331; mean = 135).

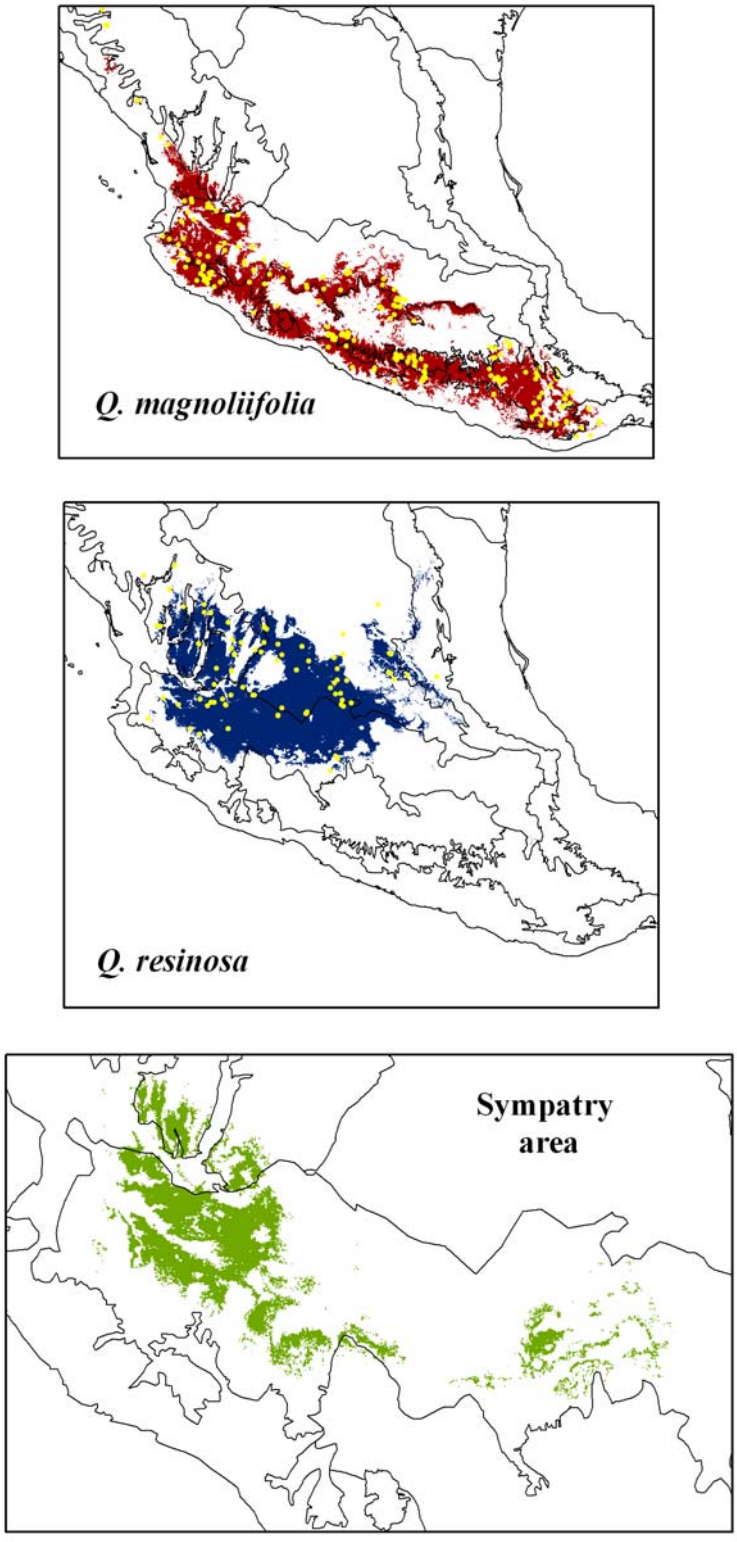

Fig. 4 Final consensus model of *Q. magnoliifolia*, *Q. resinosa* and sympatric area between the two species in Mexico, obtained with GARP and MAXENT

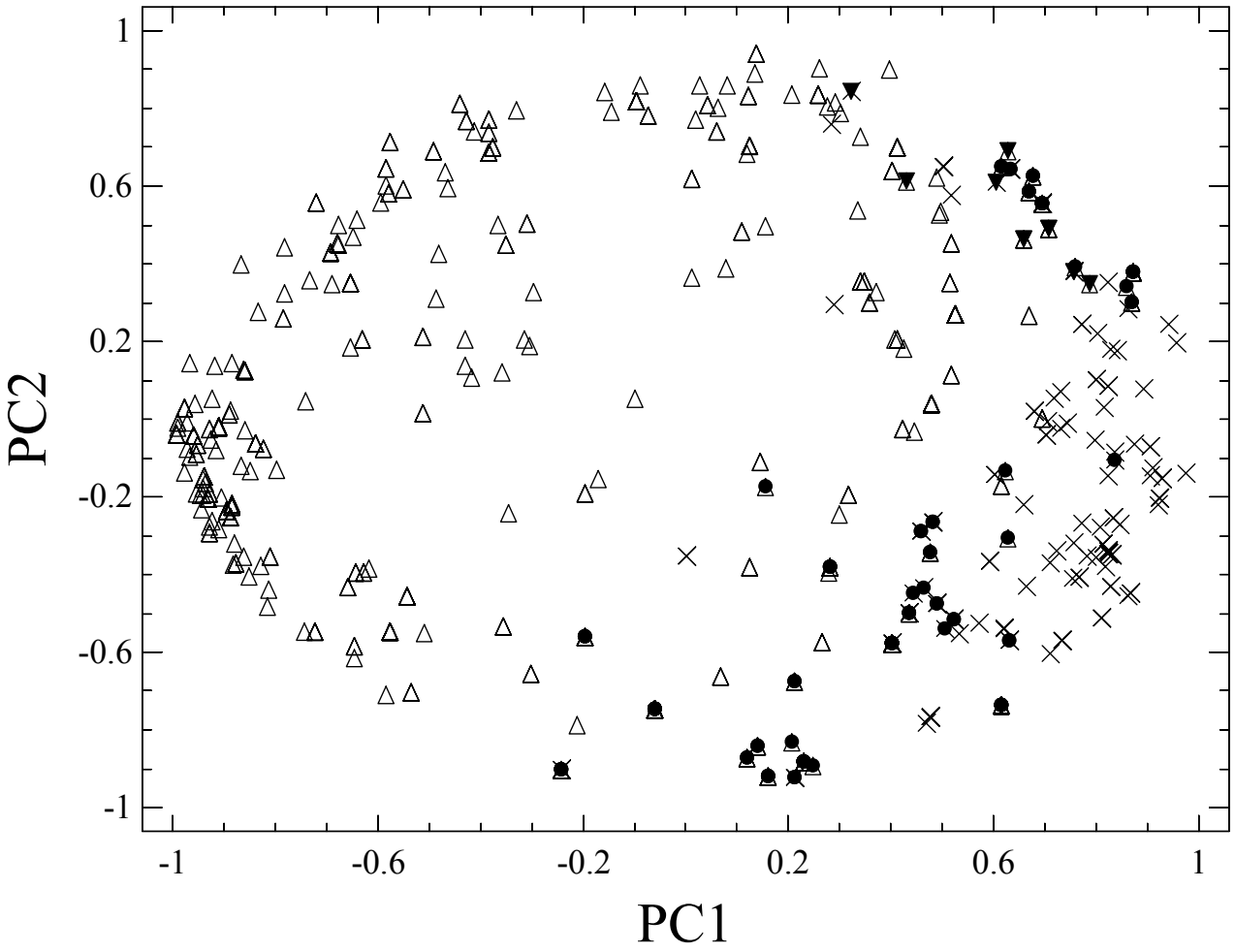

Fig. 5 Principal component analysis of the environmental features used to reconstruct the ecological niche models. PC1 explains 46.1% and PC2 explains 19.1% of total variance. *Q. magnoliifolia* in triangles, *Q. resinosa* in crosses and sympatric zone of central part of Trans-Mexican Volcanic Belt in filled inverted black triangles and sympatric zone of south of Sierra Madre Occidental and western of Trans-Mexican Volcanic Belt in filled black circles

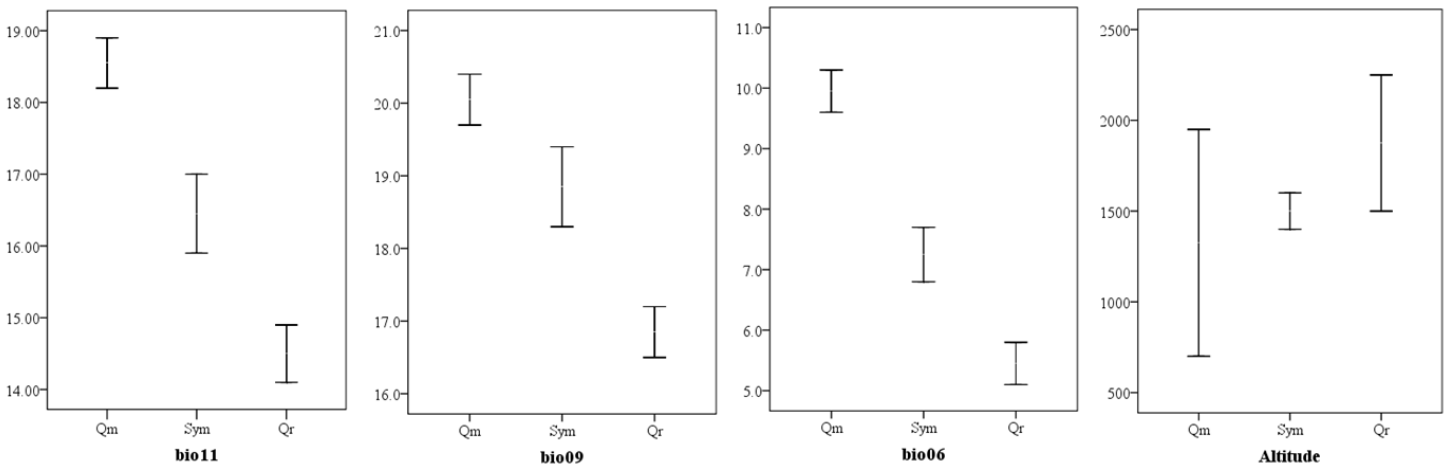

Fig. 6 Environmental features of *Q. magnoliifolia* (Qm) and *Q. resinosa* (Qr) and sympatric zone (symp) correlated with PC1 with a 95% confidence interval. Bio 11 = temperature in the coldest quarters, bio 9 = temperature in the driest quarters and bio 6 = minimum temperature of coldest month and altitude.

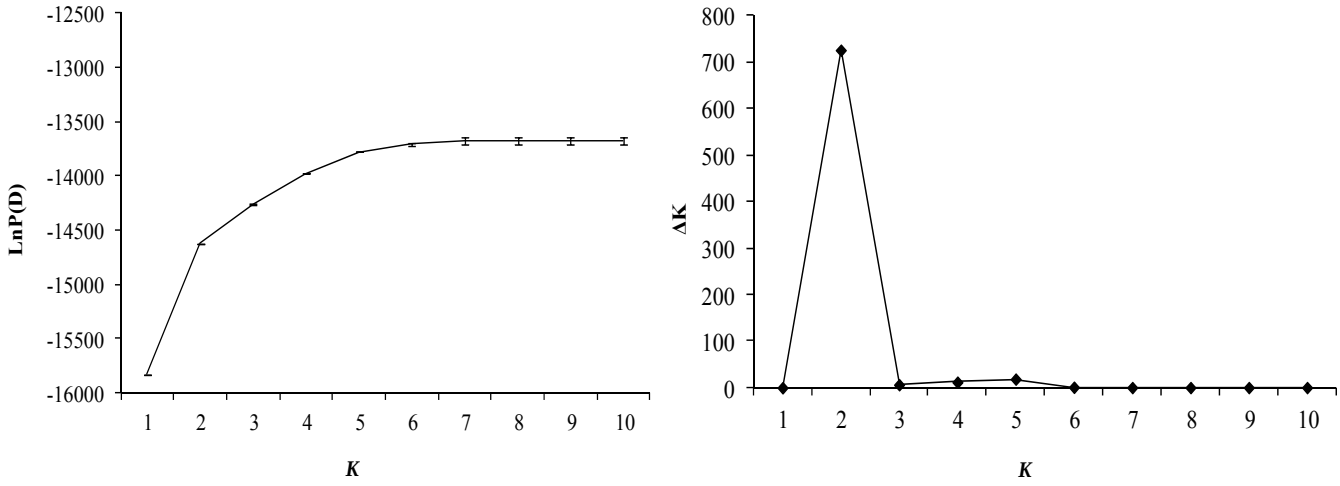

Fig. 7 Estimated genetic groups (*K*) from the Structure clustering analysis using eight loci. Mean and standard deviation of log probability of data over 10 independent runs for each *K* (above) and plot of statistics Δ*K* respect to genetic clusters *K* (from 1 to 10) (below)

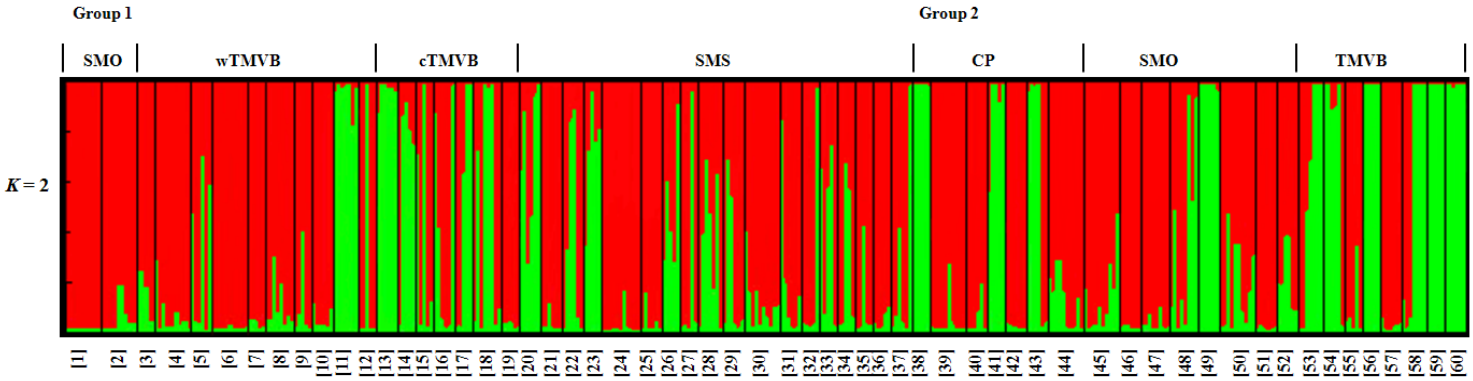

Fig. 8 Genetic structure graph for $K = 2$ using eight loci. Group 1 and group 2 represent *Q. magnoliifolia* and *Q. resinosa* defined by leaf shape analysis. Biogeographic region on top: SMO = Sierra Madre Occidental, wTMVB and cTMVB = wester and center of Trans-Mexican Volcanic Belt, SMS = Sierra Madre del Sur, CP = Central Plateau and the number of populations ordered as in Table1 on bottom.

