UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE INVESTIGACIONES EN ECOSISTEMAS Y SUSTENTABILIDAD
BIOLOGÍA EVOLUTIVA

VARIACIÓN ECOFISIOLÓGICA Y GENÉTICA ENTRE LOS ENCINOS ROJOS MEXICANOS *QUERCUS AFFINIS* SCHEIDW Y *Q. LAURINA* HUMB. ET BONPL (FAGACEAE) Y SUS HÍBRIDOS

TESIS

QUE PARA OPTAR POR EL GRADO DE:
DOCTORA EN CIENCIAS

PRESENTA:
SELENE RAMOS ORTIZ

TUTOR PRINCIPAL DE TESIS: DR. ANTONIO GONZÁLEZ RODRÍGUEZ
INSTITUTO DE INVESTIGACIONES EN ECOSISTEMAS Y SUSTENTABILIDAD, UNAM

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MÉXICO, D.F., SEPTIEMBRE 2015

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UNAM
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Ciencias Biológicas

COORDINACIÓN

Dr. Isidro Ávila Martínez
Director General de Administración Escolar, UNAM
Presente.-

Por medio de la presente, me permito informar a usted, que en reunión ordinaria del Subcomité por Campo de Conocimiento (Biología Evolutiva y Sistemática) del Posgrado en Ciencias Biológicas, se aprobó el siguiente jurado para el examen de grado de **Doctora en Ciencias** de la alumna **SELENE RAMOS ORTIZ** con número de cuenta **505016995** con la tesis titulada **"Variación ecofisiológica y genética entre los encinos rojos mexicanos *Quercus affinis* Scheidw y *Q. laurina* Humb. Et Bonlp (Fagaceae) y sus híbridos"**, bajo la dirección del **Dr. Antonio González Rodríguez**, Tutor Principal.-

Presidente:	Dr. Juan Servando Núñez Farfán
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Suplente:	Dr. Alberto Ken Oyama Nakagawa

Sin otro particular, quedo de usted.

Atentamente
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Cd. Universitaria, D.F., a 21 de agosto de 2015

Dra. María del Coro Arizmendi Arriaga
Coordinadora del Programa

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DEDICATORIA

A mí Sol

Porque en ella y a través de ella
Tuvo inicio mi vida compartida,
Convirtiendose en mi inspiración
A volar…

A mí Amaya

Porque me hizo trascender
Y aceptar por siempre,
Que mi corazón
Ande vagando fuera de mí cuerpo…

¿Has visto cómo crecen las plantas? Al lugar en que cae la semilla acude el agua: es el agua la que germina, sube al sol. Por el tronco, por las ramas, el agua asciende al aire, como cuando te quedas viendo el cielo del mediodía y tus ojos empiezan a evaporarse.

Las plantas crecen de un día a otro. Es la tierra la que crece, se hace blanda, verde, flexible. El terrón enmohecido, la costra de los viejos árboles, se desprende, regresa.

¿Lo has visto? Las plantas caminan en el tiempo, no de un lugar a otro, de una hora a otra hora. Esto puedes sentirlo cuando te extiendes sobre la tierra, boca arriba y tu pelo penetra como un manojo de raíces y toda tú eres un tronco caído.

Jaime Sabines

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RESUMEN

La hibridación es un fenómeno natural que ocurre con frecuencia en las plantas y animales, ésta implica el apareamiento exitoso en la naturaleza entre individuos de poblaciones que pertenecen a especies que son ecológica, morfológica y fisiológicamente diferentes. El género *Quercus* (encinos) destaca por una alta frecuencia de hibridación, aún entre especies lejanamente relacionadas o distintas en morfología y requerimientos ecológicos. En esta tesis se llevó a cabo un análisis de la hibridación natural en el complejo de encinos rojos mexicanos formado por *Quercus affinis* y *Q. laurina*, desde el punto de vista de la caracterización de la estructura geográfica y genética de la zona de hibridación entre estas dos especies mediante marcadores moleculares neutros (nueve microsatélites nucleares y cuatro de cloroplasto) y nueve caracteres foliares y de bellotas. Adicionalmente, se realizó una comparación del desempeño fisiológico (tasa fotosintética, conductancia estomática, transpiración, tasa de crecimiento relativo) de individuos de ambas especies e individuos híbridos en condiciones óptimas en un jardín común y bajo un experimento de estrés hídrico.

Los resultados indicaron que la estructura geográfica de la zona de hibridación presenta algunas características de una zona de tensión pero con patrones de variación heterogéneos entre los caracteres fenotípicos y genéticos, lo que sugiere introgresión diferencial; existiendo fuerzas específicas que están actuando diferencialmente sobre caracteres particulares y que la selección extrínseca (dependiente del ambiente) ha tenido un importante papel en la estructuración de esta zona de hibridación.

En el jardín común, bajo condiciones óptimas, los híbridos mostraron un desempeño fisiológico menor que las especies progenitoras. Sin embargo, en condiciones de estrés hídrico, los híbridos mostraron mayor sobrevivencia y capacidad de recuperación.

En conjunto, los resultados de la investigación sugieren que la variación espacial y temporal en las presiones de selección natural (intrínsecas y extrínsecas) han favorecido el mantenimiento de genotipos con diversos grados de mezcla genética en la zona de hibridación entre *Quercus affinis* y *Q. laurina*, y que la hibridación ha jugado un papel ecológico y evolutivo muy activo en estas especies.

Palabras clave: hibridación, *Quercus*, nicho, microsatélites, selección natural, jardín común

ABSTRACT

Hybridization is a natural phenomenon that often occurs in plants and animals; this involves the successful mating in nature between individuals from populations belonging to species that are ecologically, morphologically and physiologically different. The genus *Quercus* (oaks) is characterized by a high frequency of hybridization, even among species that are different in morphology and ecological requirements. This thesis conducted an analysis of natural hybridization in the complex formed by the Mexican red oaks *Quercus affinis* and *Q. laurina*, from the point of view of the characterization of the geographic and genetic structure of the hybrid zone between these two species by using neutral molecular markers (nine nuclear microsatellite and chloroplast four) and nine phenotypic characters leaf and acorns. Additionally, a comparison of physiological performance (photosynthetic rate, stomatal conductance, transpiration, relative growth rate) among individuals of both species and hybrids under optimal conditions in a common garden and under water stress conditions was performed.

The results indicated that the geographical structure of the hybrid zone has some characteristics of a tension zone, but with heterogeneous patterns of variation between phenotypic and genetic characters, suggesting differential introgression. Probably, there are specific forces that are differentially acting on particular characters and extrinsic selection (dependent on the environment) has an important role in structuring this hybrid zone.

In the common garden, under optimal conditions, the hybrids showed a lower physiological performance than progenitor species. However, under conditions of water stress, the hybrids showed higher survival and resilience.

Together, the research results suggest that spatial and temporal variation in the pressures of natural selection (intrinsic and extrinsic) have favored the maintenance of genotypes with varying degrees of genetic admixture in the hybrid zone between *Quercus affinis* and *Q. laurina*, and that hybridization has played a very active role in ecological and evolutionary trends of these species.

Keywords: Hybridization, *Quercus*, niche, microsatellites, natural selection, common garden

CAPÍTULO I

INTRODUCCIÓN GENERAL

La hibridación natural

La hibridación es un fenómeno natural extremadamente común en las plantas y poco común entre los animales (Harrison, 1993). Este fenómeno implica el apareamiento exitoso en la naturaleza entre individuos de poblaciones que pertenecen a especies que son ecológica, morfológica, genética y fisiológicamente diferentes (Arnold, 1992). La hibridación puede llevar a la formación de nuevas especies, a la fusión de especies existentes o afectar los niveles de variación genética, la adaptación local, y la eficacia de la selección natural (Arnold, 1992; Allendorf *et al.,* 2001; Aldrich *et al.,* 2003b; Coyne y Orr, 2004; Riesberg y Willis, 2007; Hipp y Weber, 2008; Lorenzo *et al.,* 2009).

Por definición, un híbrido es un individuo descendiente de progenitores pertenecientes a poblaciones diferenciadas en por lo menos un carácter heredable.

En general, la hibridación puede dar lugar a cinco tipos de consecuencias evolutivas: (1) completar el aislamiento reproductivo entre dos especies; (2) derivar en la formación de un zona de hibridación, donde los híbridos F1 se limitan a un área geográfica; (3) resultar en algún grado de introgresión entre las dos especies en un área más amplia donde los individuos puros pertenecientes a las especies progenitoras siguen siendo los dominantes; (4) presentar en la mayoría de los individuos de la zona híbrida morfología intermedia y/o características genéticas mixtas (Harrison, 1993; Arnold, 1997) y finalmente (5) la especiación, la cual se lleva a cabo cuando las barreras reproductivas entre ambas especies progenitoras se fortalecen (Stebbins, 1959; Grant, 1981; Rieseberg, 1997; Soltis y Soltis, 2004; Mallet, 2005).

Sin embargo, menos estudiado ha sido el caso en el que las especies se mantienen morfológicamente diferentes, sin que ocurra reforzamiento de las barreras reproductivas ni fusión de los taxa, a pesar del flujo génico recurrente entre ellas, vía hibridación e introgresión (Gömöry *et al.,* 2001; Coart *et al.,* 2002; Dodd *et al.,* 2003; Petit *et al.,* 2003; González-Rodríguez *et al.,* 2005; Muir y Schlotterer, 2005; Craft y Ashley, 2006; Lexer *et al.,* 2006; Curtu *et al.,* 2007; Gugerli *et al.,* 2007).

Otro aspecto importante del estudio de la hibridación es discernir el origen de las zonas de hibridación, las cuales se pueden definir como zonas de hibridación primarias y secundarias (Hewitt, 1988). Las zonas de hibridación primarias se forman cuando individuos de una misma especie se diferencian morfológicamente a lo largo de su distribución geográfica, debido a que la selección actúa en diferentes direcciones en un gradiente ambiental, produciendo una clina morfológica que no representa linajes evolutivos diferentes (Hewitt, 1988; Holman *et al.,* 2003; Seehausen, 2004). La hibridación por contacto secundario ocurre cuando dos especies que han permanecido geográficamente aisladas, posteriormente se encuentran en simpatría, formando una zona de contacto donde hibridan (Hewitt, 1988; Ishida *et al.,* 2003).

El género Quercus

El género *Quercus* (encinos) pertenece a la familia Fagaceae, que destaca a nivel mundial por su alta riqueza de especies, así como por su relevancia ecológica y económica. El género de los encinos es el más diverso dentro de esta familia y representa uno de los grupos de árboles caducifolios y perennifolios más ampliamente distribuido e importante en los bosques del hemisferio norte (Kaul, 1985).

El género *Quercus* se divide en dos subgéneros, *Cyclobalanopsis* y *Quercus*. Dentro de este último se reconocen cuatro secciones: *Cerris, Lobatae* (encinos rojos), *Protobalanus* (encinos intermedios) y *Quercus* (encinos blancos). Estas tres últimas secciones se encuentran en México (Nixon, 1989; Manos *et al.,* 1999).

La riqueza específica total para el género *Quercus* es de unas 400 especies en el mundo (Nixon *et al.,* 1997); otros reportan aproximadamente 531 especies (Govaerts y Frodin, 1998) siendo el más reciente de 500 especies (Manos *et al.,* 1999), se estiman cerca de 200 en el Continente Americano (McVaugh, 1974), en tanto que para México, la estimación más reciente comprende 161 especies de las que 109 son endémicas (61 de encinos rojos, 47 de blancos y una de los encinos intermedios) (Valencia-Ávalos, 2004).

Prácticamente hay encinos en todas las entidades del país, con excepción de los estados de la Península de Yucatán; en un gradiente altitudinal que va desde el nivel del mar (e.g. *Q. oleoides*) hasta por encima de los 3500 m (e.g. *Q. laurina*) (Montoya, 1966; Zavala, 1990); aunque más del 95 % de las especies mexicanas se encuentran entre los 1200 y 2800 m (Rzedowski, 1978). Además, existen encinos en lugares de clima muy diverso, desde el cálido con época seca más o menos larga, el frío semiárido extremoso y hasta en hábitats relativamente xéricos (Montoya, 1966; García-Arévalo y González-Elizondo, 1991; Zavala, 1998).

Rzedowski (1978) menciona que los encinos se encuentran mejor representados en las zonas montañosas de México, principalmente en la Sierra Madre Occidental, Sierra

Madre Oriental, Eje Neovolcánico Transversal, Sierra Madre del Sur, Sierras del Norte de Oaxaca, de Chiapas y Baja California.

Los encinos a su vez, tienen un valor ecológico importante, son pioneros de la vegetación secundaria, considerándose especies formadoras de suelo, además de albergar un gran número de plantas epífitas y animales invertebrados y vertebrados (Bello y Labat, 1987).

Muchas especies tienen importancia económica por su madera de excelente calidad, su corteza y sus frutos (bellotas). No obstante se ha hecho un mal uso de este recurso, menospreciando el valor real que representa para México y el mundo (Rzedowski, 1981; Zavala, 1990).

Diversificación del género Quercus

Las evidencias fósiles, filogenéticas y biogeográficas sugieren que la diversificación a nivel género en la familia Fagaceae ocurrió en el sureste asiático (Manos *et al.,* 2001; Manos y Standford, 2001). Un posible escenario es que las especies ancestrales de *Quercus*, probablemente perennifolias, migraron a través de los Puentes Terrestres del Atlántico Norte durante el Eoceno tardío y Oligoceno temprano hace aproximadamente 40-30 millones de años (MA), hasta alcanzar una distribución amplia y continua. Un evento inicial de vicarianza pudo haber ocurrido debido al cierre de los Puentes Terrestres del Atlántico, dejando dos grandes grupos de especies aislados en Euroasia y Norteamérica, respectivamente (Manos y Stanford, 2001). Posteriormente, los climas más fríos, estacionales y secos que se desarrollaron entre el Oligoceno y el Mioceno, hace aproximadamente 30-20 MA, favorecieron la migración y diversificación de los encinos que pertenecen al grupo de plantas con polinización por viento (Axelrod, 1983; Daghlian y Crepet, 1983; Borgardt y Pigg, 1999).

Las secciones *Quercus*, *Lobatae* y *Protobalanus* probablemente evolucionaron en las latitudes medias de Norteamérica durante el Oligoceno. Por lo que, se puede afirmar que el género colonizó México en dirección Norte-Sur desde las latitudes medias de Norteamérica, sugiriendo que la mayor parte de las especies de encinos mexicanos evolucionaron dentro de la región que hoy es nuestro país (Nixón, 1993b; Graham, 1999).

Hibridación en el género Quercus

El género *Quercus* se ha convertido en años recientes en uno de los modelos más importantes para el estudio de la hibridación en plantas (Petit *et al.,* 2003; Lexer *et al.,* 2006; Gugerli *et al.,* 2007). En los encinos, los datos morfológicos y cruzamientos controlados han sugerido hibridación generalizada, lo que ha llevado a cuestionar la utilidad del concepto de especie biológica para estos y otros taxones híbridos (Palmer, 1948; Burger, 1975; Rushton, 1993; Nixon, 2006).

Numerosos estudios a nivel de genética poblacional en los que se han utilizado marcadores moleculares neutros como son las isoenzimas y los microsatélites, han revelado que especies relacionadas de encinos generalmente muestran niveles de diferenciación genética interespecífica extremadamente bajos. Esta observación generalmente se ha explicado como un resultado de altos niveles de flujo génico entre las especies de encinos, a través de hibridación e introgresión (Whittemore y Schaal, 1991;

Ducousso *et al.,* 1993; Rushton, 1993; Bacilieri *et al.,* 1996; Gömöry *et al.,* 2001; Williams *et al.,* 2001; Coart *et al.,* 2002; Craft et al., 2002; Kremer *et al.,* 2002; Dodd *et al.,* 2003; González-Rodríguez *et al.,* 2004, 2005; Muir et al., 2004; Craft y Ashley, 2006; Hedrick, 2006; Curtu *et al.,* 2007; Gugerli *et al.,* 2007; Jost, 2008).

Aunque en algunos casos, los híbridos muestran fertilidad reducida (Rushton, 1993), en general los encinos híbridos producen semillas y polen viables (Salvini *et al.,* 2009). Las zonas de hibridación se forman con mayor frecuencia entre especies con fuertes diferencias en el requerimiento de hábitat en aquellos sitios en los que existen hábitats intermedios entre las áreas de distribución de los progenitores (Muller, 1952) por ejemplo, el papel del disturbio por acciones antropogénicas en el establecimiento de los híbridos y sus procesos evolutivos ha movido a las plantas a través de largas distancias a un ritmo sin precedentes, lo que ha llevado a aumentar las oportunidades para la hibridación.

En contraste, la introgresión puede facilitarse cuando las especies co-existen en un área con heterogeneidad ambiental a pequeña escala (Rushton, 1993; Valbuena-Carabana *et al.,* 2007).

Un gran número de estudios han examinado la tasa de hibridación en encinos blancos, mediante análisis de paternidad, utilizando semillas que provienen de la madre conocida (Bacilieri *et al.,* 1996b; Streiff *et al.,* 1999; Salvini *et al.,* 2009) o análisis de diferenciación genética entre los árboles adultos (Craft *et al.,* 2002; Muir *et al.,* 2004; Craft and Ashley, 2006; Curtu *et al.,* 2007; Valbuena-Carabaña *et al.,* 2007; Burgarella *et al.,* 2009; Cavender-Bares y Pahlich, 2009; Albarrán-Lara *et al.,* 2010). Sin embargo, pocos estudios han examinado el flujo de genes en encinos rojos, y ninguno ha estimado las tasas de hibridación (Aldrich *et al.,* 2003b; Dodd y Afzal-Rafii, 2004; González-Rodríguez *et al.,* 2004, 2005; Tovar-Sánchez y Oyama, 2004; Hipp y Weber, 2008; Peñaloza-Ramírez *et al.,* 2010).

Los trabajos enfocados en los procesos de hibridación en encinos mexicanos son aún pocos. Se ha reportado la aparición de zonas de contacto e hibridación en algunas especies tanto de encinos rojos como blancos. Nixon (1993) y Spellenberg (1996, 1998) estudiaron posibles eventos de hibridación entre los encinos blancos *Q. depressipes, Q.rugosa* y *Q. basaseachicensis*. De la misma forma, Spellenberg y Bacon (1996) reportaron zonas de contacto e hibridación entre *Q. eduardii* y *Q. conzattii* en la Sierra Madre Occidental*,* analizando caracteres morfológicos de las hojas. González-Rodríguez *et al.* (2004) utilizaron marcadores moleculares y morfológicos para discriminar entre *Q. laurina* y *Q. affini*s (especies cercanamente relacionadas) y sus híbridos, además de describir la estructura genética de la zona de hibridación entre ambas. Tovar-Sánchez y Oyama (2004) estudiaron la hibridación entre *Q. crassipes* y *Q. crassifolia* y confirmaron que el taxón *Q. × dysophylla* es producto de la hibridación entre ambas especies. Albarrán-Lara *et al*. (2010) utilizaron marcadores moleculares y morfometría geométrica foliar para describir la estructura de una zona de hibridación entre *Q. resinosa* y *Q. magnoliifolia*. Finalmente, Peñaloza-Ramírez *et al.* (2010) analizaron la estructura de una zona de hibridación triple entre *Q. hypoleucoides*, *Q. scytophylla* y *Q. sideroxyla* en la Sierra Tarahumara de México.

Análisis de la variación funcional de progenitores e híbridos

El nivel de hibridación e introgresión entre especies relacionadas es una forma potencial de respuesta evolutiva al cambio ambiental que ha sido muy poco explorada (Garroway *et al.,* 2010). La frecuencia de híbridos exitosos en la naturaleza podría verse afectada de diversas maneras por los cambios en las condiciones ambientales. Por ejemplo, el cambio climático podría alterar el grado de solapamiento espacial entre especies relacionadas, de tal manera que se reduciría o incrementaría la oportunidad para la hibridación (Garroway *et al.,* 2010). En consecuencia, este efecto de solapamiento de las áreas de distribución correspondería a un cambio en el aislamiento reproductivo debido a un aumento o reducción de la viabilidad de una especie inmigrante cuando llega a un nuevo hábitat (Nosil *et al.,* 2005).

Otro escenario podría ocurrir de un cambio en las condiciones ambientales en un área de traslape actual entre las dos especies, pudiendo aumentar o disminuir la velocidad a la que se forman los híbridos, por ejemplo, mediante el cambio de la superposición de la fenología reproductiva o alteraciones en el comportamiento de los polinizadores (Aldridge y Campbell, 2007); en este caso, el aislamiento reproductivo precigótico se vería modificado. Un último escenario que influiría en la hibridación, es si un cambio en las condiciones ambientales en una zona de solapamiento altera la capacidad relativa de los híbridos para sobrevivir y/o reproducirse en comparación con las especies progenitoras.

La reducción de la viabilidad y de la reproducción (parcial o la esterilidad completa) son dos mecanismos de aislamiento reproductivo postcigótico (Stebbins, 1947). Las combinaciones de estos mecanismos, así como los otros efectos, tales como la reducción de la producción de híbridos exitosos debido a incompatibilidades genéticas nucleares o citonucleares entre las especies divergentes o la falta de un hábitat adecuado para los híbridos (Fishman y Willis, 2001; Rieseberg y Willis, 2007; Sambatti *et al*., 2008), también pueden ocurrir y afectar su éxito.

Una forma de probar tales efectos potenciales de la variación ambiental sobre la hibridación sería manipular las condiciones ambientales en campo. Sin embargo, sólo algunos estudios han examinado esta respuesta del desempeño de los híbridos tanto en condiciones de jardín común como naturales (Williams *et al.,* 2001; Brock y Galen, 2005; Sherrard *et al.,* 2009; Campbell *et al.,* 2010; Ma *et al.,* 2010; Campbell *y* Wu, 2013)

En el caso de los encinos, hay pocos estudios sobre el desempeño de los híbridos en comparación con las especies progenitoras. Williams y Ehleringer (2000), realizaron un estudio en el que examinaron las relaciones hídricas de *Q. gambelii*, *Q. turbinella* y sus híbridos en condiciones naturales en el sureste de Utah, así como la correlación foliar entre caracteres estructurales y químicos, con el fin de discriminar entre isótopos de carbono, dado que potencialmente los híbridos pueden mostrar un menor nivel de integración funcional debido a la recombinación y el retrocruzamiento. Los resultados del estudio arrojaron que todos los caracteres evaluados en los híbridos mostraron intervalos amplios de variación sobrelapandose con valores intermedios de las poblaciones adyacentes “puras” de los progenitores. Adicionalmente, sí se observaron niveles menores de integración funcional en los híbridos que en las poblaciones puras.

