11281

UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

DOCTORADO EN CIENCIAS BIOMÉDICAS

CENTRO DE INVESTIGACIONES EN ECOSISTEMAS

ESTRUCTURA GENÉTICA DE UNA ZONA DE HIBRIDACIÓN ENTRE *QUERCUS AFFINIS* Y *Q. LAURINA*

T E S I S

QUE PARA OBTENER EL GRADO ACADÉMICO DE:

DOCTOR EN CIENCIAS

P R E S E N T A:

BIÓL. ANTONIO GONZÁLEZ RODRÍGUEZ

DIRECTOR DE TESIS: DR. ALBERTO KEN OYAMA NAKAGAWA

MORELIA, MICHOACÁN 2004

AGRADECIMIENTOS

A las personas que aportaron su trabajo, conocimiento y experiencia

Al Dr. Ken Oyama, por haber sido un verdadero maestro durante más de diez años. Gracias por la convivencia, la confianza y la amistad.

A la Dra. Susana Valencia, cuyo trabajo previo constituyó punto de partida y guía constante de esta investigación. Un agradecimiento especial a ella por toda su ayuda "taxonómica", su constante disposición a compartir ideas y, sobre todo, por su valientes y valiosas contribuciones al conocimiento de los encinos mexicanos.

A la Dra. Dulce María Arias por la generosidad con que fui recibido durante mi estancia en el CEAMISH y por su apoyo constante.

Al Dr. Luis E. Eguiarte por seguir con interés el desarrollo de esta investigación y por sus valiosas aportaciones en cada fase de la misma.

Al Dr. Miguel Lara por su disposición a participar en el comité tutoral y por las siempre amigables y agradables charlas.

Al Dr. John F. Bain y Joanne L. Golden por permitirme realizar una estancia en su laboratorio y por su ayuda durante las etapas iniciales del análisis de la variación en el ADNcp.

A los que revisaron la tesis como miembros del jurado, Dr. Daniel Piñero, Dr. Juan Núñez-Farfán, Dra. Isolda Luna, Dra. Dulce M. Arias, Dr. Luis Eguiarte, Dr. Mark Olson y Dr. Ken Oyama, por contribuir a mejorar el manuscrito de forma considerable.

A los que en diferentes momentos me apoyaron con el trabajo de campo: Susana Valencia, Euler Pedraza, Rosalinda Tapia, Miguel Angel Pérez, Alejandra Serrato, Pablo Cuevas, José Luis Ibarra y Leticia Valencia.

A Rolando Ramírez y Esaú Leyva, por enseñarme a hacer RAPDs y por ayudarme a resolver dudas técnicas.

A Nidia Pérez-Nasser, quién se encargó de innumerables aspectos logísticos y técnicos del trabajo en nuestro laboratorio. También por ser una buena amiga.

A la Sra. Dolores Lugo, por mantener nuestro laboratorio ordenado y funcional todo el tiempo. También por su constante y generosa disposición a ayudar.

A Carolina Espinosa por su amable ayuda con la realización de los trámites del posgrado.

A las Instituciones que hicieron posible el desarrollo de este trabajo.

A la Universidad Nacional Autónoma de México, por haberme dado la oportunidad de ser universitario.

Al Instituto de Ecología y al Centro de Investigaciones en Ecosistemas, que brindaron la infraestructura y el ambiente académico en el que tuve la fortuna de desarrollarme.

Al Posgrado en Ciencias Biomédicas por aceptarme como alumno de doctorado, así como por el apoyo económico que me otorgó para la realización de una estancia académica en el extranjero.

Al Consejo Nacional de Ciencia y Tecnología (CONACyT) por haberme otorgado una beca doctoral que hizo posible mi manutención durante mis estudios.

Los recursos económicos para la realización de esta investigación fueron proporcionados por la Dirección General de Apoyo al Personal Académico (DGAPA, UNAM) por medio de los proyectos IN212999 e IN229803, por el CONACYT (proyecto 38550–V) y por el Programa de Apoyo a Estudiantes de Posgrado.

A los amigos y las personas importantes...

A mis compañeros y amigos del laboratorio y del CIECo, los viejos y los recién llegados. Jenny, Luisa, Miki, Rosaura, Ivonne, Gume, Efraín, Enrique, Jenny Sotuyo, Sofía, Ale Serrato, Susana, Lety, Pepón, Benjamín, Irene, Xóchitl, Yunuen, Dolores, Ana Luisa, Juan Manuel, Sandro, Marbella, Carla, Adrián, gracias por enriquecer las horas de trabajo con su compañía.

A Quena por su generosidad. Por ese corazón enorme que llevas dentro.

A Pablo Cuevas, por lo mucho vivido juntos. Finalmente todo salió bien amigo, pero falta sin duda lo mejor. A Yuri, porque se ha convertido en buena amiga. Al buen y leal Jimmy, el otro miembro de mi familia moreliana.

A Mauricio Quesada por la complicidad lúdica y científica. A él y a Kathy Stoner por la amistad y las mil muestras de generosidad.

A Rolando Prado, por recordarme todo el tiempo algunas cosas importantes sobre la vida (aunque él seguramente nunca ha tenido tal intención).

A Omar Chassin, gracias por ser siempre buen amigo e interlocutor.
A mi viejo amigo Adrián Reyes, por el afecto que persiste a pesar de la distancia. Yo también me acuerdo del templo destruido. A Pavel, por traer un poco de su vieja magia negra a Morelia.
A Manuel Medina, por nuestra adolescencia. Por ser desde entonces ejemplo e inspiración genial.
A mis queridos Xalapeños, Areli, Lucho y Clementina. Gracias por compartir conmigo lo mejor de Xalapa.
A Marcia Tambutti, por la amistad sostenida y en crescendo.

Y más que a nadie, a mi familia. A mis padres, Leticia y Antonio, por guiarme, motivarme y apoyarme. Sobre todo por creer en mí en todo momento. Es a ellos a quienes este trabajo está dedicado.

A mi hermana Claudia, por su fortaleza, valentía, madurez y sabiduría. Ejemplo y cómplice constante. A ella y a mi cuñado Mark, quienes cariñosa pero firmemente disciplinaron mi horario durante tres meses y fomentaron en mí el gusto por las manzanas, el yogurt y el fútbol. A mi hermana Laura por su dulzura, por traer luz en su interior, por la poesía.

Theory and experiment are essential for defining the realm of the possible, but they cannot reveal the unique series of historical events leading to current patterns of diversity and adaptation. Resolution of debates about evolutionary process must ultimately emerge from careful quantitative analysis of observed patterns.

Harrison, 1990

Mientras que llenándoos va
el hacha de calvijares,
¿nadie cantaros sabrá,
encinares?

Antonio Machado

CONTENIDO

RESUMEN GENERAL

La hibridación interespecífica ocurre con una frecuencia inusualmente alta en el género *Quercus* L. (encinos, robles), y ha sido probablemente un proceso clave en la evolución de este grupo. Esta tesis se centró en el complejo formado por *Quercus affinis* y *Q. laurina*, dos encinos rojos mexicanos cercanamente relacionados y morfológicamente similares. En un estudio taxonómico previo se propuso una hipótesis de hibridación por contacto secundario entre ambos taxa, basada en patrones de distribución geográfica y variación morfológica. Las poblaciones con individuos típicos de *Q. laurina* se encuentran en la Sierra Madre del Sur y el oeste del Eje Neovolcánico Transversal, y las poblaciones con individuos típicos de *Q. affinis* en la Sierra Madre Oriental. La probable zona de hibridación, en la que existe intergradación morfológica entre ambas especies, está en la región este del Eje Neovolcánico.

El objetivo de esta investigación fue examinar la variación y diferenciación morfológica y genética en *Q. affinis* y *Q. laurina* e inferir la historia, estructura geográfica y dinámica de la zona de hibridación. Se colectaron muestras en 16 localidades (495 árboles) a lo largo de un gradiente geográfico, incluyendo poblaciones típicas de ambas especies y poblaciones situadas en la zona de hibridación. Mediante polimorfismos de ADN amplificados al azar (RAPDs) se identificaron nueve marcadores semi-diagnósticos, entre un total de 711 fragmentos amplificados, cuya frecuencia difirió significativamente entre poblaciones típicas de los dos taxa. La presencia / ausencia de estos nueve marcadores se registró posteriormente en todos los individuos de las 16 localidades. Con estos datos se calculó un índice de hibridación de máxima probabilidad, el cual mostró una transición gradual en la composición genética de las poblaciones de una especie a la otra a lo largo del gradiente macrogeográfico. Las poblaciones situadas en la zona de hibridación fueron genéticamente intermedias. Simultáneamente, se registraron en cada individuo nueve caracteres morfológicos foliares. Mediante el análisis de función discriminante se caracterizó la diferenciación en estos nueve caracteres entre ambas especies. Utilizando la función canónica discriminante obtenida se calculó un "puntaje" (score) discriminante para todos los individuos, lo que indicó que la morfología foliar también muestra un patrón de cambio gradual y continuo a lo largo del gradiente macrogeográfico, aunque de forma solo parcialmente congruente con la composición genética de las poblaciones. En particular, la proporción de individuos morfológicamente intermedios en las poblaciones fue menor a la de individuos genéticamente intermedios. En varias de las poblaciones situadas en la zona de hibridación predominó marcadamente la morfología de tipo *Q. laurina*. Como es lo esperado en zonas de hibridación secundarias, los patrones de cambio

en la frecuencia de varios de los RAPDs semi-diagnósticos a lo largo del gradiente macrogeográfico fueron paralelos entre sí o con los valores promedio de algunos de los caracteres morfológicos. Sin embargo, los patrones no fueron completamente paralelos para todos los marcadores, lo que sugiere cierto grado de introgresión diferencial.

El examen detallado de la variación foliar en las 16 poblaciones permitió distinguir dos grupos principales de poblaciones mediante un análisis de agrupamiento jerárquico (UPGMA), que fueron congruentes con la asignación taxonómica del tipo morfológico predominante en cada localidad (i.e. poblaciones de tipo *Q. affinis* y de tipo *Q. laurina*). La similitud morfológica entre las poblaciones no tuvo correlación con la distancia geográfica. Un análisis de varianza anidado mostró que la mayor proporción de la variación en los nueve caracteres foliares se encuentra dentro de las poblaciones, como variación entre individuos (28–54%, dependiendo del carácter), y variación entre hojas dentro de los individuos (17–56%). Las diferencias entre los dos grupos de poblaciones (3–27%) y entre las poblaciones dentro de los grupos (5–21%), también fueron significativas para los nueve caracteres. El patrón de cambio de cada carácter foliar fue diferente a lo largo del gradiente geográfico. La longitud del pecíolo y el número de aristas (dientes) variaron de forma particularmente marcada, lo cual posiblemente indica selección sobre estos caracteres.

Las relaciones genéticas entre las 16 poblaciones fueron analizadas utilizando 54 fragmentos de RAPDs. Un UPGMA reconoció dos grupos principales de poblaciones, uno que incluyó a poblaciones de la Sierra Madre del Sur y el oeste del Eje Neovolcánico (grupo 'suroeste'), y el otro a poblaciones de la Sierra Madre Oriental y el este del Eje Neovolcánico (grupo 'noreste'), independientemente de su morfología. La similitud genética entre las poblaciones sí mostró correlación con la distancia geográfica. Un análisis de varianza molecular (AMOVA) indicó que la mayor parte de la variación genética se encuentra dentro de las poblaciones (84%), el 5.1% ($P = 0.007$) se debe a diferencias entre las poblaciones de tipo *Q. affinis* y las de tipo *Q. laurina*, y el 10.5% ($P < 0.0001$) corresponde a la diferenciación entre poblaciones dentro de estos dos grupos. La diferenciación entre los grupos geográficos 'suroeste' y 'noreste' fue de 7.8% ($P < 0.0001$). La incongruencia entre las relaciones de similitud morfológicas y genéticas entre las poblaciones podría indicar que los marcadores moleculares neutros han experimentado considerable introgresión entre ambas especies en la zona de hibridación, mientras que la morfología foliar ha permanecido comparativamente estable.

Finalmente, se examinó la variación en el ADN de cloroplasto (ADNcp) en 39 poblaciones de *Q. affinis-Q. laurina* mediante el análisis de restricción de fragmentos amplificados (PCR-RFLP). Al contrario de lo encontrado en muchas especies de plantas de latitudes altas que se vieron

severamente afectadas por la última glaciación, la distribución de los haplotipos en estos encinos no mostró una marcada estructuración geográfica, ni se observaron patrones de variación clinal latitudinal o de otro tipo en los niveles de variación o la composición genética de las poblaciones. La diversidad genética intrapoblacional promedio ($h_S = 0.299$) y la diferenciación entre poblaciones ($G_{ST} = 0.499$) fueron, respectivamente, mayor y menor a lo reportado previamente para especies de encinos. Aparentemente, las poblaciones de *Q. affinis* y *Q. laurina* no se vieron drásticamente reducidas a pequeños refugios durante la última glaciación. Por el contrario, es probable que poblaciones grandes de estas especies hayan persistido durante varios episodios de cambio climático, y que hayan experimentado recurrentemente migraciones altitudinales y latitudinales y periodos de diferenciación seguidos de contactos secundarios.

ABSTRACT

Interespecific hybridization occurs with an unusually high frequency in the genus *Quercus* L. (oaks), and has probably been an important process in the evolution of this group. This thesis focused on the complex formed by *Quercus affinis* and *Q. laurina*, two closely related and morphologically similar Mexican red oaks. A hypothesis of hybridization through secondary contact between these taxa was proposed in a previous taxonomic study, based on patterns of geographic distribution and morphological variation. Populations with typical *Q. laurina* individuals are distributed along the Sierra Madre del Sur and the western region of the Trans-Mexican Volcanic Belt, and populations with typical individuals of *Q. affinis* are found in the Sierra Madre Oriental. The probable hybrid zone, where morphological intergradation between the two species occurs, is situated in the eastern region of the volcanic belt.

The main objective of this investigation was to examine morphological and genetic variation and differentiation in *Q. affinis* and *Q. laurina*, and to infer the history, geographic structure and dynamics of the hybrid zone. Samples were collected in 16 localities (495 trees) along a macrogeographic gradient including typical populations of both species, and populations within the hybrid zone. Randomly Amplified Polymorphic DNA (RAPD) was used to identify nine semidiagnostic markers, among a total of 711 amplified fragments, with significant differences in frequency between typical populations of the two taxa. The presence / absence of these nine markers was later scored in all individuals from the 16 localities. A maximum-likelihood hybrid index scored was calculated from these data, which indicated a gradual transition in the genetic composition of populations from one species to the other along the macrogeographic gradient. Populations within the hybrid zone were genetically intermediate. Simultaneously, nine foliar morphological characters were scored for each individual. Discriminant function analysis was used to characterize phenotypic differentiation in these nine characters between the two species. A discriminant score for each individual was calculated with the canonical discriminant function obtained, which indicated that foliar morphology also changes gradually and continuously along the macrogeographic gradient, although the pattern is only in part congruent with the change in the genetic composition of populations. In particular, the proportion of morphologically intermediate individuals in the populations was smaller than the proportion of genetically intermediate individuals. In several populations within the hybrid zone, *Q. laurina*-like morphology was predominant. As expected in secondary hybrid zones, there were parallel frequency changeovers among several of the RAPDs markers, and among RAPD markers and the mean values of several

morphological characters. However, the patterns were not completely parallel for all markers, which suggests some degree of differential introgression.