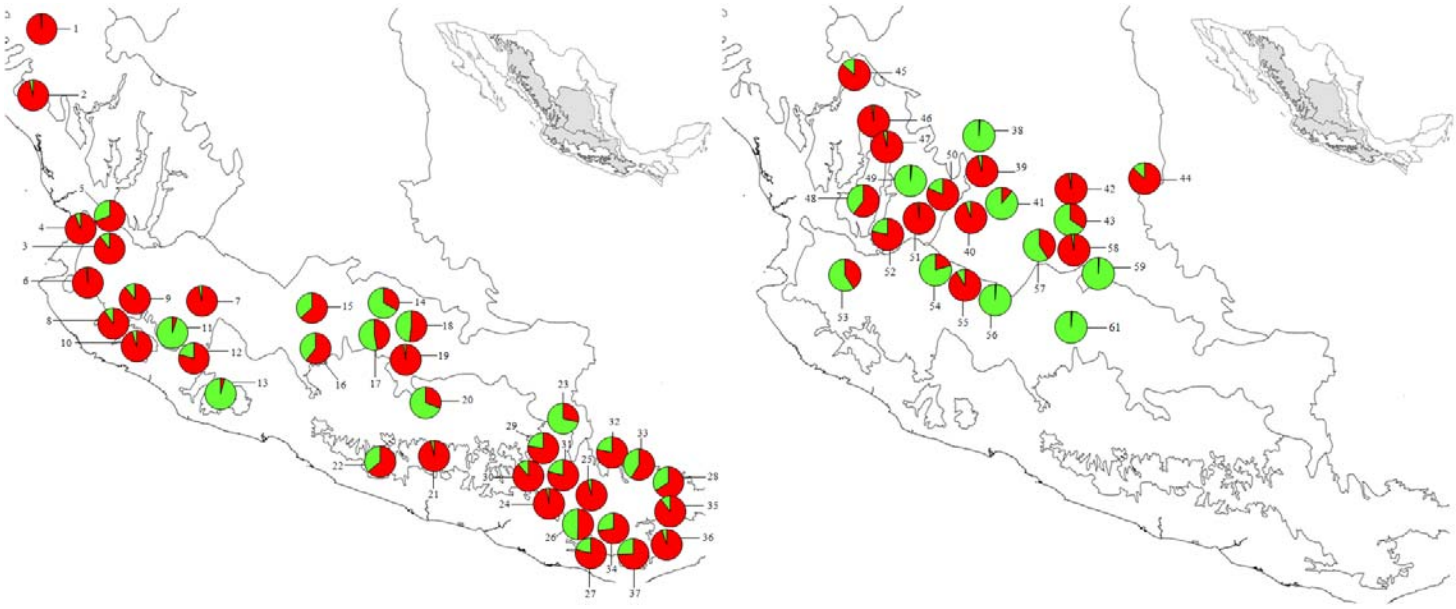

Fig. 9 Geographical distribution map of genetic admixture proportion by population of *Quercus magnoliifolia* and *Q. resinosa* obtained with Structure for $K = 2$.

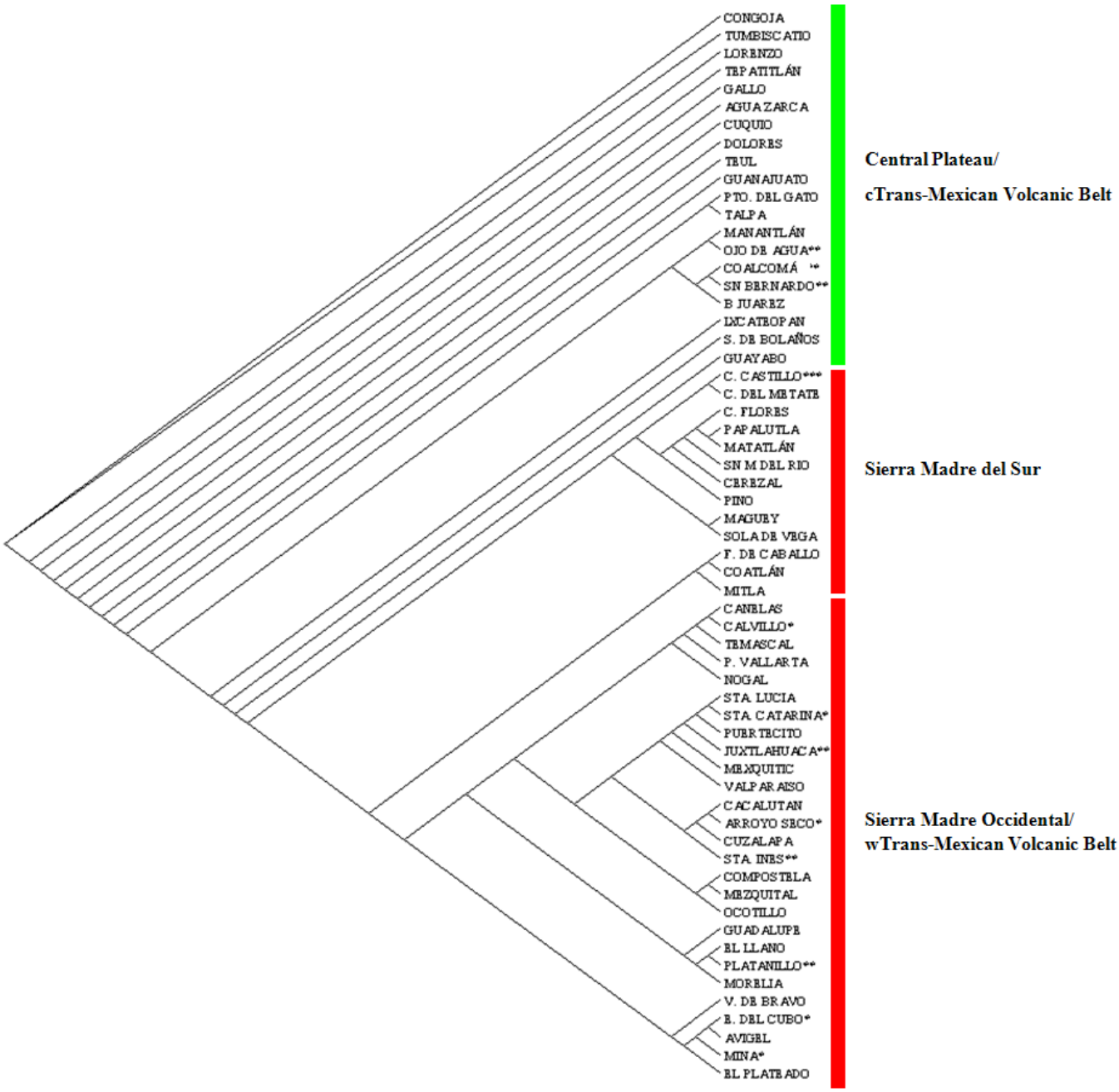

Fig. 10 Neighbour-joining tree obtained with pairwise genetic distances between populations (F_{ST}). The admixture proportion of populations cluster together were represented by red and green genotypes obtained from results of $K = 2$ from Bayesian analysis.

VII.

Ana Luisa Albarrán-Lara, Andrés Torres-Miranda, and Ken Oyama

Phylogeographic structure, demography and paleodistributional modeling indicates recurrent interspecific cytoplasmic exchange and population expansion during Quaternary between two hybridizing white oaks *Quercus magnoliifolia* and *Quercus resinosa* (Fagaceae)

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Phylogeographic structure, demography and paleodistributional modeling indicates recurrent interspecific cytoplasmic exchange and population expansion during Quaternary between two hybridizing white oaks *Quercus magnoliifolia* and *Quercus resinosa* (Fagaceae)

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Short running title: Phylogeographical structure between two hybridizing oaks.

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Abstract

We investigated the phylogeography, demography and ecological niche modeling of *Q. magnoliifolia* and *Q. resinosa* two hybridizing Mexican white oak species throughout their entire geographic distribution to know the evolutionary history. Six chloroplast microsatellites markers were amplified on a total of 397 trees. We found high number of chloroplast haplotypes in *Q. magnoliifolia* and *Q. resinosa* (56 and 34 haplotypes, respectively) with 13 shares haplotypes among species; which showed a strong phylogeographic structure. High levels of both overall diversity and within population diversity (h_T = 0.98-0.97 and h_S = 0.67-0.62), but low levels of population differentiation were found in *Q. magnoliifolia* and *Q. resinosa* (G_{ST} = 0.32 and 0.36), respectively. The genealogical distribution of the haplotypes in the network of both species showed ancient and derived shared haplotypes, which suggest recurrent inter-specific cytoplasmic introgression. All distribution models showed contraction and altitudinal ranges shift during LGM and LIG for the two species, with altitudinal downward movement at temperate sub-humid or semi-warm conditions during LGM and altitudinal upward movement during the warmer LIG. These altitudinal movements favored the formation of sympatric areas. Sympatric zones between the two species were strongly related to richness of shared haplotype. The Trans-Mexican Volcanic Belt is the largest sympatric area between *Q. magnoliifolia* and *Q. resinosa* and the biogeographic province with the highest richness of shared haplotypes currently and during the LGM. *Q. magnoliifolia* and *Q. resinosa* have different age of population expansion. The integration of phylogeographic, demographic, distribution models and palynological records allowed understand how climatic changes in the past affected the distribution range of the species.

Key words

Chloroplast microsatellites, cytoplasmic introgression, sympatric areas, population expansion, ecological niche modeling, *Quercus*.

Introduction

Phylogeography provides valuable information about the evolutionary history of species and the process that has structured the current geographical distribution of genealogical lineages (Avise 2000). Chloroplast DNA has been widely used to describe ancient and contemporary interspecific cytoplasmic exchange (Rieseberg & Soltis 1991; Whittmore & Schaal 1991; Petit *et al.* 1993; Dumolin-Lapegue *et al.* 1997; Lumaret & Jabbour-Zahab 2009). This information has been also useful for the reconstruction of historical demographic events such as population expansion and contraction, migration, colonization and recolonization in plant species (Petit *et al.* 1997; 2002; 2003; Taberlet *et al*. 1998; Grivet *et al*. 2006). Particularly in oaks, chloroplast markers are maternally inherent (Dumolin *et al.* 1995), allowing reconstruction of recent or past seed migration of introgressed genotypes, such as the interspecific chloroplast capture, where the species can retain part of the cytoplasmic genome of another species over very long periods (Petit *et al*. 1993; 1997; Olalde *et al*. 2002; González-Rodríguez *et al*. 2004; Tovar-Sánchez *et al*. 2008; Lumaret & Jabbour-Zahab 2009).

The Quaternary climatic oscillations over the past 1.6 million years have played a major role in changing both the geographical distribution and the patterns of genetic diversity within and among natural populations of plant and animal species (Hewitt 2004). The European white oaks during the major Pleistocene glaciations were

87 restricted to refuges in Spain, Italy and the Balkans (Huntley & Birks 1983; Bennett *et*
88 *al*. 1991). cpDNA surveys in European white oak species showed a complete absence of
89 specificity of the cpDNA haplotypes, a strong phylogeographic structure and low levels
90 of intrapopulation diversity (Petit *et al*. 1993; 2002; Dumolin-Lapegue *et al*. 1997;
91 Olalde *et al*. 2002). These interspecific cytoplasmatic exchanges geographically
92 structured have arisen after prolonged contact in the glacial refugia, followed by
93 postglacial recolonization routes through long-distance seed dispersal from the various
94 glacial refugias (Petit *et al*. 1993; 1997; 2002; Dumolin-Lapegue *et al*. 1997). Similar
95 pattern was found in North American white oak species where the cpDNA did not
96 reflect the species boundaries, but it is concordant with the geographic location of
97 sympatric populations (Whittemore & Schaal 1991). In Europe and North America the
98 Quaternary was characterized by many cycles of contraction and expansion of
99 geographical ranges according to climatic fluctuations: contraction of ranges to southern
100 regions during cold periods, and expansion from the leading edge during subsequent
101 warmings (Hewitt 2004). For European species, during the colonization process
102 successive bottlenecks events occurred that lead to a loss of genetic diversity in the
103 northern populations, with the exception of cold-tolerant taxa (Taberlet *et al.* 1998), but
104 at intermediates latitudes the most genetically diverse populations were found as a
105 consequence of the admixture of divergent lineages that colonized the continent from
106 separate refugia (Petit et al. 2003). However, the effects of Ice Ages on species ranges
107 varied with latitude and topography (Hewitt 2004). At lower latitudes recent climatic
108 fluctuations were less extreme than in temperate zones and forest plant species
109 experienced contraction and expansion in population size and range shifts but never
110 vanished entirely (Flenley 1998). Based on palaeoenvironmental reconstructions, the
111 climatic changes during the late Pleistocene were not as drastic in Mexico as to reduce

112 oak species to small populations isolated into a few refugia (Metcalfe *et al*. 2000).
113 Palynological records from several locations in Mexico extending back to 44 000 years
114 BP indicated than *Quercus* pollen was almost constantly present (Lozano-García &
115 Xelhuantzi-López 1997; Metcalfe *et al*. 2000). cpDNA surveys in Mexican red oak
116 species showed different patterns of interspecific cytoplasmic exchanges, low
117 phylogeographic structure and high levels of intrapopulation diversity (González-
118 Rodríguez *et al*. 2004; Tovar-Sánchez *et al*. 2008). Mexican oak species presumably
119 experienced geographical displacements according to their particular ecological
120 requirements as a result of the climatic changes, but forests were present and
121 widespread for a long time (González-Rodríguez *et al*. 2004).
122 The latitudinal and altitudinal range shifts in population species during the
123 Quaternary involved considerable demographic changes (Hewitt 2004). Population
124 expansion leaves recognizable signatures in the distribution of the number of pairwise
125 differences between cytoplasmic DNA haplotypes ("mismatch distribution") follows a
126 unimodal distribution (Rogers & Harpending 1992), that contrast to the ragged patterns
127 that would be found for a constant population size for a long time with populations
128 generating many peaks (Harpending 1994). Information on these temporal demographic
129 changes improves the inferences about the factors that promoted the genetic structuring
130 of populations (Carstens & Richards 2007). Information of the fossil record can aid in
131 reconstructing the historical distributions of some taxa. However, direct evidence of
132 past distribution and latitudinal range shifts for the majority of taxa is difficult to obtain
133 because of the lack of fossil data (Carstens & Richards 2007). Particularly, the
134 palynological records for Mexican oak species are scarce and the identification of pollen
135 grains at species level is difficult (Lozano-García & Xelhuantzi-López 1997; Metcalfe
136 *et al*. 2000). For a species without detailed fossil or palynological records,

paleodistributional modeling can provide valuable spatial-geographic data on historical distributions of species (Carstens & Richards 2007). Paleodistribution modeling use the current ecological niche of the organism that have been projected onto models of past climate using data from sites in which species are known to exist to determine the set of climatic parameters which best predict the presence of the species (Carstens & Richards 2007). The integration of phylogeographic structure data and ecological niche models can improve historical inferences about the relative importance of climatic events into the formation of current population genetic structure of a species (Carstens & Richards 2007; Jakob *et al*. 2009).

Quercus magnoliifolia Née and *Q. resinosa* Liebm. are two Mexican white oak species belonging to the series *Circinatae* and Macrophyllae, respectively (Trelease 1924). Leaf shape geometric morphometrics, ecological niche modeling and eight highly informative nuclear microsatellites (SSR) loci analyses throughout entire geographic distribution of the two species showed that *Q. magnoliifolia* and *Q. resinosa* are two different species that share alleles due to extensive inter-specific gene flow, that despite of extensive gene flow and introgression among sympatric and allopatric populations throughout their distribution range, each species maintains its morphology and ecological niche trait, possibly due to divergent selection (Albarrán-Lara *et al*. under review). Sympatric populations of these two species are distributed at south of Sierra Madre Occidental and western and center part of Trans-Mexican Volcanic Belt, whereas allopatric populations of *Q. magnoliifolia* are at northwest of Sierra Madre Occidental and Sierra Madre del Sur through an altitudinal distribution range from 400 to 2850 m and almost always in transition of tropical dry forests and pine-oak forests, and allopatric populations of *Q. resinosa* are at southeast of Sierra Madre Occidental and Central Plateau through an altitudinal distribution range from 1300 to 2800 m, and

occurs in semi-arid grasslands forming pure stands or sometimes with others oak or pine species (Fig. 1). For these two species no prior information exists about how climatic changes have affected their past distribution and the process that has structured the current geographical distribution of genealogical lineages. Thus, we studied the chloroplast DNA variation of *Q. magnoliifolia* and *Q. resinosa* throughout their entire geographic distribution in Mexico to determine the phylogeographic structure, infer historical demographic events such as population expansion and contraction, migration and colonization, and to reconstruct the present and past distribution of each species during the Last Glacial Maximum (LGM; ~20,000 years BP) and Last Interglatial Maximum (LIG; ~140,000 years BP; Otto-Bliesner et al. 2006) by modeling using MAXENT and GARP in order to understand the process that favor the cytoplasm exchange between these two hybridizing taxa. The goals of this study were to: i) determine the chloroplast genetic diversity and geographical structure of haplotypes of *Q. magnoliifolia* and *Q. resinosa* populations; ii) determine the genealogical relationship among haplotypes; iii) determine the historical demographic events of the populations of the two species; iv) reconstruct the present and past distribution of the species using MAXENT and GARP to elucidate historical species range shifts during the last climatic changes that could favor the interspecific cytoplasm exchange.