Himrane *et al.* (2004) estudiaron, bajo condiciones de jardín común, la variabilidad de caracteres morfológicos y ecofisiológicos en el híbrido *Q. subpyrenaica* y sus especies progenitoras, *Q. faginea* y *Q. pubescens*, que co -ocurren en el noreste de España y se encontró que los híbridos mostraron un amplio intervalo de valores, que abarcó e incluso superó la variación en los taxones de ambos progenitores.

Swenson *et al.* (2008) realizaron un estudio donde utilizaron datos de caracteres funcionales foliares y análisis de modelación de nicho para examinar si los factores abióticos son los responsables de la localización y estructura de una zona de hibridación entre *Q. gambelii* y *Q. grisea* en el centro y norte de Nuevo México y, en caso afirmativo, determinar qué factores son los más importantes. Los resultados apoyaron la hipótesis de que la disponibilidad de agua determina la localización y estructura de la zona de hibridación. Mostraron además que los individuos de la zona de hibridación tienen mayor eficiencia de uso de agua y tolerancia al estrés híbrido que cualquiera de las dos especies progenitoras. Finalmente, este trabajo es un buen ejemplo de la forma en que los análisis de rasgos funcionales y los modelos de nicho ecológico pueden ser utilizados en las investigaciones sobre zonas de hibridación.

En términos de desempeño, diferentes condiciones ambientales han contribuido a la identificación de los híbridos como posibles portadores de atributos que se asocian a la tolerancia a algún estrés (e.g. Troyer, 2000; Tollenaar y Lee, 2002; David *et al.,* 2007) y podrían formar parte de investigaciones más exhaustivas dirigidas a la identificación de atributos y mecanismos responsables de esta característica (Ghouil *et al.,* 2003; Aranda *et al.,* 2005a; Gandour *et al.,* 2007).

SISTEMA DE ESTUDIO

Especies de estudio

De acuerdo a la clasificación más reciente propuesta por Nixon (1993), los encinos mexicanos *Quercus affinis* Scheidw y *Q. laurina* Humb. et Bonpl (Fagaceae) pertenecen a la sección *Lobatae* (encinos rojos) del subgénero *Quercus*. Valencia-Ávalos (1995) hizo una revisión que reveló 25 nombres aplicados a especies y variedades considerados como sinónimos de *Q. laurina*, mientras que otros nueve sinónimos se han usado para nombrar a *Q. affinis,* condición que hace suponer la gran variación que se presenta en los caracteres morfológicos de dichas especies.

Valencia-Ávalos (1994) sugiere que en general estas especies se pueden diferenciar en condiciones naturales ya que *Q. laurina* tiene hojas más largas (6-15.5 cm) y anchas (3.5-6.5 cm), los peciolos son más largos (3.6-36 mm), el envés de las hojas presenta pubescencia restringida a las axilas de las nervaduras, las yemas son ovoides, la base de la hoja es atenuada o redondeada y las nervaduras son elevadas. *Quercus laurina* se considera una especie de distribución amplia media, pues se encuentra en 10 estados de México: Guerrero, Jalisco, Michoacán, Querétaro, Estado de México, Morelos, Hidalgo, Puebla, Veracruz y Oaxaca, a lo largo de la Sierra Madre del Sur y del Eje Neovolcánico Transversal y altitudinalmente está presente entre los 2440-3065 m (Valencia-Ávalos, 1994, 2004) (Fig. 1).

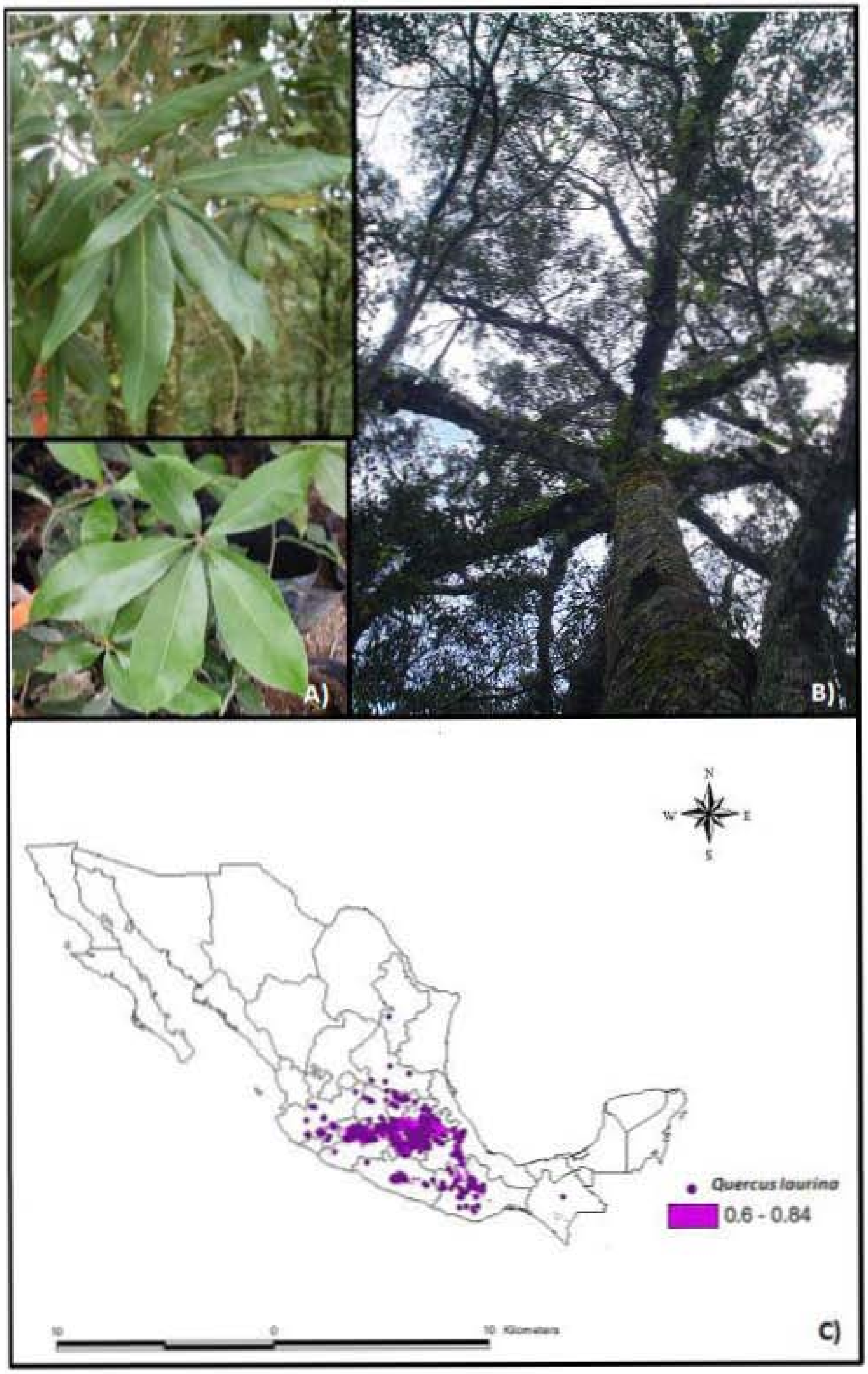

Figura 1. A) Árbol juvenil en jardín común y B) Árbol adulto en campo, representativos de la especie *Quercus laurina*, C) Mapa de distribución potencial de *Q. laurina* en México mediante el programa BIOCLIM basado en datos de colecta de los ejemplares en el herbario MEXU (Fotos S. Ramos-Ortiz).

Por su parte, *Q. affinis* se distingue de *Q. laurina* por la presencia de hojas más pequeñas (3-13.5 cm) y angostas (0.7-4.5 cm), los peciolos más cortos (3-20 mm), el envés de las hojas liso con venas poco notorias y con escasos pelos fasciculados y estipitados en las axilas de las venas y carece de pelos glandulares vermiformes. Sus yemas son conoidales y lustrosas. Se considera una especie de distribución media, pues se encuentra en ocho estados: Guerrero, Hidalgo, Nuevo León, Oaxaca, Puebla, San Luis Potosí, Tamaulipas y Veracruz, principalmente en las elevaciones de la Sierra Madre Oriental y del Eje Neovolcánico Transversal y altitudinalmente está presente entre los 1600-2800 m (Fig. 2).

Las dificultades para realizar la asignación taxonómica de los individuos se incrementa hacia el este del Eje Neovolcánico Transversal y al norte de Oaxaca, donde ambas especies se encuentran en simpatría, observándose árboles con intergradación morfológica, así como con formas de hojas atípicas, lo que sugiere que la hibridación puede explicar lo observado en estas variaciones.

La diferenciación morfológica reportada entre las poblaciones de ambas especies sugiere la posibilidad de que la adaptación a hábitats particulares puede tener un papel importante en la morfología foliar de estas especies, sobre todo porque en otros complejos de encinos se han reportado asociaciones entre fenotipos particulares y hábitats específicos (Kleinschmit *et al.,* 1995; Howard *et al.,* 1997; Bruschi *et al.,* 2003).

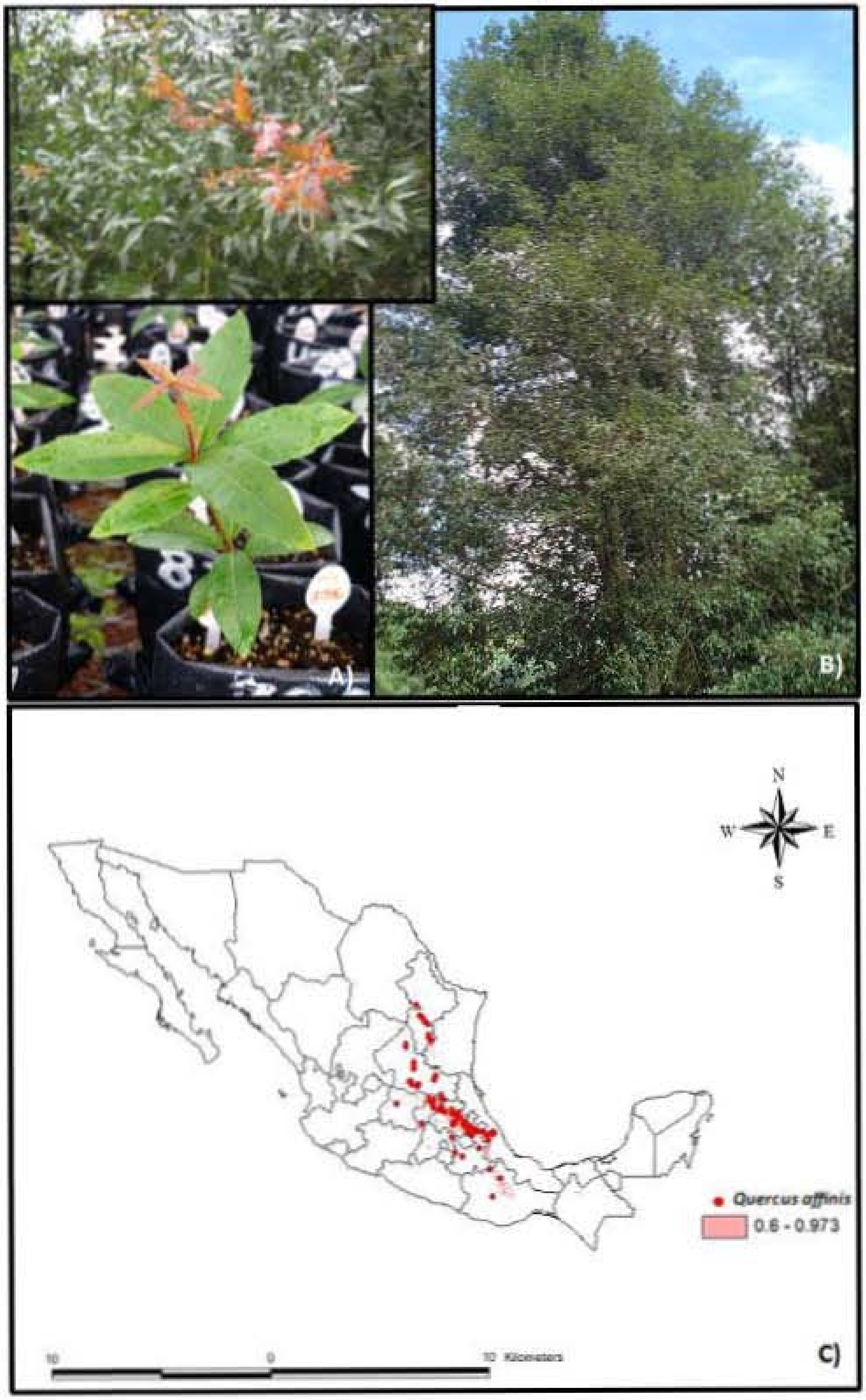

Figura 2. A) Árbol juvenil en jardín común y B) Árbol adulto en campo, representativos de la especie *Quercus affinis*, C) Mapa de distribución potencial de *Q. affinis* en México mediante el programa BIOCLIM basado en datos de colecta de los ejemplares en el herbario MEXU (Fotos S. Ramos-Ortiz).

Valencia-Ávalos (1994), propuso además una hipótesis sobre el origen de la zona de hibridación entre *Q. affinis* y *Q. laurina*, basándose en las inferencias sobre los patrones biogeográficos del género *Quercus* en México, asumiendo que probablemente los cambios climáticos ocurridos durante el Pleistoceno facilitaron periodos de contacto secundario (producto de expansiones y contracciones de las áreas de distribución de ambas especies en el norte de Oaxaca y la porción del este del Eje Neovolcánico Transversal), lo cual tuvo como resultado el rompimiento del aislamiento reproductivo.

En consecuencia, se favoreció la tendencia a la especiación de *Q. affinis* y *Q. laurina* en el caso de aislamiento en respuesta al ambiente y un incremento en la variabilidad de las especies, en el caso del contacto (zonas de hibridación) entre las dos especies.

Esta hipótesis fue puesta a prueba por González-Rodríguez *et al.* (2004, 2005) con la ayuda de caracteres morfológicos y marcadores moleculares (RAPD y PCR-RFLP del ADN de cloroplasto). Los resultados que fueron considerados evidencia para apoyar la hipótesis de Valencia-Ávalos (1994) fueron: variación espacial significativamente concordante entre los caracteres morfológicos y moleculares (como se espera en una zona de hibridación secundaria), mayor variación morfológica y genética en las poblaciones situadas en la probable zona de hibridación, y presencia de dos haplotipos aparentemente divergentes en el complejo, que podrían corresponder a cada una de las dos especies progenitoras.

Posteriormente se llevó a cabo otro estudio para poner a prueba también ésta hipótesis. Valencia-Cuevas (2006), estudió los patrones de variación morfológica foliar y genética del complejo *Q. affinis-Q. laurina*, a través de un gradiente latitudinal a nivel regional. Los resultados apoyaron nuevamente la hipótesis de Valencia-Ávalos (1994) al encontrarse que el flujo genético entre las poblaciones de este complejo ha jugado un papel importante en los patrones de variación morfológica y genética a través del gradiente latitudinal. La presencia del Eje Neovolcánico Transversal el cual funciono como un corredor que permitió parte del contacto secundario, probablemente tuvo influencia sobre los patrones geográficos de variación morfológica de tipo foliar, de manera tal que la diferenciación de las poblaciones en dos grupos morfológicos podría ser el resultado del límite biogeográfico, climático y edáfico que este sistema montañoso representa. Por otro lado, la incongruencia entre la variación morfológica foliar y la variación genética de las poblaciones sugiere la existencia de un proceso de introgresión diferencial, en donde los loci adaptativos han experimentado introgresión restringida a pesar del elevado flujo génico interespecífico e intercambio de loci neutrales entre estas especies.

Por último, Ramos-Ortiz (2007), realizó un análisis filogenético molecular de 26 especies de encinos rojos mexicanos entre las cuales se incluyó a las dos especies de estudio, utilizando AFLPs como marcadores moleculares. El análisis mostró que *Q. affinis* y *Q. laurina* no son especies hermanas (es decir, aparecen en clados distintos de la filogenia), lo que sugiere que la baja diferenciación entre ellas se puede atribuir a la hibridación, más que al hecho de que tengan variación ancestral compartida.

Por lo que se puede concluir a partir de los estudios mencionados que entre *Q. affinis* y *Q. laurina* existe clara diferenciación morfológica y genética en la mayor parte del área de distribución de ambas especies, así como evidencia de una zona de hibridación secundaria situada en el este del Eje Neovolcánico Transversal y el norte de Oaxaca. En esta zona de hibridación existe cierto grado de incongruencia entre los caracteres morfológicos y genéticos (Valencia-Ávalos, 1994; González-Rodríguez *et al.,* 2004), lo que podría explicarse considerando las evidencias que indican que cuando ocurre hibridación algunas porciones del genoma cruzan más fácilmente la barrera específica, mientras que otras lo hacen en un grado mínimo (Rieseberg *et al.,* 1996; Martinsen *et al.,* 2001). Esto quiere decir que los loci adaptativos podrían estar experimentando introgresión restringida a pesar del flujo génico interespecífico y del intercambio de loci neutrales entres estas especies.

Se esperaría que una gran porción de la variación fenotípica tuviera una base genética. Sin embargo, los caracteres morfológicos pueden estar sujetos a limitantes ambientales y de desarrollo que pueden afectar la cantidad y la dirección de la variación expresada (González-Rodríguez *et al.,* 2005); mientras que los caracteres neutrales no están sujetos a efectos ambientales (Endler, 1977).

Por lo tanto, la delimitación entre *Q. affinis* y *Q. laurina* con mayor valor biológico de que se dispone es la que se basa en caracteres morfológicos, dada que puede suponerse que éstos reflejan la divergencia adaptativa y funcional entre ambas especies, producto de diferenciación alopátrica histórica, pero hasta cierto punto mantenida actualmente en simpatría mediante selección ecológica resultado de adaptación a diferentes nichos.

En el presente estudio se pusieron a prueba las hipótesis anteriormente descritas. Como primer paso, se caracterizaron factores ambientales relevantes (principalmente climáticos) en el área de distribución de cada una de las dos especies y en la zona de hibridación. Se encontraron diferencias en algunos de estos factores entre las distintas áreas. Posteriormente, se evaluaron las diferencias morfofisiológicas entre las dos especies, las cuales a su vez estuvieron correlacionadas con las diferencias ambientales. Finalmente, se exploró la variación y la diferenciación genética relacionados con caracteres de importancia ecológica, encontrando asociaciones entre la variación morfofisiológica, la variación genética y la variación ambiental.

CAPÍTULO II

GEOGRAPHIC STRUCTURE OF GENETIC AND PHENOTYPIC VARIATION IN THE HYBRID ZONE BETWEEN *QUERCUS AFFINIS* AND *Q. LAURINA* IN MEXICO

Plant Species Biology

S. Ramos-Ortiz, K. Oyama, H. Rodríguez-Correa & A. González-Rodríguez

RESEARCH PAPER

Geographic structure of genetic and phenotypic variation in the hybrid zone between *Quercus affinis* and *Q. laurina* in Mexico

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Running title: Geographic structure of an oak hybrid zone

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ABSTRACT

Analyzing the structure of hybrid zones is important to infer their origin, dynamics and evolutionary significance. We examined the geographical structure of phenotypic and genetic variation in the contact zone between two Mexican red oaks, *Quercus affinis* and *Q. laurina*. A total of 105 individuals from seven populations were sampled along a 600 km latitudinal gradient representing the distribution area of the two species and their contact zone. Individuals were genotyped for nine nuclear and four chloroplast DNA microsatellite loci (ncSSR and cpSSR, respectively), and characterized for several leaf and acorn traits. The cpSSR data revealed extensive haplotype sharing among populations of the two species, while a Bayesian assignment analysis based on ncSSRs identified two main genetic groups, each corresponding to one of the species, and two populations in the contact zone showing evidence of admixture. The proportion of genetic ancestry in the populations was strongly associated with latitude and showed a pattern of variation with the shape of a narrow sigmoidal cline. The variation in three of the seven phenotypic traits was partially congruent with molecular variation, while the other traits did not conform to a geographical cline but instead were correlated with environmental variables. In conclusion, the hybrid zone between the two oak species has some of the characteristics of a tension zone, but heterogeneous variation across traits suggests differential introgression and the action of extrinsic selection.

Key words: cline, hybrid zone, introgression, morphological variation, microsatellites, *Quercus*.

INTRODUCTION

Natural hybrid zones form in areas where individuals from genetically differentiated populations meet, mate and produce offspring of mixed ancestry (Harrison 1990; Arnold 1997; Abbott *et al.* 2013). Hybrid zones can be structured in different ways depending on their origin and dynamics (Harrison 1990; Barton & Gale 1993; Arnold 1997; Abbott *et al.* 2013), and several conceptual frameworks have been advanced to account for the various possible scenarios. For example, sampling along geographical gradients across hybrid zones has often revealed monotonic shifts with the form of smooth sigmoid clines in allele frequencies or quantitative characters that differ between the two hybridizing taxa (Shaw *et al*. 1979; Szymura & Barton 1986, 1991; Hewitt 1993; Cruzan 2005). Theoretical analyses indicate that the relative fitness of hybrid and parental genotypes is the main factor determining the width and specific position of the clines (Barton & Hewitt 1985, 1989; Barton & Gale 1993). When the fitness of hybrid individuals is uniformly lower due to inviability or sterility arising from the incompatibility of the parental genomes (*e.g.* endogenous selection), the width of character clines will be proportional to the ratio between dispersal of parental individuals into the zone and selection against hybrids (Barton & Gale 1993). Since in this case selection is independent of the environment, the clines can move and will tend to settle in areas with natural barriers to dispersal or low population density (the tension zone model, Barton & Hewitt 1985, 1989; Barton & Gale 1993). Alternatively, if fitness variation between genotypes is the result of an external selection gradient (*e.g.* exogenous selection), cline shapes and positions for the characters under selection may reflect the underlying environmental variation (ecological gradient models, Endler 1977; Moore 1977; Rand & Harrison, 1989; Bierne *et al.* 2013). The mosaic zone model also falls in this latter category of the models (Harrison & Rand 1989; Rand & Harrison 1989; Bierne *et al.* 2013), but in this case the spatial variation in the environmental factors responsible for selection is considered to be discontinuous rather than gradual. As a result, mosaic hybrid zones are constituted by patches of parental and hybrid populations scattered across an area of overlap (Harrison & Rand 1989; Rand & Harrison 1989; Bierne *et al.* 2013), and the transitions in character states between the taxa across the zone do not have the form of smooth clines, but show a broken pattern exhibiting abrupt reversals (Harrison & Rand 1989; Shoemaker *et al*. 1996; Lexer *et al.* 2010).