Detailed examination of foliar variation in the 16 localities made it possible to distinguish two main groups of populations through a hierarchical cluster analysis (UPGMA), which were congruent with the taxonomic assignment of the predominant morphological type in the localities (i.e. *Q. affinis*-like or *Q. laurina*-like populations). The morphological similarity among populations was not correlated to geographic distances. A nested analysis of variance showed that the largest proportion of the variation in the nine foliar characters is contained within populations, as among-tree variation (28–54%, depending on the trait), and as intra-individual variation (17–56%). Differences between the two groups of populations (3–27%), and among populations within the groups (5–21%), were also significant for the nine traits. A distinct pattern of change across populations was observed for each trait. Variation was particularly pronounced along the macrogeographic gradient for petiole length and teeth number, possibly implying selection on these two traits.

Genetic relationships among the 16 populations were analyzed using 54 RAPD markers. A UPGMA recognized two main groups of populations, one formed by populations from the Sierra Madre del Sur and the western region of the volcanic belt ('south-western' group) and the other constituted by populations from the Sierra Madre Oriental and the eastern region of the volcanic belt ('north-eastern' group), independently of their foliar morphology. The genetic similarity among populations was significantly correlated to geographic distances. Analyses of molecular variance (AMOVA) indicated that most genetic variation is contained within populations (84%), that 5.1% ($P < 0.007$) is found between *Q. affinis*-like and *Q. laurina*-like population groups, and that 10.5% ($P < 0.0001$) corresponds to differentiation within these two groups. Differentiation between the 'south-western' and 'north-eastern' geographical groups was 7.8% ($P < 0.0001$). The incongruence between genetic and phenotypic relationships among populations suggests that introgression of neutral markers has been considerable between the two species in the hybrid zone, while morphological differentiation has remained comparatively stable.

Finally, chloroplast DNA (cpDNA) variation was examined in 39 populations of *Q. affinis*-*Q. laurina* using restriction analysis of PCR-amplified fragments (PCR-RFLP). In contrast to what has been found in many plant species from high latitudes that were severely affected by the last glaciation, haplotype distribution in these oaks did not show marked geographical structure. Neither latitudinal or other clinal patterns in diversity levels or genetic composition of populations were apparent. Average within-population diversity ($h_S = 0.299$) and population differentiation ($G_{ST} =$

0.499) were, respectively, higher and lower than values reported in previous studies of oak species. It is probable that *Q. affinis* and *Q. laurina* did not experience a marked restriction to one or a few refugia during the last glacial cycle. It is more likely that substantial populations persisted throughout several episodes of climatic change, but experienced recurrent latitudinal and altitudinal migrations causing periods of differentiation followed by secondary contacts.

I.

INTRODUCCIÓN GENERAL

Consideraciones generales

La hibridación natural puede definirse como la producción, en condiciones naturales, de descendencia a partir del entrecruzamiento de individuos pertenecientes a poblaciones que pueden distinguirse con base en por lo menos un carácter heredable (Harrison, 1990, 1993; Arnold, 1997). Por extensión, un híbrido es un individuo descendiente de progenitores pertenecientes a poblaciones diferenciadas en por lo menos un carácter heredable. Estas definiciones poseen dos ventajas importantes: la primera es que su aplicación no depende de ningún concepto de especie en particular y la segunda es que las poblaciones de las que provienen los individuos que se hibridan no necesariamente tienen que pertenecer a una categoría taxonómica precisa (Harrison, 1993). Por otro lado, también se debe enfatizar que en la definición de híbrido quedan incluidos no únicamente los híbridos de primera generación (F1), sino también todos los individuos con una gran variedad de genotipos que pueden producirse en posteriores generaciones (F2, F3 y retrocruzas). Las zonas de hibridación son las localidades en las que dos o más grupos de organismos genéticamente diferenciados se encuentran y forman descendencia híbrida. Finalmente, el término introgresión se refiere al flujo de genes entre dos formas diferenciadas, como producto de repetidos eventos de hibridación y retrocruzamiento (Arnold, 1997).

Los procesos de hibridación natural han llamado la atención de los biólogos durante mucho tiempo. El estudio de este fenómeno se ha llevado a cabo desde varias perspectivas. Desde el punto de vista de la taxonomía y la sistemática, se han documentado miles de casos de hibridación en prácticamente todos los grupos de plantas y animales. Muchos de estos híbridos han sido descritos y caracterizados morfológicamente y, en un número menor de casos, también mediante diversos tipos de marcadores, entre los que se encuentran los

citogenéticos, los químicos y los moleculares. Recientemente, la utilización de caracteres moleculares ha servido de igual forma para inferir en numerosos géneros de plantas la existencia de especies o grupos de especies que posiblemente se originaron a partir de un ancestro híbrido, lo cual se traduce en relaciones filogenéticas reticuladas, más que estrictamente divergentes (Rieseberg,1991; Wendel *et al.*, 1991; Sang *et al.*, 1995; Aguilar *et al.*, 1999).

Por su parte, los biólogos evolutivos han llegado a considerar a las zonas de hibridación como "laboratorios naturales" (Hewitt, 1988), o "ventanas" (Harrison, 1990), en los que es posible observar la evolución de forma privilegiada. Por ejemplo, el estudio de la hibridación es fundamental para comprender la naturaleza de las diferencias entre especies, así como las causas que originan los mecanismos de aislamiento reproductivo. Asimismo, los patrones de variación que pueden encontrarse en las zonas de hibridación han despertado el interés acerca de los procesos genéticos y ecológicos que ocurren en tales zonas. En particular, destacan las interrogantes en torno al origen, estructura interna, dinámica, y destino de las zonas de hibridación. Muchas de las respuestas a estas preguntas no han terminado de discutirse. Por el contrario, esta es un área, como otras en la biología evolutiva, en la que la utilización de marcadores moleculares ha resultado notablemente útil para reformular los viejos problemas, así como para acrecentar la calidad y cantidad de los datos empíricos disponibles (Rieseberg y Ellstrand, 1993; Rieseberg, 1995).

Aspectos teóricos

Los primeros modelos conceptuales sobre el origen y dinámica de las zonas de hibridación se elaboraron durante las primeras décadas del siglo veinte. Dobzhansky (1940) y Mayr (1942) consideraron que las zonas de hibridación son áreas de contacto secundario entre

poblaciones que previamente se han diferenciado en alopatría. De acuerdo con estos autores, la divergencia entre poblaciones representa principalmente la adquisición, vía selección natural, de combinaciones distintas de genes que "funcionan bien juntos", y que constituyen asimismo la base de la adaptación a ambientes diferentes. Por lo tanto, la hibridación no podría sino conducir a la disrupción de los genotipos coadaptados propios de los progenitores, y a la consiguiente reducción en la adecuación de los híbridos, bajo prácticamente cualquier circunstancia. Desde esta perspectiva, las zonas de hibridación son fenómenos evolutivamente transitorios, que desembocan en el "reforzamiento" de las barreras reproductivas (principalmente precigóticas), debido a la selección en contra de la hibridación (Howard, 1993).

Durante cierto tiempo existió consenso en que geográficamente las zonas de hibridación son fundamentalmente clinas en caracteres que distinguen a poblaciones con distribución contigua, y pueden por lo tanto caracterizarse mediante un muestreo a lo largo de un transecto perpendicular a la franja de contacto (Mayr, 1963; Endler, 1977; Barton y Hewitt, 1985; Hewitt, 1989; Barton 2001). Los modelos de Endler (1977) mostraron que la aparición de este tipo de clinas no requiere necesariamente de diferenciación alopátrica, sino que pueden formarse zonas de hibridación primarias cuando existe un gradiente de selección capaz de producir diferenciación genética, aún cuando no hay ninguna restricción al flujo génico entre poblaciones. Es muy difícil o imposible distinguir entre ambos escenarios históricos (*i.e.* origen primario vs. secundario) a partir del patrón contemporáneo de variación en un carácter a lo largo de un solo transecto (Endler, 1977). Sin embargo, dos líneas de evidencia se han utilizado frecuentemente como argumentos a favor de un origen por contacto secundario de una alta proporción de las zonas de hibridación. En muchas ocasiones las clinas son muy pronunciadas (el ancho de la zona de hibridación es de unas

pocas decenas o cientos de metros), y se ha sugerido que la intensidad de la selección tiene que ser muy grande para producir este tipo de patrones (Endler, 1977; Barton y Hewitt, 1985). También resulta difícil usar a la diferenciación *in situ* como una explicación para la variación concordante (clinas en la misma posición y con el mismo ancho) que se observa frecuentemente (Hewitt, 1989), para caracteres de distinto tipo y en principio independientes, como pueden ser morfológicos, cromosómicos, conductuales y moleculares (Hewitt y Barton, 1980; Hewitt, 1989; Harrison, 1990). Una tercera línea de evidencia que se ha usado en algunos casos, es la documentación directa de que la zona de hibridación es el resultado de cambios en la distribución geográfica de dos taxa después de una alteración ambiental, como las producidas por las glaciaciones (Hewitt, 1989).

Sin embargo, no todas las zonas de hibridación tienen la forma de una franja estrecha. Harrison y Rand (1989) propusieron el concepto de mosaico para aplicarlo a zonas de hibridación con una estructura geográfica más compleja en la que los recursos, hábitats o factores físicos adecuados para cada una de las formas progenitoras se encuentran entremezclados de manera discontinua. Este tipo de estructura se documentó por primera vez una zona de hibridación entre los grillos *Gryllus firmus* y *G. pennsylvanicus*, que se caracteriza por la estrecha asociación de cada especie con un cierto tipo de suelo. Esta observación se ha interpretado como evidencia de selección dependiente del ambiente (Harrison y Rand, 1989; Rand y Harrison, 1989). Por otro lado, caracteres morfológicos, aloenzimáticos y del ADN mitocondrial mostraron patrones idénticos de variación espacial, lo cual fue usado para argumentar a favor de un origen por contacto secundario (Harrison y Rand, 1989; Rand y Harrison, 1989).

Una vez formadas, la dinámica y evolución de las zonas de hibridación dependen de varios factores. En prácticamente todos los modelos, uno de los factores más importantes siempre

es el tipo e intensidad de la selección natural sobre los híbridos. A pesar de que en varios de estos modelos se supone, como pensaron inicialmente Dobzhansky y Mayr, que los híbridos inevitablemente muestran menor adecuación que los progenitores, está claro que el reforzamiento de las barreras reproductivas no es la única vía posible de evolución en las zonas de hibridación. De hecho, pueden existir indefinidamente, aun si existe una fuerte selección en contra de los híbridos cuando, por ejemplo, nuevos individuos se dispersan constantemente hacia la zona de hibridación y se entrecruzan. Este es en esencia el modelo de "zona de tensión" (Key, 1968; Barton y Hewitt, 1985, 1989). Su premisa fundamental es que, debido a causas endógenas (*i.e.* disrupción de los genomas de los progenitores), la adecuación de los híbridos es reducida, por lo que la selección natural debe actuar siempre en su contra, independientemente del ambiente en el que se encuentren. Según Barton y Hewitt (1985) "la mayoría de los fenómenos conocidos como zonas de hibridación son clinas mantenidas por el balance entre la dispersión y la selección en contra de los híbridos". Este modelo explica la estructura geográfica y genética que se observa en algunas zonas de hibridación bien estudiadas. El que estas zonas puedan permanecer como franjas estrechas con numerosos caracteres distintos variando a lo largo de ellas según un mismo patrón difícilmente puede atribuirse a la selección producida por factores ecológicos, que en todo caso produciría clinas con distinta forma y posición para caracteres diferentes (Barton y Hewitt, 1985; Barton y Gale, 1993; Hewitt, 1988, 1989). Una de las propiedades más notables de las zonas de tensión es que pueden moverse de lugar, debido a que la selección que opera en ellas es independiente de las condiciones ambientales locales. De acuerdo con el modelo, quedarán estacionadas en áreas de baja densidad poblacional, puesto que la dirección neta de dispersión de los progenitores será de las áreas de mayor densidad hacia las de menor densidad poblacional (Barton y Hewitt, 1985; Hewitt, 1988,

1989). También se puede esperar, a partir de la inmigración constante de los progenitores, y de la selección en contra de los híbridos, que en las zonas de tensión exista desequilibrio de ligamiento entre los alelos provenientes de cada forma progenitora, así como desequilibrio citonuclear (Barton y Hewitt, 1985; Barton y Gale, 1993, M. Arnold, 1992; J. Arnold, 1993; Barton 2001).

Algunos modelos alternativos han considerado otras posibles dinámicas. Existe la posibilidad de que la adecuación relativa de los híbridos sea en mayor medida un resultado de causas exógenas que endógenas. En este caso, se espera que las clinas para los caracteres bajo selección se asocien con un gradiente ambiental o con un área de transición ecológica. Otros caracteres no seleccionados seguirán patrones de variación diferentes, a menos que se encuentren ligados con los caracteres bajo selección (Barton y Hewitt, 1985; Hewitt, 1988, 1989; Barton 2001). Si la adecuación de los híbridos es menor a la de los progenitores, este tipo de zonas de hibridación requiere, al igual que las zonas de tensión, de la inmigración constante de los progenitores para permanecer estables (Endler, 1977; Barton y Hewitt, 1985; Hewitt, 1988, 1989; Harrison, 1990; Barton 2001). En cambio, un escenario diferente resulta cuando los progenitores muestran una menor adecuación que los híbridos en la zona de hibridación, como puede ocurrir, según algunos autores (Anderson, 1948; Heiser, 1973; Moore, 1977; Grant, 1981), en áreas ecológicamente perturbadas o ecotonos. Puesto que los híbridos son seleccionados a favor, este tipo de zonas de hibridación no depende directamente de la dispersión de los progenitores para persistir. Sin embargo, la superioridad de los híbridos se encuentra confinada a estos hábitats particulares, por lo que no pueden establecerse fuera del área de hibridación. Por esta razón a este se le llama el modelo de la "superioridad restringida del híbrido" (Moore, 1977).