Material and Methods

Sampling populations

A total of 397 white oak trees were collected from 61 populations of *Q. magnoliifolia* and *Q. resinosa* covering the whole distribution range (Table 1; Fig. 1). Of these, 392 individuals from 60 populations had been included previously in an analysis of

introgressive hybridization between these two species using leaf shape morphological traits, ecological differentiation and nuclear microsatellites (Albarrán-Lara *et al*. under review). For each population 10 white oak trees separated by at least 20 m were randomly selected. From each individual, fresh leaves were stored at -80°C in the laboratory for the genetic analysis. Herbarium specimens from each tree was identified and deposited at Herbarium of Facultad de Ciencias, UNAM. Based on previously leaf shape morphometric analysis each individual was classified as *Q. magnoliifolia* and *Q. resinosa* (Table 1). To determine the biogeographic province to belong each population we follow Morrone (2005) with modifications on base of Ferrusquía-Villafranca (1993) (Table 1).

DNA extraction and chloroplast microsatellites genotyping

DNA isolation was performed using a cetyltrimethyl ammonium bromide (CTAB) protocol with an additional phenol-chloroform cleaning step (Lefort & Douglas 1999). All isolated DNAs were diluted with deionized water to a final concentration of 20 ng/µL and storage at -20°C. Six chloroplast microsatellites were amplified in multiplex PCR reactions: Ccmp10 (Weising & Gardner 1999), µdt1, µd3, µdt4, µcd4, y µcd5 (Deguilloux *et al*. 2003). PCR was performed using the QIAGEN Multiplex PCR kit (QIAGEN) in 5 µL reactions as follows: 1X Multiplex PCR Master Mix, 2 µM each primer, deionized water, and 20 ng DNA (Albarrán-Lara *et al*. 2010). The thermal cycling program was run on an Applied Biosystems thermocycler. The program consisted of one cycle at 95°C for 15 min and then 35 cycles, each of 95°C for 30 sec, annealing at 50°C for 1:30 min and extension at 72°C for 1 min. A final extension at 60°C for 30 min was included. Multiplex PCR products were diluted 1:1 in deionized water and run in an ABI-PRISM 3100-Avant sequencer with the GeneScan-500 LIZ size standard included (Applied Biosystems). Fragment sizing analysis was performed

using Peak Scanner software v1.0 (Applied Biosystems). We verified and corrected the individual genotype assignment through the six chloroplast microsatellite loci at least four times.

Genetic diversity and structure

Each allelic size combination across the six chloroplast microsatellites loci comprised a haplotype. The number of haplotypes (N_h) were calculated using ARLEQUIN version 3.0 (Excoffier *et al*. 2005), the number of private haplotypes (i.e. haplotypes present in only one population) (N_{hp}), the frequency of the most common haplotype between the two species ($f\alpha$) were calculated for each population. The effective number of haplotypes (n_e) by population was calculated as $n_e = 1/ (\Sigma pi^2)$, where p_i is the frequency of the *i*-th haplotype using LMSE program (Navascués *et al*. 2009). The gene diversity within populations (h_S) and allelic richness (A) were obtained by rarefaction due to differences in sample size by populations using RAREFAC program (http://www.pierroton.inra.fr/genetics/labo/software/). The average genetic distances among individuals D^2_{sh} (Goldstein *et al*. 1995) applied to plastid microsatellite loci was calculated by population using LMSE program (Navascués *et al*. 2009).

The average gene diversity within populations (h_S), the overall diversity (h_T), and the coefficients of differentiation among populations G_{ST}, which assume identical correlation for all alleles and R_{ST}, which takes into account the similarity between haplotypes (haplotypes with similar alleles size are more closed related) were estimated by species using the program CPSSR (http://www.pierroton.inra.fr/genetics/labo/Software/PermutCpSSR). Significance was obtained after 1 000 random permutations of haplotypes identities. If R_{ST} is significantly higher than G_{ST}, indicate that the species presents phylogeographical structure; it means

235 that similar haplotypes are geographically closer than less related ones (Pons & Petit
236 1996).

237 Hierarchical partition of total gene diversity was made at two levels: the first one
238 was between species, among populations within species and within populations and the
239 second one was among provinces of each species, among populations within provinces
240 and within province obtained using analyses of molecular variance (AMOVA) using the
241 program ARLEQUIN version 3.0 (Excoffier *et al*. 2005). Significance was tests with 10
242 000 permutations.

243 For each species genealogical relationship among cpDNA haplotypes were
244 reconstructed using median-joining networks with Network 4.5.1.6 (Bandelt *et al*.
245 1999). This method combines the minimum spanning trees with a maximum parsimony
246 search to add a few consensus sequences (i.e., median vectors) in the cases of extant
247 unsampled sequences or extinct ancestral haplotypes (Bandelt *et al*. 1999). Haplotypes
248 geographic location in the network were represented in four different color based on
249 their biogeographic province: white color for Sierra Madre Occidental, black color for
250 Trans-Mexican volcanic belt, grey color for Sierra Madre del Sur and dark grey color
251 for Central Plateau. The thirteen shared haplotypes were represented in thirteen different
252 colors.

253 To represent on a map the geographic distribution of haplotypes located at
254 different biogeographic province of each species populations we used different colors
255 jointly with different texture to differentiate between haplotypes. In the case of *Q.*
256 *magnoliifolia* haplotypes located at northwest of Sierra Madre Occidental, Trans-
257 Mexican Volcanic Belt and Sierra Madre del Sur were represented with fuchsia, purple
258 and orange colors, respectively, whereas, the haplotypes of *Q. resinosa* located at east
259 of Sierra Madre Occidental, Central Plateau and Trans-Mexican Volcanic Belt were

represented with lime green, blue and sky blue colors, respectively. Shared haplotypes between the two species were represented with solid color the same ones used in the networks.

The genetic discontinuities "barriers" between the 61 populations of both species were analyzed with the Monmonier's maximum difference algorithm implemented in the program BARRIERS version 2.2 (Manni *et al*. 2004). The genetic barriers are associate with the highest rate of change in a given genetic distance matrix (see Manni *et al*. 2004). An average square distance (Goldstein *et al*. 1995, Slatkin 1995) matrix was used. The statistical support of each predicted barriers was obtained by resampling individuals within populations in order to obtained 100 bootstrap replicated of each genetic distance matrix using MSA program version 4.05 (Dieringer & Schlötterer 2002).

Inferences of historical population demography

Population growth was tested using the neutrality test F_S (Fu 1997) and distribution of pairwise differences often called "mismatch distributions" (Rogers & Harpending 1992) were carried out at populations of each species using the program Arlequin version 3.0 (Excoffier *et al*. 2005). In order to use Arlequin version 3.0 for the analyses of demographic expansion, the chloroplast microsatellites (cpSSR) data were binary coded, representing for each locus the number of repeats of the largest variant with "1" and replacing the absent repeats of shorter variant with "0" follows Navascués *et al.* (2006). Significance of F_S values was tested using 10 000 bootstraps. The neutrality test F_S (Fu 1997) tends to be negative when there is an excess of recent mutations (an excess of rare alleles), indicating population growth (Fu 1997). To evaluate the fit of the observed distributions of mismatches to those distributions expected under the model of

284 population expansion we used the raggedness index of Harpending (1994), also
285 implemented in the program Arlequin 3.0 (Excoffier *et al*. 2005).
286 The distribution of pairwise differences (difference in number of repeats)
287 between individuals within populations was used to estimate the three demographic
288 parameters of Roger & Harpending (1992): $\tau = 2\mu t$, $\theta_0 = 2\mu N_0$ and $\theta_1 = 2\mu N_1$, where τ is
289 a mutational time scale, considered an estimate of the time of expansions, μ is the
290 mutation rate, t is the number of generations since expansion, θ is the effective
291 population size scaled by the mutation rate, N_0 and N_1 are the population sizes before
292 and after expansion. All these demographic parameters were obtained by populations of
293 each species using maximum pseudo-likelihood estimation procedure for population
294 growth which take into account a mutation model with homoplasy (i.e. a stepwise
295 mutation model) common for cpSSR implemented in the program LMSE (Navascués *et*
296 *al*. 2009). The time of the population expansion was obtained using the formula [$t = (\tau /$
297 $2l\mu)\ g$] (Navascués et al. 2006), where τ was the average values overall populations
298 species and morphotectonic provinces of each species; l was the number of cpSSR loci
299 and μ was the mutation rate per locus and g was the generation times. We using two
300 mutation rates (μ)1 x 10^{-5} and 1 x 10^{-4} per locus per generation and considering two
301 generation times (g) for trees between 25 and 100 years (Navascués et al. 2006; 2009).
302 The calculations of maximum age of population expansion was made assumed (low μ =
303 10^{-5} and high g = 100 years) and the minimum age was assumed (high $\mu = 10^{-4}$ and low
304 g = 25 years).

305

306 *Reconstruction of present and past distribution of species using ecological niche*
307 *modeling*

308 *Occurrence and environmental data*

We compiled herbaria data for *Q. magnoliifolia* and *Q. resinosa*. Occurrence information of species was used in the form of unique latitude–longitude combinations gathered from our own fieldwork and online available herbarium information, especially from Global Biodiversity Information Facility (Gbif Accesed). We first removed duplicate records for the same species collected at the same site. Thus we obtained 462 unique records for *Q. magnoliifolia* and 136 for *Q. resinosa*. These localities cover the entire distribution ranges of the taxa.

Environmental scenarios both currents and past were represented by a series of 19 variables summarizing aspects of climate (appendix 1). Present climates were drawn from the WorldClim database (Hijmans *et al.* 2005); we used two general circulation model outputs for the Last Glacial Maximum (LGM), about 21000 years BP: the Community Climate System Model (CCSM3; http://www.ccsm.ucar.edu/ Kiehl & Gent 2004) and the Model for Interdisciplinary Research on Climate (MIROC, version 3.2; www.ccsr.u-tokyo.ac.jp/;hasumi/MIROC/). The CCSM3 and MIROC layers were resampled from their original resolution using cubic convolution implemented in ArcGIS 9.2 (ESRI 2008). We used the two LGM scenarios (CCSM3 and MIROC) to reduce methodological biases of climate-models implementation and to detect consistencies in the resulted geographic patterns via a consensus approach (Jakob et al. 2009).

We also used the environmental data for Last Interglacial Maximum (LIG), about 140 000 years BP, which were derived from simulations under the CCSM3 general circulations model (Otto-Bliesner *et al*. 2006).

Ecological Predictive Models

To obtain more robust models of ecological niche of each species we used the Genetic Algorithm for Rule-set Production (GARP; Stockwell & Noble 1992; Stockwell & Peters 1999) implemented in DESKTOPGARP v. 1.1.6 (http://www.nhm.ku.edu/desktopgarp/), and the maximum entropy machine learning algorithm in MAXENT 3.3.1 (Phillips *et al*. 2004, 2006) via a consensus approach. A fundamental condition for projecting niche models to a different time periods is that the models predict the habitat of species in the present time (Nogués-Bravo *et al*. 2009). The procedure proposed by Albarrán-Lara et al. (in press) was followed to obtain the ecological niche models of *Q. magnoliifolia* and *Q. resinosa* and validate their accuracy.

Testing the role of sympatry on richness of shared haplotypes in the time

The shared haplotypes present in at least three populations were used to modeling their current and past distribution using GARP. Of the 13 shared haplotypes only nine [H9, H10, H11, H12, H20, H34, H35, H36 and H58] had more than three records. The nine models were added in only one final model to determine the spatial distribution of shared haplotypes richness. Sympatric areas were obtained intersect the final consensus model of each species. These procedures were used to locate the geographic regions with highest richness in shared haplotypes and to test if this richness is associated with the sympatric areas.

To quantify altitudinal changes in the distribution range of both species, as in the regions with highest richness in shared haplotypes and simpatric areas we intersected all distribution models (current, LGM and LIG) with raster climatic chart (García 1998) and a raster digital elevation model. The altitudinal ranges were defined with the 90% of data.

Results

Genetic diversity and structure

From the six chloroplast microsatellites loci we obtained 29 and 24 alleles of which μdt3 and μdt1 loci were highly polymorphic with seven and six alleles for *Q. magnoliifolia* and *Q. resinosa*, respectively. The combination of alleles from the six loci resulted in a total of 103 haplotypes of which 56 haplotypes were to *Q. magnoliifolia*, 34 haplotypes were to *Q. resinosa* and 13 haplotypes were shared between the two species (Fig. 2). Haplotype diversity by population is shown in Table 1. The highest number of haplotypes (N_h) by population was five. The highest number of private haplotypes (N_{hp}) by population was two. The frequency of the most common haplotype ($f\alpha$) (haplotype nine; H9) range from 0.12 to 0.10. The effective number of haplotypes (n_e) range from 2.96 to 0.40. The highest within population diversity (hs) value was 0.91. The highest allelic richness (A) value was 3.0. The lowest values of diversity were in four populations (16, 23, 34 and 38) of *Q. magnoliifolia* which had only one haplotype. The populations that showed the highest mean pairwise distance (mean D^2_{sh}) were Manantlán and Ixcateopan (0.78 and 0.73; populations 11 and 20, respectively) at Trans-Mexican Volcanic Belt, Cerro del Metate (0.67; population 36) at Sierra Madre del Sur of *Q. magnoliifolia*, whereas the highest mean pairwise distance for *Q. resinosa* were Estancia del Cubo (0.45; population 43) and Dolores (0.39; population 44) at Central Plateau (Table 1). The frequency and biogeographic province location of the 13 shared haplotypes between *Q. magnoliifolia* and *Q. resinosa*, showed that the haplotype nine (H9) was the most frequent and widespread distributed (Table 2).

High levels of total cpDNA diversity (h_T = 0.98 and 0.97) and relatively high levels of average gene diversity within populations (h_S = 0.67 and 0.62) were found in *Q. magnoliifolia* and *Q. resinosa*, respectively. R_{ST} values showed significant higher

levels of population differentiation than G_{ST} values for *Q. magnoliifolia* (R_{ST} = 0.73 > G_{ST} = 0.32; $p < 0.01$) and *Q. resinosa* (R_{ST} = 0.83 > G_{ST} = 0.36; $p < 0.01$). This result showed a strong phylogeographic structure for the populations of the two species. Analyses of molecular variance (AMOVA) indicated that most of the genetic variation was among populations within species (60%; F_{SC} = 0.624) than between species (3.6 %; F_{CT} = 0.036) (Table 3). Partition of genetic variation by biogeographic provinces of each species indicated that most of the genetic variation was among populations within provinces (60.4 %; F_{SC} = 0.594 and 66%; F_{SC} = 0.675) than among provinces (-1.73%; F_{CT} = -0.017 and 3%; F_{CT} = 0.028) for *Q. magnoliifolia* and *Q. resinosa*, respectively (Table 3).

The haplotypes network of *Q. magnoliifolia* and *Q. resinosa* showed a star-like shape, most of the haplotypes are closed related to each other with only one mutational step separating them in the network and only five haplotypes [H13, H23, H51, H56 and H67] had more than two mutational steps (Fig. 3). Of the 13 shared haplotypes between the two species, four [H9, H12, H35 and H36] are ancestral for the two species, whereas, the other nine haplotypes were derived. The distribution of the shared haplotypes suggests recurrent cytoplasmic introgression along different periods of time and the genealogical relationships between the haplotypes showed strong phylogeographic structure where the haplotypes closed related are also geographically closer (Fig. 3).

The six genetic discontinuities "Barriers" detected by the Monmonier's maximum differences algorithm with more than 60% bootstrap support showed that the main differences were among populations within rather than between species (Fig. 2) supporting the AMOVA results. The first barrier (B1) with a 97% of bootstrap support separated the populations of *Q. resinosa* (50, 51 and 52) located at south of Sierra

Madre Occidental from populations (39 and 41) located at Central Plateau. The second barrier (B2) with an 89% of bootstrap support showed isolation in population nine of *Q. magnoliifolia* at Trans-Mexican Volcanic Belt from neighboring populations (3, 7, 8, 10 and 11). The third barrier (B3) with a 72% of bootstrap support was among populations of *Q. resinosa* (45 from 43 and 44) at Central Plateau. The fourth and fifty barriers (B4 and B5) with a 67% and 66% of bootstrap support separated the population of *Q. magnoliifolia* (24 from 30 and 31) and (23 from 29 and 33) at Sierra Madre del Sur. The sixth barrier (B6) with a 63% of bootstrap support showed isolation in population (40 from 39 and 41, 42] of *Q. resinosa* at Central Plateau.