The degree of 'coincidence' (*e.g.* same position) and 'concordance' (*e.g.* same shape) among clines for different characters also depend on the intensity of selection. With strong selection against hybrids or hybrid unfitness resulting from the combined effects of many loci distributed throughout the genome, little recombination occurs between the parental genomes and significant linkage disequilibria is observed among taxon specific markers in the centre of the zone (Barton & Hewitt 1985; 1989; Barton & Gale 1993). So, strong coincidence and concordance of clines are expected under the tension zone model (Barton & Hewitt 1985; 1989; Barton & Gale 1993), and also under ecological gradient models when there is strong environmental selection against hybrids (Kruuk *et al*. 1999). However, if selection against hybrids is weak, different characters will respond independently of each other rather than as a single unit to evolutionary forces. In this case, the noncoincidence of clines indicates the action of differential extrinsic selection pressures on characters, but could also result from genetic drift (Durrett *et al.* 2000). Similarly, the nonconcordance of clines reveals differences in the strength of selection, with steeper clines indicating stronger selection against introgression of a trait, while wider clines indicate weaker selection (Barton & Gale 1993; Durrett *et al.* 2000; Cruzan 2005).

The genus *Quercus* L. is a model system to study hybridization in plants (Gugerli *et al*. 2007; Valbuena-Carabaña *et al*. 2007), but despite the great number of morphological, ecological, physiological and genetic studies of oak hybridization recently performed (*e. g.* Dumolin-Lapègue *et al*. 1999; Himrane *et al*. 2004; Scareli-Santos *et al*. 2007; Hipp & Weber 2008; Cavender-Bares & Pahlich 2009; Ito 2009; Lepais *et al*. 2009; Albarrán-Lara et *al*. 2010; Gailing *et al*. 2012; Lagache *et al*. 2013), the geographic and genetic structure of few oak hybrid zones have been formally characterized (*e. g.* Howard *et al*. 1997; Dodd & Afzal-Rafii 2004; Sánchez de Dios *et al*. 2006). Such studies may help to uncover the processes influencing oak hybridization and its importance as a source of novel genotypes contributing to the adaptive evolution and diversification of the genus. For example, some previous analyses of oak hybrid zones (Howard *et al*. 1997; Dodd & Afzal-Rafii 2004; Sánchez de Dios *et al*. 2006; Peñaloza-Ramírez *et al*. 2010) have revealed a clear influence of environmental variation on the distribution of parental and hybrid genotypes. Furthermore, some of these hybrid zones were explicitly characterized as having a mosaic structure.

In Mexico, there are approximately 81 species of white oaks (*Quercus*, subgenus *Quercus*, section *Quercus*) and 76 species of red oaks (section *Lobatae*) (Valencia 2004). This extremely high diversity offers a unique opportunity to study genetic interactions among taxa with various degrees of genetic divergence, as well as to examine hybrid zones with different characteristics and histories. In this study, we focus on the hybrid zone between two Mexican red oaks, *Quercus affinis* Scheidw. and *Quercus laurina* Humb. et Bonpl. It was previously shown that there is morphological and genetic intergradation between the two species across a broad geographical area, pattern that was better explained by a scenario of secondary contact and hybridization than by alternative hypotheses (Valencia 1994; González-Rodríguez *et al*. 2004, 2005; González-Rodríguez & Oyama 2005). In this study, we obtained morphological, molecular and climatic data for oak populations sampled along a latitudinal gradient across the hybrid zone between the two species, to evaluate if it can be better described by the tension zone model or by an ecological gradient model. Specifically, we determined: i) the geographical pattern of genetic and morphological variation, ii) if morphological characters and molecular markers follow congruent patterns of variation or not, and iii) if there is an association of morphological and/or genetic variation with climatic variables. In this way we expect to gain insight into the processes underlying the structuring of the hybrid zone between these two oak species.

MATERIALS AND METHODS

Study populations and sampling procedure

Samples were collected from 105 individuals of the *Quercus affinis*-*Q. laurina* complex in seven localities along a latitudinal gradient from the south of Oaxaca state (16.06° N and 96.48° W) to the north of Hidalgo state (20.62° N and 98.6° W) (Table 1, Fig. 1). This latitudinal gradient encompassed the distribution area of *Q. laurina* in southern Oaxaca (populations Suchixtepec and Lachao), the contact zone which is situated in northern Oaxaca, southern Puebla and central Veracruz (populations Pápalo, Zoquitlán and Tonayán), and the distribution area of *Q. affinis* in northern Puebla and northern Hidalgo (populations Zacatlán and Tizapán) (González-Rodríguez *et al*. 2004). An important feature of the present sampling of study populations in the seven localities along the latitudinal gradient is that they were chosen at random with respect to foliar morphology and not on the basis of the previous identification of sites where intermediate forms occur, as in the earlier studies (González-Rodríguez *et al*. 2004). At each site, 15 randomly chosen individuals were sampled keeping minimum distances of 50 m between consecutive trees.

From each individual a few young intact leaves were collected, transported on ice, and then stored in the laboratory at -80 °C for molecular analysis. In addition, three branches per individual were collected and pressed to prepare herbarium specimens for morphological analysis. Mature acorns were also sampled (50-200 per individual) from five individuals of each population.

Nuclear and chloroplast microsatellites

DNA extraction was performed from 120 mg of leaf tissue using the protocol designed by Lefort & Douglas (1999). Nine primer pairs previously designed to amplify nuclear microsatellites (ncSSRs) in *Q. rubra* (Aldrich *et al*. 2002) were selected on the basis of preliminary trials. We set up multiplexed reactions with three groups of primers organized according to the size of alleles, the fluorescent label and the temperature of optimal alignment. The first group was constituted by the primers quru-GA-0A01 and quru-GA-0C11. The second group included quru-GA-0I01, quru-GA-0M05, and quru-GA-0M07. The third group was formed by quru-GA-1C08, quru-GA-1F07, quru-GA-2F05, and quru-GA-2M04. All these loci are (GA) n repeats.

We also analyzed four chloroplast DNA microsatellites (cpSSRs) using multiplexed reactions with primer pairs arranged into two groups. The first group was formed by primers cmcs6, cmcs7 and cmcs12 (Sebastiani *et al*. 2004) and the second group by primers udt1 only (Deguilloux *et al*. 2003).

In all cases PCR reactions were performed using the QIAGEN multiplex PCR kit in a final volume of 5 µL containing 1X multiplex master mix, 40 ng of DNA, 2 µM of each primer and deionized H_2O. The thermal cycling program consisted of one cycle at 95 °C for five min and then 40 cycles, each at 95 °C for 1 min, annealing for 1 min 30 s and extension at 72 °C. Annealing temperatures were 45, 50 and 58 °C for the first, second and third nuclear primer pair groups, respectively, and 48 °C for the two groups of cpSSRs. A final extension at 72 °C for 15 min was included. PCR products were combined with the GeneScan -500 LIZ size standard and ran in an ABI PRISM 3100-Avant sequencer (Applied Biosystems, USA). The sizes of the fragments were analyzed with the program Peak Scanner v.1.0 (Applied Biosystems, USA).

Genetic diversity and structure

To describe the patterns of genetic diversity in each of the seven sampled populations, we calculated the number of alleles (N_A), the effective number of alleles (N_E), the observed heterozygosity (H_O) and the expected heterozygosity (H_E) with the software GenAlex v. 6.5 (Peakall & Smouse 2006). The program MICROCHECKER (van Oosterhout *et al.* 2004) was used to test for the presence of null alleles or scoring errors due to stuttering or large allele dropout. Since all loci except 2M04 showed signs of the presence of null alleles, the FreeNA software (Chapuis & Estoup 2007) was used to estimate the frequency of null alleles for each locus and population with the Expectation Maximization Algorithm (Dempster *et al.* 1977). This program was also used to estimate overall and pairwise differentiation (F_{ST}) values among populations with the method of Weir (1996) and implementing the ENA correction to account for null alleles (Chapuis & Estoup 2007). A bootstrapping procedure over loci with 1000 replicates was performed to obtain F_{ST} values and their 95% confidence intervals. The inbreeding coefficient (F_{IS}) was also estimated while simultaneously considering the presence of null alleles with INEST (Chybicki & Burczyk 2009), using the individual inbreeding model (IIM).

Genetic relationships among all individuals were determined by first estimating an individual by individual pairwise matrix of genetic distances in GenAlex v. 6.5 (Peakall & Smouse 2006) with the method of Smouse & Peakall (1999). The matrix was analyzed with a Principal Coordinates Analysis (PcoA) and individual score values on the first two coordinates were graphed to depict genetic similarity patterns (Orloci 1978; Peakall & Smouse 2006).

Multilocus ncSSR genotypes were analyzed with the model-based Bayesian procedure implemented in the STRUCTURE 2.3.1 program (Pritchard *et al*. 2000; Falush *et al*. 2003; Hubisz *et al*. 2009) to determine the genetic composition of individuals and populations in terms of their inferred ancestry. Simulations were run using the admixture model without prior population information. The program was set to run with values for the number of assumed genetic clusters (*K*) from one to eight, with ten independent runs for each *K*. In all cases, the length of the burn-in period was 100,000 iterations, followed by 10^6 Markov chain Monte Carlo repetitions. The number of uppermost inferred genetic clusters maximizing the probability of the data was identified following the method described by Evanno *et al*. (2005) to compute ΔK, an ad hoc quantity that is a good predictor of the real number of genetic clusters.

For the cpSSRs, the partitioning of genetic diversity was calculated by obtaining the average value of genetic diversity within populations (h_S), the total genetic diversity (h_T) and the coefficient of differentiation for ordered and unordered alleles (G_{ST} and N_{ST} respectively) with the SPAGeDi program (Hardy & Vekemans 2002). If N_{ST}, which takes into account the genetic distance between haplotypes, is significantly higher than G_{ST}, then there is phylogeographic structure in the populations. A haplotype network was constructed in NETWORK 4.6.0 (Bandelt *et al*. 1995) using the Median-Joining method, which combines the topology of the minimum spanning tree with a maximum parsimony search to identify and remove unnecessary links between haplotypes.

Morphological measurements

Previous studies have identified several morphological characters that are useful to differentiate *Q. affinis* and *Q. laurina*. In general, the leaves of *Q. laurina* are longer, wider with longer petioles and fewer leaf-margin teeth than the leaves of *Q. affinis* (Valencia 1994; González-Rodríguez & Oyama 2005). In this study, morphological measurements were taken in ten randomly chosen, fully extended, undamaged leaves from each individual. Leaf size was measured as lamina area (LA), and was obtained by scanning the leaves and then analyzing the images with the program SHAPE 3.1 (Iwata & Ukai 2002). We also obtained the dry mass (DM) of each leaf with an analytical balance and specific leaf area (SLA) was obtained by dividing LA (cm^2) by the DM (g). To analyze differences in the shape of the leaves we used a geometric morphometrics approach based on a quantitative evaluation of the contour shape of each leaf with elliptic Fourier descriptors (EFD) (Viscosi *et al.* 2009). For this purpose we employed the SHAPE 3.1 program, which performs a principal components analysis to summarize the information contained in the EFD, so that the scores of principal components can be used as observed values of morphological traits in subsequent analysis (Iwata & Ukai 2002). Finally, in a sample of 20 acorns from each individual (five individuals per population) we separated the cup from the nut, and determined the length (AL) and the fresh weight of the nut (AM).

These traits were compared among populations with one-way analyses of variance (ANOVA) followed by Tukey-Kramer multiple comparison tests to identify the populations showing significant differences. These analyses were conducted using JMP 8 (SAS Institute, Cary, NC, USA).

Associations among genetic, morphological and climatic variables

To characterize variation in environmental factors across the latitudinal gradient, we obtained bioclimatic variables for the seven localities from the WorldClim database (Hijmans *et al*. 2005). To reduce redundancy among variables in the dataset we estimated pairwise correlation coefficients among all variable pairs. We dropped one of the variables from a pair whenever the correlation between them was high (≥ 0.8). In all cases the variable retained was the more general of the two (for example annual mean temperature instead of maximum temperature of warmest quarter). In this way, we used for subsequent analysis altitude (m), annual mean temperature (AMT, °C), isothermality (IT, without units, it is the ratio between the mean diurnal temperature range and the mean annual temperature range), mean temperature of driest quarter (MTDQ, °C), annual precipitation (AP, mm), precipitation seasonality (PS, coefficient of variation).

We determined if the genetic composition of the populations and the morphological variation are associated with geographic and climatic variables. First, we tested for bivariate correlations between the populations mean values of the morphological traits and the proportion of genetic ancestry in each population (calculated from STRUCTURE) with latitude, longitude and altitude, and with the climatic variables. However, given in some cases the existence of correlations between climatic and geographic variables, we used stepwise multiple regression models to determine the combination of independent variables that best predicted each response variable. These analyses were conducted with the JMP 8 software (SAS Institute, Cary, NC, USA).

Secondly, for those morphological and genetic variables that showed significant association with geographical variables, we fitted models of sigmoid clines, as are commonly found in some types of hybrid zones (see Introduction). For this purpose, we employed the HZAR package (Derryberry *et al.* 2014) for the R programming environment (R-Development-Core-Team 2014). In these analyses, the individual values of the morphological variables were first transformed to range between zero and one, with values closer to zero found in *Q. laurina* individuals. The proportion of genetic ancestry was not transformed since it was already expressed in this scale. For quantitative traits, HZAR tests five different models depending on whether the model includes symmetric exponential tails on both sides of the curve, no tails, only a right or a left tail or asymmetric tails (Derryberry *et al.* 2014). All clines were defined in a space between zero (population Suchixtepec) and 600 km (population Tizapán). To fit the models, the Metropolis-Hasting algorithm was used, as implemented in HZAR, with a length of the Markov chain Monte Carlo search of 100,000 and a burn-in period of 10,000. For each of the variables analyzed, the corrected Akaike Information Criterion for the resulting five models was recorded and the model with the lowest score was selected to infer cline width and center. The analyses were repeated three times to ensure convergence of the parameter estimates. Comparison of cline center and width across variables was performed using confidence intervals, defined as 2 log-likelihood scores around the mean.

RESULTS

Nuclear microsatellites

The mean number of alleles per locus (N_A) within populations ranged from 7.8 to 10.4 and the mean number of effective alleles (N_E) was between 4.4 and 6.7. The values of H_O were between 0.541 and 0.741, and H_E was between 0.752 and 0.838. Populations Tizapán and Zacatlán (*Q. affinis*) had the lowest genetic diversity values, and population Pápalo (contact zone) had the highest values, but a clear geographical pattern in genetic diversity was not observed (Table 2).

According to the MICROCHECKER software, null alleles were present in all populations. The analysis with INEST indicated that inbreeding coefficients were not significantly different from zero when estimated jointly with the frequency of null alleles and ranged from $F_{IS} = 0.01$ (Lachao) to 0.05 (Zoquitlán).

After the ENA correction implemented in FreeNA, the value of overall genetic differentiation among the seven populations was estimated to be $F_{ST} = 0.068$, while the uncorrected value was $F_{ST} = 0.076$. The bootstrapped value using the ENA correction was $F_{ST} = 0.046$ with a 95% confidence interval between -0.046 and 0.139. Pairwise genetic differentiation values (corrected) ranged between 0.11 (between populations Suchixtepec and Zacatlán) and 0.03 (between populations Zoquitlán and Pápalo) (Table S1).

The principal coordinates analyses based on pairwise genetic distances among all individuals indicated a clear separation between the genotypes of individuals from allopatric populations of both parental species (Fig. 2). Populations from the contact zone showed an overlap with both parental species but were more similar to one or the other depending on their position along the latitudinal gradient. Individuals from population Pápalo were similar to individuals from populations Suchixtepec and Lachao, while individuals from population Zoquitlán had a wide range of genotypes that were similar to one species or the other but with some tendency towards intermediacy. Meanwhile, most individuals from population Tonayán were similar to *Q. affinis* individuals, but a few clustered closer to *Q. laurina* individuals.

The multilocus analysis using STRUCTURE, followed by the computation of ΔK, revealed that the number of genetic clusters best fitting the data was $K = 2$ (Fig. 3). The memberships of populations in each of the two genetic clusters changed along the latitudinal gradient from an almost complete proportion of membership of populations Suchixtepec and Lachao in one of the genetic clusters (0.958 and 0.967, respectively), to an almost complete proportion of membership of populations Zacatlán and Tizapán in the other genetic cluster (0.951 and 0.894). Populations Zoquitlán and Tonayán showed clear admixture between the two genetic groups (Fig. 3).

Chloroplast microsatellites

In total, 35 haplotypes were identified in the 105 individuals analyzed (Fig. 4). Within populations, between 8 and 11 haplotypes were observed, with haplotype diversity values (h_S) in the range of 0.90 and 0.96 (Table 2). The mean (standard error) of h_S was 0.93 (0.009), and the total haplotype diversity (h_T) was 0.997 (0.009). Population differentiation for ordered alleles (N_{ST}) was 0.27 and for unordered alleles (G_{ST}) was 0.038. The permutation test for the comparison of N_{ST} and G_{ST} was significant ($P < 0.001$), indicating that more closely related haplotypes tend to occur together in the same populations, or in other words that there is phylogeographic structure.

Genetic, morphological and climatic variables

The morphological traits examined showed significant variation among populations except for specific leaf area and the second principal component (PC2) derived from the elliptic Fourier descriptors (EFD) (Table 3). These two characters were excluded from further analysis. Pairwise correlations using individual values of the morphological traits and genetic ancestry showed that in general, the morphological traits were not correlated among themselves, with the exception of acorn length and acorn mass (as was expected), and acorn mass and the PC4 (Table 4). The genetic ancestry of the individuals was significantly correlated with the PC4, the PC5 and acorn mass (Table 4).

Bivariate correlations of the mean values of morphological traits and the genetic composition of the populations with the geographic and climatic variables revealed significant relationships between the morphological PC1 and altitude, and the PC3 and annual precipitation (Table 5). The PC4, acorn length and the genetic ancestry of the populations were correlated with latitude, longitude and isothermality, while acorn mass was correlated with latitude and isothermality (Table 5). However, after taking into account correlations among geographic and climatic variables (using stepwise regressions), latitude was the only significant explanatory variable in the case of PC4, acorn mass and genetic ancestry, while only isothermality was significant in the case of acorn length (Table S2).

The plots of the maximum-likelihood clines fitted with HZAR for acorn mass, PC4 and the proportion of genetic ancestry are presented in Fig. 6. The estimated parameters for each cline are shown in Table S3. Cline centers were similar for the PC4 and the genetic ancestry of the populations, while the cline center for acorn mass was somewhat displaced to the left (Fig. 6; Table S3). The width of the cline was narrow in the case of the genetic ancestry of the populations but broad for PC4 and acorn mass.

DISCUSSION

The populations sampled in this study are situated along a latitudinal gradient intersecting the contact zone between the red oaks *Quercus affinis* and *Q. laurina*. The results are consistent with a hypothesis of secondary contact and hybridization between previously differentiated species (Valencia 1994; González-Rodríguez *et al*. 2004) as follows: the clustering of ncSSR genotypes identified two distinct genetic groups, each corresponding to one of the species, and showed that the transition from one species to the other occurs over a geographical area that extends from the south of Puebla to Central Veracruz (Figs. 1 and 3); a Principal Coordinates Analysis indicated clear genetic differentiation between individuals from populations situated at both ends of the latitudinal gradient representing the two species, and genetic intermediacy in some individuals from populations within the contact zone (Fig. 2).

The analysis of the hybrid zone structure revealed that the proportion of genetic ancestry of populations, acorn mass and the PC4 of foliar morphology could be fitted to models of geographic sigmoid clines (Fig. 6, Table S3). These models suggested that the clines for the PC4 and acorn mass were significantly wider than the cline for genetic ancestry, and that the cline center for acorn mass was shifted to the left with respect to the other two. According to hybrid zone theory, narrow coincident (i. e. in the same position) and concordant (i. e. with the same width) clines are evidence for a tension zone and suggest that intrinsic selection is strong against hybrids (Barton & Hewitt 1985, 1989; Barton & Gale 1993). The proportion of genetic ancestry of the populations, based on the multilocus analysis of nine ncSSRs situated on different chromosomes showed a narrow cline, suggesting that at the genome-wide level there is some degree of selection against hybridization between the two oak species analyzed here. In contrast, the wider clines for the PC4 and acorn mass indicate comparatively weaker selection against introgression of these two phenotypic traits and the specific genes underlying them. Furthermore, the shifted center of the cline for acorn mass suggests that not only intrinsic factors but also extrinsic selection could be acting on this trait. However, the stepwise regression models (Table S2) indicated that latitude was the only significant explanatory variable for variation in acorn mass, discarding an effect of the climatic variables considered in this study. Therefore, other environmental variables could be exerting a selection pressure on acorn mass. Alternatively, drift or the movement of the hybrid zone can explain the shifted clines (Durrett *et al.* 2000).

On the other hand, four of the morphological traits evaluated (leaf area, the PC1 and the PC3 of foliar morphology, and acorn length) did not show latitudinal clinal variation. This non-clinal variation reflects the association of the traits with environmental factors that do not follow a latitudinal gradient. Particularly, the PC1, the PC3 and acorn length were significantly correlated with altitude, annual precipitation and isothermality, respectively (Table 5). These associations could be due to local adaptation and, if this is the case, would indicate that extrinsic selective factors are playing a significant role in structuring this hybrid zone, as outlined in the ecological gradient models. Nevertheless, it could be that these traits are responding to environmental conditions through phenotypic plasticity, without necessarily implying the action of selection. Therefore, common garden experiments would be crucial to determine the degree of phenotypic plasticity in morphological traits and to evaluate the performance of parental and hybrid individuals under homogeneous conditions.

Overall, the results of the analysis of ncSSRs and phenotypic traits suggest that different characters and genomic regions are under the action of different forces. In this oak hybrid zone it is likely that, despite some degree of intrinsic selection against hybrids, this is not strong enough to preclude some admixed genotypes from being successful. Consequently, recombination among parental gene pools can occur and processes such as neutral introgression, drift, movement of the zone, or environmental selection, may independently influence the dynamics of different genomic regions, resulting in discordant patterns among characters (Barton 1993; Jaarola *et al*. 1997; Brumfield *et al*. 2001).