Además de ocasionar el reforzamiento de las barreras reproductivas entre dos taxa, o de permanecer relativamente estables durante prolongados periodos de tiempo como resultado de distintas combinaciones de procesos, las zonas de hibridación pueden derivar también en la fusión de las poblaciones progenitoras, o en la formación de nuevos linajes evolutivamente estables. Por ejemplo, si entre dos taxa se forma una franja angosta de hibridación por contacto secundario, pero no existen diferencias en adecuación entre progenitores e híbridos, se obtendrá una clina que tenderá a difundirse gradualmente, y cuyo ancho será una función del tiempo transcurrido desde el contacto secundario y de la magnitud de la dispersión (Barton y Hewitt, 1985; Hewitt, 1988, 1989; Barton, 2001). El resultado final será una población homogénea en la que, entre otras cosas, habrá aumentado la variabilidad genética.

El modelo recientemente propuesto de las "novedades evolutivas" (Arnold, 1997) ha hecho énfasis en las vías a través de las cuales las zonas de hibridación pueden ser sitios de origen de nuevos taxa, distintos de ambas formas progenitoras. Los modelos de este tipo son muy necesarios considerando la gran cantidad de especies de animales (Bullini, 1994; Dowling y Secor, 1997) y plantas (Rieseberg, 1997) que probablemente se originaron a través de procesos de hibridación. En este caso, el factor determinante es nuevamente la intensidad y naturaleza de la selección actuando sobre los híbridos. Si la selección endógena en contra no es demasiado fuerte (como sería en el caso de completa esterilidad o inviabilidad de los híbridos), pueden ponerse de manifiesto diferencias en adecuación entre individuos híbridos producidas por causas exógenas. Lo anterior, aunado a la posibilidad de formación de numerosos genotipos recombinantes en sucesivas generaciones de hibridación y retrocruza proporciona la oportunidad de que ocurran procesos selectivos. De esta manera, es de esperarse que aumente la frecuencia de ciertos genotipos híbridos con

adecuación particularmente alta, mismos que puedan incluso colonizar exitosamente hábitats novedosos y constituir a la larga linajes evolutivamente estables.

Estudios de hibridación en plantas

Los estudios empíricos de zonas de hibridación han estado en su mayor parte guiados por los modelos mencionados. A partir de la búsqueda de los patrones de variación esperados bajo diferentes escenarios, es posible realizar algunas inferencias sobre el origen y dinámica de zonas particulares. Aunque el punto de partida de la mayor parte de estos estudios es una hipótesis de hibridación entre dos taxa basada en caracteres morfológicos, el uso de marcadores para determinar los patrones de variación genética (*i.e.* estructura genética) en las zonas de hibridación, es en ocasiones la única forma posible de detectar eventos que ocurren con una frecuencia baja, o procesos que de otra forma no pueden observarse.

Existe una cantidad notablemente menor de trabajos sobre la estructura genética de zonas de hibridación en plantas que en animales, lo que resulta irónico, puesto que la hibridación ocurre en plantas con una frecuencia muy alta, y tradicionalmente se ha considerado mucho más importante en la evolución de este grupo que en la de los animales (Dowling y Secor, 1997). La causa es que el estudio de la hibridación en plantas se ha realizado preponderantemente desde la perspectiva de la taxonomía y la sistemática. Sin embargo, un número creciente de estudios han puesto a prueba recientemente las predicciones de algunos de los modelos mencionados arriba en zonas de hibridación de plantas. Algunos ejemplos notables incluyen estudios en grupos como *Aesculus* (dePamphilis y Wyatt, 1990), *Artemisia* (Freeman *et al.*, 1995; Graham *et al.*, 1995; Wang

et al., 1997), *Helianthus* (Beckstrom-Sternberg *et al.*, 1991; Dorado *et al.*, 1992; Rieseberg *et al.*, 1998), *Iris* (Cruzan y Arnold, 1993, 1994; Young, 1996; Burke *et al.*, 2000), *Pinus* (Watano *et al.*, 2004), *Populus* (Paige *et al.*, 1991; Martinsen *et al.*, 2001) y *Salix* (Hardig *et al.*, 2000), entre otros. De acuerdo con algunos autores, las zonas de hibridación en plantas se caracterizan generalmente, en comparación con los animales, por la frecuente formación de poblaciones híbridas segregantes (también llamadas "hybrid swarms", "enjambres híbridos"), y por una estructura geográfica menos definida (Paige *et al.*, 1991; Harrison, 1993). No obstante, en ocasiones se ha reportado variación clinal, a veces muy marcada (Heywood, 1986; dePamphilis y Wyatt, 1990; Cruzan y Arnold, 1993; Graham *et al.*, 1995; Young, 1996; Villani *et al.*, 1999), y a escalas espaciales que van desde unos cuantos metros hasta cientos de kilómetros. La estructura genética se caracteriza por desequilibrio de ligamiento nuclear y citonuclear (Paige *et al.*, 1991; Cruzan y Arnold, 1993; 1994, Rieseberg *et al.*, 1996; Villani *et al.*, 1999). Las asociaciones genotipo-ambiente, que han sido detectadas en varias ocasiones, ponen de manifiesto la clara influencia de la selección exógena sobre la estructura de algunas zonas (Heywood, 1986; Cruzan y Arnold, 1993).

Las plantas poseen por lo general dos vías diferentes a través de las que puede ocurrir flujo génico: la dispersión de polen y la dispersión de semillas. Los genomas citoplásmicos (*e.g.* mitocondrias y cloroplastos) se heredan a través del progenitor materno en la mayor parte de las angiospermas, lo que significa que su dispersión puede ocurrir únicamente a través de las semillas. Por esta razón, el uso simultáneo de marcadores nucleares y citoplásmicos permite detectar posibles asimetrías en la dirección en que ocurre hibridación (*e.g.* cuando una de las "formas" actúa con mayor frecuencia como progenitor

materno o paterno que la otra). De la misma forma, cuando existe introgresión, se puede evaluar la medida relativa en que la dispersión de semillas y de polen contribuye al flujo génico entre ambos taxa.

Los estudios filogeográficos en plantas, recientemente con gran auge, han mostrado que el uso de marcadores citoplásmicos (principalmente del ADN de cloroplasto, ADNcp), además de permitir inferencias sobre patrones históricos de migración, colonización y demografía (McCauley 1995; Ennos *et al.*, 1999), también puede revelar eventos históricos y contemporáneos de hibridación e introgresión difíciles de detectar mediante análisis morfológicos o marcadores nucleares (Rieseberg y Soltis, 1991; Rieseberg *et al.*, 1996). En grupos como *Eucalyptus* (McKinnon *et al.*, 2001; 2004), *Packera* (Bain y Jansen 1996; Golden y Bain 2000) y *Lithocarpus* (Cannon y Manos, 2003), entre otros, se ha encontrado que con una alta frecuencia las especies comparten los mismos haplotipos de ADNcp. En tales grupos las barreras reproductivas interespecíficas se encuentran pobremente desarrolladas, y la escasa diferenciación en el ADNcp se ha atribuido comúnmente a la hibridación y posterior introgresión citoplásmica, aunque en algunos casos puede tratarse también de polimorfismos ancestrales compartidos (Golden y Bain 2000; Byrne *et al.*, 2002). En ocasiones se ha documentado el caso extremo que es la completa sustitución de los genomas citoplásmicos de un taxón por los genomas citoplásmicos de un segundo taxón con el cual se hibrida, fenómeno que se ha llamado "captura citoplásmica" (Rieseberg y Soltis, 1991; Rieseberg *et al.*, 1996). Por último, la distribución de los haplotipos citoplásmicos dentro y fuera de una zona de hibridación permite en ocasiones, junto con otros datos, inferir su probable origen. Esto se debe a que en una zona secundaria se puede esperar la presencia de los haplotipos citoplásmicos propios de ambas o de una de las formas progenitoras, mientras que en una zona primaria se espera la presencia de haplotipos

ancestrales de los haplotipos *derivados* propios de los progenitores (Beckstrom-Strenberg *et al.*, 1991). Este enfoque permitió recientemente establecer que una clina morfológica formada supuestamente por el contacto secundario entre *Eucalyptus populnea* y *E. brownii* es en realidad una zona de intergradación primaria (Holman *et al.*, 2003).

La hibridación en el género Quercus

El género *Quercus* L. (encinos, robles), destaca por una frecuencia inusualmente alta de hibridación natural. Las especies de encinos son interfértiles en muchas combinaciones, y pueden formarse híbridos inclusive entre especies considerablemente diferenciadas morfológica y fisiológicamente (Stebbins, 1950, Grant 1981, Hardin 1975). Este escaso desarrollo de las barreras reproductivas llamó la atención de Darwin (1859), quien usó a los encinos como ejemplo de especiación incipiente en su argumentación en contra de la inmutabilidad de las formas biológicas. Posteriormente, el género ha destacado en las discusiones en torno a la importancia de la introgresión en la evolución de las plantas (Anderson, 1949; Whittemore y Schaal, 1991; Rieseberg y Wendel, 1993) y a los factores ecológicos que limitan o facilitan la hibridación (Muller, 1952). Incluso se han propuesto conceptos de especie distintos al biológico para ser aplicados al caso de *Quercus* (Burger, 1975; Van Valen, 1976; Nixon y Wheeler 1990). Por otro lado, la hibridación ha sido constantemente utilizada como explicación, al menos parcial, de las dificultades taxonómicas que presenta el género (Nixon, 1993; Manos *et al.*, 1999).

A pesar de lo anterior, se han realizado pocos análisis genéticos en las zonas de hibridación en encinos. La hibridación ha sido inferida típicamente con base en caracteres morfológicos, algunos de los cuales pueden presentar considerable plasticidad o ser interpretados equívocamente (Rieseberg, 1995). No obstante, se puede enfatizar que la

hibridación ha sido confirmada en todos los trabajos que han utilizado marcadores genéticos. Mediante el análisis de patrones de restricción del ADNcp, se ha puesto de manifiesto que los encinos figuran como algunos de los ejemplos más notables de introgresión citoplásmica. Whittemore y Schaal (1991) descubrieron que cuando se encuentran en simpatría poblaciones de *Q. alba*, *Q. macrocarpa*, *Q. michauxii*, *Q. stellata*, *Q. virginiana* o *Q. emoryi*, comparten el mismo haplotipo de cloroplasto a escala local, con una fuerte estructuración entre localidades. El mismo fenómeno ha sido reportado también en varias especies de encinos europeos (Dumolin-Lapègue *et al.* 1999; Belahbib *et al.* 2001; Petit *et al.* 2002a, b). En otros estudios, se ha determinado la frecuencia y el patrón asimétrico de hibridación entre *Q. robur* y *Q. petraea* mediante isoenzimas (Bacilieri *et al.*, 1996) y entre *Q. grisea* y *Q. gambelli* mediante RAPDs. El análisis de la estructura de la zona de hibridación entre estas dos últimas especies mostró que es de tipo mosaico (Howard *et al.*, 1997). A partir de estos trabajos se ha hecho también evidente que es difícil encontrar marcadores diagnósticos o especie-específicos como un prerrequisito para el análisis de las zonas de hibridación entre especies de encinos cercanamente relacionadas. Por ejemplo, Howard *et al.* (1997) tuvieron que ensayar 700 primers para encontrar 14 bandas diagnósticas o especie-específicas de *Q. grisea* y *Q. gambelli*.

II.

ANTECEDENTES

Taxonomía, filogenia y biogeografía histórica del género Quercus

El género *Quercus* incluye unas 500 especies de árboles y arbustos cuya distribución abarca gran parte del Hemisferio Norte (Nixon, 1993; 1997). Sus representantes son con frecuencia elementos dominantes en los bosques templados y también se les encuentra en bosques mesófilos, subtropicales, tropicales, e incluso en matorrales xerófitos (Jones, 1986; González-Rivera, 1993; Zavala-Chávez, 1998). *Quercus* se encuentra incluido en la familia Fagaceae, orden Fagales, que se ubica en el clado de las Eurosids I (APG II, 2003). La familia Fagaceae se caracteriza por la presencia de una estructura leñosa denominada cúpula que rodea la base de una o varias flores. Después de la fertilización, el ovario se desarrolla en un fruto indehiscente, unilocular y con una sola semilla denominado nuez (Nixon, 1989; Borgardt y Pigg, 1999). En el caso de *Quercus* la cúpula presenta escamas, tiene forma de copa, carece de valvas y engloba completamente o rodea la base de un solo fruto. En conjunto, la cúpula y la nuez constituyen la bellota, que es el carácter que diagnostica al género (Nixon 1989; Borgardt y Pigg, 1999). Otras características biológicas relevantes de los encinos son la polinización por viento, el entrecruzamiento obligado y el periodo de vida muy largo, que puede sobrepasar los 400 años en algunas especies (Kaul, 1985; 1986).

De acuerdo con la clasificación taxonómica más actual y análisis filogenéticos recientes, el género *Quercus* se divide en dos subgéneros, *Cyclobalanopsis* y *Quercus* (Nixon, 1993; Manos *et al.*, 1999; Manos y Stanford, 2001; Manos *et al.*, 2001). A su vez, el subgénero *Quercus* incluye cuatro secciones: *Cerris*, *Lobatae* (encinos rojos), *Protobalanus* (encinos intermedios) y *Quercus* (encinos blancos). La distribución geográfica de las especies del subgénero *Cyclobalanopsis* es en el sureste de Asia, mientras que la sección *Cerris* tiene una distribución Euroasiática, la sección *Quercus* se encuentra

en Norteamérica y Euroasia, la sección *Lobatae* se encuentra únicamente en Norteamérica y la sección *Protobalanus* se restringe exclusivamente al oeste de Norteamérica. La hipótesis filogenética obtenida por Manos y Stanford (2001) para los subgéneros y secciones de *Quercus*, así como las áreas geográficas ocupadas por cada grupo se muestran en la Fig. 1.