Inferences of historical population demography

Significant negative values of F_S neutrality test supported population expansion in all populations of *Q. resinosa* and almost all populations of *Q. magnoliifolia* except in four populations (16, 23, 34 and 38) (Table 4). Mismatch distributions also supported population expansions because in all cases the Raggedness index did not show significant differences of the observed distributions of mismatches to those distributions expected under the model of population expansion (i.e. a unimodal distribution), except in five populations (16, 23, 34, 38 and 15) of *Q. magnoliifolia* which showed a bimodal distribution indicating constant population size (Table 4). The demographic parameters calculated by populations of each species showed that the effective population size values after expansion (θ_1) indicated a growth for all populations and the estimates of the time of expansions (τ) showed that Ixcateopan (τ = 11.607; population 20 at Sierra Madre del Sur) was the population with the highest value for *Q. magnoliifolia* populations, whereas, Dolores (τ = 4.853; population 44 at Central Plateau) was the population with the highest value for *Q. resinosa* populations (Table 4). The maximum age of population expansion for *Q. magnoliifolia* populations was 1.4×10^6 years

(assumed $\tau = 1.764$ with a low $\mu = 10^{-5}$ and high $g = 100$ years) and the minimum age of 36×10^3 years (high $\mu = 10^{-4}$, low $g = 25$ years) and for *Q. resinosa* the maximum age of population expansion was 1.2×10^6 years (assumed $\tau = 1.481$, with a low $\mu = 10^{-5}$ and high $g = 100$ years) and the minimum age of 30×10^3 years (high $\mu = 10^{-4}$, low $g = 25$ years).| Our results suggest that the oldest expansion for *Q. magnoliifolia* morphotectonic provinces occurred at the Sierra Madre del Sur (SMS) between 1.66×10^6 (low $\mu = 10^{-5}$ and high $g = 100$ years) and 42×10^3 years (high $\mu = 10^{-4}$ and low $g = 25$ years) and for *Q. resinosa* the oldest expansion occurred at Central Plateau of Mexico (CPMx) between 1.5×10^6 (low μ and high g) and 38×10^3 (high μ and low g). The most recent population expansion for the two species occurred at Trans-Mexican volcanic belt (TMVB) between 1.16×10^6 (low μ and high g) and 29×10^3 (high μ and low g) for *Q. magnoliifolia* and between 96×10^3 (low μ and high g) and 24×10^3 (high μ and low g) for *Q. resinosa.*

Reconstruction of past distribution of species using ecological niche modeling

Ecological models and projections

The current and past final consensus model for *Q. magnoliifolia* and *Q. resinosa* are given in Fig. 4. Final consensus models of richness of shared haplotypes and sympatric area are shown in Fig. 5. These models are quite accurate representations of the current geographical distribution of both species. In almost all cases, the chi square was statistically significant ($\chi^2_{0.05}$ (9) = 18.3) indicated that all predicted models were not due to chance.

The intersection of all distribution models with a climatic chart showed that *Q. magnoliifolia* has a warm subhumid to semiwarm subhumid climate characterized by their tolerance to minimal precipitation in driest month (4.7-5.3 mm), higher

456 temperatures in both quarter most driest (19.5°-20.2°C), and quarter most coldest
457 (18.0°-18.6°C), while, *Q. resinosa* has a semiwarm subhumid to temperate subhumid
458 climate, characterized by tolerate precipitation in driest month (5.6-6.9 mm), lower
459 temperatures in both quarter most driest (16.4°-17.2°C) and quarter most coldest (14.1°-
460 14.8°C). The intersection of all distribution models with a raster digital elevation model,
461 showed changes in the altitudinal distribution ranges between current → LGM and
462 LGM → LIG for both species (Fig. 6). The percentage of distribution area gains and
463 losses during the LGM and LIG models showed contractions in the distribution area of
464 the two species (Fig. 6).

465 Based on current predictions models, there was a sympatry zone between *Q.*
466 *magnoliifolia* and *Q. resinosa* at south of Sierra Madre Occidental, western and center
467 part of Trans-Mexican Volcanic Belt and south of Central Plateau (Figs. 4 and 5). In the
468 Last Glacial Maximum (LGM), the distribution models for *Q. magnoliifolia* was similar
469 to the current distribution, except for a contraction of ~22%, mainly occurred at the
470 north of Sierra Madre Occidental and Sierra Madre del Sur, showed that suitable
471 conditions of niche was present at surrounding areas of south of Trans-Mexican
472 Volcanic Belt and north of Sierra Madre del Sur, which implied a downward altitudinal
473 migration of 162 to 300 m overall distribution (Fig. 6). In the case of *Q. resinosa*, its
474 area of distribution at the LGM was similar to current distribution, but with an
475 important contraction of 60%, at east of Sierra Madre Occidental, west and east of
476 Central Plateau and center of Trans-Mexican Volcanic Belt, which implied a downward
477 altitudinal migration of 230 to 340m overall distribution (Figs. 6). The sympatric zone
478 between the two species for the LGM was restricted at south of Sierra Madre
479 Occidental, western and small areas at center of Trans-Mexican Volcanic Belt,
480 specifically in the highlands of Jalisco, southeast of Nayarit and Sierra of the Huicholes

481 and small areas at south of Central Plateau, with a reduction of ~42% in relation with
482 current simpatry. The distribution models for Last Interglacial Maximum (LIG) of *Q.*
483 *magnoliifolia* showed a reduction of ~47%, mainly at Oaxaca in the Sierra Madre del
484 Sur, Sierra Madre Occidental and centre of Trans-Mexican Volcanic Belt, which
485 implied a downward altitudinal migration of 38 to 123m, showed expansions at lower
486 altitudes through western of their distribution. The LIG models for *Q. resinosa* showed
487 a reduction of ~64%, their distribution was quite different to the current distribution
488 involved latitudinal migrations in its range from the Central Plateau to Sierra Madre del
489 Sur but maintenance at western part of Trans-Mexican Volcanic Belt, with an upward
490 altitudinal migration of 100 to 300m. In the LIG models, the most important sympatric
491 area between the two species was the Sierra Madre del Sur and small areas at western
492 and south of Trans-Mexican Volcanic Belt, between 1200 to 1900 masl.
493 The distribution models showed that currently the highest richness in shared
494 haplotypes is at Trans-Mexican Volcanic Belt, Guanajuato state at the Central Plateau
495 and Oaxaca state at Sierra Madre del Sur (Fig. 5). According to the models of LGM the
496 highest richness in shared haplotypes were present at western and south of Trans-
497 Mexican Volcanic Belt, south of Sierra Madre Occidental, small areas at Sierra Madre
498 del Sur and small area at south of Central Plateau, across an altitudinal range from 950-
499 1600 masl. The LIG models showed that the most diversity in shared haplotypes were at
500 Central Valleys of Oaxaca at Sierra Madre del Sur, across an altitudinal range from
501 1250 to 1900 masl.

502 Models comparison of richness of shared haplotype vs. predicted sympatric
503 zones showed a strong relation between richness of shared haplotype and sympatric
504 zones between the two species. The extension area of shared haplotypes richness
505 overlapping with the sympatric area has been change through the time (Fig. 6).

506 Currently, the 49% of total area of shared haplotype richness overlap with the sympatric
507 area, during the LGM 41% and 89% for the LIG. The Trans-Mexican Volcanic Belt was
508 the biogeographic province with the highest richness of shared haplotypes and the
509 largest area of sympatry between the two species (Fig. 5).

510 **Discussion**

511 We analyzed phylogeographic, demography and ecological niche models in two
512 hybridizing Mexican white oak species, *Q. magnoliifolia* and *Q. resinosa* throughout
513 distribution range. We found high cpDNA haplotype diversity, high levels within and
514 total diversity (56-34 haplotypes; h_S = 0.67-0.62 and h_T = 0.98-0.97) for *Q.*
515 *magnoliifolia* and *Q. resinosa*, with 13 shared haplotypes between them. Similar genetic
516 diversity levels were observed in other oak species of North America, as the endemic to
517 California white oak *Q. lobata* (30 haplotypes; h_S = 0.285 and h_T = 0.979) (Grivet et al.
518 2006), and in the Mexican red oak hybrid complex formed by *Q. hypoleucoides*, *Q.*
519 *scytophylla* and *Q. sideroxyla* (h_S = 0.671-0.708-0.695 and h_T = 0.932-0.972-0.981,
520 respectively) with 14 shared haplotypes between (Peñaloza-Ramírez et al. in press), but
521 comparatively higher levels than eight European white oak species (h_S = 0.025–0.183
522 and h_T = 0.635–0.847) with an extensive cytoplasmic exchanges between sympatric
523 species (Petit et al. 2002), and two distantly related European white oak species *Q.*
524 *suber* and *Q. ilex* (h_S = 0.043-0.034 and h_T = 0.827-0.926) with a dominance
525 replacement of *Q. suber* cpDNA by that of *Q. ilex* (Lumaret and Jabbour-Zahab 2009).
526 Palaeoclimatic records from Mexico indicate that the effects of glaciations over the Late
527 Pleistocene and Holocene produced climatic changes less drastic and more
528 heterogeneous than in other parts of the northern hemisphere (Metcalfe et al. 2000), as
529 to reduce oak species to small populations isolated into a few refugia (González-
530 Rodríguez et al. 2004), which could be explain the high genetic diversity found in these

Mexican white oak species. The low frequency of sharing haplotypes between *Q. magnoliifolia* and *Q. resinosa* contrasts with the extensive sharing cytoplasmic exchange between white oak species in Europe (Petit *et al*. 1993; 1997; 2002; Dumolin-Lapegue *et al*. 1997; Olalde *et al*. 2002; Lumaret & Jabbour-Zahab 2009), which could be explain because in Mexico, the past climatic changes implied altitudinal and latitudinal range shift that favor the sympatric zones between oak species (González-Rodríguez et al. 2004; Tovar-Sánchez et al. 2008; Peñaloza-Ramírez et al. in press) and between *Pinus* species (Moreno-Letelier and Piñero 2009), while, in Europe implied prolonged contact in the glacial refugia.

Lower population differentiation was found in *Q. magnoliifolia* (G_{ST} = 0.32) and *Q. resinosa* (G_{ST} = 0.36) comparatively with other oak species as, Mexican red oaks complex *Q. affinis*-*Q. laurina* (G_{ST} = 0.499) (González-Rodríguez *et al*. 2004), North America red oak *Q. rubra* (G_{ST} = 0.46) (Magni *et al*. 2005) and white oak *Q. lobata* (G_{ST} = 0.709) (Grivet et al. 2006) and European white oak species (G_{ST} = 0.781–0.961 for eight species) (Petit et al. 2002), *Q. suber* and *Q. ilex* (G_{ST} = 0.948-0.963) (Lumaret & Jabbour-Zahab 2009), but comparatively higher than the Mexican red oak complex of *Q. hypoleucoides*, *Q. scytophylla* and *Q. sideroxyla* (G_{ST} = 0.280, 0.271 and 0.291) (Peñaloza-Ramírez et al. in press). Comparatively, Mexican oaks present the lowest G_{ST} values, then the northern oaks and European oaks are the higher differentiate, reflecting the contrast effect of the last climatic changes over the evolutionary history of these species. In Mexico, during the Last Glacial Maximum (LGM) the temperature decrease 8°C, precipitation increase and there was a decline in vegetation strips to ca. 1000m (Bradbury, 1997; Metcalfe, 2006; Caballero et al. 2010). These changes based on palaeobotanic records produced a downward altitudinal migration and widespread distributions of temperate species (Metcalfe et al., 2002; Lozano-García et al. 2002;

556 Piperno et al., 2007; Caballero et al. 2010), as has been observed in *Pinus strobiformis*
557 in Mexico, where low genetic differentiation suggested genetic connectivity as a result
558 of population expansions during the last glacial stages (Moreno-Letelier and Piñero
559 2009). Another example is *Picea chihuahuana*, which fossil pollen records from the
560 LGM suggest a much wider distribution throughout northern and central Mexico
561 (Metcalfe et al., 2002; Lozano-García et al., 2005), and genetic data indicate a lack of
562 phylogeographic structure and low genetic differentiation (Jaramillo-Correa et al. 2006).
563 So, the climate changes may be expected to affect the genetic composition of species,
564 depending on their ecological affinities (Moreno-Letelier and Piñero 2009). In this
565 sense, *Q. magnoliifolia* has a warm subhumid to semiwarm subhumid climate and *Q.*
566 *resinosa* has a semiwarm subhumid to temperate subhumid climate, and during glacial
567 stages the temperature decrease and precipitation increase, which could be contracted
568 the habitat availability of these species. Also, the distribution models showed
569 contraction across their distribution range during the LIG and LGM for the two species,
570 but maintenance in situ populations in different distribution parts and with downward
571 altitudinal movement (Fig. 5). Thus, the low genetic differentiation and the strong
572 phylogeographic structure observed in *Q. magnoliifolia* and *Q. resinosa* could be
573 explained because the last climatic changes contracted the distribution range of these
574 two species, but maintenance populations in different regions with downward altitudinal
575 migrations allowed to increase habitat availability and formed sympatric zones between
576 them. Whereas, in North America palynological evidence indicates that during the
577 LGM, *Q.* rubra had one major distribution, resulting in both modest movement
578 northwards when climate improved and a lack of phylogeographic structure (Magni et
579 al. 2005), and in Europe the white oaks, were largely restricted to southern refugias
580 during the LGM increasing the genetic differentiation and experienced extensive

581 postglacial recolonization, resulting in a high genetic differentiation and strong
582 phylogeographic structure (Dumolin-Lapegue et al. 1997; Petit et al. 2002; Lumaret &
583 Jabbour-Zahab 2009).

584 Recurrent interspecific cytoplasmatic exchange between *Q. magnoliifolia* and *Q.*
585 *resinosa* can be observed in the network of both species. In theory, the internal
586 haplotypes with high frequency that have more connections to other haplotypes and are
587 geographically widespread can be considered as ancestral (Matos & Schaal 2000;
588 Moreno-Letelier and Piñero 2009). Haplotypes [H9, H20, H12, H58 and H34] are
589 ancestral and shared between the two species, and their positions in the networks
590 suggest cytoplasmic capture by gene flow than shared ancestral polymorphism. The
591 closed related haplotypes [H36, H35 and H10] and [H49 and H11] are ancestral in the
592 network of one specie and derived in the network of the other, whereas, [H14, H2 and
593 H3] were derived in the network of both species, which is also evidence of interspecific
594 gene flow. These shared ancestral and derived haplotypes between *Q. magnoliifolia* and
595 *Q. resinosa*, evidence recurrent cytoplasmic exchange by interspecific gene flow at
596 different periods of time.

597 All distribution models showed altitudinal range shift for *Q. magnoliifolia* and
598 *Q. resinosa* during the LGM and LIG, with downward altitudinal movement during the
599 LGM and upward altitudinal movement during warm climate of LIG, with the exception
600 of *Q. magnoliifolia*, which must to move downward due to *Q. resinosa* occupied higher
601 altitudes, thus, these altitudinal movement favored the formation of sympatric areas
602 (Fig. 5). Also, the all distribution models showed contractions in the distribution area of
603 the two species during the LGM, with a strong contraction during LIG. The sympatric
604 areas has been lowest compare to current distribution areas of the two species, during
605 the LGM the sympatric area contracted, whereas, during the LIG was presented the

606 largest sympatric areas between the two species, mainly due to latitudinal movement of
607 *Q. resinosa* from Central Plateau to Sierra Madre del Sur and western of Trans-Mexican
608 Volcanic Belt (Fig. 5). The distribution area of diversity of shared haplotype, showed an
609 increase from the LIG reaching its maximum areas currently (Figs. 3 and 5). Thus, the
610 potential sympatric zones for the two species are strongly related to diversity of shared
611 haplotype. The altitudinal ranges shift observed in the distribution models for *Q.*
612 *magnoliifolia* and *Q. resinosa*, could be validated by palinological records dated from
613 the LGM which showed a decline in vegetation strips to ca. 1000 m (Caballero et al.
614 2010). Palynological evidence (22,000-18,000 yr BP) suggests that the period of
615 maximum cold at Sierra Madre Occidental (Ortega-Rosas et al. 2008), center of Trans-
616 Mexican Volcanic Belt and Central Plateau occurred a forests expansion predominantly
617 of *Pinus* and in very low proportions of *Picea* pollen, this conifer is a boreal taxa
618 currently found at altitudes ranging from 2000 to 3700 meters, suggest southward
619 expansion, and thus the existence of cold weather (Metcalf et al. 2000; Lozano-García
620 *et al*., 2005; Caballero et al. 2010). During the LGM, at south of Trans-Mexican
621 Volcanic Belt and north of Sierra Madre del Sur (i.e. Central Balsas), palinological
622 records showed *Podocarpus* pollen, a coniferous tree which suggest wetter areas,
623 temperatures 4–5°C cooler than today and the Balsas teosintle, largely absent below
624 400–500 m today, could have descended into lower-lying tropical areas (Piperno et al.
625 2007). Thus, palynological records support the contraction of *Q. magnoliifolia* and *Q.*
626 *resinosa*, at center of Trans-Mexican Volcanic Belt, north and western of Sierra Madre
627 Occidental, also support the downward altitudinal migration of both species during the
628 LGM. The altitude of the equilibrium line (ALE) of glaciers during the LGM suggests
629 relatively more humid environments in the eastern and western versus center of Trans-
630 Mexican Volcanic Belt which was dry (Caballero et al. 2010). This climatic condition

631 explains the persistence of *Q. magnoliifolia* and *Q. resinosa* at western of Trans-
632 Mexican Volcanic Belt, where sympatric area occurred.