In contrast with ncSSRs, cpSSR variation did not conform to a pattern of differentiation between the populations of the two species and intermediacy of contact zone populations, but instead indicated extensive haplotype sharing between the two species across the whole study area. Similar widespread cytoplasmic introgression has been commonly found among oak species, resulting in patterns more related to the geographical location of the populations than to species boundaries (Whittemore & Schaal 1991; Dumolin-Làpegue *et al.* 1999; Curtu *et al.* 2007). In our case, it is possible that historical shifts, expansions and contractions of the range of both species and in the position of the hybrid zone have resulted in multiple instances of haplotype exchange among populations as proposed for Mediterranean oaks (Jiménez *et al.* 2004).

An aspect of interest that should be considered to understand the patterns of variation documented here is the age of the hybrid zone (*i.e.* Zeng *et al.* 2011). It has been previously hypothesized that this hybrid zone may be as old as the beginning of the Pleistocene (Valencia 1994; González-Rodríguez *et al*. 2004), but evidence for this is still lacking. However, such evidence would have to come from the analysis of molecular markers, since the paleontological record is not very useful for this purpose in areas of high oak diversity, as southern Mexico. An interesting difference between our results and previous findings in temperate oak species using similar cpDNA microsatellite markers (e.

g. Grivet *et al.* 2006; Magri *et al.* 2007; Marsico *et al.* 2009) is the higher haplotype variation and lower differentiation observed in *Q. affinis*-*Q. laurina* populations. This pattern can be explained considering that in Mexico oak species have probably maintained comparatively larger population sizes and relatively more stable distribution ranges for several glacial cycles in comparison to some of their counterparts from more northern latitudes, which were severely restricted to refugia during the last glacial cycle. A similar argument has been used to explain the large amount of haplotype diversity in the Californian oak *Q. lobata* in comparison with European white oaks (Grivet *et al*. 2006). In Mexico, forests along the Sierra Madre Oriental and Sierra Madre del Sur experienced downward altitudinal migrations and expansions during glacial maxima with higher connectivity among populations than during interglacials (Ramírez-Barahona & Eguiarte 2013; Ornelas *et al*. 2013). Therefore, it is likely that historical effective population sizes for species such as *Q. affinis* and *Q. laurina* have been continuously large during their evolutionary history, and that recurrent upwards and downwards altitudinal migrations contributed to cpDNA exchange among populations, as previously mentioned. However, this interpretation must be taken with caution, since phylogeographic inference in this study system can be confounded by homoplasy in the cpDNA microsatellites and also by genetic exchange from other species. *Q. affinis* and *Q. laurina* inhabit species-rich oak communities with up to five or six species coexisting at a local scale, and some degree of cytoplasmic introgression beyond these two species cannot be discarded.

In conclusion, the data herein presented indicate that the hybrid zone between *Q. affinis* and *Q. laurina* shows the characteristics of a secondary contact zone with evidence of the presence of two distinct genetic pools and a zone of admixture. The proportion of genetic ancestry in the populations showed a geographical pattern of variation with the shape of a narrow sigmoid cline, as expected in a tension zone, but some phenotypic traits showed wider and displaced clines, indicating comparatively weaker selection on these traits or the action of extrinsic selection. Other traits had non-clinal variation and were significantly correlated with environmental factors that are independent of the latitudinal gradient, pattern that can be explained by extrinsic selection, plastic responses or a combination of both. It seems like different characters and genomic regions are under the action of different forces in this oak hybrid zone, but common garden experiments are necessary to compare the performance of parental and hybrid individuals and to determine the degree of phenotypic plasticity of the characters evaluated.

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Figure legends

FIG.1. Map showing the seven sampling localities. Red symbols represent *Quercus laurina* populations, green symbols represent contact zone populations and blue symbols represent *Q. affinis* populations. The numbering of populations is as in Table 1.

FIG. 2. Principal Coordinates Analysis depicting genetic relationships among individuals of sampled populations. The first axis accounted for 24.2% of the variation and the second axis for 20.4% of the variation. Red symbols represent *Q. laurina* individuals, green symbols represent *Q. affinis* individuals and blue symbols individuals from the contact zone.

FIG. 3. (A). Genetic assignment of individuals and populations according to the Bayesian method implemented in STRUCTURE. Each thin vertical line represents an individual and the proportion of each color represents the proportion of ancestry in each of the two genetic groups inferred. Populations are separated by black lines. (B). Estimated number of genetic groups (*K*) derived from the Bayesian clustering analysis. The magnitude of ΔK was estimated using the method of Evanno *et al*. (2006).

FIG. 4. Median-joining haplotype network for the 35 haplotypes identified. Each circle represents an individual haplotype and circle size is proportional to the frequency of the haplotype. Different colours indicate the presence of the haplotypes in *Q. affinis* populations, *Q. laurina* populations or contact zone populations.

FIG. 5. Plots of the maximum-likelihood clines and observed data over the 95% credible cline region (in gray). (A) PC4 of leaf morphology; (B) Acorn mass; (C) Proportion of genetic ancestry in the populations. To ease comparisons, data for PC4 and acorn mass were transformed to range between zero and one.

TABLE 1. Geographical and climate information for the seven study localities.

Number	Locality	State	Lat/ Long	Altitude (m)	AMT (°C)	IT	MTDQ (°C)	AP (mm)	PS
Q. laurina									
1	Suchixtepec	Oaxaca	16.06°/ 96.48°	1962	15.1	0.79	14.5	1556	100
2	Lachao	Oaxaca	16.22°/ 97.13°	1974	17.0	0.74	15.7	1324	103
Contact zone									
3	Pápalo	Oaxaca	17.85°/ 96.8°	2778	11.8	0.71	11.2	2044	80
4	Zoquitlán	Puebla	18.28°/ 97.08°	2597	12.9	0.70	12.0	1296	83
5	Tonayan	Veracruz	19.72°/ 96.9°	2068	14.6	0.61	12.9	1572	64
Q. affinis									
6	Zacatlán	Puebla	19.9°/ 97.95°	2043	15.7	0.65	13.7	1090	79
7	Tizapán	Hidalgo	20.62°/ 98.6°	2085	13.6	0.62	12.1	1492	80

AMT, annual mean temperature; IT, isothermality; MTDQ, mean precipitation of the driest quartet; AP, annual precipitation; PS, precipitation seasonality.

TABLE 2. Genetic diversity parameters for nuclear and chloroplast DNA microsatellites scored in seven populations of the *Quercus affinis-Q. laurina* complex in Mexico.

Population	Nuclear microsatellites				Cp DNA microsatellites	
	N_A	N_E	H_E	H_O	Nh	h_S
Suchixtepec	9.000 (0.898)	5.396 (0.614)	0.792 (0.026)	0.659	9	0.90 (0.024)
Lachao	8.111 (0.873)	5.591 (0.885)	0.778 (0.036)	0.681	11	0.95 (0.024)
Pápalo	10.444 (0.648)	6.742 (0.595)	0.838 (0.021)	0.741	10	0.95 (0.027)
Zoquitlán	8.222 (0.465)	5.104 (0.332)	0.797 (0.013)	0.578	11	0.96 (0.024)
Tonáyan	8.778 (0.862)	5.706 (0.837)	0.765 (0.061)	0.667	9	0.93 (0.024)
Zacatlán	7.778 (0.909)	4.398 (0.482)	0.752 (0.025)	0.63	8	0.90 (0.024)
Tizapán	7.778 (0.641)	4.831 (0.578)	0.769 (0.026)	0.541	9	0.93 (0.024)

N_A, mean number of alleles per locus; N_E, mean number of effective alleles per locus; H_E, mean expected heterozygosity; H_O, mean observed heterozygosity; *Nh*, number of haplotypes; h_S, haplotype diversity. Standard errors are indicated in parenthesis.

TABLE 3. Population means of leaf and acorn morphological traits along the latitudinal gradient. Standard errors are shown in parenthesis. Different letters indicate significant differences ($P < 0.05$) after an ANOVA and a Tukey-Kramer post-hoc test.

Morphological traits	Populations						
	Suchixtepec	Lachao	Pápalo	Zoquitlán	Tonayán	Zacatlán	Tizapán
LA (cm^2)	9.26 ^{a} (0.67)	6.26 ^{b} (0.67)	7.09 ^{ab} (0.67)	6.17 ^{b} (0.67)	6.84 ^{ab} (0.67)	7.26 ^{ab} (0.67)	5.99 ^{b} (0.67)
SLA (cm^2/gr)	50.13 ^{a} (2.12)	45.68 ^{a} (2.12)	53.87 ^{a} (2.12)	48.91 ^{a} (2.12)	51.48 ^{a} (2.12)	50.47 ^{a} (2.12)	50.57 ^{a} (2.12)
PC1	-3.92 x 10$^{-2\ cd}$ (1 x 10^{-2})	-4.94 x 10^{-2} ^{d} (1 x 10^{-2})	3.35x 10$^{-2\ ab}$ (1 x 10^{-2})	8.71 x 10$^{-2\ a}$ (1 x 10^{-2})	9.02 x 10$^{-3\ bc}$ (1 x 10^{-2})	-1.86 x 10^{-2} ^{bcd} (1 x 10^{-2})	-2.34 x 10$^{-2\ cd}$ (1 x 10^{-2})
PC2	3.43 x 10$^{-3\ a}$ (3 x 10^{-3})	-7.73 x 10^{-4} ^{a} (3 x 10^{-3})	-2.34 x 10^{-3} ^{a} (3 x 10^{-3})	-1.61x 10$^{-3\ a}$ (3 x 10^{-3})	5.91 x 10$^{-3\ a}$ (3 x 10^{-3})	-2.53 x 10$^{-3\ a}$ (3 x 10^{-3})	-2.06 x 10$^{-3\ a}$ (3 x 10^{-3})
PC3	-9.19 x 10$^{-3\ b}$ (4 x 10^{-3})	7.91 x 10^{-3} ^{ab} (4 x 10^{-3})	-7.99 x 10^{-3} ^{b} (4 x 10^{-3})	2.62 x 10^{-3} ^{ab} (4 x 10^{-3})	-7.17 x 10$^{-4\ ab}$ (4 x 10^{-3})	1.18 x 10$^{-2\ ab}$ (4 x 10^{-3})	-4.46 x 10$^{-3\ a}$ (4 x 10^{-3})
PC4	4.85 x 10$^{-3\ ab}$ (2 x 10^{-3})	7.35 x 10$^{-3\ a}$ (2 x 10^{-3})	1.14 x 10^{-3} ^{ab} (2 x 10^{-3})	-7.18 x 10^{-4} ^{b} (2 x 10^{-3})	-3.61 x 10$^{-4\ ab}$ (2 x 10^{-3})	-2.52 x 10$^{-3\ bc}$ (2 x 10^{-3})	-9.74 x 10$^{-3\ c}$ (2 x 10^{-3})
PC5	8.36 x 10$^{-4\ ab}$ (1 x 10^{-3})	3.40 x 10$^{-3\ a}$ (1 x 10^{-3})	3.70 x 10^{-3} ^{ba} (1 x 10^{-3})	-1.89 x 10^{-3} ^{b} (1 x 10^{-3})	-7.97 x 10$^{-4\ ab}$ (1 x 10^{-3})	-3.30 x 10$^{-3\ b}$ (1 x 10^{-3})	-1.95 x 10$^{-3\ b}$ (1 x 10^{-3})
AL (cm)	1.22 ^{b} (0.10)	1.37 ^{ab} (0.10)	1.35 ^{ab} (0.10)	1.39 ^{ab} (0.10)	1.47 ^{a} (0.10)	1.36 ^{ab} (0.10)	1.53 ^{a} (0.10)
AM (gr)	0.91 ^{bc} (0.07)	0.83 ^{c} (0.07)	1.12 ^{abc} (0.07)	0.83 ^{c} (0.07)	1.25 ^{a} (0.07)	1.17 ^{ab} (0.07)	1.37 ^{a} (0.07)

LA, leaf area; SLA, specific leaf area; PC1-PC5, the first principal components describing leaf shape derived from elliptic Fourier analysis; AL, acorn length; AM, acorn mass.

TABLE 4. Correlation coefficients among pairs of morphological traits and among morphological traits and individual proportion of genetic ancestry. Values in boldface are significant after a Bonferroni correction.

	Leaf area	PC1	PC3	PC4	PC5	Acorn length	Acorn mass	Genetic ancestry
Leaf area	-							
PC1	0.06	-						
PC3	-0.16	0.02	-					
PC4	-0.07	-0.16	0.08	-				
PC5	0.10	0.002	0.02	0.24	-			
Acorn length	0.06	0.12	-0.002	-0.25	-0.05	-		
Acorn mass	0.17	-0.007	-0.12	**-0.32**	-0.20	**0.55**	-	
Genetic ancestry	0.11	0.09	-0.16	**-0.36**	**-0.40**	-0.26	**-0.40**	-

TABLE 5. Correlation coefficients of morphological traits and the proportion of genetic ancestry in the populations with geographical and climatic variables. Values in boldface are significant after a Bonferroni correction.

	Lat	Long	Alt	AMT	IT	MTDQ	AP	PS
Leaf area	-0.47	0.54	-0.25	0.15	0.15	0.3	0.18	0.32
PC1	0.2	0.2	**0.84**	-0.75	-0.17	-0.74	0.18	-0.46
PC3	0.18	-0.34	-0.28	0.59	-0.24	0.42	**-0.81**	0.009
PC4	**-0.91**	**0.81**	-0.08	0.43	**0.78**	0.63	0.08	0.62
PC5	-0.73	0.58	0.23	-0.009	0.59	0.2	0.64	0.5
Acorn length	**0.87**	**-0.83**	0.004	-0.07	**-0.89**	-0.35	-0.3	-0.62
Acorn mass	**0.83**	-0.64	0.03	-0.25	**-0.78**	-0.48	0.12	-0.7
Genetic ancestry	**0.9**	**-0.83**	-0.36	0.11	**-0.83**	-0.18	-0.43	-0.56

Lat, latitude; Long, longitude; Alt, altitude; AMT, annual mean precipitation; IT, isothermality; MTDQ, mean temperature of the driest quartet; AP, annual precipitation; PS, precipitation seasonality.

TABLE S1. Pairwise differentiation (F_{ST}) values corrected for null alleles among the seven oak populations included in the study.

	Suchixtepec	Lachao	Pápalo	Zoquitlán	Tonayán	Zacatlán	Tizapán
Suchixtepec	-						
Lachao	0.065	-					
Pápalo	0.038	0.053	-				
Zoquitlán	0.064	0.058	0.033	-			
Tonayán	0.110	0.055	0.075	0.042	-		
Zacatlán	0.111	0.105	0.088	0.067	0.057	-	
Tizapán	0.088	0.080	0.073	0.066	0.063	0.037	-

Table S2. Results of the multiple regression analysis of the PC4, acorn length, acorn mass and the proportion of genetic ancestry in the populations, on spatial and climate variables of the seven collection sites.

	Estimate	Standard Error	*t*	*P*
PC4				
Intercept	0.16	0.06	2.76	0.05
Latitude	-0.006	0.002	-3.59	**0.02**
Isothermality	-0.0008	0.0004	-1.88	0.13
Acorn length				
Intercept	-4.55	3.34	-1.36	0.24
Longitude	-0.07	0.03	-2.12	0.10
Isothermality	-0.01	0.004	-3.05	**0.04**
Acorn mass				
Intercept	-1.13	0.66	-1.71	0.15
Latitude	0.12	0.04	3.38	**0.02**
Proportion of genetic ancestry				
Intercept	-3.41	0.83	-4.09	**0.009**
Latitude	0.21	0.04	4.57	**0.006**

TABLE S3. Parameter estimates for the cline models obtained with the HZAR software for the morphological traits and the proportion of genetic ancestry in the populations.

Traits	Cline parameters	
	Center	Width
PC4	323.2 (262.5-387.4)	575 (388.4-629.8)
Acorn mass	236.5 (175.6-317.4)	431.5 (253.5-629.7)
Genetic ancestry	313.5 (311.2-337.1)	15.3 (14.6-100.3)

Two log-likelihood support limits are shown in parenthesis. Cline width corresponds to 1/maximum slope. Cline center is the distance in km from the first locality.

FIGURE 1.

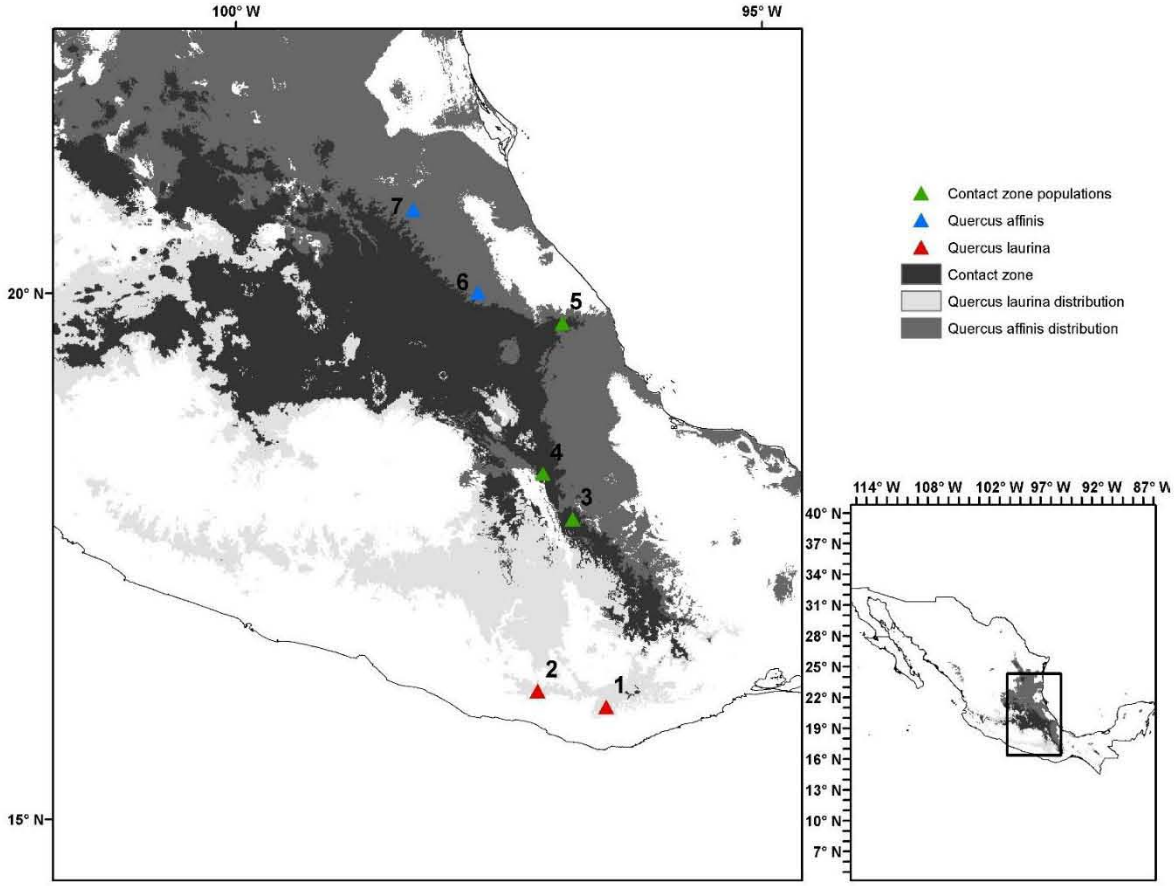
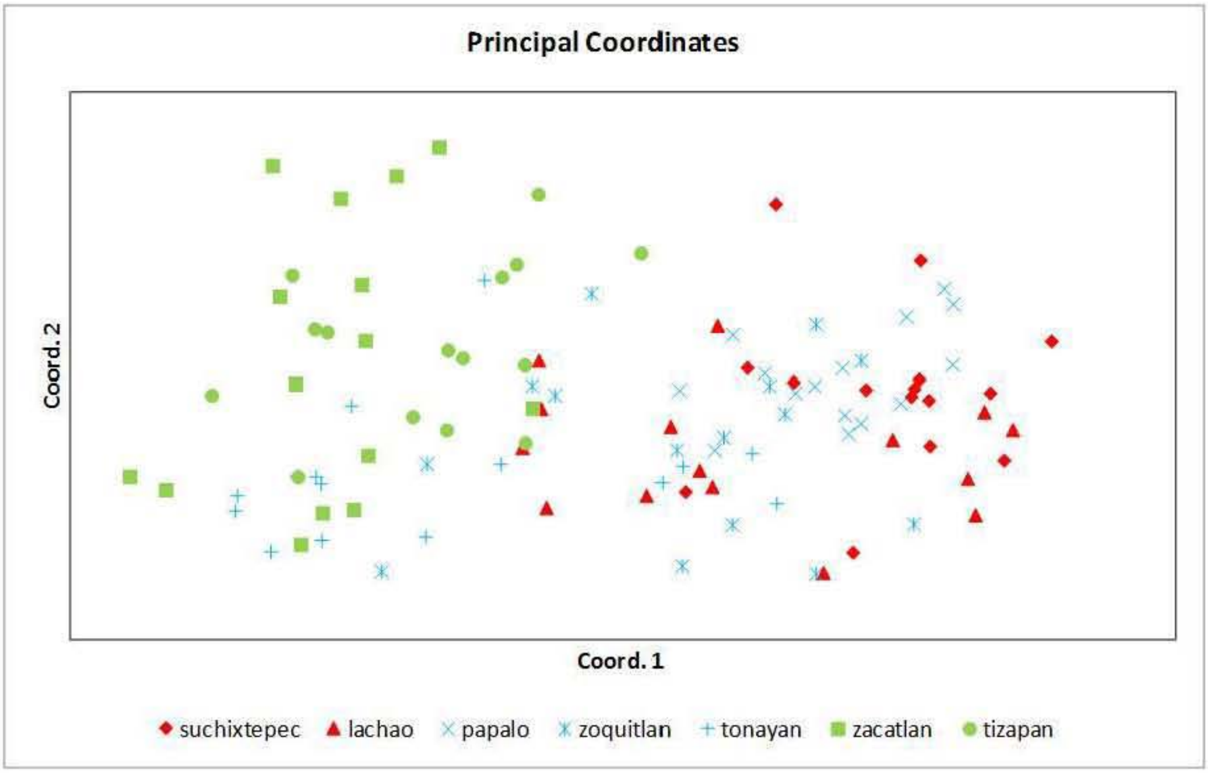

100° W
95° W
20° N
15° N
7
6
5
4
3
2
1
Contact zone populations
Quercus affinis
Quercus laurina
Contact zone
Quercus laurina distribution
Quercus affinis distribution
114° W
108° W
102° W
97° W
92° W
87° W
40° N
37° N
34° N
31° N
28° N
25° N
22° N
19° N
16° N
13° N
10° N
7° N

FIGURE 2.