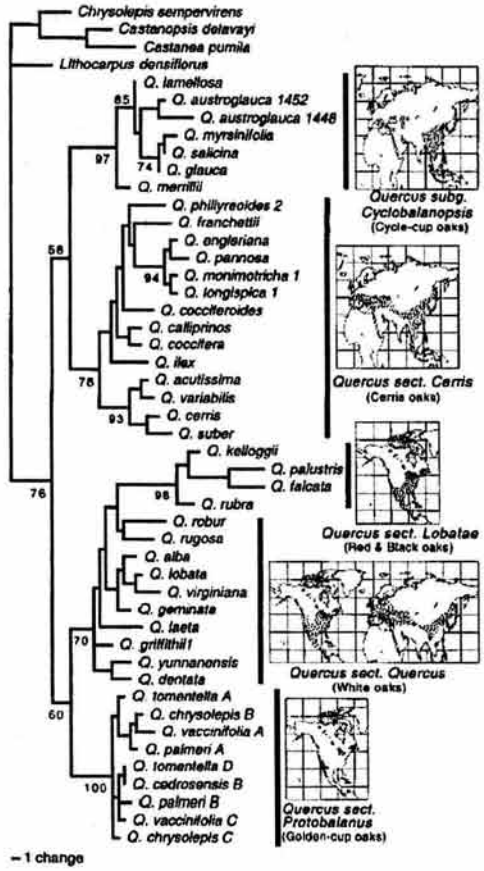


Figura 1. Hipótesis filogenética para los subgéneros y secciones del género *Quercus* y área de distribución geográfica de cada grupo. *Tomado de Manos y Stanford, 2001.*

Las evidencias fósiles, filogenéticas y biogeográficas sugieren que la diversificación a nivel de género en la familia Fagaceae ocurrió en el sureste asiático (Manos *et al.*, 2001; Manos y Stanford, 2001). Un posible escenario es que, posteriormente, las especies ancestrales de *Quercus*, probablemente perennifolias, migraron a través de los Puentes Terrestres del Atlántico Norte (PTAN) durante el Eoceno tardío y el Oligoceno temprano (hace 40-30 millones de años) hasta alcanzar una distribución amplia y continua. Un evento inicial de vicarianza pudo haber ocurrido debido al cierre de los PTAN (Manos y Stanford, 2001), dejando dos grandes grupos de especies aislados en Euroasia y Norteamérica, respectivamente. Posteriormente, los climas más fríos, estacionales y secos que se desarrollaron entre el Oligoceno y el Mioceno (hace 30-20 millones de años) favorecieron la migración y diversificación de grupos de plantas con polinización por viento, como los encinos (Axelrod, 1983; Daghlian y Crepet, 1983; Borgardt y Pigg, 1999). La evidencia fósil disponible indica que los grupos infragenéricos dentro de *Quercus* ya habían aparecido hace aproximadamente 25 millones de años (Oligoceno Superior) (Axelrod, 1983; Daghlian y Crepet, 1983; Borgardt y Pigg, 1999). Es decir, que para ese entonces ya existían los dos grandes clados que se observan en la Fig. 1: por un lado el grupo estrictamente Euroasiático formado por *Cyclobalanopsis* y *Cerris* y, por el otro, el clado formado por las secciones *Quercus*, *Lobatae* y *Protobalanus*, las cuales probablemente se originaron en Norteamérica (Manos *et al.*, 1999; Manos y Stanford, 2001).

Por su parte, análisis filogenéticos y biogeográficos realizados para la sección *Quercus* (encinos blancos), sugieren que tras haberse originado en Norteamérica, algunas especies de este grupo migraron hacia Asia y después hacia Europa a través del puente de Bering durante el Oligoceno (Manos y Stanford, 2001). Según estos autores, es probable que únicamente especies deciduas de la sección *Quercus* hayan conseguido migrar hacia

Asia en esa época, como lo sugirió inicialmente Axelrod (1983). Esta hipótesis es congruente con la gran riqueza de especies de encinos blancos en América (125 spp.), en contraste con las 20 spp. que se encuentran en Eurasia. El episodio de migración debió terminar a causa de la disminución de la temperatura global que inició hace aproximadamente 15 millones de años y que provocó la extinción de las poblaciones de *Quercus* cercanas al puente de Bering (White *et al.*, 1997). El tiempo de divergencia entre las especies americanas y euroasiáticas estimado a partir de la comparación de secuencias no codificantes del ADNcp es de 17 millones de años, el cual es congruente con los datos paleoclimáticos (Manos y Stanford, 2001).

Los encinos en México

Actualmente, México constituye el centro de diversidad mas importante para el género *Quercus* en el Hemisferio Occidental (Nixon, 1993). De las aproximadamente 225 especies de encinos que existen en América, entre 135 y 150 están presentes en México (Nixon, 1993). El número de endemismos es de 86, 40 de los cuales tienen una distribución restringida a un solo estado (González-Rivera, 1993). Del número total de especies, 55 son encinos rojos (41 endémicos), 76 encinos blancos (44 endémicos) y cuatro encinos intermedios (Nixon, 1993). Sin embargo, la mayor parte de los aspectos micro- y macro-evolutivos de los encinos mexicanos se desconocen por completo. Como se mencionó anteriormente, las secciones *Quercus*, *Lobatae* y *Protobalanus* probablemente evolucionaron en las latitudes medias de Norteamérica durante el Oligoceno. El registro fósil de polen muestra que la dispersión del género *Quercus* ocurrió progresivamente hacia el sur en México y Centroamérica desde el Mioceno (hace 20 millones de años) hasta hace 330,000 años, tiempo en que hizo su aparición en Colombia (Graham, 1999). De acuerdo

con estos datos, los encinos siguieron un patrón de dispersión similar al de otros muchos géneros de árboles de origen templado (*e.g. Abies*, *Alnus*, *Picea*, *Pinus*, *Populus*, *Ulmus*) que eran raros o prácticamente no se encontraban en el norte de México a principios del Mioceno (hace 23 millones de años), pero cuya presencia se incrementó en esta región durante la transición del Mioceno temprano al tardío (hace 15 millones de años) y que finalmente se volvieron muy abundantes en el Plioceno (hace 5 millones de años), tiempo en el que llegaron a Centroamérica, aunque con una diversidad decreciente hacia el sur (Graham, 1999). De forma muy significativa, estos eventos de dispersión de los elementos templados hacia el sur coincidieron con disminuciones progresivas de la temperatura global que iniciaron hace 15 millones de años (Graham, 1999).

Por lo tanto, se puede afirmar que el género *Quercus* colonizó México en dirección Norte-Sur desde las latitudes medias de Norteamérica. Sin embargo, la alta proporción de endemismos, así como las probablemente lejanas relaciones filogenéticas entre las especies de *Quercus* endémicas de México y las especies presentes en el este de los Estados Unidos (Nixon, 1993), sugieren que la mayor parte de las especies de encinos mexicanos evolucionaron dentro de la región que hoy es nuestro país.

El estudio de los encinos mexicanos es muy importante por varias razones. La primera es que, como acabamos de mencionar, una fracción muy significativa de las especies de este género se encuentra en México. Se trata además de un grupo con una enorme importancia ecológica y económica en nuestro país. El estudio de la hibridación en encinos es relevante porque existen razones suficientes para pensar que este ha sido un proceso clave en la evolución del género, incluyendo probablemente la diversificación que tuvo en nuestro país. Además, el estudio de la hibridación puede ayudar a comprender los complejos patrones de variación morfológica que han dificultado la taxonomía de *Quercus*,

lo cual a su vez ha entorpecido e inhibido la realización de otro tipo de estudios. Para el presente trabajo se eligió como sistema de estudio un complejo formado por dos especies de encinos rojos mexicanos, *Q. affinis* y *Q. laurina*. Estas especies fueron estudiadas previamente desde el punto de vista taxonómico, y se propuso una hipótesis de hibridación por contacto secundario entre ambos taxa (Valencia, 1994). El objetivo principal de la investigación fue examinar los patrones de variación y diferenciación morfológica y genética en *Q. affinis* y *Q. laurina*, e inferir la historia, estructura geográfica y dinámica de la zona de hibridación.

Sistema de estudio

Quercus affinis y *Q. laurina* son dos especies de encinos mexicanos incluidos en la serie Lanceolatae del subgénero *Erythrobalanus*, o encinos rojos *sensu* Trelease (1924). Sin embargo, en la actualidad, los "encinos rojos" han sido agrupados en la sección *Lobatae* del subgénero *Quercus* (Nixon, 1993).

Quercus laurina fue incialmente descrita como especie nueva por Humboldt y Bonpland (1809), y *Q. affinis* por Scheidweiler (1837), quién indicó a través del epíteto la semejanza que tiene esta especie con *Q. laurina*. Un total de 25 taxa, entre especies y variedades, publicados posteriormente por diversos autores, se consideran actualmente sinónimos de *Q. laurina*, y nueve taxa de *Q. affinis* (Valencia, 1994). Esta gran cantidad de sinonimias se debe probablemente a la considerable variación presente en la mayor parte de los atributos morfológicos de ambas especies. Tras diversas modificaciones, la serie Lanceolate, de incluir en un inicio 15 taxa (Trelease, 1924), ha pasado a incluir solo tres, que son *Q. affinis*, *Q. laurina* y *Q. acatenangensis* (Valencia, 1994). Esta última es una especie descrita por Trelease y ubicada originalmente en la serie Acatenangensis, pero que

de acuerdo con Valencia (1994) presenta más similitudes morfológicas con *Q. affinis* y *Q. laurina* que con las otras especies de la serie Acatenangensis.

De acuerdo con Valencia (1994) y una revisión de herbario realizada por el autor, las formas asignables a *Q. affinis* se distribuyen en los estados de Nuevo León, Tamaulipas, San Luis Potosí, Hidalgo, Puebla, Veracruz, Oaxaca y Guerrero, principalmente en las elevaciones de la Sierra Madre Oriental y del Eje Neovolcánico Transversal. Por su parte *Q. laurina* se encuentra en 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. Altitudinalmente, *Q. affinis* está presente entre los 1600-2800 m, y *Q. laurina* entre los 2440-3065 m. Ambas especies son simpátricas en numerosas localidades, especialmente en la porción este del Eje Neovolcánico y el norte de Oaxaca (Valencia, 1994; ver Fig. 2).

A pesar de que no han dejado de considerarse especies válidas, la delimitación taxonómica entre *Q. affinis* y *Q. laurina* no es clara. Este problema fue estudiado en detalle por Valencia (1994), quien encontró, mediante un análisis de fenología, anatomía de madera, arquitectura foliar y polen, escasos caracteres que permiten diferenciar consistentemente a ambas especies (Tabla 1). Sin embargo, los ejemplares de *Q. affinis* procedentes del norte de la Sierra Madre Oriental son fácilmente distinguibles de los de *Q. laurina* de la Sierra Madre del Sur (Fig.3a). En cambio, las dificultades para realizar la asignación taxonómica se incrementan hacia el este del Eje Neovolcánico Transversal y el norte de Oaxaca (Fig. 3b). A partir de estas evidencias, Valencia (1994) consideró que *Q. affinis* y *Q. laurina* son dos especies cercanamente relacionadas, entre las que una alta frecuencia de hibridación ha oscurecido las diferencias en aquellas zonas en las que se traslapan sus áreas de distribución.

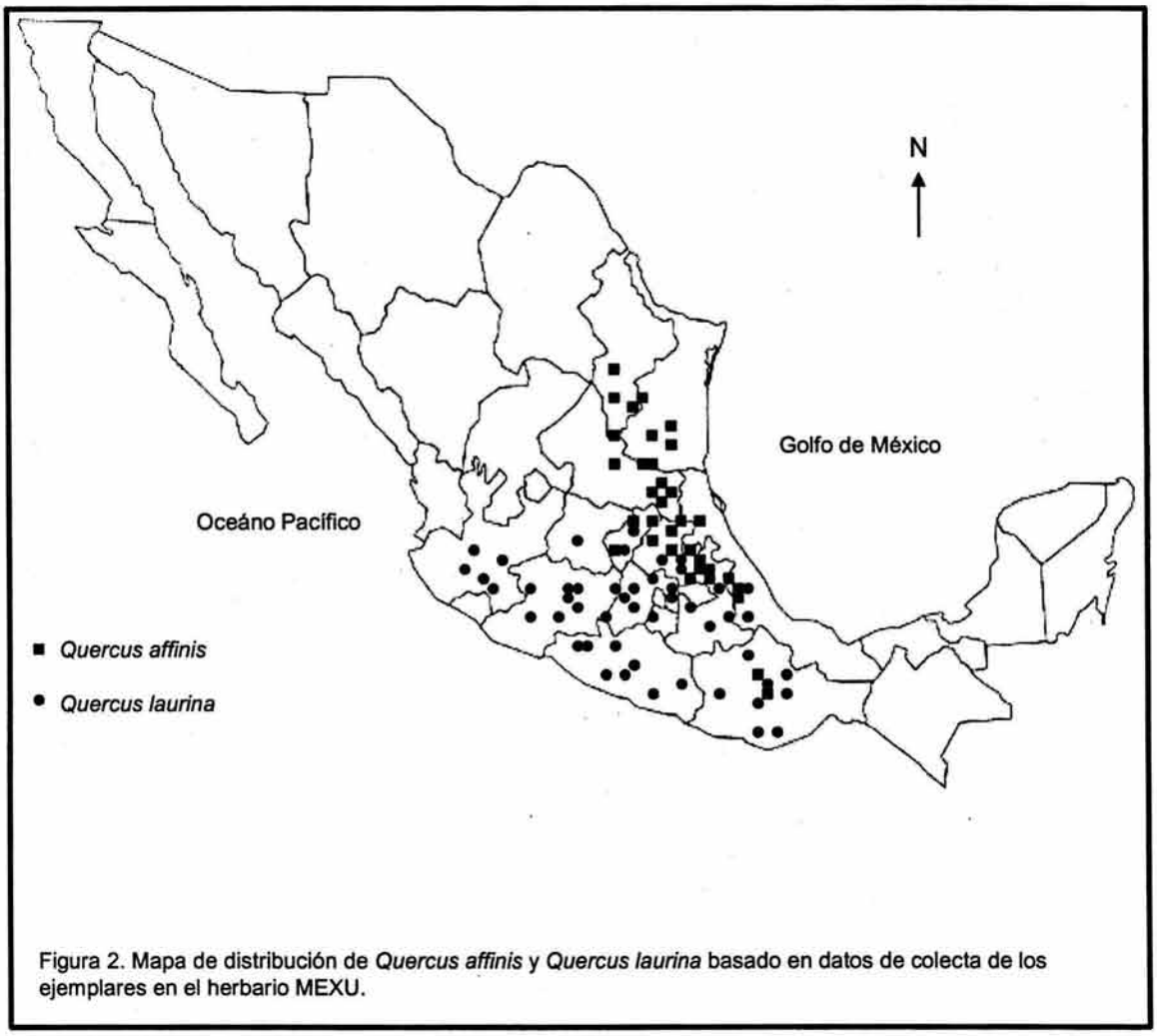


Figura 2. Mapa de distribución de *Quercus affinis* y *Quercus laurina* basado en datos de colecta de los ejemplares en el herbario MEXU.

Tabla 1. Principales caracteres morfológicos que distinguen a *Q. affinis* y *Q. laurina* (Valencia, 1994).