633 The Trans-Mexican Volcanic Belt is the biogeographic region with the highest
634 number of shared haplotypes and the largest sympatric area between *Q. magnoliifolia*
635 and *Q. resinosa*, currently and during the LGM. The Trans-Mexican Volcanic Belt is
636 the highest mountain range in Mexico (Rzedowski 1978). Nixon (1993) considers it to
637 be a center of diversity for *Quercus*. The Trans-Mexican Volcanic Belt has been
638 considered crucial to the evolutionary history of the genera *Pinus* and *Quercus* since
639 these mountains connect the biotas of the Sierra Madre Occidental and the Sierra Madre
640 Oriental (Styles 1993). The high levels of volcanic activity in the area gave rise to many
641 microhabitats that allowed radiation and speciation of taxa (Challenger 1998).
642 Similarly, the Trans-Mexican Volcanic Belt has been reported as historical and
643 contemporary sympatric area that favor the hybridization and cytoplasmic introgression
644 between Mexican red oaks species as *Q. affinis* and *Q. laurina* (González-Rodríguez et
645 al. 2004) and *Q. crassifolia* and *Q. crassipes* (Tovar-Sánchez et al. 2008).

646 Demographic analyses showed expansions in all populations of *Q. magnoliifolia*
647 and *Q. resinosa*, also, the network of both species showed a star-like shape that suggests
648 population expansion resulting in new recent haplotypes due to an excess of recent
649 mutations (or excess of rare alleles). Similarly, Cannon & Manos (2003) found a star-
650 like shape network in Southeast Asia stone oaks (*Lithocarpus*), Magni *et al*. (2005)
651 found a star-like shape network in North American populations of *Quercus rubra*,
652 which after LGM theirs populations experienced population expansion. In Mexico, the
653 Holocene chronology in palynological record between ca. 10,000 and ca. 5000 yr BP
654 indicate greater abundances of *Quercus* interpreted as evidence of dry, warm and sub-
655 humid climate (Metcalf et al. 2000; Caballero et al. 2010). These data support the

hypothesis of during Holocene including current climate favor the demographic expansion of *Q. magnoliifolia* and *Q. resinosa* which has a warm subhumid and temperate subhumid climate. The time of expansion parameter (τ) and mean pairwise distance (mean D^2_{sh}) showed that the populations at Trans-Mexican Volcanic Belt for *Q. magnoliifolia* (Manantlán and Ixcateopan; populations 11 and 20) and Central Plateau for *Q. resinosa* (Estancia del Cubo and Dolores; populations 43 and 44), were the populations with major pairwise differences, suggest that they were the first populations in expanding.

In conclusion, our data indicate recurrent interspecific cytoplasmatic exchange between *Q. magnoliifolia* and *Q. resinosa*. During the LGM cycles *Q. magnoliifolia* and *Q. resinosa* moved to lower altitudes at temperate sub-humid or semi-warm conditions and during the warmer LIG moved to higher altitudes and these altitudinal movements favored the formation of sympatric areas. The potential sympatric zones for the two species are strongly related to diversity of shared haplotype, which showed that the Trans-Mexican Volcanic Belt is the biogeographic province with the highest number of shared haplotypes and the largest sympatric area between *Q. magnoliifolia* and *Q. resinosa*, currently and during the LGM. Demographic analysis showed populations expansion and palinological records and current distribution models indicate that Holocene period favor *Quercus* species. The integration of phylogeographic, demographic, distribution models and palynological records allowed understand how climatic changes in the past affected the distribution range of the species.

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Table 1. Morphological identification, morphotectonic province, geographical location and estimation of cpDNA diversity for *Quercus magnoliifolia* and *Q. resinosa* populations in Mexico. SMOc = Sierra Madre Occidental, TMVB = Trans-Mexican Volcanic Belt, SMS = Sierra Madre del Sur, CP = Central Plateau. *N* = simple size, *Nh* = number of haplotypes, *Nhp* = number of private haplotypes, *fα* = frequency of the most common haplotype between the two species, n_e = effective number of haplotypes, h_S = gene diversity within populations, *A* = allelic richness, D^2_{sh} = average genetic distances among individuals.

Species/ Morphotectonic Province/ Populations	Latitude N /Longitude W	*N*	*Nh*	*Nhp*	*fα*	n_e	h_S	*A*	D^2_{sh}
Q. magnoliifolia									
SMOc									
1.Canelas	25.12/-106.50	10	4	1	0.00	1.31	0.53 (0.18)	1.5	0.12
2.Sta. Lucia	23.45/-105.85	10	5	1	0.10	2.96	0.82 (0.10)	2.5	0.13
TMVB									
3.Cacalutan	21.08/-104.23	5	2		0.60	0.81	0.60 (0.18)	1.0	0.20
4.Compostela	21.22/-104.80	10	4		0.30	2.96	0.82 (0.07)	2.4	0.09
5.Ocotillo	21.27/-104.65	6	4		0.33	1.99	0.87 (0.13)	2.7	0.08
6.Pto. Vallarta	20.43/-105.28	10	3	2	0.00	1.01	0.38 (0.18)	1.0	0.26
7.Guadalupe	19.87/-103.46	5	3	1	0.00	1.22	0.80 (0.16)	2.0	0.18
8.El Llano	19.75/-104.77	8	4	1	0.00	2.41	0.82 (0.10)	2.4	0.07
9.Casimiro Castillo	19.70/-104.38	5	3	2	0.00	0.97	0.70 (0.22)	2.0	0.04
10.Cuzalapa	19.45/-104.52	6	4	1	0.17	1.99	0.87 (0.13)	2.7	0.09
11.Manantlán	19.60/-104.22	7	4	2	0.00	2.00	0.81 (0.13)	2.4	0.78
12.Nogal	19.32/-104.13	5	2		0.00	0.81	0.60 (0.18)	1.0	0.20
14.Puerto del Gato	19.48/-100.37	5	2		0.00	0.60	0.40 (0.24)	1.0	0.03
15.Morelia	19.66/-101.17	5	4	2	0.00	1.64	0.90 (0.16)	3.0	0.14
16.Guayabos	19.23/-101.33	6	1		0.00	0.47	0	0.0	0.00

17.Benito Juárez	19.35/-100.40	5	2		0.00	0.81	0.60 (0.18)	1.0	0.05
18.Valle de Bravo	19.25/-100.13	8	3		0.38	1.89	0.75 (0.10)	1.9	0.14
19.Temascaltepec	19.05/-100.07	5	3	1	0.00	1.22	0.80 (0.16)	2.0	0.35
20.Ixcateopan	18.55/-99.70	6	4	1	0.00	1.99	0.87 (0.13)	2.7	0.73
SMS									
13.Coalcoman	18.73/-103.27	6	4	1	0.33	1.99	0.87 (0.13)	2.7	0.14
21.Filo de Caballo	17.78/-99.70	6	3	1	0.00	1.33	0.73 (0.16)	1.8	0.10
22.Platanillos	17.47/-100.58	6	3		0.00	1.60	0.80 (0.12)	2.0	0.29
23.Chila de las Flores	17.95/-97.88	5	1		0.00	0.40	0	0.0	0.00
24.Magueyal	17.18/-97.78	11	3		0.00	1.81	0.66 (0.11)	1.6	0.09
25.Sta Inés del Monte	16.95/-96.85	6	3	1	0.00	1.33	0.73 (0.16)	1.8	0.15
26.Sn Bernardo	16.85/-96.92	5	4	1	0.00	1.64	0.90 (0.16)	3.0	0.70
27.Ojo de Agua	16.42/-97.08	5	4	1	0.00	1.64	0.90 (0.16)	3.0	0.08
28.Mitla	16.93/-96.30	7	5	1	0.00	2.96	0.91 (0.10)	3.0	0.11
29.Papalutla	17.73/-97.90	6	2		0.00	0.89	0.53 (0.17)	1.0	0.18
30.Juxtlahuaca	17.48/-98.02	10	3		0.60	1.51	0.60 (0.13)	1.4	0.04
31.Pinos	17.23/-97.72	6	4		0.17	1.60	0.80 (0.17)	2.5	0.12
32.Cerezal	17.27/-96.53	5	2		0.40	0.81	0.60 (0.18)	1.0	0.05
33.Sn Miguel del Rio	17.32/-96.55	5	4		0.00	1.64	0.70 (0.22)	2.5	0.10
34.Sola de Vega	16.58/-96.93	5	1		0.00	0.40	0	0.0	0.00
35.Matatlán	16.83/-96.35	5	3	2	0.00	0.97	0.70 (0.22)	2.0	0.19
36.Cerro del Metate	16.25/-96.53	5	3		0.00	0.97	0.70 (0.22)	2.0	0.67
37.Coatlán	16.28/-96.68	6	3		0.50	1.33	0.73 (0.16)	2.0	0.18
38.Sto. Reyes	16.48/-96.98	5	1		0.00	0.40	0	0.0	0.00
Q. resinosa									
CP									
39.La Congoja	22.18/-102.52	5	2		0.00	0.81	0.60 (0.18)	1.0	0.20
40.Calvillo	21.92/-102.58	10	2	1	0.00	0.80	0.20 (0.15)	0.5	0.02
41.Arroyo Seco	21.72/-102.63	6	3	1	0.00	1.00	0.60 (0.22)	1.7	0.17

42.Cerro de los Gallos	21.67/-102.22	5	2		0.60	0.81	0.60 (0.18)	1.0	0.05
43.Estancia del Cubo	21.38/-101.12	6	2		0.50	1.00	0.60 (0.13)	1.0	0.45
44.Dolores	21.17/-101.12	6	3	1	0.00	1.33	0.73 (0.16)	1.8	0.39
45.Minas	21.68/-100.05	10	2		0.00	0.98	0.36 (0.16)	0.8	0.03
58.Sta. Catarina	20.90/-101.05	6	3		0.00	1.33	0.73 (0.16)	1.8	0.15
59.Guanajuato	20.88/-101.15	7	5		0.29	2.96	0.91 (0.1)	3.0	0.07
60.Agua Zarca	20.77/-101.02	6	2		0.00	0.89	0.53 (0.17)	1.0	0.04
SMOc									
46.Mezquital	23.45/-104.35	10	5	1	0.00	3.68	0.87 (0.07)	2.8	0.08
47.Valaparaiso	22.67/-103.77	6	3		0.00	1.60	0.80 (0.12)	2.0	0.07
48.Mexquitic	22.52/-103.77	8	4	2	0.00	1.43	0.64 (0.18)	1.9	0.08
49.Sierra de Bolaños	21.88/-103.87	8	3		0.63	1.32	0.61 (0.16)	1.5	0.07
50.San Lorenzo	21.97/-103.20	6	2		0.00	0.89	0.53 (0.17)	1.0	0.04
51.El Plateado	21.92/-103.03	10	4		0.00	2.70	0.80 (0.09)	2.3	0.07
52.Puertecito	21.68/-103.17	5	3	1	0.00	1.22	0.80 (0.16)	2.0	0.13
53.Teul de González	21.40/-103.52	6	2		0.00	0.89	0.53 (0.17)	1.0	0.18
TMVB									
54.Talpa	20.40/-104.88	7	3	1	0.71	1.01	0.52 (0.21)	1.4	0.11
55.Cuquio	21.02/-103.00	6	3	1	0.67	1.00	0.60 (0.22)	1.7	0.10
56.Avigel	20.87/-102.80	5	2		0.00	0.60	0.40 (0.24)	1.0	0.03
57.Tepatitlán	20.87/-102.77	5	3		0.00	0.97	0.70 (0.22)	2.0	0.04
61.Tumbiscatio	19.59/-101.11	6	3	2	0.00	1.00	0.60 (0.22)	1.7	0.08

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Table 2. Frequency and biogeographic provinces location of the 13 shared haplotypes between *Q. magnoliifolia* and *Q. resinosa*.

Shared	Haplotypes	*Q. magnoliifolia*			*Q. resinosa*		
haplotypes	colors	SMOc	TMVB	SMS	SMOc	TMVB	CP
H2	Green	1	-	-	-	-	8
H3	Sky blue	1	-	-	-	-	2
H9	Orange	1	12	14	5	5	12
H10	Lime green	-	8	1	-	-	1
H11	Fuchsia	-	2	1	1	-	-
H12	Lavander	-	3	-	2	5	7
H14	Grey blue	-	8	-	-	-	9
H20	Light pink	-	6	-	-	-	3
H34	Dark yellow	-	-	4	-	-	2
H35	Plum	-	6	-	-	1	-
H36	Turquoise	-	1	-	-	4	-
H49	Brown	-	-	2	-	1	-
H58	Yellow	-	-	3	-	-	1
Total		3	46	25	8	16	45

883 Table 3. Hierarchical analysis of molecular variance (AMOVA) based on F_{ST} calculated between species and among morphotectonic

884 provinces within species.

Source of variation	d.f.	SS	Variance components	Percentage of variation	*F-statistics*
Among species	1	20.11	0.064	3.620	F_{CT} = 0.036**
Among populations within species	59	442.00	1.056	60.10	F_{SC} = 0.624***
Within populations	336	214.22	0.638	36.28	F_{ST} = 0.637***
Total	396	6776.34	1.757		
Q. magnoliifolia					
Among provinces	2	14.55	-0.030	-1.73	F_{CT} = -0.017^{ns}
Among populations within provinces	35	256.1	1.055	60.43	F_{SC} = 0.594***
Within populations	204	147.2	0.721	41.30	F_{ST} = 0.587***
Total	241	417.83	1.75		
Q. resinosa					
Among provinces	2	19.641	0.046	2.84	F_{CT} = 0.028^{ns}
Among populations within provinces	20	151.69	1.054	65.57	F_{SC} = 0.675***
Within populations	132	67.061	0.508	31.60	F_{ST} = 0.684***
Total	154	238.39	1.608		

885 F_{CT} = differentiation among species/provinces; F_{SC} = differentiation among populations within species/provinces; F_{ST} = differentiation

886 among populations among species/provinces; *$P < 0.05$; ***$P < 0.001$.