Principal Coordinates
Coord. 2
Coord. 1
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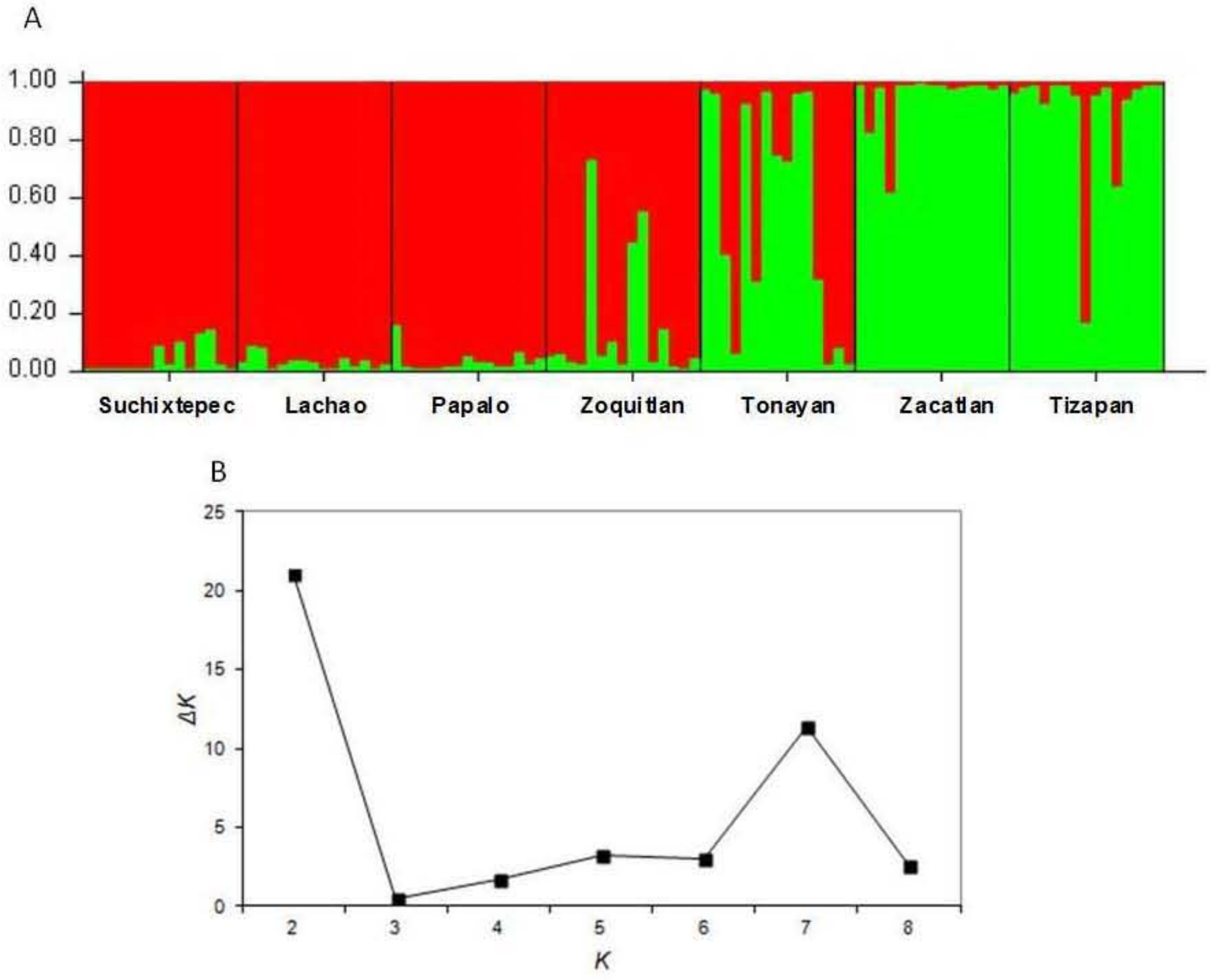
▲ lachao
× papalo
⋇ zoquitlan
+ tonayan
■ zacatlan
● tizapan

FIGURE 3.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Suchixtepec
Lachao
Papalo
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Tonayan
Zacatlan
Tizapan
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20
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10
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ΔK
2
3
4
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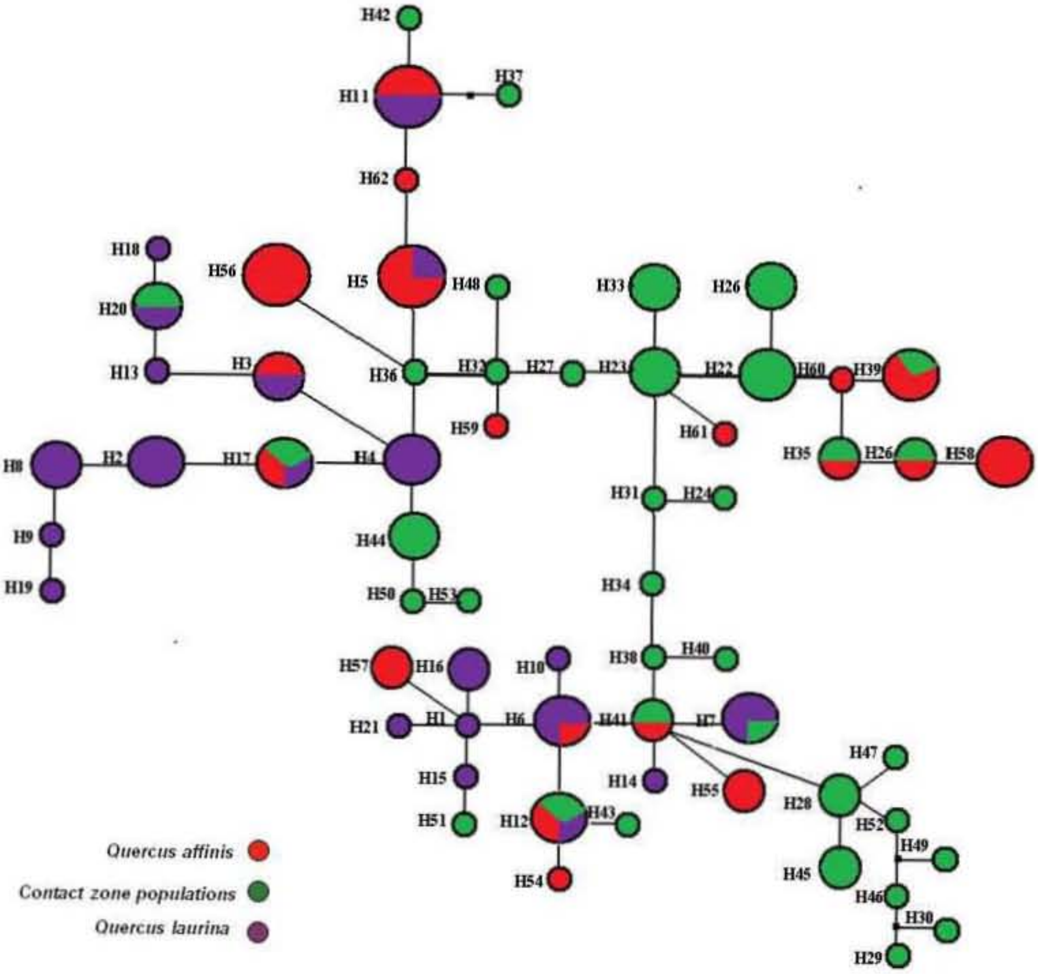
6
7
8
K

FIGURE 4.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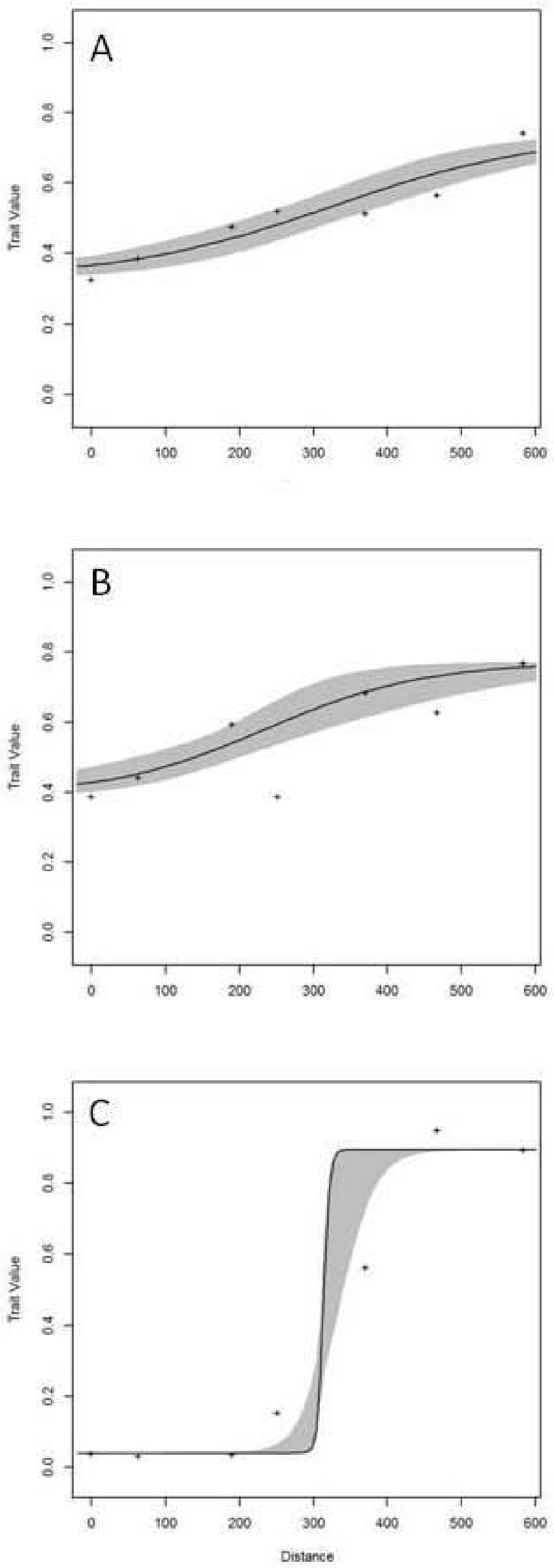
H10
H38
H40
H21
H1
H6
H41
H7
H47
H15
H14
H55
H28
H51
H12
H43
H52
H49
H54
H45
H46
H30
H29
Quercus affinis ●
Contact zone populations ●
Quercus laurina ●

FIGURE 5.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Trait Value
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0.2
0.4
0.6
0.8
1.0
0
100
200
300
400
500
600
B
Trait Value
0.0
0.2
0.4
0.6
0.8
1.0
0
100
200
300
400
500
600
C
Trait Value
0.0
0.2
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0.6
0.8
1.0
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200
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400
500
600
Distance

CAPÍTULO III

DIFFERENTIAL PERFORMANCE OF *QUERCUS AFFINIS*, *Q. LAURINA* AND HYBRID SEEDLINGS UNDER DIFFERENT WATER CONDITIONS

Para ser enviado a la revista Tree Phisiology

Selene Ramos-Ortiz, Fernando Pineda-García, Ken Oyama, Jeannine Cavender-Bares & Antonio González-Rodríguez

Full title:

Differential performance of *Quercus.affinis*, *Q. laurina* (Fagaceace) and hybrid seedlings under different water conditions

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Abstract

The level of hybridization and introgression between related species is a potential form of evolutionary response to environmental change that has been largely unexplored. The frequency of successful hybrids in nature may be affected in different ways by changes in environmental conditions. In the case of oaks, there are few studies on the performance of hybrids compared to the parental species. In this paper, we focused on the analysis of morphological and ecophysiological traits related to the performance of seedlings of two Mexican red oaks, *Quercus affinis* and *Q. laurina* and their hybrids collected along a latitudinal gradient, under optimal conditions and under water stress.

A total of 90 individuals from six locations where analyzed for several morphological and physiological traits. The results indicated that under optimal conditions, populations displayed significant variation at several important functional traits (LA: leaf area; LDMC: leaf dry matter content; SD: stomatal density; RGR: relative growth rate; *Amax*: maximum photosynthetic rate; *gs*: stomatal conductance rate; *E:* transpiration rate; *WUEi*: water-use efficiency; *PhiPS2*: photochemical quenching of PSII; *NPQ*: non photochemical quenching), but we did not observe a clear pattern of differentiation between populations of the two parental species and intermediacy of the populations within the contact zone.

Depending on the trait, populations from within the contact zone displayed character values that were identical, intermediate or extreme in comparison with parental species populations. The results of the drought simulation experiment also revealed significant variation among populations in their capacity to retain physiological functions through prolonged water deficit and in survival rate after the cessation of drought. From our results we can conclude that under one specific type of environmental stress (drought stress) some individuals from a hybrid population (Zoquitlán) had a superior performance in comparison to all other populations studied.

Therefore, different environmental conditions could contribute to the identification of hybrids as potential carriers of attributes that are associated with tolerance to some type of environmental stress. However, it must be kept in mind that under natural conditions, hybrid performance relative to parental species is also likely to depend on several local abiotic and biotic factors. Hence, reciprocal transplant experiments are necessary to broaden our understanding of the hybridization-fitness link.

Key words: contacts zone, fitness, drought stress, *Quercus affinis*, *Quercus laurina*, survival.

Introduction

Natural hybridization may contribute to adaptation and / or speciation in one of two ways. First, introgression can lead to the transference of the adaptations of one taxon to another, perhaps allowing for the expansion of the range of the introgressed populations (Gibbs 1968, Birch and Vogt 1970). In the other way, hybridization can lead to the founding of new evolutionary lineages (Arnold 1997, Rieseberg 1997).

Even though little is known about the existence of successful hybrids in nature, a large number of works on natural hybridization has accumulated over the years. Most of these studies have focused on the inference of evolutionary relationships among populations based on the ability (or inability) to hybridize (*e.g.,* Wiegand 1935, Gillet 1966, Heiser et al. 1969), or on understanding the mechanisms that limit gene flow (*e.g,* Ball and Jameson 1966, Hopper and Burbidge 1978, Shaw and Wilkinson 1980, Howard 1986, Rand and Harrison 1989, Howard and Waring 1991). In relatively fewer studies hybridization has been considered a widespread process with potentially important evolutionary outcomes (*e.g.*, Anderson 1948, Anderson and Stebbins 1954, Lewontin and Birch 1966, Wang et al. 1997), another example is when hybridization between species has led to an enhancer of the invasion of some genotypes (Ellstrand and Schierenbeck 2000, Lee 2002, Lambrinos 2004, Schierenbeck and Aïnouche 2005, Suarez and Tsutsui 2008, Schierenbeck and Ellstrand 2009).

In general, hybrid performance may be influenced by either endogenous or exogenous selection. Endogenous selection arises as a result of the incompatibility of the parental genomes and acts against hybrid genotypes regardless of the environment in which they occur (Schulte-Merker et al. 1997, Pogson 2001, Colosimo et al. 2005, Wood et al. 2008, Bierne et al. 2011). In contrast, exogenous selection refers to performance differences among genotypes depending on specific environmental conditions. The pattern generally observed in most studies of hybrids is that their performance is much lower or zero compared to that of their parents. However, it is known that a fraction of hybrid genotypes can have higher performance than their parental counterparts under certain conditions (*e.g*., Emms and Arnold 1996, Rieseberg et al. 1996, Wang et al. 1997, Burke et al. 1998, Jiang et al. 2000). In fact, studies documenting that hybrid genotypes exhibit a wide range of fitness values are becoming increasingly common. Interestingly, it has been shown that in many cases the successful hybrid genotypes show phenotypic characters that are new or transgressive respective to their parental species, and are able to occupy novel or extreme environments (*e. g*., Lewontin and Birch 1966, Grant 1981, Templeton 1981, Büerkle et al. 2000)

Therefore, the comparative analysis of the performance of parental and hybrid genotypes over different environmental conditions can contribute to the identification of those hybrids that are possible carriers of attributes that are associated with tolerance or resistance to a range of stress factors (*e.g*., Troyer 2000, Tollenaar and Lee 2002, David et al. 2007), and could be part of more thorough investigations aimed at identifying the specific attributes and mechanisms of tolerance or resistance (Ghouil et al. 2003, Aranda et al. 2005a, Gandour et al. 2007).

For *Quercus* species little is known about population differences in phenotypic traits with potential adaptive value. Results of analysis of some provenance trials show significant differences in growth and survival between provenances of *Q. ilex* and *Q. suber* (Almeida et al. 2006, Dettori et al. 2006, Gandour et al. 2007). Also, even though it is widely understood that hybridization and hybrids zones are common among oaks (Trelease 1924, Palmer 1948, Whittemore and Schaal 1991, Spellenberg and Bacon 1996, Dumolin-Lapegue et al. 1999, Petit et al. 2004, Cristofolini and Crema 2005, Craft and Ashley 2006), very few studies have examined and compared the performance of hybrid and parental individuals in hybridizing oak species. Such studies are fundamental to assess the potential evolutionary role of hybridization in oak evolution.

For example, Williams and Ehleringer (2000) conducted a study that examined water relations of Quercus gambelii, Q. turbinella and hybrids under natural conditions in southeastern Utah, and the correlation between leaf structural and chemical characteristics, in order to discriminate between carbon isotope, as potentially hybrids can exhibit a lower level of functional integration due to recombination and backcrossing. The results of the study showed that variation within the hybrid populations spanned the mean values observed for these traits in parental taxa from adjacent "pure" populations of each species. In another study, Himrane et al. (2004) analyzed under common garden conditions, the variability of morphological and ecophysiological traits in the hybrid *Quercus subpyrenaica* and its assumed parental species *Q. faginea* and *Q. pubescens*, which co-occur in northeastern Spain. In both set of traits (*e. g.* morphological and ecophysiological traits) the hybrid taxon showed a wide range of values, which spanned and even exceeded the variation of the parental taxa. However, these two studies did not explicitly compare the performance of hybrid and parental individuals. Swenson et al. (2008) used empirical data and niche modeling to examine whether abiotic factors are responsible for the location and structure of an oak hybrid zone between *Q. gambelii* and *Q. grisea* in central and northern New Mexico. Leaf trait analyses and ecological niche models both supported the hypothesis that water availability determines the location and structure of the hybrid zone, and that hybrid individuals have greater water use efficiency and are able to tolerate a higher level of water stress than both parental species. These findings highlighted the general importance of environmental factors in determining hybrid zone location and structure.

Furthermore, they demonstrate how functional trait analyses and predictive ecological niche models can be used in hybrid zone research.

In this study, we focused on the analysis of morphological and ecophysiological traits related to the performance of seedlings of two Mexican red oaks, *Quercus affinis* and *Q. laurina* and their hybrids, under common garden conditions. It was previously shown that there is morphological and genetic intergradation between the two species across a broad geographical area, pattern that can be explained by a scenario of secondary contact and hybridization (Valencia-Ávalos 1994, González-Rodríguez et al. 2004 2005, González-Rodríguez and Oyama 2005; Ramos-Ortiz et al. 2015). But the variation in functional traits of individuals originating from different populations located in the distribution area of the two species and the hybrid zone has not been analyzed and compared. The aim of this study was to evaluate the performance between pure species individuals of *Quercus laurina* and *Q. affinis* and their hybrids, in optimal conditions and under water stress. Specifically, we addressed the following questions: 1) Is there functional variation between *Q. affins*, *Q. laurina* and hybrid individuals under optimal and drought conditions? 2) How is variation at functional attributes related to the capacity to survive drought? 3) Is functional variation among individuals related to climatic conditions at their localities of origin?

Materials and methods

Study system

According to the most recent classification proposed by Nixon (1993), the Mexican oaks *Quercus affinis* and *Q. laurina* belong to the section *Lobatae* (red oaks), of the *Quercus* subgenus. The representative populations of *Q. laurina* occur at an altitudinal range from 2000 to 3065 m along the Sierra Madre del Sur and the west of the Trans-

Mexican Volcanic Belt (TMVB). In turn, *Q. affinis* is mainly distributed in the Sierra Madre Oriental, Sierra of Oaxaca and part of the TMVB at altitudes ranging from 1600 to 2800 m.

Both species have a sympatric distribution in the eastern region of the TMVB and, in this region individuals with varying degrees of morphological intergradation are frequent (Valencia-Ávalos 1994). Several molecular and multivariate morphological studies have indicated patterns consistent with a zone of secondary contact and hybridization between the two species in this area. Furthermore, the hybrid zone has been characterized as showing a mosaic structure with a clear influence of climatic variables (González-Rodríguez et al. 2004, 2005, Ramos-Ortiz et al. 2015).

Acorn sampling and germination

Acorns were collected from six populations along a latitudinal gradient from the south of Oaxaca state (16° 06’ N and 96° 28’ W) to the north of Hidalgo state (20° 37’ N and 98° 36’ W), representing *Q. laurina* (population Suchixtepec), the contact zone (populations Pápalo, Zoquitlán and Tonayán), and *Q. affinis* (population Tizapán and Tonayán) (Figure 1, Table 1). These populations have been previously characterized using nuclear and chloroplast microsatellites and a series of phenotypic traits in wild collected specimens (Ramos-Ortiz et al. 2015). Unfortunately, it was not possible to obtain acorns from a second *Q. laurina* population because in the season collecting acorns were not developped and ripe for germination (Lachao in Ramos-Ortiz et al. 2015). At each population we sampled five individual trees and collected 50-200 acorns per tree.

All collected seeds were sown in a substrate containing 25% perlite, 25% vermiculite and 50% peatmoss in 30 x 40 cm perforated plastic bags. The distribution of the bags was randomized in a greenhouse located in the city of Morelia, Michoacán State (19° 69’ N and 101° 26’ W, 1920 m). Bags were watered to field capacity once per week during the germination and growth stages. The substrate had enough water retention capacity to maintain adequate humidity levels during that period. Seedlings were grown for two years under the same conditions. The position of the containers within the greenhouse was reshuffled every two or three months.