Carácter	Especie de encino	
	Q. affinis	*Q. laurina*
Ramillas	De 1-2.5 (-2.8) mm de diámetro, laxamente pubescentes a casi glabras	De 0.9-2 (-3.7) mm d diámetro, pubescentes o glabrescentes
Yemas	Generalmente conoidales, ovoides, 2-4 (-5.5) mm de largo, (0.5-)1-1.5 (-2) mm de diámetro	En su mayoría ovoides o globosas, rara vez conoidales, de (1-)1-3.5 (-4) mm de largo, 1-2 (-2.5) mm de diámetro
Hojas maduras	Angostamente lanceoladas, elípticas o elíptico-lanceoladas, 3.12-3.56 veces más largas que anchas	Lanceoladas u ovado-lanceoladas, elípticas o elíptico-lanceoladas, en su mayoría 2.67-3.11 veces más largas que anchas
Grosor de la vena media	Moderado con relación al ancho de la hoja	Delgado con relación al ancho de la hoja
Ángulo de divergencia de las venas secundarias	Agudo-estrecho a moderado	Agudo-amplio
Curso de las venas secundarias	Recto u ocasionalmente curvo abrupto	Curvo uniforme
Glándulas	Sin pelos glandulares vermiformes	Ocasionalmente con pelos glandulares vermiformes
Nervaduras en el envés	Nervaduras secundarias y terciarias planas e inconspicuas	Nervaduras secundarias y terciarias formando un retículo mas o menos conspicuo
Tricomas	Número de radios promedio por pelo fasciculado estipitado: 13.7 (7-26)	Número de radios promedio por pelo fasciculado estipitado: 10.7 (7-16)

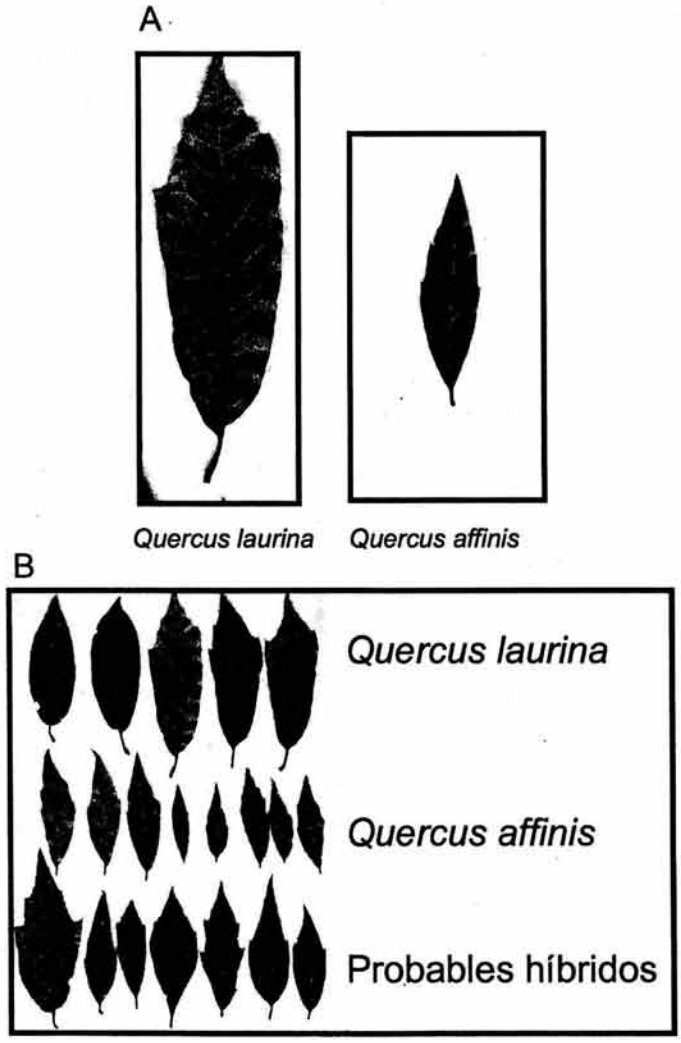


Figura 3. A. Morfología foliar representativa de *Quercus affinis* y *Quercus laurina*. B. Ejemplos de la variación foliar presente en poblaciones representativas de *Q. laurina*, *Q. affinis* y una población situada en la probable zona de hibridación.

Valencia (1994) propuso un posible escenario histórico para el origen del complejo *Q. affinis–Q. laurina*, el cual está basado preponderantemente en inferencias realizadas a partir de los patrones biogeográficos del género *Quercus* en México, la distribución geográfica de la variación morfológica que se observa actualmente en este complejo y las reconstrucciones paleoclimáticas disponibles. Puesto que el género *Quercus* se dispersó de norte a sur en México y dado que en la Sierra Madre Occidental no existen poblaciones de *Q. affinis–Q. laurina*, Valencia (1994) sugirió que la distribución ancestral de las poblaciones que dieron origen a este complejo fue principalmente en la Sierra Madre Oriental, aunque tal vez alcanzaron una distribución casi continua hasta Chiapas y Guatemala a finales del Mioceno (hace 5 millones de años). Posteriormente, y tal vez favorecido por condiciones climáticas favorables, pudo ocurrir un evento de migración hacia el norte a lo largo de la Sierra Madre del Sur. De esta manera, hacia el Plioceno medio o tardío (hace 3.5–2 millones de años) habrían quedado en relativo aislamiento los tres grupos de poblaciones que darían origen a las especies que forman la serie Lanceolate: *Q. acatenangensis* en Chiapas y Guatemala, *Q. affinis* en la Sierra Madre Oriental, y *Q. laurina* en la Sierra Madre del Sur. Las zonas de hibridación entre *Q. affinis* y *Q. laurina* se habrían formado por recurrentes contactos secundarios producto de expansiones y contracciones de las áreas de distribución de ambas especies en el norte de Oaxaca y la porción este del Eje Neovolcánico Transversal, posiblemente desde inicios del Pleistoceno (1.8–1.6 millones de años). Aunque no existen datos que indiquen directamente la serie de diferentes eventos postulados, las diversas evidencias indirectas reunidas por Valencia (1994) hacen de este escenario una hipótesis verosímil que merece ponerse a consideración. En particular, los trabajos realizados recientemente en encinos europeos han puesto de manifiesto la forma extremadamente rápida y dramática en que puede modificarse el área

de distribución de estas especies como respuesta a los cambios climáticos (Petit *et al.*, 2002a, b).

Estructura del estudio

La tesis está formada por cuatro capítulos. En el primero se presenta un análisis morfológico y genético (mediante RAPDs) de la hibridación entre *Quercus affinis* y *Q. laurina*. Las preguntas particulares de este capítulo fueron: (1) ¿Son los patrones de variación morfológica y genética consistentes con una hipótesis de hibridación por contacto secundario entre estas especies?, (2) ¿cuál es el grado de congruencia entre los patrones morfológicos y genéticos?, y (3) ¿cuál es la estructura geográfica de la zona de hibridación? Para contestarlas, primero se caracterizó la diferenciación fenotípica entre poblaciones morfológicamente representativas de ambas especies situadas fuera de la zona de hibridación y se identificaron fragmentos de RAPDs que mostraron marcadas diferencias en frecuencia entre estas poblaciones. Posteriormente se utilizaron estos caracteres para analizar la estructura de la variación morfológica y genética en poblaciones de *Q. affinis–Q. laurina* situadas a lo largo de un gradiente geográfico incluyendo el área de distribución de cada especie y la zona de hibridación.

En el segundo capítulo se estudia con detalle la variación foliar en este complejo de encinos, para identificar y comparar los patrones de variación de caracteres individuales al nivel de las poblaciones a lo largo del gradiente geográfico, determinar las relaciones de similitud generales entre las poblaciones y evaluar si están asociadas con la distancia geográfica. Se presenta un análisis de varianza anidado para cuantificar cómo se encuentra repartida la variación morfológica entre diferentes niveles jerárquicos, incluyendo especies, poblaciones, individuos y hojas. Finalmente, se determinó si las poblaciones con un posible

origen híbrido son más variables que las no híbridas, como se ha observado en otros sistemas y lo predicen algunas consideraciones teóricas.

Para el tercer capítulo se realizó un análisis de la genética de poblaciones de *Q. affinis-Q. laurina* mediante 54 marcadores de RAPDs. Los objetivos específicos fueron analizar los patrones de variación y diferenciación genética entre especies, poblaciones dentro de especies, e individuos dentro de poblaciones, así como determinar la asociación entre estos patrones y factores geográficos.

Finalmente, en el cuarto capítulo se estudió la variación en el ADNcp con el objetivo de obtener una perspectiva filogeográfica del complejo *Q. affinis–Q. laurina*, para realizar inferencias sobre la dinámica espacio-temporal de estos encinos, en términos de cambios históricos en la distribución y los tamaños poblacionales producto de las alteraciones climáticas en la historia geológica reciente. Además, nuevamente se pone a prueba la hipótesis de que la zona de hibridación entre las dos especies se originó por contacto secundario, escenario bajo el cual se espera encontrar haplotipos divergentes en las poblaciones con supuesto origen híbrido. Finalmente, se evaluó el grado de introgresión citoplásmica entre ambas especies.

III.

Antonio González-Rodríguez, Dulce M. Arias, Susana Valencia, and Ken Oyama

Morphological and RAPD analysis of hybridization between *Quercus affinis* and *Q. laurina* (Fagaceae), two Mexican red oaks.

American Journal of Botany, 91: 401–409. 2004

American Journal of Botany 91(3): 401–409. 2004.

MORPHOLOGICAL AND RAPD ANALYSIS OF HYBRIDIZATION BETWEEN *QUERCUS AFFINIS* AND *Q. LAURINA* (FAGACEAE), TWO MEXICAN RED OAKS[1]

ANTONIO GONZÁLEZ-RODRÍGUEZ,[2,5] DULCE M. ARIAS,[3]
SUSANA VALENCIA,[4] AND KEN OYAMA[2]

[2]Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México, Antigua Carretera a Pátzcuaro No. 8701, Col. Ex-Hacienda de San José de la Huerta, Morelia, 58190 Michoacán, México; [3]Centro de Educación Ambiental e Investigación Sierra de Huautla, Universidad Autónoma del Estado de Morelos, Av. Universidad 1001, Col. Chamilpa, Cuernavaca, 62210, Morelos, México; and [4]Departamento de Biología, Facultad de Ciencias, Universidad Nacional Autónoma de México, México, D.F. 04510, México

Quercus affinis and *Q. laurina* are two closely related Mexican red oaks with partially overlapping distributions. Within the area of overlap, there are localities where morphological intergradation occurs. A previous hypothesis explained this pattern as a result of secondary contact between the two species, followed by hybridization and introgression. This possibility was analyzed here by examining foliar and genetic variation in 16 localities situated along a macrogeographic gradient, which included morphologically representative populations of both species and populations from within the area of overlap. Maximum-likelihood hybrid index scores calculated from nine semi-diagnostic RAPD markers indicated a shift in the genetic composition of populations from one species to the other along the macrogeographic gradient, with genetically intermediate populations situated in the area of overlap. Foliar variation followed a partially congruent pattern, but *Q. laurina*-like morphology predominated in some of the genetically intermediate populations. There were several instances of correlated frequency changeovers of single RAPD markers and morphological characters along the macrogeographic gradient and a few cases of markedly parallel patterns between markers. The results were interpreted as consistent with a hypothesis of secondary contact between the two oak species that has resulted in some differential introgression among markers.

Key words: hybrid zones; hybridization; *Quercus affinis*; *Quercus laurina*; RAPD markers.

Hybridization and introgression are considered important influences in the adaptive evolution and diversification of many plant groups (Rieseberg and Ellstrand, 1993; Rieseberg and Wendel, 1993; Arnold, 1997). The genus *Quercus* (the oaks) has long been considered a group with an unusually high frequency of interspecific hybridization (Burger, 1975; Van Valen, 1976). Although morphological patterns of variation that support or are consistent with hybridization between many oak species pairs have been reported (Palmer, 1948; Cooperrider, 1957; Brophy and Parnell, 1974; Hardin, 1975; Jensen et al., 1993; Bacon and Spellenberg, 1996), detailed studies of the patterns and frequency of interspecific nuclear and cytoplasmic genetic exchange have focused on only a few species complexes, notably European and North American white oaks. The results of these studies include the demonstration of extensive local sharing of cytoplasmic haplotypes (i.e., cytoplasmic introgression) among sympatric white oak species (Whittemore and Schaal, 1991; Dumolin-Lapègue et al., 1999), low genetic differentiation between possibly hybridizing species as measured by allozymes and nuclear molecular markers (Hokanson et al., 1993; Kleinschmit et al., 1995; Samuel et al., 1995; Bodénès et al., 1997; Howard et al., 1997; Bruschi et al., 2000), direct evidence of considerable asymmetric pollination of *Q. robur* progenies by *Q. petraea* in a local stand (Bacilieri et al., 1996b), and the documentation of a mosaic hybrid zone between *Q. grisea* and *Q. gambelli* in New Mexico (Howard et al., 1997).

Red oaks are restricted to the New World and are in general thought to hybridize more extensively than white oaks (Guttman and Weigt, 1989; Jensen et al., 1993), but fewer detailed studies of hybridization processes have been conducted on this group (Jensen et al., 1993; Bacon and Spellenberg, 1996). Mexico is considered the center of diversity of *Quercus* in the Western Hemisphere, with an estimated total number of species between 135 and 150 and 86 endemics (Nixon, 1993a). Of these, 55 species are red oaks, with 41 of them endemic. Diverse topography, climate, and habitat probably exerted an important influence in the process of radiation and maintenance of oak species diversity in this region (Nixon, 1993a). Paleobotanical evidence suggests that the cooler, drier, and more variable climates that developed after the Eocene-Oligocene transition in North America encouraged the evolution and migration of *Quercus* (Daghlian and Crepet, 1983; Borgardt and Pigg, 1999). The history of oaks in Mexico was probably characterized by range shifts, expansions, and contractions, a product of climatic changes producing the opportunity for periods of differentiation followed by secondary contact between taxa (Bacon and Spellenberg, 1996). However, very few studies have been conducted on population genetics, hybridization processes, and speciation of Mexican oaks.

Recently, several red oak complexes have been identified in Mexico by specialists (Valencia, 1994; Bacon and Spellenberg, 1996). One of these complexes is formed by the closely related species *Quercus affinis* Scheidweiler and *Q. laurina* Humboldt

[1] Manuscript received 8 April 2003; revision accepted 18 September 2003.

The authors thank Luis E. Eguiarte and Miguel Lara for useful comments throughout the development of this work; Euler Pedraza, Rosalinda Tapia, and Pablo Cuevas-Reyes for field assistance; Nidia Pérez-Nasser, Esaú Leyva, and Rolando Ramirez for laboratory assistance. Comments by Daniel J. Howard and Pauline Garnier-Géré greatly improved our first version of the manuscript. Financial support was provided by DGAPA, UNAM (grant IN212999 to K. O.) and CONACyT (grant 38550-V to K. O.). A. G. R. was supported by a doctoral scholarship from CONACyT.