887 Table 4. Parameters of population growth obtained with neutrality test (Fu 1997) and distribution of pairwise differences often called

888 "mismatch distributions" (Harpending 1994) and demographic expansion parameters calculated for *Q. magnoliifolia* and *Q. resinosa*

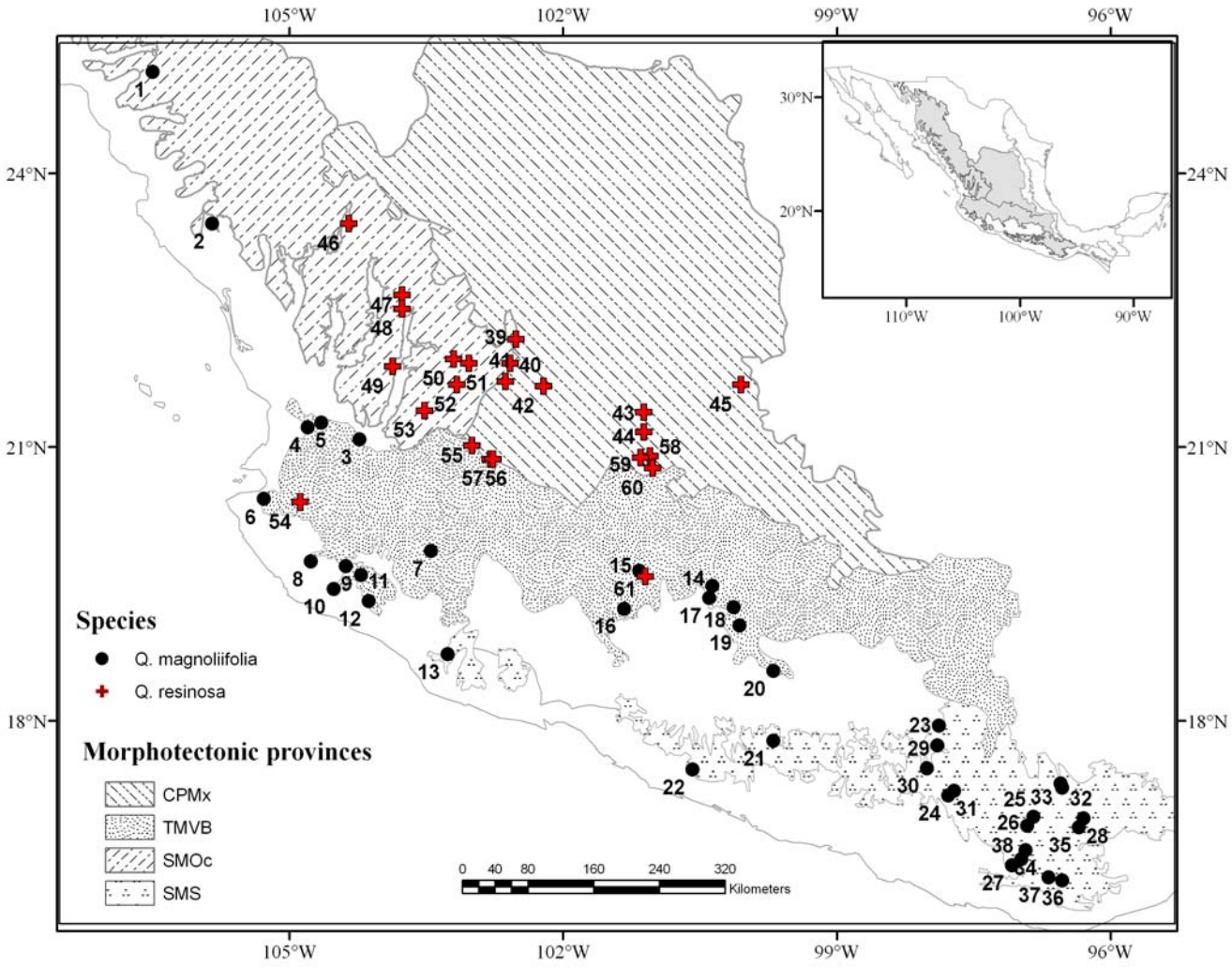
889 populations.

Species/ Morphotectonic Provinces/ Populations	Fu (1997)		Harpending (1994)		Demographic expansion parameters		
	Fu's F_S	$p(F_S)$	RI	$p(RI)$	θ_0	θ_1	τ
Q. magnoliifolia							
SMOc							
1.Canelas	-12.034	0.000	0.247	0.347	2.991	$1.2x10^{-3}$	$3.8x10^{-4}$
2.Sta. Lucia	-9.567	0.000	0.048	0.908	$9.60x10^{-8}$	5.478	4.717
TMVB	-25.753	0.000	0.0125	0.697	1.123	$1.21x10^{13}$	1.392
3.Cacalutan	-4.292	0.000	0.880	0.160	$1.26x10^{-5}$	3.605	1.706
4.Compostela	-11.771	0.000	0.080	0.726	$3.12x10^{-5}$	$4.08x10^{13}$	1.902
5.Ocotillo	-5.946	0.000	0.311	0.312	$1.34x10^{-5}$	$3.86x10^{13}$	1.332
6.Pto. Vallarta	-12.808	0.000	0.515	0.367	5.008	0.018	0.014
7.Guadalupe	-3.578	0.001	1.320	0.068	$1.22x10^{-4}$	$3.9x10^{10}$	1.851
8.El Llano	-9.602	0.000	0.203	0.313	$2.21x10^{-5}$	$3.93x10^{13}$	1.351
9.Casimiro Castillo	-5.409	0.000	0.350	0.510	$4.98x10^{-6}$	$4.29x10^{10}$	0.857
10.Cuzalapa	-5.016	0.000	0.116	0.674	$1.90x10^{-14}$	$2.82x10^{13}$	1.843
11.Manantlán	-3.081	0.023	0.327	0.150	10.709	0.010	0.001
12.Nogal	-4.292	0.000	0.880	0.159	$1.26x10^{-5}$	3.605	1.706
14.Puerto del Gato	-7.582	0.000	0.200	0.942	$1.33x10^{-5}$	$1.4x10^{12}$	0.414
15.Morelia	-2.862	0.009	1.070	0.014	$3.77x10^{-5}$	$6.81x10^{13}$	2.678
16.Guayabos	3.400	1.000	0.000	0.000	0.000	10000	0.000

17.Benito Juárez	-6.274	0.000	0.400	0.372	$5.76x10^{-6}$	$4.16x10^{12}$	0.632
18.Valle de Bravo	-8.566	0.000	0.070	0.889	$6.85x10^{-5}$	$2.82x10^{13}$	1.712
19.Temascaltepec	-2.680	0.013	0.280	0.514	$4.71x10^{-5}$	6.334	4.321
20.Ixcateopan	-2.270	0.047	0.880	0.052	$3.08x10^{-5}$	14.554	11.607
SMS	-25.500	0.000	0.010	0.768	0.847	$1.29x10^{13}$	1.995
13.Coalcoman	-4.660	0.000	0.133	0.633	$4.90x10^{-4}$	$5.23x10^{9}$	2.113
21.Filo de Caballo	-5.946	0.000	0.080	0.903	$6.08x10^{-5}$	$4.10x10^{13}$	1.332
22.Platanillos	-4.172	0.002	0.218	0.515	$5.73x10^{-5}$	5.288	3.780
23.Chila de las Flores	3.500	1.000	0.000	0.000	0.000	10000	0.000
24.Magueyal	-16.814	0.000	0.036	0.992	0.001	$9.27x10^{12}$	1.199
25.Sta Inés del Monte	-5.148	0.000	0.169	0.595	$8.35x10^{-5}$	$2.39x10^{13}$	1.759
26.Sn Bernardo	-1.633	0.082	0.170	0.756	9.526	$2.25x10^{10}$	0.019
27.Ojo de Agua	-3.578	0.001	0.150	0.767	$2.08x10^{-17}$	$3.23x10^{10}$	1.843
28.Mitla	-5.584	0.000	0.222	0.196	$5.66x10^{-5}$	$1x10^{14}$	2.534
29.Papalutla	-6.350	0.000	0.787	0.153	$1.30x10^{-5}$	2.246	1.724
30.Juxtlahuaca	-17.361	0.000	0.133	0.660	$1.97x10^{-5}$	$2x10^{10}$	0.781
31.Pinos	-4.660	0.000	0.102	0.792	$1.49x10^{-15}$	5.936	2.707
32.Cerezal	-6.274	0.000	0.400	0.374	$5.76x10^{-6}$	$4.16x10^{12}$	0.632
33.Sn Miguel del Rio	-3.304	0.004	0.130	0.775	$4.70x10^{-5}$	$6.65x10^{13}$	2.112
34.Sola de Vega	3.500	1.000	0.000	0.000	0.000	10000	0.000
35.Matatlán	-3.578	0.001	0.230	0.622	$4.84x10^{-16}$	2.858	3.252
36.Cerro Metate	-2.116	0.035	0.410	0.318	6.562	0.001	$1.2x10^{-4}$
37.Coatlán	-4.891	0.000	0.240	0.457	$2.23x10^{-5}$	4.716	2.622
38.Sto. Reyes	4.000	1.000	0.000	0.000	0.000	10000	0.000
Q. resinosa							
CP	-25.873	0.000	0.0071	0.978	$8.9x10^{-5}$	11.03	10.39
39.La Congoja	-4.292	0.000	0.880	0.155	$1.26x10^{-5}$	3.605	1.706
40.Calvillo	-28.462	0.000	0.400	0.223	$5.99x10^{-6}$	$7x10^{10}$	0.203

41.Arroyo Seco	-5.148	0.000	0.347	0.319	$4.10x10^{-6}$	3.031	2.847
42.Cerro de los Gallos	-6.274	0.000	0.400	0.374	$5.76x10^{-6}$	$4.16x10^{12}$	0.632
43.Estancia del Cubo	-4.660	0.000	0.880	0.056	$1.57x10^{-5}$	3.609	3.670
44.Dolores	-3.709	0.004	0.720	0.088	$1.56x10^{-5}$	6.670	4.853
45.Minas	-23.035	0.000	0.210	0.495	$6.63x10^{-6}$	$1.76x10^{12}$	0.366
58.Sta. Catarina	-5.148	0.000	0.169	0.588	$6.99x10^{-5}$	$1.89x10^{13}$	1.755
59.Guanajuato	-6.316	0.000	0.150	0.460	$1.22x10^{-4}$	$4.04x10^{10}$	1.999
60.Agua Zarca	-8.984	0.000	0.289	0.413	$7.50x10^{-6}$	$3.36x10^{12}$	0.558
SMOc							
46.Mezquital	-11.363	0.000	0.104	0.503	$6.58x10^{-5}$	$4.49x10^{13}$	2.055
47.Valaparaiso	-6.350	0.000	0.253	0.383	$1.28x10^{-5}$	$2.37x10^{13}$	1.170
48.Mexquitic	-9.456	0.000	0.140	0.958	1.291	$1.3x10^{12}$	0.214
49.Sierra de Bolaños	-10.797	0.000	0.159	0.666	$3.45x10^{-5}$	$8.7x10^{12}$	1.048
50.San Lorenzo	-8.984	0.000	0.289	0.406	$7.50x10^{-6}$	$3.36x10^{12}$	0.558
51.El Plateado	-14.651	0.000	0.253	0.144	$1.28x10^{-5}$	$2.54x10^{13}$	1.170
52.Puertecito	-3.901	0.000	0.120	0.832	$1.46x10^{-14}$	$1.16x10^{13}$	1.583
53.Teul de González	-6.350	0.000	0.787	0.154	$1.30x10^{-5}$	2.246	1.724
TMVB	-26.840	0.000	0.030	0.844	$1.33x10^{-16}$	50316.3	2.82
54.Talpa	-7.946	0.000	0.685	0.149	$8.49x10^{-6}$	1.920	2.247
55.Cuquio	-5.946	0.000	0.880	0.086	$1.19x10^{-4}$	52881.7	1.332
56.Avigel	-7.582	0.000	0.200	0.941	$1.33x10^{-5}$	$1.4x10^{12}$	0.414
57.Tepatitlán	-5.409	0.000	0.350	0.510	$4.97x10^{-6}$	$3.95x10^{10}$	0.857
61.Tumbiscatio	-6.578	0.000	0.062	0.990	$1.59x10^{-14}$	$2x10^{12}$	1.090

890 FIGURES



891 Fig. 1 Map of the morphotectonic province of sampled populations of *Q. magnoliifolia*

892 and *Q. resinosa* in Mexico.

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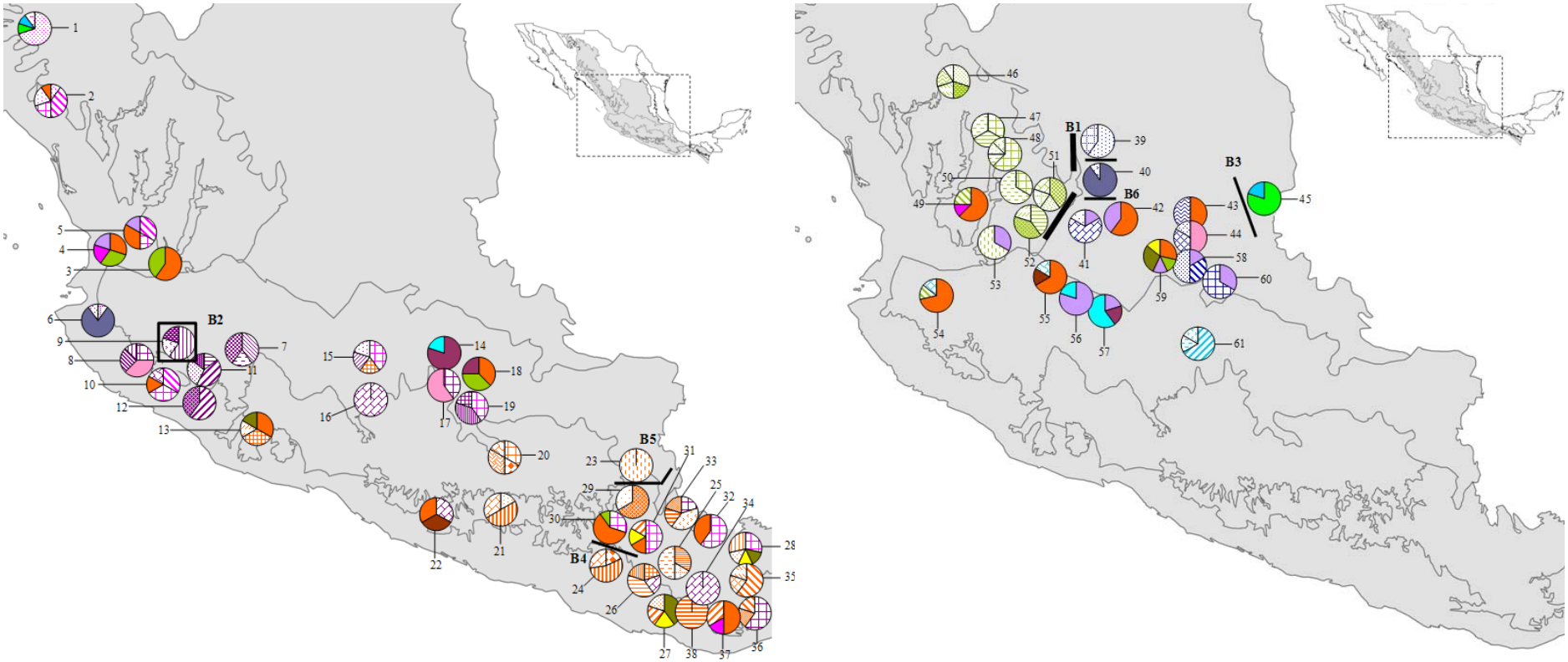

Fig. 2 Distribution of haplotypes of *Q. magnoliifolia* (on top) and for *Q. resinosa* (on bottom). Shared haplotypes are represented with solid colors and haplotypes located at different morphotectonic regions were represented with different color and textures. Haplotypes of *Q. magnoliifolia* located at SMOc (fuchsia), TMVB (purple) and SMS (orange) and haplotypes of *Q. resinosa* located at SMOc (lime green), CP (blue) and TMVB (sky blue). The six genetic barriers (B1 to B6) with a bootstrap support more than 60% are also represented in a map (see the text for more details).