Morphological analysis of two year old seedlings

After two years of growth we sampled 10 fully developed leaves from 10 randomly chosen individuals per population. Fresh leaves were scanned and weighted with an analytical balance, and then weighted again after drying. For each leaf we recorded the leaf area (LA; cm^2) and calculated the specific leaf area (SLA; cm^2/gr) and the leaf dry matter content (LDMC; is the ratio between the dry weight and the fresh weight).

We examined stomatal density (SD) in three leaves of five randomly chosen individuals per population. The abaxial surface of the leaves was coated with clear varnish and, after drying; the layers were removed, transferred to microscopic slides and capped with coverslips. Preparations were examined with a light microscope (Leica DMLB, equipped with a Leica DC200 digital camera) with a magnification of 100X. Stomata were counted directly on the images obtained from each sample.

Finally, in three randomly chosen individuals per population we examined the variation in stem density (StD, mg mm^{-3}) which is the ratio of the dry mass of a section of the main stem of a plant divided by the volume of the same section before drying. In our case ~ 10-cm-long sections were cut from main stems near the base of the stem, oven dried at 70 °C for 72 h and then weighted in an analytical balance. Volume was calculated using the Archimedes principle using a graduated test tube with water. A low stem density (with large vessels) is usually correlated to fast growth, because of cheap volumetric construction costs and a large hydraulic capacity, whereas a high stem density (with small vessels, thickened vessel walls and fibers) leads to a high survival, because of biomechanical and hydraulic safety, resistance against pathogens, herbivores or physical damage (Pérez-Harguindeguy et al. 2013).

Relative growth rate

We measured the height of 25 randomly chosen individuals per population after the first and after the second year of growth and estimated the relative growth rate (RGR) as follows:

$$RGR = (\ln P_2 - \ln P_1) / (t_2 - t_1)$$

Where P_1 and P_2 are the height of the plant at time 1 and 2 (t1 and t2, respectively) (Pérez-Harguindeguy et al. 2013). RGR is the exponential increase in size relative to the size of the plant at the start of a given time interval. Expressed in this way, growth rates can be compared among species and individuals that differ widely in size (Hunt et al. 2002, Pérez-Harguindeguy et al. 2013).

Gas exchange rates and chlorophyll fluorescence

Maximum photosynthetic rate (*Amax*, µmol $CO_2/m^{-2}/s^{-1}$), stomatal conductance (*gs*, mmol $CO_2/m^{-2}/s^{-1}$) and transpiration rate (*E*, mmol $H_2O/m^{-2}/s^{-1}$) were measured in June of 2010 in ten randomly chosen individuals from each population and three randomly chosen leaves per individual. Water use efficiency (*WUEi* mmol/mol^{-1}) was calculated as *Amax*/*gs*. All measurements were made with a portable gas exchange system (LI-6400, Li-Cor, Lincoln, Nebraska, USA) with saturating light (PAR=1500) using the red–blue LED attachment of the LI-6400. All herein reported gas exchange measurements were made in the green-house between 1200 and 1400 hours (most stressful period when population differences are expected to be expressed more strongly) in clear days. Individuals were measured in random order with respect to population of origin.

Also, chlorophyll fluorescence functions: *PhiPS2* (photochemical quenching of PSII, 1600 µmol/ m^{-2}/ s^{-1}) and *NPQ* (non-photochemical quenching) were measured in dark adapted leaves, choosing one leaf per individual and five randomly chosen individuals per population.

Drought resistance experiment

After the previously described measurements were completed, fifteen randomly chosen individuals from each population were subjected to a drought simulation study by maintaining them without watering for 21 days. At three times during this period (at days 7, 14 and 21) we measured *Amax, g*s, *E* and *WUEi* between 1100 and 1300 hrs. After the 21-day period the plants were watered again and survival of each individual was assessed.

Data Analysis

Statistical significance of variation among populations in morphological and physiological traits was assessed with one-way analyses of variance (ANOVA). A principal components analysis (PCA) was used to summarize variation at the different traits. For the results of the drought resistance experiment a two-way analysis of variance was used, with population and day of measurement as main factors. Survival rate after 21 days of drought was compared among populations with a chi-square test.

We used linear regressions to explore whether population mean values of morphological and physiological traits correlate with the values of the 19 bioclimatic variables at the collection localities. Climate data were obtained from the WorldClim database (Hijmans et al. 2005) (http://biogeo.berkeley.edu/worldclim/worldclim.htm).

Results

Significant differences among populations were observed for LA, LDMC, SD and RGR, while SLA and StD did not vary significantly (Table 2). In general, *Q. affinis* populations (Tizapán and Zacatlán) showed a higher LA, followed by Tonayán (hybrid zone), Suchixtepec (*Q. laurina*) and Zoquitlán and Pápalo (hybrid zone). LDMC was highest in Pápalo (hybrid zone) lowest in Zoquitlán (hybrid zone) and intermediate in the other populations. SD was highest in the geographically intermediate populations Tonayán and Zoquitlán (hybrid zone) and lower towards both ends of the geographical gradient. RGR was higher in Tonayán and Pápalo (hybrid zone), followed by Zacatlán (*Q. affinis*) and Zoquitlán (hybrid zone), and finally by the southernmost and northernmost populations, Suchixtepec (*Q. laurina*) and Tonayán (*Q. affinis*).

Populations also differed in all the physiological traits evaluated (gas exchange and chlorophyll fluorescence functions) (Table 3). For *Amax*, *gs* and *E*, the Pápalo population had the lowest values, followed by Suchixtepec, Zoquitlán, Tonayán, Zacatlán and Tizapán. In contrast *WUEi* was higher in Suchixtepec, followed by Tizapán, Zacatlán, Tonayán, Pápalo and Zoquitlán. Populations Tizapán, Zacatlán and Tonayán had the higher *PhiPS2*, followed by Zoquitlán, Suchixtepec and Pápalo, while *NPQ* was higher in Tonayán, followed by Suchixtepec, Zoquitlán, Tizapán, Zacatlán and Pápalo.

According to the Principal Components Analysis, the first and the second main axis respectively explained 23% and 21% of the total variation. The variables with the highest loadings on the first principal component (PC1) were *WUEi* and *NPQ*, while for the PC2 the variables with the highest loadings were *gs* and *E*. The scatter plot of individual scores on the first two PCs (Figure 2) showed that the most distinct populations are the two southernmost populations Suchixtepec and Pápalo on one side and population Tonayán (from the contact zone) on the other side. Individuals from the other three populations were similar and clustered together in the center of the plot.

We found a significant negative correlation between mean *E* and annual precipitation ($R^2 = 0.88$; $P = 0.005$). Also, *Amax* was positively correlated with temperature seasonality ($R^2 = 0.79$; $P = 0.017$) and RGR was negatively correlated with precipitation seasonality ($R^2 = 0.70$; $P = 0.038$) (Figure 3).

The results of the drought resistance experiment indicated a significant effect of population on all the physiological variables evaluated (*Amax*, *gs*, *E* and *WUEi*) (Table 4, Figure 4). The day of measurement (7, 14 and 21 after water suppression) also had a significant effect on all variables except *WUEi*. Finally, the interaction of population and day of measurement was only significant for *WUEi.* In general, all populations showed a marked decrease in photosynthetic capacity (*Amax*) after 14 days of drought and after 21 days only individuals from population Zoquitlán (hybrid zone) showed positive values of *Amax*. The negative values in the other populations indicate a higher rate of respiration than CO_2 capture. A similar pattern was also found for *gs*, *E* and *WUEi*, indicating that population Zoquitlán has a higher capacity of maintaining physiological functions under drought conditions. This population also had a 100% survival rate after the experiment, followed by population Pápalo with a 93.3% survival rate (Figure 5). Variation in survival rate was significant according to the chi-square test. Finally, there was a negative correlation between population survival rate and mean leaf area ($R^2 = 0.81$; $P = 0.015$) (Figure 6).

Discussion

The results of this study indicated that, under homogeneous conditions, populations of the *Quercus affinis-Q. laurina* complex, collected along a latitudinal gradient, displayed significant variation at several important functional traits. Of all traits evaluated only two, specific leaf area (SLA) and stem density (StD) did not vary significantly. Noticeably, traits also differed in the patterns of variation displayed along the latitudinal gradient. For example, the maximum photosynthetic rate (*Amax*) increased from south to north in a more or less clinal pattern, whereas other traits (stomatal density, RGR, *WUEi*) showed maximum values at geographically intermediate populations, while populations at both ends of the gradient did not differ significantly. Finally, values of the other traits fluctuated along the gradient without a clear pattern. Principal components analysis, used to summarize the overall variation at the different traits, indicated that the three most distinct populations are Suchixtepec, Pápalo (the two southernmost populations) and Tonayán (in the contact zone). Individuals from the other three populations were similar and clustered together in the center of the scatterplot. Therefore, for the traits analyzed, we did not observe a clear pattern of differentiation between populations of the two parental species and intermediacy of the populations within the contact zone. On the contrary, depending on the trait, populations from within the contact zone displayed character values that were identical, intermediate or extreme in comparison with parental species populations.

Areas of secondary contact and hybridization are of interest because a wide array of new, recombinant genotypes are produced and provide a source of variability upon which selection can act (Arnold 1997, Rieseberg et al. 1999, 2003). A crucial aspect to the understanding of the evolution of hybrid populations and to ultimately explain the creative evolutionary role of hybridization is the assessment of how this recombination of genotypes is expressed in the phenotypic characters (Rieseberg et al. 1999 2003). Although the non-intermediacy of hybrids have been found in many previous studies, in most cases only morphological characters have been evaluated in wild collected individuals (Anderson 1948, Potts and Reid 1985, Grant 1989, Rieseberg and Carney 1998, Nuñez-Castillo et al. 2010). The expression of morphological and physiological traits under controlled conditions has been examined less frequently (Cullis et al. 2006, Poorter 2012, Juenger 2013, Tardieu 2013, Mohamed 2014), but such studies are crucial to evaluate the degree to which these traits are under genetic control. A few common-garden experiments have demonstrated that hybrids can outperform their parental species, lending support to the idea that hybridization facilitates invasion, survival and/or range expansion (Vilà and D'Antonio 1998, Campbell et al. 2006, Whitney et al. 2006, 2010*a*, Ridley and Ellstrand 2009).

Furthermore, we observed that some physiological traits at the seedlings stage were related with the environment that adult trees experience in the field. We detected a positive relation between *Amax* and temperature seasonality. Particularly, sites with more variation in temperature are also the ones with higher average temperature. Given that photosynthesis is a process highly sensitive to temperature, the pattern detected in our work suggests that sites with higher temperature offer ideal conditions for the photosynthetic enzymes to work (Berry and Bjorkman 1980, Cunningham and Read 2002, Hjelm and Ogren 2003, Hikosaka et al. 2006, Angert 2006, Körner 2007). Further research on the temperature optimum of photosynthetic activity needs to be conducted in populations of the *Quercus affinis*-*Q. laurina* complex to confirm this hypothesis. On the other side, we observed a negative relationship between transpiration rate and total annual precipitation. This suggests that *Quercus* populations from areas with less soil water availability minimize water loss via a reduced transpiration rate, which could allow them to maintain plant water status and to be less sensitive to drought (Walter 1971, Lauenroth 1979, Sala et al. 1988, Beatley 1974, Comstock and Ehleringer 1992). Finally a negative correlation was observed between relative growth rate and precipitation seasonality. This relationship implies that areas with less variation in water availability offer a more stable environment round-year for realized plant growth (Beatley 1974, Comstock and Ehleringer 1992, Venable 2007, Huxman et al. 2008). Altogether, these relations indicate that environment is a mayor selective force guiding differentiation among *Quercus* populations (Dudley 1996, Ackerly et al. 2000, Arntz and Delph 2001, Heschel et al. 2002, Heschel and Riginos 2005, Donovan et al. 2007, Agrawal et al. 2008). Furthermore, previous evidence reveals that at least two populations (Zoquitlán and Tonayán) significantly differ at the genetic level (Ramos-Ortiz et al. 2015). Overall, these results indicate that genetic admixture and environmental selection have both contributed to the variation patterns observed at functional traits across this hybrid zone.

The results of the drought simulation experiment also revealed significant variation among populations in their capacity to retain physiological functions through prolonged water deficit and in survival rate after the cessation of drought. The only population that showed positive values of *Amax*, the highest *gs*, *E*, *WUEi* and a 100% survival rate after 21 days of drought was Zoquitlán, which is located in the center of the contact zone and has a majority of hybrid individuals (Ramos-Ortiz et al. 2015). Interestingly, a few previous studies conducted in different plant species, including oaks (*e.g.* Swenson et al. 2008), have found that interspecific hybrids can be more drought resistant than pure parental individuals. For example, in the subalpine herbs *Ipomopsis aggregata* (Polemoniaceae), *I. tenuituba¸* and their natural hybrids, Campbell and Wendlandt (2013) found that, under simulated conditions of reduced precipitation in a common garden experiment, the hybrids increased water use efficiency and produced more flowers than the two parental species. In fact, in several instances interspecific hybrids have been identified as possible carriers of attributes that are associated with some stress tolerance under different environmental conditions (*e.g,* Troyer 2000, Tollenaar and Lee 2002, David et al. 2007). This observation may deserve more extensive research aimed at identifying the specific attributes and mechanisms responsible for this characteristic (Ghouil et al. 2003, Aranda et al. 2005a, Gandour et al. 2007).

An interesting result of our experiment is that survival rate was negatively correlated with leaf area, providing some insight about the traits associated with the variation in drought resistance in *Q. affinis*-*Q. laurina* seedlings. Theoretically, leaf size has been considered a link with heat dissipation (Cunningham et al. 1999, Wright and Westoby 1999, Cornelissen et al. 2003, McDonald et al. 2003). Leaves of small size have a reduced boundary layer which facilitates leaf cooling through heat convection and minimizes transpiration as a mechanism of heat loss. Overall this suggests that *Quercus* population with smaller leaves could maintain water status during drought which has consequences to plant survival. To our knowledge this is the first time evidencing that leaf dimension determines plant survival during drought.

Leaf area was not significantly correlated with any of the 19 bioclimatic variables. Therefore, the reasons for a reduced leaf area in populations within the contact zone in comparison with the parental species populations are not clear. However, the appearance of phenotypic traits with extreme values is well documented in hybrid zones, resulting mainly from the action of complementary genes (Rieseberg et al. 2003).

Therefore, from our results we can conclude that under one specific type of environmental stress (drought stress) some individuals from a hybrid population (Zoquitlán) had a superior performance in comparison to the other populations along the geographical gradient represented in this study. However, it must be kept in mind that under natural conditions, hybrid performance relative to parental species is also likely to depend on several local abiotic and biotic factors such as antagonistic and mutualistic interactions (Campbell and Snow 2007, Mercer et al. 2007). For example, although wild radish (*Raphanus raphanistrum*) were more fecund than crop x wild radish hybrids grown in common garden under noncompetitive conditions, hybrids were less negatively affected by intense competition (Campbell and Snow 2007). Similarly, although *Carpobrotus* hybrids were more resistant to herbivory than their parental taxa across three common gardens, total greater biomass of hybrids was only observed in certain habitats (Vilà and D' Antonio 1998). Such genotype by environment interactions often impacts the success of hybrids (Arnold et al. 1999, Mercer et al. 2006, Arnold and Martin 2010). Therefore, reciprocal transplant experiments are necessary to broaden our understanding of the hybridization-fitness link.

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de Vos, N. Buchmann, G. Funes, F. Quetier, J. G. Hodgson, K. Thompson, H. D. Morgan, H. ter Steege, M. G. A. van der Heijden, L. Sack, B. Blonder, P. Poschlod, V. Vaieretti, G. Conti, A. C. Staver, S. Aquino, J. H. C. Cornelissen. New handbook for standardised measurement of plant functional traits worldwide. Aust. J. Bot. 61:167–234.

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Figure legends

Fig.1 Map showing the seven sampling localities. Red symbols represent *Quercus laurina* populations, green symbols represent contact zone populations and blue symbols represent *Q. affinis* populations. The numbering of populations is as in Table 1.

Fig. 2 Scatter plot depicting individual scores from a Principal Components Analysis based on morphological and physiological traits of *Quercus affinis*-*Q. laurina* individuals from six populations in a common garden.

Fig. 3 Linear regressions of climatic variables at the collection localities and physiological traits of the seedlings in a common garden. (A) Temperature seasonality vs. maximum photosynthetic rate (*Amax*), (B) Annual precipitation vs. transpiration rate (*E*), (C) Precipitation seasonality vs. relative growth rate (RGR).

Fig. 4 Mean values of *Quercus affinis*-*Q. laurina* individuals from the six study populations of (A) maximum photosynthetic rate (*Amax*), (B) stomatal conductance (*gs*), (C) water use efficiency (*WUEi*) and, (D) transpiration rate (*E*) at days 0, 7, 14 and 21 after the initiation of simulated drought.

Fig. 5 Percentage of individuals surviving of *Quercus affinis*-*Q. laurina* individuals from the six study populations after 21 days of drought.

Fig. 6 Correlation between mean leaf area (LA) and percentage survival of *Quercus affinis*-*Q. laurina* individuals from the six study populations.

Table 1. Geographical information for the six study populations of the *Quercus affinis*-*Q. laurina* complex along a latitudinal gradient.

Code	Locality	State	Longitude / Latitude	Altitude (m)
	Q. laurina			
1	Suchixtepec	Oaxaca	16° 06’/ 96° 28’	1962
	Contact zone			
2	Pápalo	Oaxaca	17° 51’ / 96° 48’	2778
3	Zoquitlán	Puebla	18°17’ / 97°05’	2597
4	Tonayan	Veracruz	19°43’ / 96°54’	2068
	Q. affinis			
5	Zacatlán	Puebla	19° 54’/ 97° 57’	2043
6	Tizapán	Hidalgo	20° 37’ / 98° 36’	2085

Table 2. Population means (standard error) of morphological traits and relative growth rate of seedlings of *Quercus affinis* (Zacatlán and Tizapán), *Q. laurina* (Suchixtepec) and contact zone (Pápalo, Zoquitlán and Tonayán) in a common garden. Different letters indicate significant differences after an analysis of variance and a Tukey-Kramer post-hoc test.

	Population					
Morphological traits	**Suchixtepec**	**Pápalo**	**Zoquitlán**	**Tonayán**	**Zacatlán**	**Tizapán**
LA(cm^2)	6.310^{bcd}	4.009^{d}	5.688^{cd}	7.479^{bc}	8.045^{b}	9.863^{a}
	(1.139)	(0.277)	(0.323)	(0.277)	(0.445)	(0.388)
SLA(cm^2 / gr)	153.409^{a}	96.925^{a}	122.236^{a}	94.495^{a}	93.261^{a}	92.664^{a}
	(47.102)	(8.578)	(13.763)	(11.504)	(7.195)	(6.335)
LDMC	0.470^{bc}	0.745^{a}	0.442^{c}	0.496^{b}	0.489^{b}	0.508^{b}
	(0.0136)	(0.0105)	(0.00865)	(0.00865)	(0.00865)	(0.00865)
SD	54.3^{bc}	63.00^{bc}	104.18^{a}	112.69^{a}	81.6^{ab}	46.44^{c}
	(4.422)	(1.179)	(2.786)	(15.982)	(1.493)	(5.740)
RGR (cm / day)	0.0005^{c}	0.0008^{b}	0.0006^{bc}	0.0009^{a}	0.0007^{abc}	0.0005^{c}
	(2.38E-05)	(8.27E-05)	(5.18E-05)	(8.51E-05)	(6.77E-05)	(4.45E-05)
StD (mg / mm^3)	0.5319^{a}	0.5442^{a}	0.5144^{a}	0.6500^{a}	0.4808^{a}	0.6153^{a}
	(0.016368)	(0.03720)	(0.032109)	(0.05746)	(0.04393)	(0.00274)

Notes: LA: leaf area, SLA: specific leaf area, LDMC: leaf dry matter content, SD: stomatal density, RGR: relative growth rate, StD: stem density.

Table 3. Population means (standard error) of physiological traits of seedlings of *Quercus affinis* (Zacatlán and Tizapán), *Q. laurina* (Suchixtepec) and contact zone (Pápalo, Zoquitlán and Tonayán) in a common garden. Different letters indicate significant differences after an analysis of variance and a Tukey-Kramer post-hoc test.

Trait	**Population**					
	Suchixtepec	**Pápalo**	**Zoquitlán**	**Tonayán**	**Zacatlán**	**Tizapán**
Amax	3.097^{b}	3.692^{b}	5.510^{a}	5.363^{a}	5.260^{a}	5.710^{a}
	(0.2120)	(0.1657)	(0.1860)	(0.2605)	(0.1510)	(0.3283)
Gs	0.0822^{ab}	0.0746^{ab}	0.0704^{ab}	0.0667^{b}	0.0923^{a}	0.0731^{ab}
	(0.0172)	(0.0059)	(0.0030)	(0.0050)	(0.0085)	(0.0043)
E	1.593^{b}	0.141^{c}	1.770^{ab}	1.518^{b}	2.046^{a}	1.685^{b}
	(0.1827)	(0.0076)	(0.0392)	(0.0707)	(0.1119)	(0.0610)
WUEi	63.042^{bc}	56.130^{c}	83.015^{ab}	97.444^{a}	72.548^{bc}	86.267^{ab}
	(10.161)	(5.732)	(4.852)	(4.849)	(3.561)	(3.747)
PhiPS2	0.030^{ab}	0.029^{b}	0.040^{a}	0.023^{b}	0.030^{b}	0.041^{a}
	(0.0031)	(0.0023)	(0.0029)	(0.0021)	(0.0013)	(0.0023)
NPQ	2.481^{ab}	1.531^{d}	2.355^{ab}	2.538^{a}	2.048^{c}	2.238^{bc}
	(0.084)	(0.059)	(0.045)	(0.047)	(0.048)	(0.043)

Notes: Amax: maximum photosynthetic rate, *gs*: stomatal conductance rate, *E:* transpiration rate, *WUEi*: water-use efficiency, *PhiPS2*: photochemical quenching of PSII, *NPQ*: non photochemical quenching.

Table 4. Significance levels for the variation in four physiological traits in a drought simulation experiment. Factors evaluated were the population of origin of the seedlings and the day of measurement (0, 7, 14 and 21 days of drought).