[5] E-mail: agrodrig@oikos.unam.mx.

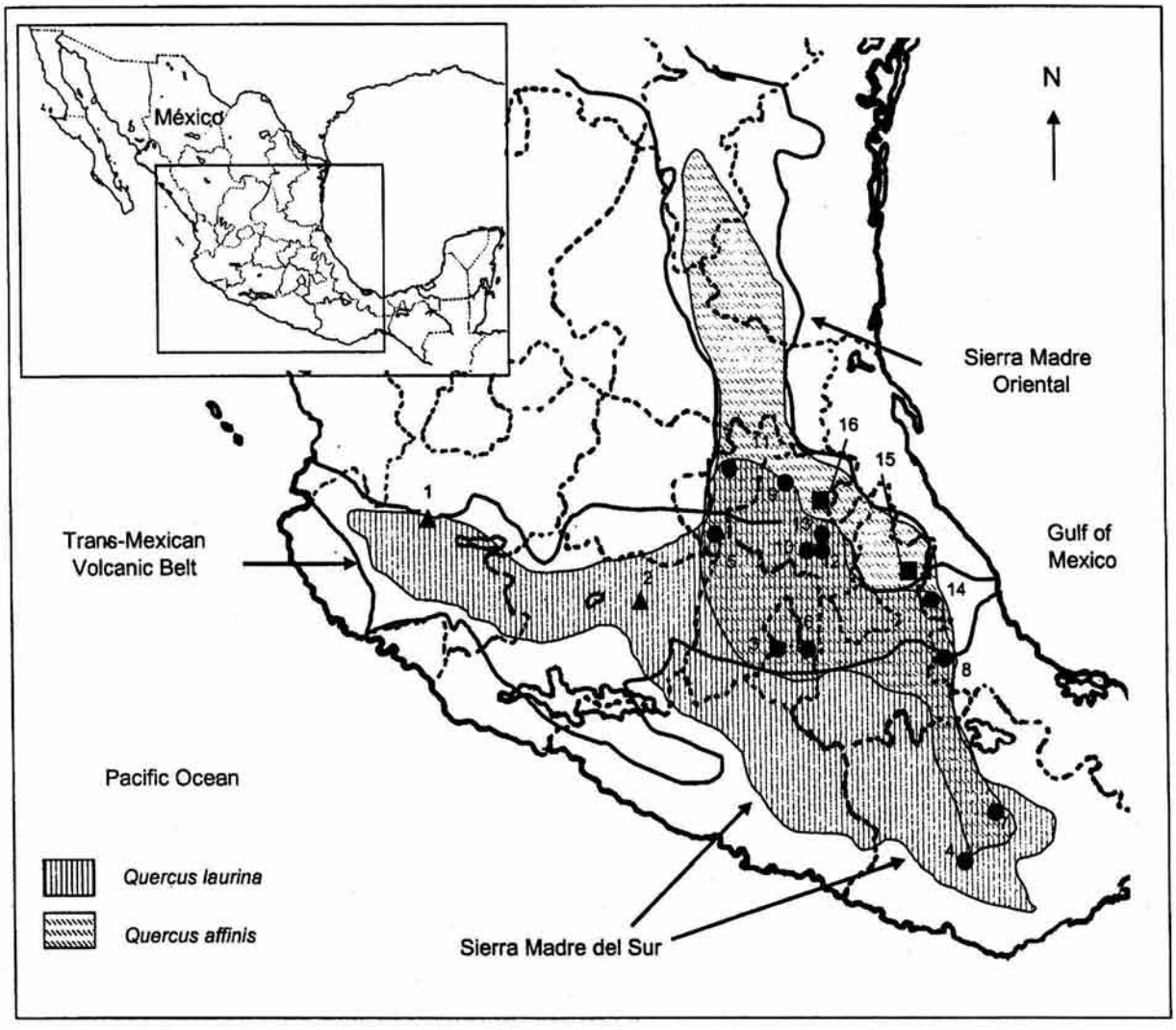


Fig. 1. Map of the geographical range of *Quercus affinis* and *Q. laurina* and the locations of the sampled populations. Squares symbolize morphologically representative populations of *Q. affinis*, triangles symbolize morphologically representative populations of *Q. laurina*, and circles symbolize populations in which morphological intergradation occurs. Numbers next to each symbol correspond to those given in Table 1.

et Bonpland (subgenus *Quercus*, section *Lobate*; Nixon, 1993b), which have partially overlapping distributions and show morphological intergradation within the region of overlap, but otherwise remain distinct in localities outside of this area. The objective of this study was to gain insight into the origin and structure of this hybrid zone.

The two species in this complex show a wide range of morphological variation and are difficult to delimit taxonomically. *Quercus laurina* probably also hybridizes with at least four other red oak species besides *Q. affinis* (*Q. crassifolia*, *Q. crassipes*, *Q. mexicana*, and *Q. rubramenta*). According to a recent systematic study, 25 taxa, including species and varieties, are synonymous with *Q. laurina* and nine with *Q. affinis* (Valencia, 1994). An analysis of phenology, wood anatomy, foliar architecture, and pollen ultrastructure revealed that few characters are consistently differentiated between both species across the whole range of their geographical distribution (Valencia, 1994). Morphological intergradation occurs in localities situated in the eastern portion of the Trans-Mexican Volcanic Belt and northern Oaxaca, while individuals from populations outside of these areas usually can be unambiguously determined (Valencia, 1994). Morphologically representative populations of *Q. laurina* are distributed along elevations of the Sierra Madre del Sur and the western region of the volcanic belt, at altitudes that vary between 2440 and 3065 m, and morphologically representative populations of *Q. affinis* are along the Sierra Madre Oriental, with an altitudinal range of 1600–2800 m (Fig. 1). From this pattern of morphological geographic variation, *Q. affinis* and *Q. laurina* were hypothesized to be two closely related species that may have diverged in isolation (*Q. affinis* in the Sierra Madre Oriental and *Q. laurina* in the Sierra Madre del Sur) probably during the mid- or late Pliocene and entered into secondary contact in the volcanic belt after a period of range expansion favored by climatic conditions at the beginning of the Pleistocene. The climatic pulsations of the Pleistocene probably determined recurrent

periods of secondary contact and periods of range contraction and divergence (Valencia, 1994). According to this hypothesis, as a result of frequent hybridization and introgression during periods of secondary contact, the morphological differences between the two species have been obscured in some localities (Valencia, 1994).

However, morphological intergradation may arise from secondary contact between differentiated populations or be primary in origin (Durrett et al., 2000). In the second case, morphological and genetic intermediacy represents the ancestral state, and apparent parents are the result of divergence from a common gene pool (Beckstrom-Sternberg et al., 1991). Although sometimes it may be difficult to discriminate between either scenario (Endler, 1977), features of hybrid zones such as parallel patterns in the changeovers from the character states of one parental population to those of the other for several presumably independent characters across the area of intergradation and nonrandom associations among markers derived from the same parental population in the center of the zone (i.e., linkage disequilibrium) are generally considered strong indications of an origin via secondary contact (Durrett et al., 2000). Molecular markers can provide a large number of neutral and independent characters that are extremely useful in the genetic analysis of hybrid zones (Rieseberg and Ellstrand, 1993). Compared to allozymes, markers such as AFLP or RAPD are more appropriate for the study of oak hybrid zones, because they have usually provided better discrimination between closely related, hybridizing oak species (Bodénès et al., 1997; Coart et al., 2002). RAPD markers have already been used in the study of oak hybrid zones (Howard et al., 1997).

In this study we measured foliar attributes to characterize phenotypic differentiation between isolated populations of *Q. affinis* and *Q. laurina*, identified several RAPD markers that showed substantial frequency variation between these populations, and then used these traits to assess the structure of morphological and genetic variation at a macrogeographic level, which included the distribution area of both species and the intergradation zone. The particular goals were (a) to determine if the hypothesis of a secondary hybrid zone between *Q. affinis* and *Q. laurina* is supported, (b) to assess the degree of congruence between morphological and molecular variation, and (c) to gain insight into the macrogeographic spatial structure of the intergradation zone.

MATERIALS AND METHODS

Selection of localities and sampling procedure—Examination of 431 specimens from 154 different localities deposited at the National Herbarium of Mexico (MEXU) aided in identifying populations morphologically representative of *Q. affinis* and *Q. laurina*, as well as potential sites of morphological intergradation between them. Two populations considered morphologically representative of each species were sampled: *Q. laurina* from localities in the states of Jalisco (population 1, Tequila) and Michoacán (population 2, Mil Cumbres) in the western portion of the volcanic belt and *Q. affinis* from Hidalgo (population 16, Zacualtipán) and Puebla (population 15, Zacapoaxtla) in the southern region of the Sierra Madre Oriental morphotectonic province (Ferrusquía-Villafranca, 1993). Fourteen other populations situated along the volcanic belt (most of them in the eastern region) and northern Oaxaca with various degrees of morphological intergradation were also chosen for sampling (Table 1, Fig. 1). The populations were numbered according to a geographic gradient that reflects their position relative to the distribution areas of the two species and the intergradation zone. This was done by jointly considering localities' latitude and longitude. In this way populations follow and order from more western–southern populations to more eastern–northern populations.

TABLE 1. Locality name, state, geographic coordinates, and sample size for the collecting sites.

Locality number and name	State	Latitude, N/ longitude, W	Sample size
Q. laurina sites			
1 Tequila	Jalisco	20°50′/103°48′	22
2 Mil Cumbres	Michoacán	19°40′/100°55′	20
Mixed sites			
3 Cuernavaca	Morelos	19°05′/99°15′	32
4 Santa Inés	Oaxaca	17°03′/96°55′	26
5 Amealco	Querétaro	20°10′/100°20′	21
6 Ozumba	México	19°05′/98°42′	31
7 Llano de Flores	Oaxaca	17°30′/96°30′	22
8 Puerto Aire	Veracruz	18°45′/97°30′	37
9 Jacala	Hidalgo	20°50′/99°05′	31
10 El Chico	Hidalgo	20°05′/98°40′	35
11 Pinal de Amoles	Querétaro	21°01′/99°40′	30
12 Cerro Navajas	Hidalgo	20°12′/98°30′	40
13 El Zembo	Hidalgo	20°15′/98°32′	45
14 Jalacingo	Veracruz	19°30′/97°15′	34
Q. affinis sites			
15 Zacapoaxtla	Puebla	19°50′/97°40′	34
16 Zacualtipán	Hidalgo	20°39′/98°40′	35

In each locality, 3–5 young intact leaves for molecular analysis and two randomly chosen branches for morphological analyses were collected from each individual. Sampling was performed by randomly choosing an adult tree every 100 or 200 m (depending on the specific locality) along a transect. Twenty to 45 trees were sampled per locality. Leaves for molecular analyses were immediately frozen in liquid nitrogen and the branches were pressed as herbarium specimens. In total, 495 individuals were sampled.

RAPD markers—Markers that could differentiate between the two species were developed through randomly amplified polymorphic DNA (RAPD). DNA of individuals from a morphologically representative population of each species (Zacualtipán [16] and Tequila [1], respectively; Table 1) was amplified using 131 10-bp random oligonucleotides (Series A–L; Operon Technologies, Alameda, California, USA). Satisfactory amplifications were obtained with 94 of these primers, from which 79 gave reproducible results, according to a second round of assays. In total, 711 fragments were consistently amplified in both series of assays. From these 711 bands nine, produced by seven primers, had substantial differences in frequency between the two species (Table 2). The reproducibility of these nine markers was corroborated in a third round of assays, and their presence/absence was later scored in all individuals.

DNA was isolated using the procedure proposed by Lefort and Douglas (1999) with only minor modifications. Isolated DNA was stored in deionized water at −20°C. The concentration of DNA in solution was determined with a DNA fluorometer (Hoefer Pharmacia Biotech, San Francisco, California, USA), following procedures supplied by the manufacturer. Amplification reactions were carried out in a 25-μL mix containing 10 ng template DNA, 50 mM KCl, 10 mM Tris-HCl; pH 9, 0.1% Triton x-100, 2 mM $MgCl_2$, 0.1 mM each dNTP, 0.2 μM of a single 10-mer primer, and 1 unit *Taq* polymerase (Gibco/Invitrogen, San Diego, California, USA). The thermal cycling program was run on a MJ Research (Waterton, Massachusetts, USA) thermal cycler. The program consisted of 45 cycles, each at 94°C for 1 min, annealing at 36°C for 1 min, and extension at 72°C for 2 min. A final extension at 72°C for 15 min was included.

The amplification products were electrophoresed in 1.5% (m/v) agarose gels with TBE buffer at 200 V for 2 h and stained with ethidium bromide. Gels with amplification fragments were visualized and photographed under UV light. Molecular mass of the RAPD bands was estimated by reference to a 123-bp ladder (Gibco/Invitrogen, San Diego, California, USA), with aid of

TABLE 2. Frequency of nine semidiagnostic RAPD markers in morphologically representative populations of *Q. affinis* and *Q. laurina*.

Population	Marker								
	A5-7	A7-9	A8-1	A10-3	B17-4	B17-6	C9-3	C9-5	I7-2
Q. affinis									
Zacualtipán	0.750	0.188	1.000	1.000	0.844	0.750	0.781	0.000	0.531
Q. laurina									
Tequila	0.048	0.619	0.500	0.550	0.000	0.300	0.150	0.500	0.150

the Alpha Ease Version 4 program (Alpha Innotech, San Leandro, California, USA).

Morphological analyses—The following morphological variables were quantified in 10 randomly chosen leaves from each individual: total length (TL), lamina length (LL), petiole length (PL), maximal width (MW), distance from the base of the leaf to the point of maximal width (PMW), and teeth number (TN). Additionally, the ratios of PL/TL, MW/LL, and PMW/LL were calculated. Individual tree means were obtained for each variable and used in further analysis.

Data analysis—Because none of the markers identified as useful was completely diagnostic, a maximum likelihood estimate of hybrid index scores was used, instead of the conventional arithmetic index employed when completely diagnostic markers are available. The algorithm used was the one developed by Hardig et al. (2000) specifically for RAPD markers (M. Morgan, Washington State University, personal communication). The program standardizes the resulting scores to range between zero and one. In the program, populations Tequila and Zacualtipán represented *Q. laurina* and *Q. affinis*, respectively. The frequency of the nine RAPD markers in these two populations thus constituted the end points for calculating hybrid index scores of all plants. These two populations constitute geographical extremes, are morphologically representative of their respective species, and had the largest frequency differences in the nine RAPD markers.