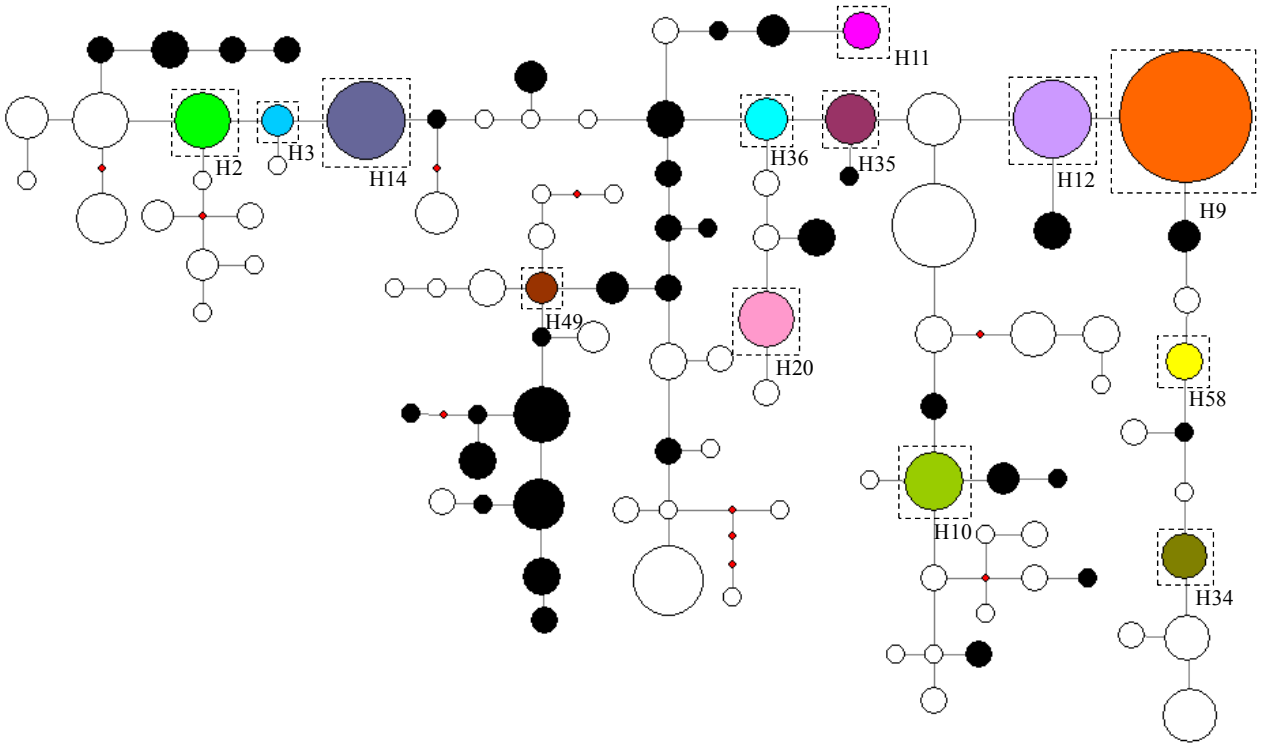

Fig. 3 Haplotype network of *Q. magnoliifolia* (on top) and for *Q. resinosa* (on bottom). Each haplotype is represented by a circle and their frequency over all populations is proportional to size. Haplotypes located at SMOc (white color), TMVB (black color), SMS (grey color) and CP (dark grey color).

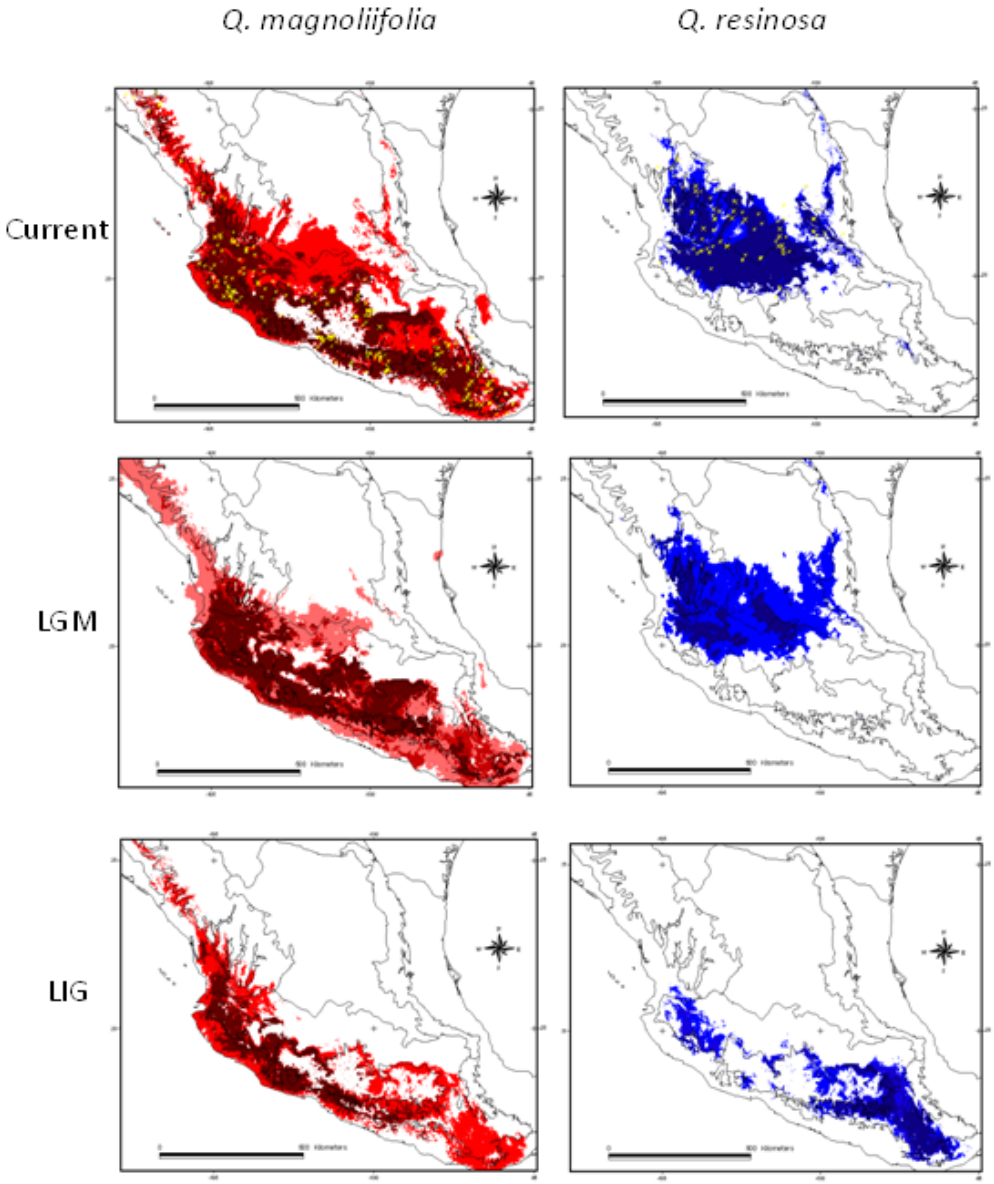

Fig. 4 Distribution models of *Q. magnoliifolia* and *Q. resinosa* currently and during the Last Glacial Maximum (LGM) and Last Interglacial (LIG).

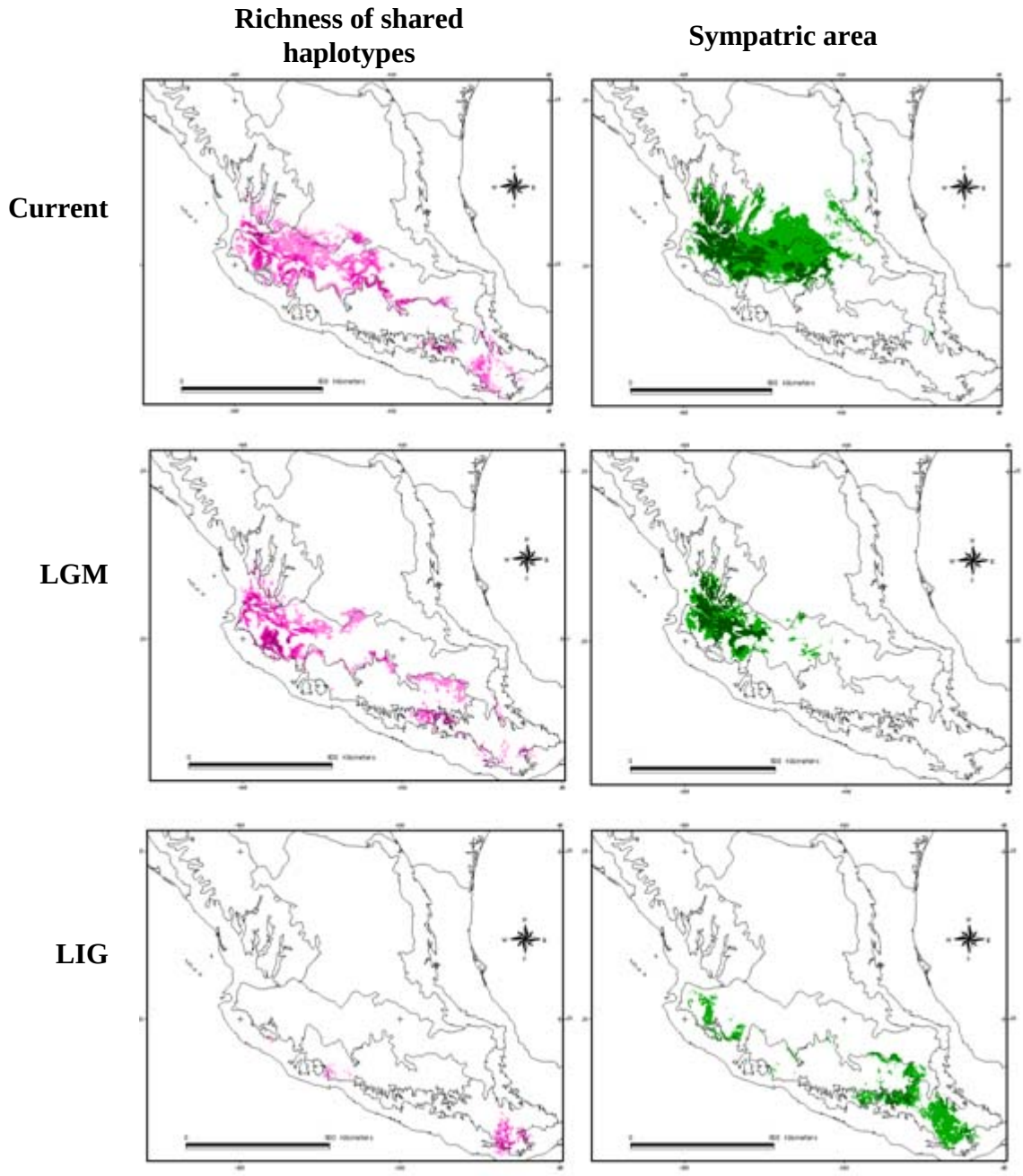

Fig. 5 Distribution models of richness of shared haplotypes currently and during LGM and LIG obtained from nine shared haplotypes between *Q. magnoliifolia* and *Q. resinosa* and distribution models of sympatric area between species.

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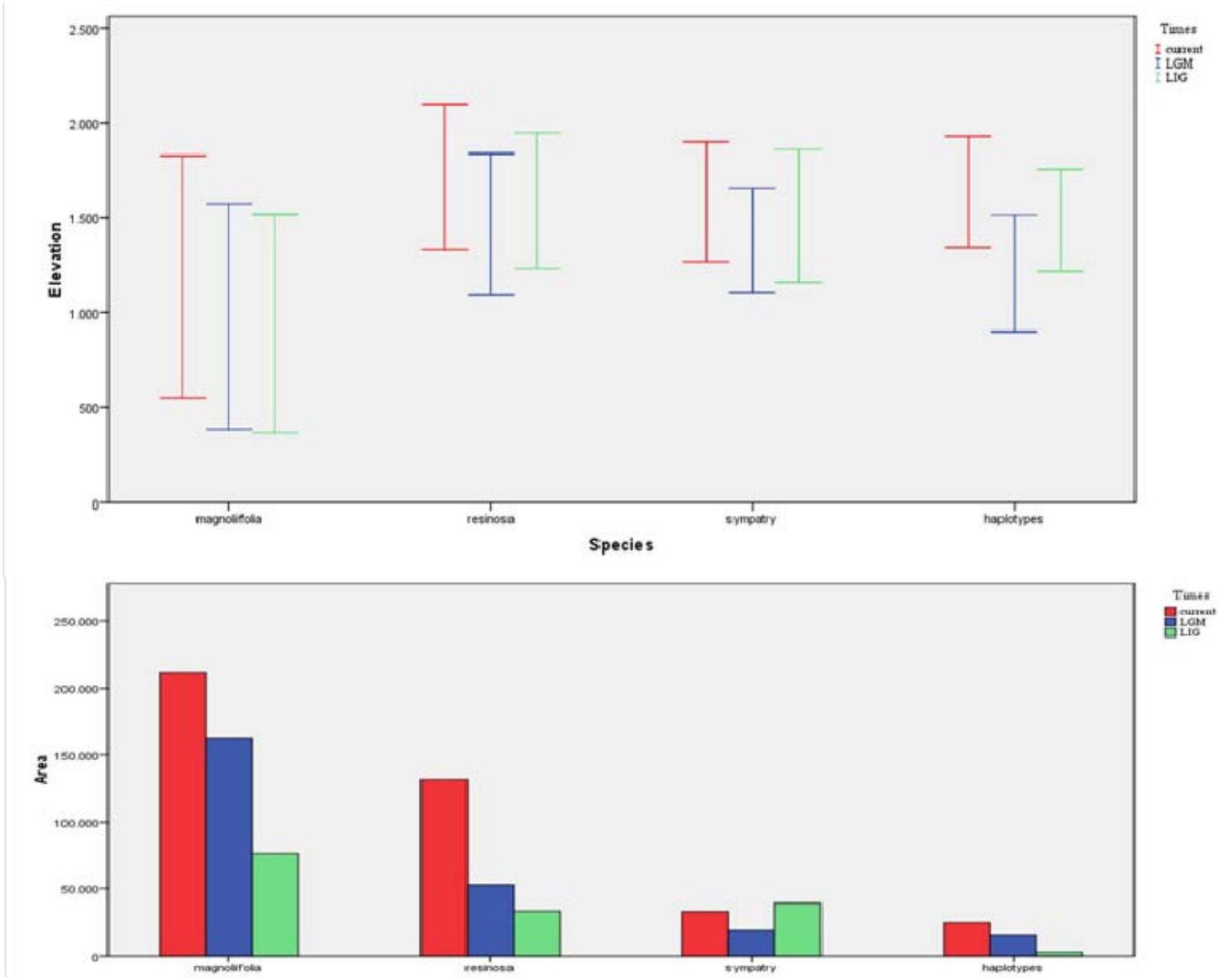

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963 Fig. 6 Intersection of all distribution models with a raster digital elevation model

964 (above), and percentage of gains and losses distribution area during the LGM and LIG

965 models (below).

VIII. DISCUSIÓN GENERAL

Cada uno de los tres capítulos de la tesis nos permitió contestar preguntas específicas que refieren a diferentes escalas geográficas y de tiempo (histórico y contemporáneo), los cuales muestran que los patrones de estructura genética y variación morfológica es diferente dentro de una zona hibrida que a lo largo de toda la distribución geográfica de las especies. Los patrones de flujo génico inter-específico entre *Q. magnoliifolia* y *Q. resinosa* muestran niveles de introgresión nuclear mucho más altos que a nivel del ADN de cloroplasto (ADNcp), a lo largo de su distribución en México. Estos patrones de flujo génico contrastan con los encontrados en especies de *Helianthus*, en donde el flujo génico citoplasmático es aproximadamente 10 veces mayor al flujo génico nuclear, incluso marcadores citoplasmáticos foráneos son algunas veces encontrados a cientos de kilómetros de donde está la zona híbrida actualmente, en ausencia de introgresión nuclear (Rieseberg et al. 1990, 1991). En plantas, la introgresión es a menudo más fácilmente observado para genes de herencia materna que para genes de herencia biparental (Arnold 1997). Los altos niveles de introgresión nuclear que citoplasmática entre *Q. magnoliifolia* y *Q. resinosa*, pueden deberse a su historia evolutiva durante los cambios climáticos pasados (Fig. 4, cap. VII).

Hay tres tipos de desviación de la simétrica bilateral un organismo: la asimetría fluctuante (FA), la asimetría direccional (DA) y la antisimetría (AS) (Van Valen 1962; Palmer and Strobeck 1986; 1992). La FA es la desviación aleatoria de la simetría bilateral perfecta, se mide como la variación entre el dado izquierdo-derecho (I-D) esta diferencia se distribuyen cerca de cero. La DA ocurre cuando un lado de un carácter es consistentemente más grande que el otro, la diferencia entre izquierda-derecha (ID) se distribuyen alrededor de una media que es significativamente mayor o menor que cero.