Trait	**Population**	**Day of measurement**	**Population* day of measurement**
Amax	<0.0001	<0.0001	0.89
gs	<0.0001	<0.0001	0.85
E	0.0006	<0.0001	0.79
WUEi	0.0005	0.47	0.0001

Notes: Amax: maximum photosynthetic rate, *gs:* stomatal conductance rate, *E:* transpiration rate, *WUEi:* water-use efficiency.

FIGURE 1.

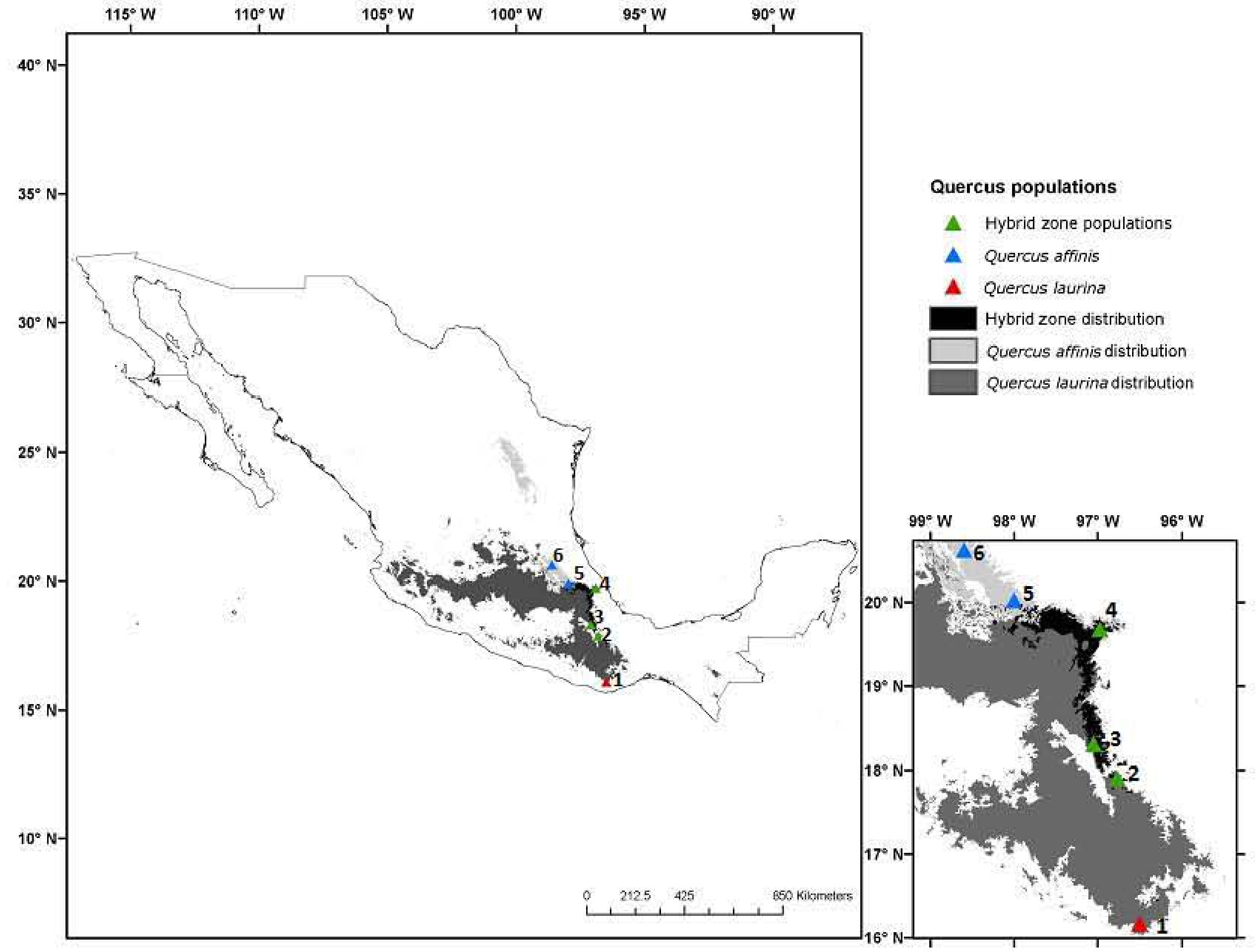

115° W
110° W
105° W
100° W
95° W
90° W
40° N
35° N
30° N
25° N
20° N
15° N
10° N
6
5
4
3
2
1
0
212.5
425
850 Kilometers
Quercus populations
Hybrid zone populations
Quercus affinis
Quercus laurina
Hybrid zone distribution
Quercus affinis distribution
Quercus laurina distribution
99° W
98° W
97° W
96° W
20° N
19° N
18° N
17° N
16° N

FIGURE 2.

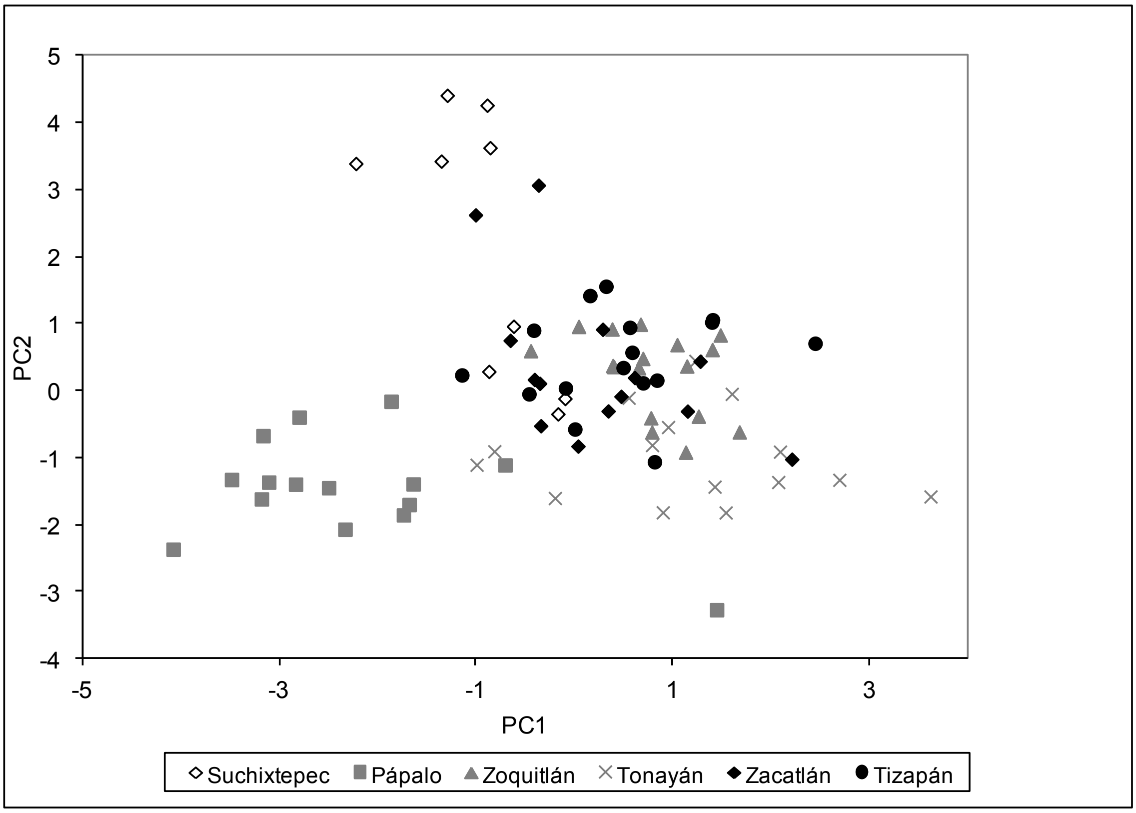

PC2
5
4
3
2
1
0
-1
-2
-3
-4
-5
-3
-1
1
3
PC1
Suchixtepec
Pápalo
Zoquitlán
Tonayán
Zacatlán
Tizapán

FIGURE 3.

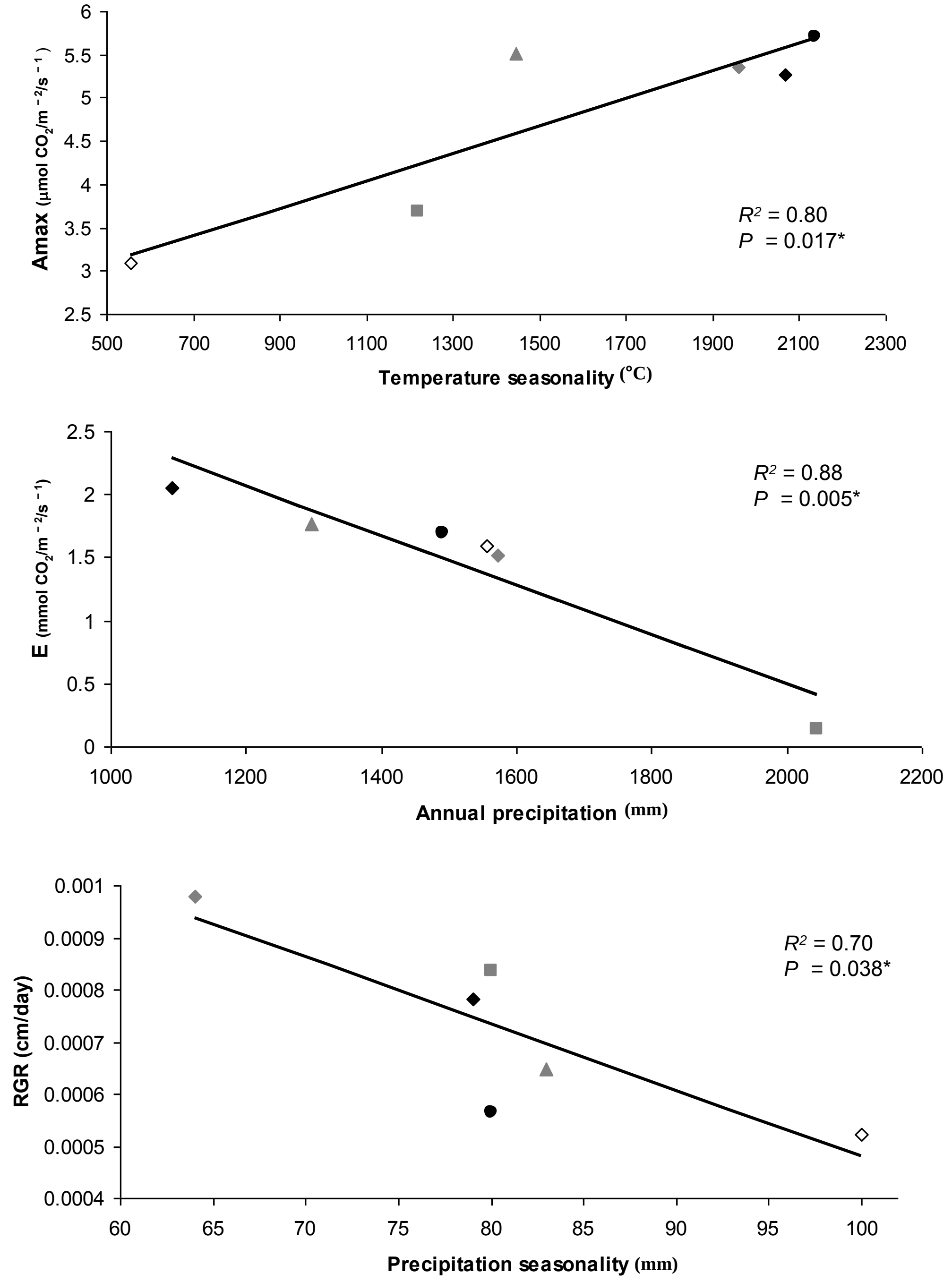
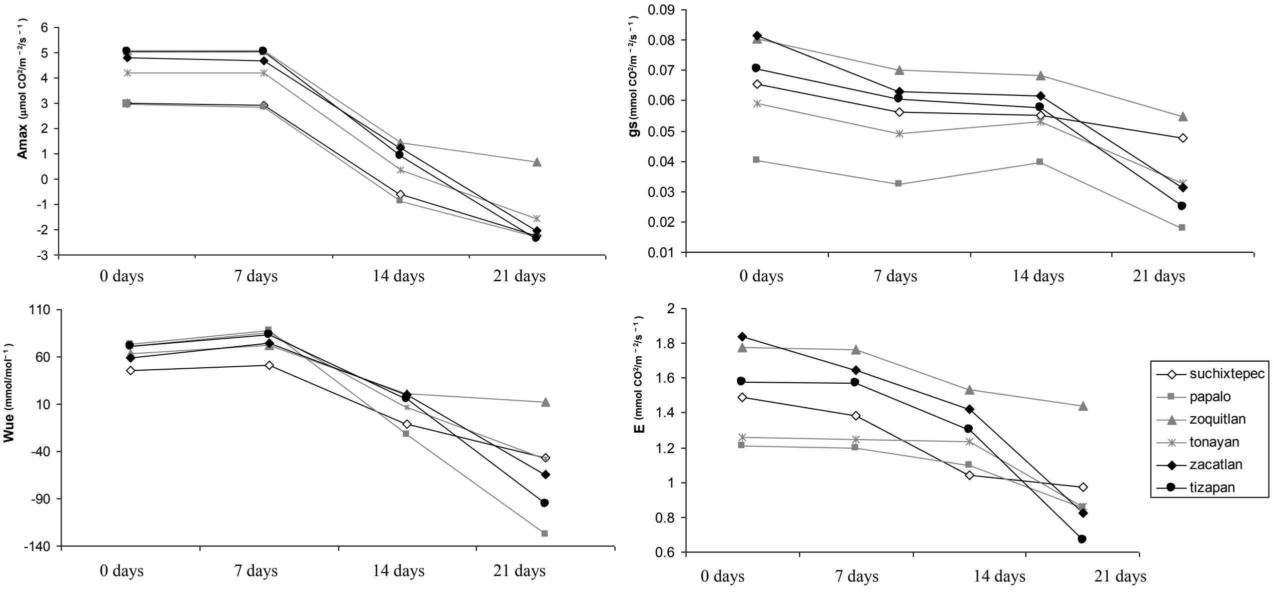

Amax (µmol CO$_2$/m^{-2}/s^{-1})
6
5.5
5
4.5
4
3.5
3
2.5
500
700
900
1100
1300
1500
1700
1900
2100
2300
Temperature seasonality (°C)
R^2 = 0.80
P = 0.017*
E (mmol CO$_2$/m^{-2}/s^{-1})
2.5
2
1.5
1
0.5
0
1000
1200
1400
1600
1800
2000
2200
Annual precipitation (mm)
R^2 = 0.88
P = 0.005*
RGR (cm/day)
0.001
0.0009
0.0008
0.0007
0.0006
0.0005
0.0004
60
65
70
75
80
85
90
95
100
Precipitation seasonality (mm)
R^2 = 0.70
P = 0.038*

FIGURE 4.



Amax (µmol CO²/m⁻²/s⁻¹)
6
5
4
3
2
1
0
-1
-2
-3
0 days
7 days
14 days
21 days
gs (mmol CO²/m⁻²/s⁻¹)
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0 days
7 days
14 days
21 days
Wue (mmol/mol⁻¹)
110
60
10
-40
-90
-140
0 days
7 days
14 days
21 days
E (mmol CO²/m⁻²/s⁻¹)
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0 days
7 days
14 days
21 days
suchixtepec
papalo
zoquitlan
tonayan
zacatlan
tizapan

FIGURE 5.

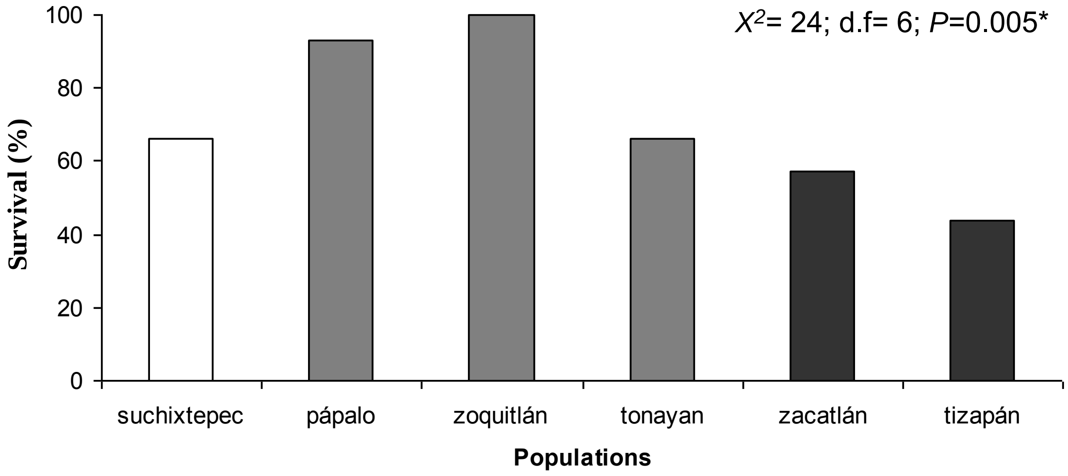
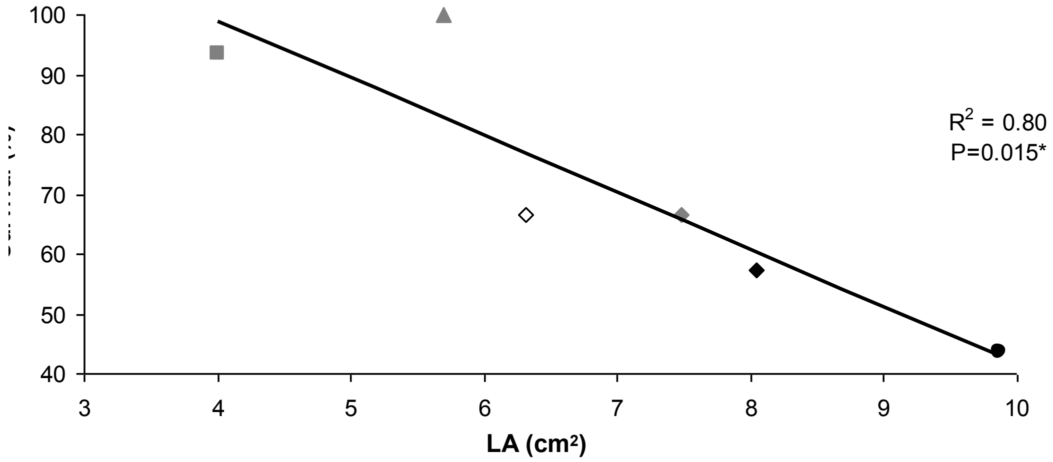

X^2= 24; d.f= 6; P=0.005*
100
80
60
40
20
0
Survival (%)
suchixtepec
pápalo
zoquitlán
tonayan
zacatlán
tizapán
Populations

FIGURE 6.



CAPÍTULO IV

DISCUSIÓN GENERAL Y CONCLUSIONES

La presente tesis consistió de dos estudios, los cuales permitieron abordar preguntas sobre la hibridación natural del complejo de encinos rojos mexicanos formado por *Quercus affinis* y *Q. laurina*. En el primer estudio (capítulo II) se analizó el complejo desde el punto de vista de la caracterización de la estructura geográfica y genética de la zona de hibridación entre estas dos especies mediante marcadores moleculares neutros (nueve microsatélites nucleares y cuatro de cloroplasto) y nueve caracteres fenotípicos foliares y de las bellotas. En el segundo estudio (capítulo III), se realizó una comparación del desempeño fisiológico de individuos de ambas especies e individuos híbridos en condiciones óptimas en un jardín común y bajo un experimento de estrés hídrico. En la Fig. 1 se propone un mapa conceptual que integra los principales conceptos que sirvieron de base para el diseño global de la tesis.

Estructura geográfica y asociaciones fenotípicas y genéticas con gradientes climáticos en la zona de hibridación entre Quercus affinis y Q. laurina

En el capítulo II, las poblaciones muestreadas se encontraron a lo largo de un gradiente geográfico que atraviesa la zona de hibridación entre *Quercus affinis* y *Q. laurina*. Estas poblaciones fueron escogidas al azar, tomando en cuenta estudios previos de caracteres morfológicos (Valencia-Ávalos, 1994; González-Rodríguez *et al.,* 2004a). Los resultados del presente estudio fueron consistentes con la hipótesis de contacto secundario y la hibridación entre especies previamente diferenciadas. En particular, el análisis de coordenadas principales indicó clara diferenciación genética entre individuos de poblaciones situadas en ambos extremos del gradiente geográfico que representa las dos especies y algunos individuos genéticamente intermedios en las poblaciones dentro de la zona de hibridación. La transición de una especie a la otra se produce en una zona geográfica que se extiende desde el norte del Estado de Oaxaca a Veracruz y Puebla.

Algunas características morfológicas (*e. g*. masa de la bellota, y el componente 4 de la morfología foliar), mostraron una correlación significativa con la asignación genética de los individuos según un análisis bayesiano, aunque en la mayoría de los caracteres morfológicos analizados no fue así. Además, no hubo muchos caracteres que mostraran patrones paralelos (e. g. correlación significativa) de cambio a lo largo del gradiente geográfico.

Las zonas de hibridación mantenidas por un equilibrio entre la selección en contra de los híbridos y la dispersión, comúnmente muestran clinas estrechas y marcadamente paralelas para la mayoría de los caracteres. La amplitud de la zona de hibridación entre *Q. affinis* y *Q. laurina* podría sugerir una combinación de alta dispersión y débil selección en contra de los híbridos. Pocos estudios han evaluado directamente el desempeño de los individuos híbridos y sus especies progenitoras en *Quercus* (ver capítulo III). Los datos obtenidos en dichos estudios han mostrado, en lugar de un desempeño uniformemente menor de los híbridos, valores que pueden ser más altos, equivalentes, intermedios o menores que los de las especies progenitoras (Williams y Ehleringer, 2000; Williams et al 2001; Himrane *et al.,* 2004). Por lo tanto, es probable que en la naturaleza algunos híbridos entre especies de encino no experimenten una fuerte selección negativa. Nuestros propios resultados obtenidos en el capítulo III apoyaron este punto de vista y mostraron que los individuos de las poblaciones de la zona de hibridación pueden ser superiores a las especies puras bajo condiciones de estrés hídrico.