Discriminant function analysis (Tabachnick and Fidell, 1989) was used to assess multivariate morphological differentiation between representative populations of *Q. affinis* and *Q. laurina*. Individuals from populations Tequila and Zacualtipán were first analyzed to obtain a canonical discriminant function. Discriminant scores calculated with this function were then obtained for trees from all populations.

To identify geographical patterns of variation in the genetic and morphological composition of populations, product-moment correlations (Sokal and Rohlf, 1995) were calculated for the populations' hybrid index scores and morphological discriminant scores with each localities' latitude and longitude.

We calculated pairwise correlations among the frequencies of the RAPD markers on a population-by-population basis, as well as among frequencies of RAPD markers and mean values of morphological characters to determine possible associations in the patterns of change of these presumably independent characters across the area of intergradation that may indicate an origin for this area from secondary contact between the two oak species. To better visualize the patterns, plots for the frequency of markers that had high correlation coefficients were constructed, with populations following their order in the macrogeographic gradient. To ease the comparison between RAPD marker frequencies and morphological characters in these plots, values of morphological characters were transformed to range between zero and unity, with values close to zero representing *Q. laurina* and values closer to one representing *Q. affinis*.

To test for linear associations between RAPD markers and morphological variables at the level of individual trees, a multiple regression analysis was performed between maximum likelihood hybrid index scores and the set of morphological variables. Additionally, each morphological variable was regressed against the set of RAPD markers. These analyses were performed on the total set of individuals over all populations and then separately within each population.

RESULTS

Histograms of the maximum likelihood hybrid index scores for each population are presented in Fig. 2A. Individuals from the Tequila population had scores that ranged from 0.00 to 0.50, with a mean of 0.24 (SD 0.153). Three individuals from this population had hybrid index scores that deviated towards the hybrid condition by more than one standard deviation from the population mean. Two of these individuals lacked the two markers (A7–9 and C9–5) semi-diagnostic of *Q. laurina* and at the same time possessed three markers semi-diagnostic of *Q. affinis*. The hybrid index score of the third individual was 0.50; this individual possessed markers A7–9 and C9–5 and also harbored six of the seven markers semi-diagnostic of *Q. affinis*. Individual hybrid index scores in population Zacualtipán ranged from 0.69 to 1.00, with a mean of 0.88 (SD 0.098). Five individuals from this population had scores that deviated more than one standard deviation below the mean. These individuals lacked three or four of the semi-diagnostic markers of *Q. affinis*, but did not possess any semi-diagnostic marker of *Q. laurina*.

The frequency distributions of hybrid index scores in all other populations were to some degree intermediate between those of populations Tequila and Zacualtipán. A transition can be observed in Fig. 2A from frequency distributions situated towards the left of the graphs that characterized populations collected in the western region of the volcanic belt or southern Oaxaca to the more intermediate distributions in populations collected from the intergradation zone to frequency distributions shifted to the right side of the graphs characteristic of populations from the eastern region of the volcanic belt or southern Sierra Madre Oriental. A significant but very weak product-moment correlation between hybrid index scores and latitude was detected ($r = 0.106$; $P = 0.030$). This correlation was clearer for longitude ($r = -0.346$; $P < 0.0001$).

The first canonical function derived from discriminant analysis on the morphological traits explained 100% of the variation and provided highly significant discrimination (Wilks' lambda = 0.105; df = 18; $P < 0.001$) between *Q. laurina* individuals from population Tequila and *Q. affinis* individuals from population Zacualtipán. The standardized canonical discriminant function coefficient of each morphological variable is given in Table 3. The variable with the highest coefficient was petiole length. Scores for trees from population Tequila calculated with this canonical discriminant function ranged from −5.911 to −2.320, while *Q. affinis* trees from population Zacualtipán had scores that varied between 0.108 and 4.945. Figure 2B shows the frequency distributions of morphological discriminant scores in all populations. Individuals with scores similar to those observed in morphologically representative populations of *Q. laurina* were preponderant in more western and/or southern populations, with an increasing proportion of

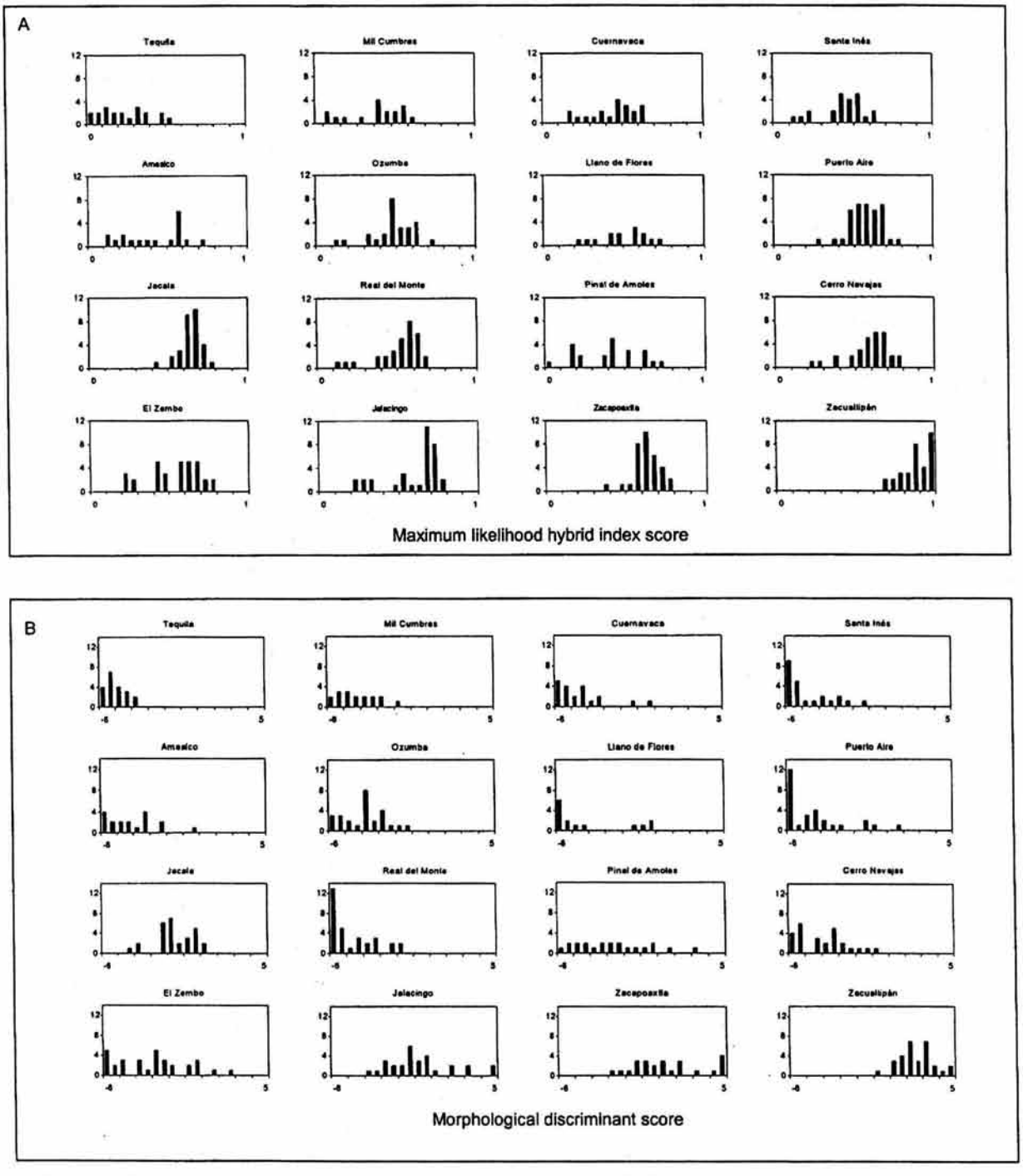


Fig. 2. Frequency distribution in each population of *Q. affinis–Q. laurina* of (A) maximum-likelihood hybrid index scores and (B) morphological canonical discriminant function scores. The *y*-axis represents the number of individuals.

intermediate individuals towards the intergradation zone and a majority of individuals with *Q. affinis* morphology in northeastern populations.

However, the frequency distributions of maximum likelihood hybrid index scores and morphological discriminant scores were only weakly congruent. A regression analysis indicated a low but significant global linear relationship between both variables ($R^2 = 0.179$; $F_{1,493} = 85.29$; $P < 0.0001$). In most populations, the proportion of morphologically intermediate individuals was smaller than the proportion of genetically intermediate individuals, even in the intergradation zone. *Quercus laurina*-like morphology seems to predominate in

TABLE 3. Standardized canonical discriminant function coefficients.

Morphological variable	Coefficient
Total length (TL)	−1.065
Lamina length (LL)	−0.441
Petiole length (PL)	3.045
Maximal width (MW)	−0.183
Distance from base to point of maximal width (PMW)	−0.667
Teeth number (TN)	0.791
Petiole length/total length (PL/TL)	−2.573
Maximal width/lamina length (MW/LL)	−0.591
Distance from base to point of maximal width/lamina length (PMW/LL)	0.199

these cases. Morphological discriminant scores were significantly correlated to latitude ($r = 0.182$; $P = 0.0001$) but not to longitude ($r = -0.004$; $P = 0.93$).

Pairwise correlations of the frequency of RAPD markers across populations are presented in Table 4. All correlations were of the expected sign, that is, negative frequency correlations among semidiagnostic markers of the two species and positive correlations among markers of the same species. Eleven of 36 (31%) of these comparisons were significant. As expected from these correlations, the patterns of frequency changeover on a population-by-population basis among several RAPD fragments were relatively parallel (Fig. 3). Significant associations were also found between the frequency of individual RAPD markers and population mean values of morphological characters (Table 5). Marked patterns of parallel change were found between the frequency of marker A5–7 and mean teeth number and between marker B17–4 and mean petiole length (Fig. 3).

Over all populations, the regression analysis indicated a significant linear association between the maximum likelihood hybrid index scores and the set of morphological variables, although the correlation coefficient was low (multivariate $R^2 = 0.251$; $F_{9,485} = 14.17$; $P < 0.0001$). All morphological variables also had significant relationships with the set of RAPD markers (Table 6). Correlations within populations were in general nonsignificant. In populations Amealco [5], Cerro Navajas [12], Jacala [9], and Puerto Aire [8], one morphological variable (different in each case) and four in the case of population Jalacingo [14], were significantly correlated to RAPD variation.

DISCUSSION

The results of this study support the occurrence of morphological and genetic intergradation between *Q. affinis* and *Q. laurina*. There is also evidence of correlated frequency changeovers among RAPD markers and among RAPD markers and morphological characters on a population-by-population basis along the macrogeographic gradient represented in this study. These patterns seem consistent with a secondary contact hypothesis for the origin of the intergradation area between these two oak species. In primary zones, parallel frequency changes are expected for traits responding similarly to selection gradients, but not for neutral markers (Durrett et al., 2000). Leaf morphology might certainly experience natural selection by the environment, but under a primary origin scenario, to explain the correlated changeover patterns observed here would require physical linkage of most RAPD markers with loci controlling foliar traits, which seems improbable a priori. Although congruent in general, the frequency changeovers of only a few marker pairs had high correlation coefficients and were patently parallel. This could indicate that some differential introgression between markers has occurred after secondary contact. Several studies have shown that differential introgression of neutral markers in secondary zones can occur and depends on linkage to loci under selection and time since contact (Durrett et al., 2000). Localized and parallel introgression of all species-specific markers is expected in very recent contact zones (Rieseberg and Ellstrand, 1993; Durrett et al., 2000). In taxa with a longer history of interaction, chromosome segments containing genes with advantageous or neutral effects in heterospecific backgrounds may have a more widespread introgression, while negatively selected ones will be impeded from crossing species' boundaries and will remain localized. This may create nonconcordant patterns of variation among different markers (Martinsen et al., 2001). In the original hypothesis of a secondary contact between *Q. affinis* and *Q. laurina*, and based on biogeographic considerations and paleobotanical evidence, Valencia (1994) postulated that genetic interactions between these two species probably date from the beginning of the Pleistocene. Even if this estimation

TABLE 4. Correlation coefficients between frequencies of pairs of RAPD markers across populations. Asterisks indicate significant values ($P < 0.05$).

	A05-7	A07-9	A08-1	A10-3	B17-4	B17-6	C09-3	C09-5	I07-2
A05-7	—	−0.056	0.531*	0.588*	0.657*	0.180	0.264	−0.395	0.674*
A07-9		—	−0.159	−0.403	−0.324	−0.283	−0.310	0.146	−0.393
A08-1			—	0.871*	0.712*	0.409	0.238	−0.571*	0.322
A10-3				—	0.728*	0.486	0.165	−0.448	0.483
B17-4					—	0.576*	0.213	−0.260	0.502*
B17-6						—	0.201	−0.226	0.536*
C09-3							—	−0.120	0.386
C09-5								—	−0.353
I07-2									—

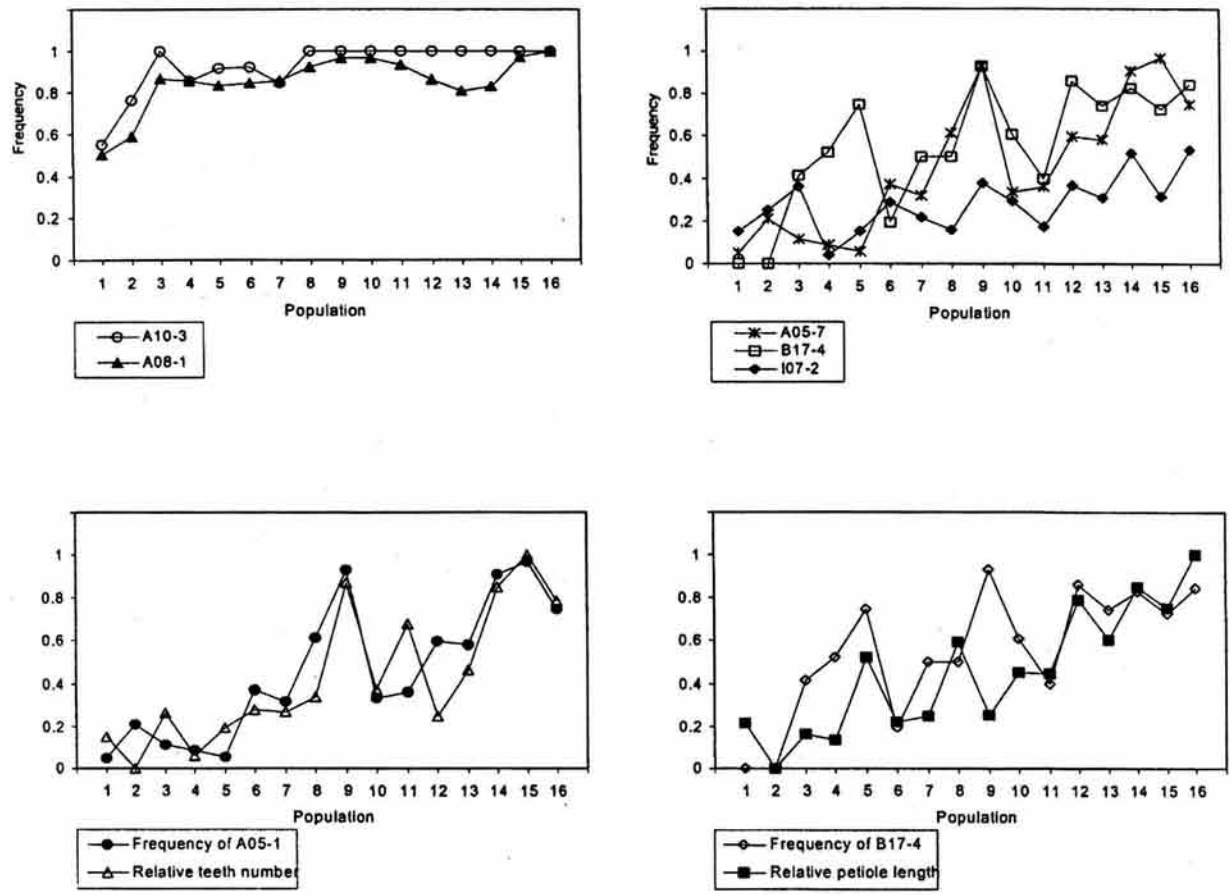


Fig. 3. Plots of the frequency of RAPD markers and RAPD markers and mean value of morphological characters with similar patterns of changeover on a population-by-population basis along the macrogeographic gradient.

is not very accurate, it nevertheless seems like the hybrid zone between these two species is not very recent. It is thus probable that enough time has elapsed for some differential introgression to occur.