La AS es una situación rara, donde la asimetría está presente normalmente, se caracteriza por una distribución platikurtica (amplio pico unimodal) o bimodal de las diferencias de izquierda-derecha (LR) cerca de una media de cero (Van Valen, 1962; Palmer y Strobeck 1986, 1992). La AF corresponde a una variación aleatoria, por lo que, se puede utilizar para medir la inestabilidad del desarrollo, mientras que DA se consideren inapropiados como descriptores de la estabilidad del desarrollo, porque implica asimetrías con base genética que puede ser adaptativo (Van Valen, 1962; Palmer y Strobeck 1986, 1992). Entre los factores que pueden generar inestabilidad durante el desarrollo son los factores exógenos (p. ej. estrés ambiental) y endógenos (p. ej. disrupción del complejo de genes). La hibridación genera disrupción del complejo coadaptado de genes de cada uno de los linajes involucrado, lo cual puede generar inestabilidad en el desarrollo (ID) (Soulé 1967; Levin 1970; Graham and Felley 1985; Wilsey et al. 1998; Hochwender and Fritz 1999; Siikämaki 1999), y dependiendo de la cercanía filogenética entre las especies parentales los híbridos van a presentar mayor o menor ID, es decir especies cercanamente relacionadas menor AF y especies lejanas mayor AF (Markow 1995; Alibert and Auffray 2003).

En el primer capítulo encontramos que la asimetría observada en los individuos de *Q. magnoliifolia* y *Q. resinosa*, en la zona hibrida del volcán de Tequila, se debía a la asimetría fluctuante (AF) y no a la DA o a la AS. Además encontramos que el efecto de la hibridación sobre la asimetría fluctuante (AF) tiene una base genética, en donde, el nivel de asimetría fluctuante foliar tuvo una correlación más alta con la proporción de ancestría genética de cada individuo que con la morfología de las hojas o la altitud. Los valores de AF más bajos fueron en los individuos parentales, después las retrocruzas y los valores más altos de AF fueron en los híbridos F1, apoyando la hipótesis genética,

debido a que las retrocruzas es producto de la recombinación de un individuo híbrido F1 con uno de los parentales, por lo que, la información genética del parental aumenta y con ello disminuye la disrupción de los genes coadaptados. Por lo tanto, la AF observada en *Q. magnoliifolia* y *Q. resinosa* en la zona híbrida del volcán de Tequila está más relacionada con la hibridación que con las condiciones ambientales. Patrones similares fueron encontrados en *Betula pubescens*, *B. nana* and *B. pendula* (Wilsey et al. 1998), *Lychnis alpina* y *L. viscaria* (Siikamäki 1999), *Salix sericea* y *S. eriocephala* (Hochwender and Fritz 1999), en donde los valores más altos de AF fueron debidos a la hibridación que al estrés ambiental. Nuestro trabajo es el primero realizado en especies de encinos que aborda el efecto de la hibridación sobre la inestabilidad del desarrollo, a pesar de que los encinos modelos idóneos para estudiar la hibridación e introgresión (Futuyma 2005).

Las gradillas de deformación de la asimetría fluctuante de la forma de la hoja (Fig. 5, cap. V), es una opción gráfica de mostrar en que parte de la hoja la asimetría fluctuante esta actuando y con que intensidad. Los círculos rojos son cada uno de los 29 puntos homólogos o landamarks utilizados en el análisis de la variación morfológica. Las flechas azules indican la dirección y la intensidad de la asimetría, es decir cuando varias flechas señalan hacia un mismo lado indican un alto grado de asimetría en esa parte del carácter analizado.

Los niveles de hibridación e introgresión, variación morfológica y la estructura genética es diferente dentro de la zona híbrida del volcán de Tequila que a lo largo de toda la distribución de las especies (ver capítulo dos). En la zona híbrida del volcán de Tequila, encontramos individuos morfológica y genéticamente identificados como puros *Q. magnoliifolia*, *Q. resinosa*, híbridos F1 e introgresos, lo cual, apoya la hipótesis de

hibridación por contacto secundario. En la zona híbrida la morfología se correlacionó fuertemente con la altitud, mostrando una clina morfológica, es decir, la selección exógena está determinando el establecimiento fenotípico de los parentales en los extremos, en donde *Q. magnoliifolia* ocupa las altitudes más bajas, *Q. resinosa* las altitudes más altas y a altitudes intermedias se encuentran los fenotipos intermedios. Lo cual contrasta con la débil correlación entre los genotipos (puros e híbridos) y la altitud, sin embargo, los genotipos de *Q. resinosa* predominaron a grandes altitudes (1600 a 2100m) y los genotipos de *Q. magnoliifolia* a lo largo de todo el gradiente (1400 a 2100m), sin embargo, el mayor número de genotipos se encontró a bajas altitudes entre 1600 a 1700m, lo cual sugiere que la selección exógena no es tan fuerte dentro de la zona, posiblemente porque estas especies cubren estas altitudes a lo largo de su distribución en México. Lo que es sumamente interesante es que el número de genotipos híbridos F1 y retrocruzas supera por mucho el número de genotipos parentales puros, además estos genotipos híbridos están bien distribuidos a lo largo del todo el gradiente, lo cual sugiere que los genotipo híbridos son mucho más aptos que los parentales dentro de la zona de hibridación. Ecológicamente el volcán de tequila está formado por un bosque de pino-encino muy conservado y geológicamente el volcán de Tequila es considerado un volcán joven activo recientemente (Ferrusquía-Villafranca 1993), formado durante un periodo de vulcanismo silícico en la Faja Volcánica Trans-Mexicana (FVTM) durante el Mioceno tardío y Plioceno tardío (7 Ma) (Rossotti et al. 2002). Estas características especiales de tipo de suelo, tipo de vegetación y que fue un hábitat relativamente reciente para estas especies permitió el establecimiento de la zona híbrida.

En cambio en el segundo capítulo, el análisis morfológico de las 60 poblaciones de *Q. magnoliifolia* y *Q. resinosa*, a lo largo de toda su distribución en México, no muestra individuos con características morfológicas intermedias, a pesar de que el análisis genético muestra altos niveles de flujo génico inter-específico (Fig. 3, cap. 2). La ausencia de individuos morfológicamente intermedios fuera de la zona de hibridación puede deberse a su baja adecuación fenotípica y ser seleccionados en contra, debido principalmente a que estas dos especies son ecológicamente diferentes y están bien adaptadas a las condiciones climáticas y ecológicas presentes a lo largo de su distribución en México (Figs. 5 y 6, cap. 2). El mantenimiento de la diferenciación morfológica y ecológica entre especies que presentan altos niveles de introgresión, ha sido ampliamente explicado desde un punto de vista genómico (Bodénès *et al*. 1997; Wu 2001; Petit *et al*. 2003; Scotti-Saintagne *et al.* 2004; Minder & Widmer 2008), en la cual pequeñas secciones del genoma asociados con la diferenciación inter-específica se mantiene aisladas del flujo génico e introgresión debido a la selección divergente, mientras que las otras partes del genoma son altamente porosas a la introgresión (Bodénès *et al*. 1997; Wu 2001; Petit *et al*. 2003; Scotti-Saintagne *et al.* 2004; Minder & Widmer 2008). La diferenciación morfológica y ecológica de *Q. magnoliifolia* y *Q. resinosa*, a pesar de los altos niveles de flujo génico inter-específico e introgresión, puede ser explicado por la selección divergente en un limitado número de loci los cuales acumulan divergencia manteniendo los límites entre las especies. En el caso de los encinos es muy frecuente encontrar discrepancia entre los caracteres morfológicos que se mantiene diferentes y los marcadores moleculares que presentan altos niveles de introgresión reflejando bajos niveles de divergencia genética entre las especies (Kremer *et al*. 2002; Dodd & Afzal-Raffi 2004; González-Rodríguez *et al*. 2004; Salvini *et al*. 2009).

La diferenciación inter-específica fue baja entre poblaciones geográficamente cercanas que entre poblaciones distantes, es decir, las poblaciones de *Q. magnoliifolia* y *Q. resinosa*, cercanas geográficamente son más parecidas entre ellas que entre sus con-específicos más lejanos. Este patrón sigue el modelo de flujo génico de aislamiento por distancia (Tabla 4, cap. 2). Este patrón de aislamiento por distancia apoya la hipótesis de que *Q. magnoliifolia* y *Q. resinosa*, comparten un gran número de alelos debido al flujo génico inter-específico más que a polimorfismos ancestrales compartidos. Recientemente se ha discutido el papel del flujo génico intra-específico en la modulación de la introgresión (Petit y Excoffier 2009), debido a la disrupción de un patrón normal de apareamiento tal vez debido a la baja densidad de sus compañeros con-específicos (Hubbs 1955), se favorece el flujo génico inter-específico.

los barrios más fríos y secos (BIO BIO 11 y 9), y la temperatura mínima del mes más frío (BIO 6)

the temperature in the coldest and driest quarters (BIO 11 and BIO 9), and the minimum temperature of the coldest month (BIO 6).

La variación de temperatura es la variable ambiental que separa el nicho de *Q. magnoliifolia* y *Q. resinosa* (Fig. 6). El nicho ecológico de *Q. magnoliifolia* muestra que es una especie que tolera altas temperaturas en la estación más fría y seca y no tiene estacionalidad marcada. Estas características son congruente con la descripción de Rzedowski (1978), el cual considera a *Q. magnoliifolia* como una especie de transición ecológica entre los bosques tropicales secos y los bosques de pino-encino. El nicho ecológico de *Q. resinosa* muestra que es una especie tolerante a bajas temperaturas en la estación más fría y seca y tiene estacionalidad marcada. Estas características son

congruentes con la descripción del hábitat de McVaugh (1974), el cual considera que *Q. resinosa* habita en matorrales xerófilos y bosques de pino-encino.

Lo novedoso de nuestro trabajo es que al integrar la información del modelado de nicho ecológico de las especies junto con nuestros datos genéticos de la proporción de mezcla entre las especies, nos permitió entender que la distribución geográfica de las especies y las características ambientales están moldeando los patrones de introgresión entre *Q. magnoliifolia* y *Q. resinosa* en México(Figs. 4, 8, 9 y 10).

Altos niveles de diversidad genética total fueron encontrados en las poblaciones de *Q. magnoliifolia* y *Q. resinosa*, a lo largo de su distribución, lo cual puede ser explicado por la introgresión, debido a nuevas combinaciones de alelos o a la introducción de alelos de una especie en el reservorio genético de la otra especie (Stebbins 1959, Lewontin y Birch 1966; Rieseberg 1997).

Durante los últimos 400000 años el clima en la Tierra se ha caracterizado por tener fluctuaciones muy marcadas en ciclos de ~100000 años. Estos ciclos se manifiestan con la alternancia de etapas más frías que el clima actual (en promedio, 8 °C menos), conocidas como glaciales, y etapas en las que el clima es similar o un poco más cálido (2 ° a 3 °C mayor) que el presente, conocidas como interglaciales (Caballero et al. 2010). El llamado Último Máximo Glacial (UMG, ~20,000 años AP) representa el momento más reciente en el que los grandes glaciares alcanzaron sus máximos volúmenes (Mix *et al*., 2001), se caracterizó también por un descenso en el nivel del mar de ~130 m en relación al actual (Clark *et al*., 2009). El Último Interglacial Máximo (UIM; ~140,000 años AP; Otto-Bliesner et al. 2006) se caracterizó por un clima cálido o más cálido que el presente (~ 2 a 3°C más caliente) (Caballero et al. 2010), representada por un bajo volumen global de hielo y un nivel del mar alto (Kukla, 2002).

En el tercer capítulo, los modelos de distribución muestran que los cambios climáticos pasados durante el UMG y UIM afectaron la distribución altitudinal de *Q. magnoliifolia* y *Q. resinosa*, con movimientos hacia altitudes más bajas durante el UMG y movimientos hacia altitudes más altas durante los periodos más cálidos del UIM con excepción de *Q. magnoliifolia* (Fig. 3, cap. 3), la cual tuvo que moverse a altitudes aun más bajas durante el UIM, debido a que *Q. resinosa* ocupó las altitudes más altas durante este periodo. Estos movimientos altitudinales favorecieron la simpatría entre las especies. Los modelos de distribución también muestran contracción en el área de distribución de *Q. magnoliifolia* y *Q. resinosa*, durante el UGM y UIM (Fig. 3, cap. 3), siendo el UGM el periodo de mayor contracción en la simpatría de las especies, caso contrario, durante el UIM se presentó la mayor área de simpatría entre las especies, debido principalmente al movimiento latitudinal de *Q. resinosa* de la Planicie Central a la Sierra Madre del Sur y oeste de la FVTM (Fig. 5, cap. 3). En el caso de las áreas de distribución de la diversidad de haplotipos compartidos, muestran un incremento desde el UIM alcanzando su máxima área de distribución actualmente (Figs. 3 y 5, cap. 3). Las areas potenciales de simpatría estuvieron estrechamente relacionadas con la diversidad de haplotipos compartidos. Nuestros resultados contrastan con los encontrados en otras especies de encinos rojos Mexicanos como son, el complejo *Q. hypoleucoides*, *Q. scytophylla* and *Q. sideroxyla*, en los cuales sus modelos de distribución muestran expansión en sus áreas de distribución durante el UGM y una reducción durante los periodos cálidos actuales (Peñaloza-Ramírez et al. in press), en el complejo *Q. affinis*-*Q. laurina*, la variación del ADN de cloroplasto (ADNcp) muestran una débil estructura filogeográfica y una completa ausencia de especificidad de los haplotipos del ADNcp, lo cual sugiere que durante el UGM experimentaron expansiones de rango, debido a que son especies de climas templado, por lo cual fueron

favorecidas (González-Rodríguez *et al*. 2004). Los cambios climáticos pasados pudieron llegar a afectar la composición genética de las especies, en función de sus afinidades ecológicas (Moreno-Letelier y Piñero 2009). En este sentido, *Q. magnoliifolia* es una especie de clima cálido subhúmedo a semicálido subhúmedo, y *Q. resinosa* es una especie de clima semicálido subhúmedo a templado subhúmedo, mientras que durante las fases glaciales hubo descenso de la temperatura, aumento de las precipitaciones en algunas regiones, y con un clima frio y seco en el centro de México, lo cual podría estar explicando las contracciones en las áreas de distribución, los cambios altitudinales, la fuerte estructura filogeográfica y el bajo número de haplotipos compartidos. En Europa evidencias de la variación del ADNcp, muestran que muchas especies comparten una gran cantidad de haplotipos, ya sea entre especies filogenéticamente cercanas como son *Fraxinus angustifolia* y *F. excelsior*, lo cual sugiere hibridación ya sea en refugios glaciales y/o durante la recolonización posglacial (Heuertz et al. 2006).

Los datos filogeográficos (red de haplotipos en forma de estrella) y demográficos de *Q. magnoliifolia* y *Q. resinosa*, muestran expansión poblacional (Fig. 3, cap. 3). La transición del UGL al Holoceno es hacia un clima más cálido, por lo que, las características climáticas de estas especies, así como los modelos de distribución actual soportan la hipótesis de crecimiento demográfico.

En conclusión, el presente trabajo es el primer trabajo realizado entre dos especies de encinos blancos mexicanos, *Q. magnoliifolia* y *Q. resinosa* a lo largo de toda su distribución, utilizando morfometría geométrica, modelado de nicho ecológico y marcadores moleculares nucleares y citoplasmáticos, con el objetivo de conocer la historia evolutiva histórica y contemporánea de estas dos especies. *Q. magnoliifolia* y

Q. resinosa son dos especies morfológica y ecológicamente diferentes que mantienen su identidad a pesar de tener altos niveles de flujo génico inter-específico e introgresión, debido posiblemente a la selección divergente. Los patrones de variación morfológica y los patrones de flujo génico intra- e inter-específicos fueron diferentes dentro de la zona de hibridación que a lo largo de su distribución geográfica. La baja diferenciación genética entre *Q. magnoliifolia* y *Q. resinosa* se debe a altos niveles de flujo génico inter-específicos debido al aislamiento por distancia. Encontramos niveles mucho más altos de introgresión nuclear que citoplasmática. Cada uno de los tres capítulos hace contribuciones importantes e innovadoras en el estudio de las especies de encinos así como de la dinámica de las zonas hibridas e introgresión y del efecto de los cambios climáticos pasados en la distribución de la diversidad genética y geográfica de las especies y como estos cambios influyeron en los patrones de intercambio citoplasmático inter-especifico. La hibridación e introgresión es una fuente importante de variación genética que les ha ayudado a las especies de encino a responder ante los cambios climáticos pasado y muy posiblemente ayude a responder ante los cambios climáticos futuros, en donde especies con afinidades climáticas más cálidas posiblemente se vean aun más favorecidas como es el caso de *Q. magnoliifolia* y *Q. resinosa.*

IX. REFERENCIAS GENERALES

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