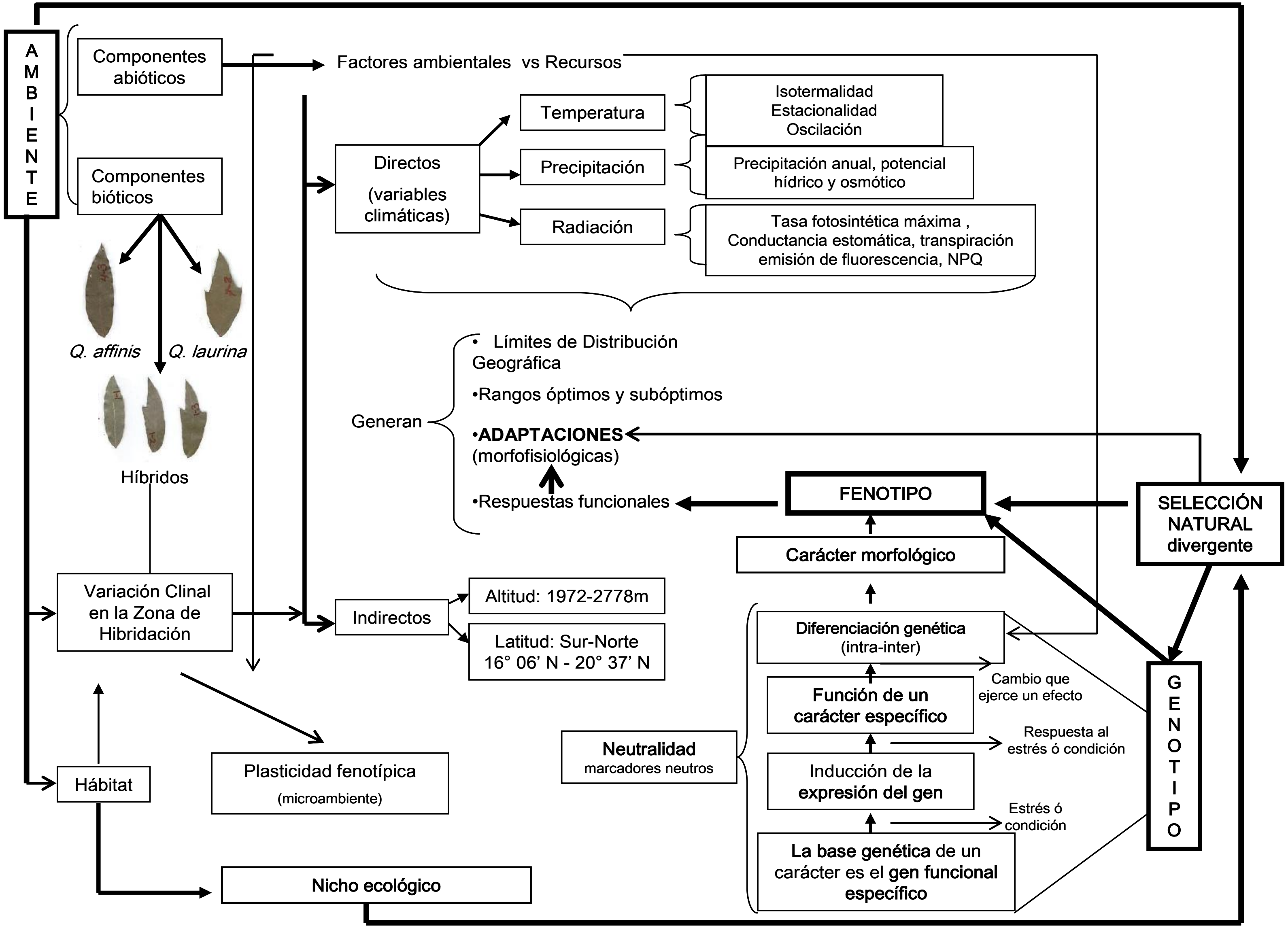


Fig. 1. Mapa conceptual que engloba todos los conceptos aplicados al proyecto de investigación.

Por otra parte, una asociación entre la variación genética y fenotípica y algunas variables climáticas es evidente en la zona de hibridación entre *Q. affinis* y *Q. laurina*. La isotermalidad, una variable relacionada con la temperatura parece tener la mayor influencia en la variación morfológica, seguida por una variable relacionada con la precipitación (precipitación anual). La proporción de ascendencia genética poblacional estuvo también fuertemente asociada con la isotermalidad. Varios estudios previos han mostrado una considerable correspondencia entre la forma de la hoja y el clima dentro de las especies de *Quercus* (e. g., Castro-Díez *et al.,* 1997; Royer *et al.,* 2008; Uribe-Salas *et al.,* 2008; Sork *et al.,* 2010).

En general, nuestros resultados apoyan la idea de que la selección natural (intrínseca como extrínseca), puede estar actuando sobre una serie de caracteres fenotípicos y regiones genómicas y por lo tanto contribuye a formar la estructura de la zona de hibridación entre *Q. affinis* y *Q. laurina*. Aunque si bien es cierto que la plasticidad fenotípica puede explicar algunas de las asociaciones observadas entre los caracteres morfológicos y las variables climáticas, los resultados del capítulo III, indicaron que varios caracteres ecofisiológicos expresados en condiciones de jardín común mostraron correlaciones significativas con variables climáticas de las localidades de origen de los individuos.

Un aspecto de interés para comprender los patrones de variación que reportamos en este estudio es la edad de la zona de hibridación. La gran diversidad de haplotipos encontrados en este estudio con los microsatélites ADNcp probablemente indica la antigüedad de las poblaciones contemporáneas de *Q. affinis* y *Q. laurina*. En México, los bosques templados a través de la Sierra Madre Oriental y la Sierra Madre del Sur probablemente experimentaron migraciones altitudinales mas que latitudinales durante los máximos glaciales, teniendo mayor conectividad entre las poblaciones en este periodo que durante los interglaciares (Ornelas *et al.,* 2013; Ramírez-Barahona y Eguiarte, 2013). Por lo tanto, es probable que el tamaño efectivo de la población histórica para especies tales como *Q. affinis* y *Q. laurina* han sido continuamente grandes durante su historia evolutiva, y que migraciones recurrentes altitudinales han contribuido al intercambio de ADNcp entre las poblaciones, lo que explica la falta de estructura filogeográfica.

De manera complementaria, los modelos de distribución potencial histórica para *Q. affinis* y *Q. laurina* para las condiciones del último máximo glacial sugieren la permanencia de ambas especies en básicamente las mismas regiones que ocupan hoy en día, aunque ocupando una mayor área que en el presente (Fig. 2).

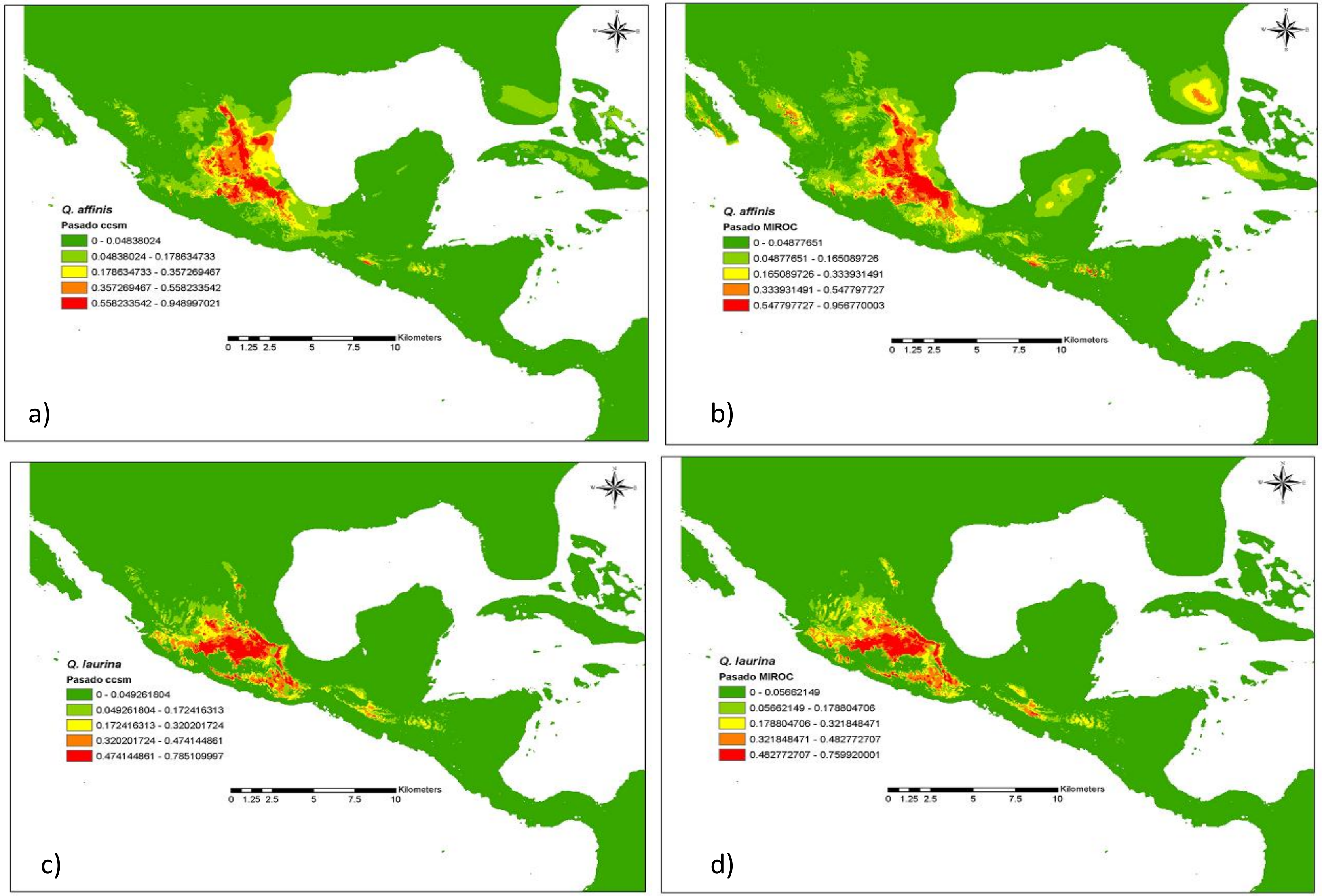


Fig. 2. Mapas que muestran la probabilidad de ocurrencia (verde= 0- 0.2, rojo =0.8 – 1) para *Quercus affinis* y *Q. laurina* en el último máximo glacial (21 Ka) según los modelos CCSM (a, c) y MIROC 3.2 (b, d).

En resumen, los datos presentados en este primer estudio indican que la zona de hibridación entre Q. *affinis* y *Q. laurina* se asemeja a una zona de tensión en lugar de un mosaico, existiendo algunos patrones heterogéneos de variación entre los caracteres moleculares y fenotípicos. Aunado a trabajos previos (Dodd y Afzal –Rafii, 2004; Sánchez de Dios *et al.,* 2006; Peñaloza-Ramírez *et al.,* 2010) nuestro estudio presenta resultados que apoyan la idea de que la selección opera manteniendo una diversidad de genotipos (incluyendo los tipos progenitores) en los complejos de encinos híbridos (Petit *et al.,* 2003; Dodd y Afzal-Rafii, 2004; Lexer *et al.,* 2006).

Desempeño fisiológico del complejo Q. affinis-Q.laurina

En el capítulo III, se evaluó el desempeño entre los individuos de las especies progenitoras de *Quercus affinis*, *Q. laurina* y sus híbridos en condiciones óptimas y bajo estrés hídrico. La variación que existe en las características funcionales de los individuos procedentes de diferentes poblaciones ubicadas en el área de distribución de las dos especies y la zona híbrida como tal, no había sido analizada y mucho menos comparada. Por lo cual, es de suma importancia conocer si existe variación funcional entre *Q. affinis* y *Q. laurina* e individuos híbridos tanto en condiciones óptimas como de sequía, saber cómo es la variación en los atributos funcionales relacionados con la capacidad de sobrevivir a la sequía, y finalmente si las condiciones climáticas locales originales están relacionadas con la variación funcional poblacional.

Numerosos estudios ecofisiológicos han permitido identificar caracteres asociados a un mayor desempeño en condiciones ambientales específicas (Grime, 1977; Ackerly *et al.,* 2000). Por ejemplo, las especies de plantas en ambientes secos tienen una tendencia a presentar menor área foliar específica (SLA) y una mayor eficiencia en el uso del agua (*WUEi*) que especies en ambientes húmedos (Smith *et al.,* 1997; Reich *et al.,* 1997; Diefendorf *et al.,* 2010).

La estacionalidad de la precipitación tiene una mayor influencia en la humedad y la disponibilidad de otros recursos del suelo que la cantidad total de precipitación anual, siendo esta variable climática probablemente más importante en la determinación del desempeño de las plantas (Huxman *et al.,* 2004; Reynolds *et al.,* 2004; Schwinning y Sala, 2004). A su vez, la variable de temperatura durante períodos de crecimiento en las plantas también influye directa o indirectamente en los procesos fisiológicos en ambientes secos (Comstock y Ehleringer, 1992; Huxman *et al.,* 2008; Collins *et al.,* 2010); la fotosíntesis puede estar fuertemente influenciada por la temperatura, ya que las especies presentan diferentes temperaturas óptimas, por lo tanto, varían en su capacidad individual para aclimatarse al cambio (Berry y Bjorkman, 1980; Comstock y Ehleringer, 1992). Es decir, los patrones específicos de temperatura y precipitación influyen fuertemente en el éxito de las especies y sus híbridos, de tal manera que es necesario conocer y comprender de qué forma los llevan a cabo para inferir la variación, análisis y comparación de estas características funcionales.

La mejor manera de probar tales efectos potenciales de la variación ambiental en la hibridación sería manipulando las condiciones ambientales en el campo. Sin embargo, sólo unos pocos estudios recientes han examinado la respuesta del desempeño de híbridos tanto en jardín común como en condiciones naturales

(Williams *et al.,* 2001; Brock y Galeno, 2005; Sherrard *et al.,* 2009; Campbell *et al.,* 2010; Ma *et al.,* 2010; Campbell y Wendlandt, 2013). En términos de desempeño, diferentes condiciones ambientales han y forman parte de una investigación más amplia cuyo objetivo es identificar los atributos y mecanismos responsables de esta característica (Ghouil *et al.,* 2003; Aranda *et al.,* 2005a; Gandour *et al.,* 2007).

De la misma forma, en el presente estudio todos los caracteres evaluados presentaron significancia poblacional excepto SLA (área foliar específica) y StD (densidad estomática), mostrando diferencias en los patrones de variación distribuidos a lo largo del gradiente latitudinal. Por lo cual, en todos los caracteres analizados, no se observó un claro patrón de diferenciación entre poblaciones de las especies progenitoras y de las poblaciones de la zona de contacto. Es decir, se encontró que algunos de los híbridos presentan caracteres fisiológicos intermedios y otros una marcada tendencia unidireccional hacia algunas de las especies progenitoras. La posible explicación a esto es que los híbridos pueden expresar un mosaico de caracteres funcionales de los progenitores, que depende de la característica evaluada y la interacción entre su expresión genética y el ambiente (Rieseberg y Carney, 1998).

No fue posible determinar la generación a la que pertenecen los individuos híbridos. Se ha mencionado que los híbridos de la generación F_1 presentan características intermedias a los progenitores, pero que al retrocruzarse van adquiriendo mayor número de características de uno de los progenitores en las generaciones subsecuentes (Anderson, 1948; Potts y Reid, 1985; Grant, 1989; Rieseberg y Carney, 1998).

Por otra parte, también se han observado diferencias poblacionales en la plasticidad de algunos caracteres morfológicos y fisiológicos en otras especies del género *Quercus* (*e.g*. Balaguer *et al.,* 2001; Gratani *et al.,* 2003). Los resultados obtenidos con respecto a los caracteres a nivel de hoja (intercambio de gases y emisión de fluorescencia) y su correlación con las variables ambientales (estacionalidad de la temperatura, la precipitación anual y la precipitación estacional) arrojaron una correlación significativa con tres caracteres fisiológicos (*Amax:* Tasa fotosintética máxima; *E:* Tasa de transpiración y *RGR*: tasa de crecimiento relativo) importantes respectivamente.

Es necesario mantener un nivel adecuado de agua para el crecimiento de las plantas (Landis *et al.,* 1989; Lopushinsky, 1990) debido a su influencia en la mayoría de los procesos fisiológicos, tales como en la tasa fotosintética máxima, respiración, síntesis química, división celular, elongación de las hojas, transporte esencial y termorregulación, los procesos en función del nivel alcanzado en la escasez de agua, pueden afectar el desempeño de las plantas (Rojas *et al.,* 2003).

Por lo tanto, el papel del agua en los cambios de las plantas, además de servir para realizar su metabolismo y disminución en su contribución a la sequía es inducido para reducir el crecimiento, la inducción de la aparición de las yemas foliares y poner en marcha mecanismos de resistencia a las bajas temperaturas (Joly, 1985; Timmer y Armstrong, 1989; Landis *et al.,* 1992).

Para hacer frente a la sobrevivencia y crecimiento, las especies de encinos cuentan con mecanismos de tolerancia y/o la evitación del estrés hídrico (Levitt, 1980; Kramer, 1983; Valladares *et al.,* 2004) estas estrategias, por otra parte, no son excluyentes. Por lo tanto , los caracteres funcionales y morfológicos relacionados con el control de la pérdida de agua, la eficiencia de los recursos, el crecimiento y la morfología (macro y micro) son de vital importancia para el éxito biológico (desempeño) de las especies y sus híbridos citas

En el experimento de sequía (0, 7, 14 y 21 días) se observó un patrón de disminución en todas las características fisiológicas, cuando las plantas permanecieron más tiempo sin agua, las diferencias fisiológicas se fueron reduciendo, de una manera mas pronunciada al final del cuarto tiempo (21 días). Las plantas presentaron altos niveles de estrés hídrico (ver Tabla 4 en el capítulo III), la única población que mostró valores positivos en *Amax* (Tasa fotosintética máxima), *gs* (Tasa de conductancia estomática), *E* (Tasa de transpiración), *WUEi* (Uso eficiente del agua) y 100% en la tasa de sobrevivencia después de 21 días de sequía fue Zoquitlán, localizada al centro de la zona de contacto su inversión preferencial posiblemente en el sistema radicular y de su tasa de crecimiento de las plantas después de la germinación permitió mantener un mejor estado fisiológico durante el período de sequía que las otras poblaciones (Dell y Havel, 1989), esta resistencia debe considerar la procedencia de las especies, en este caso híbrido (Calamassii, 2001; Cregg y Zhang, 2001; Martínez *et al.,* 2002, Swenson *et al.,* 2008), como resultado, la selección se realiza en favor de las plantas más tolerantes a las bajas condiciones de disponibilidad de agua, se sugiere por lo tanto, que las presiones selectivas diferenciales sometidas por la variación del clima y la influencia antropogénica entre los sitios son probablemente una de las principales causas de la diferencia en esta población híbrida, tal vez se incluyen mecanismos de evasión por esta población (*e.g.,* algunas barreras bioquímicas preformadas como compuestos fenólicos, saponinas, leticinas ó barreras estructurales o físicas como mayor presencia de tricomas, cera cuticular, grosor de la cutícula y de la pared celular, forma, tamaño y comportamiento de los estomas, lenticelas, tejido interno de la planta, suberificación).

Experimentos de jardín común han demostrado que los híbridos pueden superar a sus especies progenitoras, favoreciendo la idea de que la hibridación facilita la invasión, sobrevivencia y/o rango de expansión, por ejemplo, *Carpobrotus* spp. (Vilà y D'Antonio, 1998), *Raphanus* spp. (Campbell *et al.,* 2006; Ridley y Ellstrand, 2009), *Raphanus raphanistrum* (Campbell and Snow 2007), *Helianthus* spp. (Whitney *et al.,* 2006, 2010a).

Otro ejemplo que apoya nuestros resultados con respecto al desempeño de los híbridos es el estudio de Campbell y Wendlandt (2013), donde examinaron los componentes del desempeño de *Ipomopsis aggregata* (Polemoniaceae), *I. tenuituba* y sus híbridos naturales en jardín común, sus resultados sugieren con respecto al aumento de las sequías severas, la posibilidad de que la frecuencia del desempeño híbrido sea más común.

Dicho de esta manera, el desempeño híbrido probablemente dependa de los factores bióticos y abióticos locales como la competencia, herbivoría y también al estrés ambiental (Campbell y Snow, 2007; Mercer *et al.,* 2007), por lo tanto es necesario, llevar a cabo experimentos manipulados en poblaciones de origen con condiciones locales con el fin de ampliar nuestra comprensión entre la hibridación y su relación con el desempeño.

CONCLUSIONES

Existen pocos estudios que han profundizado en el análisis de la dinámica y las consecuencias de la hibridación en el género *Quercus*; en el complejo de encinos rojos mexicanos formado por *Quercus affinis-Q. laurina*, se llevó a cabo un análisis de este tipo desde el punto de vista de la caracterización de la estructura geográfica y genética de la zona de hibridación. Indicando que esta zona se asemeja a una zona de tensión, existiendo patrones heterogéneos de variación entre los caracteres moleculares y fenotípicos.

La comparación del desempeño fisiológico de individuos de ambas especies e individuos híbridos en condiciones óptimas en un jardín común y bajo un experimento de estrés hídrico, mostraron que los híbridos presentan un desempeño fisiológico menor que las especies progenitoras en condiciones óptimas (algunos de los híbridos presentan caracteres fisiológicos intermedios y otros una marcada tendencia unidireccional hacia algunas de las especies progenitoras), la posible explicación a esto es que los híbridos pueden expresar un mosaico de caracteres funcionales de los progenitores, que depende de la característica evaluada y la interacción entre su expresión genética y el ambiente.

Bajo estrés hídrico, se observó un patrón de disminución en todas las características fisiológicas, cuando las plantas permanecieron más tiempo sin agua excepto en una población intermedia (esta resistencia debe considerar la procedencia de las especies, en este caso híbrido), las diferencias fisiológicas para ésta sugieren que las presiones selectivas diferenciales sometidas por la variación del clima y la influencia antropogénica son probablemente una de las principales causas de la diferencia, tal vez incluyendo mecanismos de evasión. Por lo tanto, se sugiere que los híbridos pudieran superar a sus especies progenitoras, favoreciendo la idea de que la hibridación facilita la invasión, sobrevivencia y/o rango de expansión.

Finalmente, nuestro estudio presenta resultados que apoyan la idea de que la selección opera manteniendo una diversidad de genotipos (incluyendo los tipos progenitores) en los complejos de encinos híbridos.

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