In this study, we observed significant global multivariate correlations between phenotypic and molecular variation. However, these correlation coefficients were quite low, and for most populations, the degree of genetic and morphological intermediacy indicated by frequency distributions of hybrid index scores and morphological discriminant scores (Fig. 2A and B) were only partially congruent. In general, the number of morphologically intermediate individuals is smaller than the number of genetically intermediate individuals and, in fact, clear incongruence is observed in populations Cerro Navajas [12], Puerto Aire [8], and El Chico [10], in which nearly all individuals appeared genetically intermediate or even closer to *Q. affinis*, but were morphologically more similar to isolated *Q. laurina*. Other studies of hybridization between oak species have also found incongruence or only partial congruence between morphological and molecular variation. For example, although nuclear and cytoplasmic markers support extensive genetic exchange between the two species in the *Q. petraea-*

TABLE 5. Correlation coefficients between populations' mean values of morphological variables (MV) and RAPD marker frequencies. Asterisks indicate significant values ($P < 0.05$). See Table 3 for explanations of abbreviations.

	RAPD markers								
MV	A05-7	A07-9	A08-1	A10-3	B17-4	B17-6	C09-3	C09-5	I07-2
TL	−0.563*	0.376	−0.565*	−0.419	−0.661*	−0.278	−0.586*	0.320	−0.408
LL	0.526*	0.341	−0.563*	−0.379	−0.623*	−0.253	−0.550*	0.369	−0.363
PL	−0.664*	0.459	−0.481	−0.549*	−0.699*	−0.324	−0.594*	0.111	−0.577
MW	−0.345	0.198	−0.204	−0.082	−0.186	−0.001	−0.443	0.579*	−0.381
PMW	−0.675*	0.189	−0.480	−0.306	−0.530*	0.045	−0.483	0.456	−0.399
TN	0.858*	−0.159	0.599*	0.567*	0.610*	0.097	0.259	−0.596*	0.618*
PL/TL	−0.011	−0.037	0.152	0.175	0.239	0.156	−0.096	0.465	−0.187
MW/LL	−0.594*	0.383	−0.283	−0.449	−0.545*	−0.215	−0.532*	−0.120	−0.547*
PMW/LL	−0.603*	−0.032	−0.215	−0.141	−0.248	0.297	−0.274	0.378	−0.349

TABLE 6. Squared multiple correlations between single morphological variables and the set of RAPD markers. See Table 3 for explanations of abbreviations.

Morphological variable	R^2	$F_{9,485}$	P
TL	0.107	4.99	<0.001
LL	0.100	4.69	<0.001
PL	0.128	5.92	<0.001
MW	0.089	4.25	<0.001
PMW	0.123	5.17	<0.001
TN	0.269	13.27	<0.001
PL/TL	0.120	5.55	<0.001
MW/LL	0.044	2.54	0.008
PMW/LL	0.095	4.51	<0.001

Q. robur complex, several studies have found that morphologically intermediate individuals are very infrequent (Bacilieri et al., 1996a; Kremer et al., 2002). Possible explanations for this phenomenon include maternal effects (i.e., hybrids are more similar to the species of their maternal parent), as well as selection against intermediate forms (Bacilieri et al., 1996a; Kremer et al., 2002). In a mixed stand of *Q. lobata* and *Q. douglasii*, Craft et al. (2002) found phenotypically intermediate trees that had little evidence of mixed ancestry according to microsatellites. On the other hand, only one of four trees with the highest probability of hybrid ancestry was intermediate in appearance. In contrast, a remarkably high correspondence between morphological variables and genetic markers was found in a hybrid zone between *Q. gambelli* and *Q. grisea* in New Mexico (Howard et al., 1997). It is possible that the result was obtained because a highly optimized set of discriminant markers was used and the data were subjected to a canonical correlation analysis, which resulted in a high correlation between both first canonical variates on markers and morphological traits.

Incongruence between morphology and molecular markers has been observed many times in plant hybrid zones (Rieseberg and Ellstrand, 1993), and in general, introgression of morphological characters is more restricted than introgression of molecular markers (Rieseberg and Wendel, 1993). It is thought that recombination of adaptively relevant morphological or physiological characters with a polygenic basis may result in individuals with unfit phenotypes, while this is not expected for individuals that combine neutral markers from different species (Shoemaker et al., 1996). Several times it has been asserted that hybridizing oak species are capable of remaining morphologically or ecologically different in the face of considerable introgression (Whittemore and Schaal, 1991; Howard et al., 1997) and this may be also occurring in the case of *Q. affinis* and *Q. laurina*, despite a probably ancient event of secondary contact between them. It is possible that species distinctness in hybridizing oaks is maintained because natural selection operates against the exchange of genes that constitute the basis of functional divergence (i.e., differential adaptation) between species, while considerable gene flow can occur at the rest of the genome, as suggested by Wu (2001) in the genic view of the process of speciation. Which ecological factors, as well as which traits and genes may account for the functional divergence between the two oaks studied here merits considerable future attention.

Another major theme in the literature on hybrid zones concerns the organization of such areas as simple clines or geographically more complex mosaics (Rand and Harrison, 1989). In the first case, a gradual transition is observed between the character states typical of each parental population (Barton and Hewitt, 1989). In our case, frequency distributions of hybrid index scores and morphological discriminant scores were significantly correlated to geographic coordinates and change (although with weak concordance, as discussed before) from isolated populations of *Q. laurina* to isolated populations of *Q. affinis* with a series of more or less intermediate populations in between. This would argue in favor of a clinal structure for this hybrid zone. However, the frequencies of single RAPD markers as well as the values of morphological variables seem to follow a more complex pattern of change across localities than what would be expected for a clinal hybrid zone. In general, mosaic zones can be characterized as patches of pure species populations and mixed populations scattered across a zone of overlap (Howard et al., 1997). At this moment, we cannot firmly argue in favor of such structure for the hybrid zone between *Q. affinis* and *Q. laurina*, because we primarily focused on populations that previously showed some morphological evidence of intergradation. The sampled populations are in fact scattered among populations that were judged to be pure according to the appearance of herbarium specimens, but it would nevertheless be necessary to include a sample of such populations in a larger survey using molecular markers to better understand the structure of this hybrid zone.

LITERATURE CITED

ARNOLD, M. L. 1997. Natural hybridization and evolution. Oxford University Press, New York, New York, USA.

BACILIERI, R., A. DUCOUSSO, AND A. KREMER. 1996a. Comparison of morphological characters and molecular markers for the analysis of hybridization in sessile and pedunculate oak. *Annales des Sciences Forestieres* 53: 79–91.

BACILIERI, R., A. DUCOUSSO, R. J. PETIT, AND A. KREMER. 1996b. Mating system and asymmetric hybridization in a mixed stand of European oaks. *Evolution* 50: 900–908.

BACON, J. R., AND R. SPELLENBERG. 1996. Hybridization in two distantly related Mexican black oaks *Quercus conzattii* and *Quercus eduardii* (Fagaceae: *Quercus*: Section *Lobatae*). *Sida* 17: 17–41.

BARTON, N. H., AND G. M. HEWITT. 1989. Adaptation, speciation and hybrid zones. *Nature* 341: 497–503.

BECKSTROM-STERNBERG, S. M., L. H. RIESEBERG, AND K. DOAN. 1991. Gene lineage analysis in populations of *Helianthus niveus* and *H. petiolaris* (Asteraceae). *Plant Systematics and Evolution* 175: 125–138.

BODÉNÈS, C., S. JOANDET, F. LAIGRET, AND A. KREMER. 1997. Detection of genomic regions differentiating two closely related oak species *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. *Heredity* 78: 433–444.

BORGARDT, S. J., AND K. B. PIGG. 1999. Anatomical and developmental study of petrified *Quercus* (Fagaceae) fruits from the Middle Miocene, Yakima Canyon, Washington, USA. *American Journal of Botany* 86: 307–325.

BROPHY, W. B., AND D. R. PARNELL. 1974. Hybridization between *Quercus agrifolia* and *Quercus wislizenii* (Fagaceae). *Madroño* 22: 290–302.

BRUSCHI, P., G. G. VENDRAMIN, F. BUSSOTTI, AND P. GROSSONI. 2000. Morphological and molecular differentiation between *Quercus petraea* (Matt.) Liebl. and *Quercus pubescens* Willd. (Fagaceae) in northern and central Italy. *Annals of Botany* 85: 325–333.

BURGER, W. C. 1975. The species concept in *Quercus*. *Taxon* 24: 45–50.

COART, E., V. LAMOTE, M. DE LOOSE, E. VAN BOCKSTAELE, P. LOOTENS, AND I. ROLDÁN-RUIZ. 2002. AFLP markers demonstrate local genetic differentiation between two indigenous oak species [*Quercus robur* L. and *Quercus petraea* (Matt.) Liebl.] in Flemish populations. *Theoretical and Applied Genetics* 105: 431–439.

COOPERRIDER, M. 1957. Introgressive hybridization between *Quercus macrocarpa* and *Quercus velutina* in Iowa. *American Journal of Botany* 44: 804–810.

CRAFT, K. J., M. V. ASHLEY, AND W. D. KOENIG. 2002. Limited hybridization between *Quercus lobata* and *Quercus douglasii* (Fagaceae) in a mixed

stand in central and coastal California. *American Journal of Botany* 89: 1792–1798.

DAGHLIAN, C. P., AND W. L. CREPET. 1983. Oak catkins, leaves and fruits from the Oligocene Catahoula Formation and their evolutionary significance. *American Journal of Botany* 70: 639–649.

DUMOLIN-LAPEGUE, S., A. KREMER, AND R. J. PETIT. 1999. Are chloroplast and mitochondrial DNA variation species independent in oaks? *Evolution* 53: 1406–1413.

DURRETT, R., L. BUTTEL, AND R. HARRISON. 2000. Spatial models for hybrid zones. *Heredity* 84: 9–19.

ENDLER, J. A. 1977. Geographic variation, speciation and clines. Princeton University Press, Princeton, New Jersey, USA.

FERRUSQUIA-VILLAFRANCA, I. 1993. Geology of Mexico: a synopsis. *In* T. P. Ramammoorthy, R. Bye, A. Lot, and J. Fa [eds.], Biological diversity of Mexico: origins and distribution, 3–107. Oxford University Press, New York, New York, USA.

GUTTMAN, S. I., AND L. A. WEIGT. 1989. Electrophoretic evidence of relationships among *Quercus* (oaks) of eastern North America. *Canadian Journal of Botany* 67: 339–351.

HARDIG, T. M., S. J. BRUNSFELD, R. S. FRITZ, M. MORGAN, AND M. ORIANS. 2000. Morphological and molecular evidence for hybridization and introgression in a willow (*Salix*) hybrid zone. *Molecular Ecology* 9: 9–24.

HARDIN, J. W. 1975. Hybridization and introgression in *Quercus alba*. *Journal of the Arnold Arboretum* 56: 336–363.

HOKANSON, S. C., J. G. ISEBRANDS, R. J. JENSEN, AND J. F. HANCOCK. 1993. Isozyme variation in oaks of the Apostle Islands in Wisconsin: genetic structure and levels of inbreeding in *Quercus rubra* and *Q. ellipsoidalis* (Fagaceae). *American Journal of Botany* 80: 1349–1357.

HOWARD, D. J., R. W. PRESZLER, J. WILLIAMS, S. FENCHEL, AND W. J. BOECKLEN. 1997. How discrete are oak species? Insights from a hybrid zone between *Quercus grisea* and *Quercus gambelli*. *Evolution* 51: 747–755.

JENSEN, R. J., S. C. HOKANSON, J. G. ISEBRANDS, AND J. F. HANCOCK. 1993. Morphometric variation in oaks of the Apostle Islands in Wisconsin: evidence of hybridization between *Quercus rubra* and *Q. ellipsoidalis* (Fagaceae). *American Journal of Botany* 80: 1358–1366.

KLEINSCHMIT, J. R. G., R. BACILIERI, A. KREMER, AND A. ROLOFF. 1995. Comparison of morphological and genetic traits of pedunculate oak (*Q. robur* L.) and sessile oak (*Q. petraea* (Matt.) Liebl.). *Silvae Genetica* 44: 256–269.

KREMER, A., ET AL. 2002. Leaf morphological differentiation between *Quercus robur* and *Quercus petraea* is stable across western European mixed oak stands. *Annals of Forest Science* 59: 777–787.

LEFORT, F., AND G. C. DOUGLAS. 1999. An efficient micro-method of DNA isolation from mature leaves of four hardwood tree species *Acer*, *Fraxinus*, *Prunus* and *Quercus*. *Annals of Forest Science* 56: 259–263.

MARTINSEN, G. D., T. G. WHITHAM, R. J. TUREK, AND P. KEIM. 2001. Hybrid populations selectively filter gene introgression between species. *Evolution* 55: 1325–1335.

NIXON, K. C. 1993a. The genus *Quercus* in Mexico. *In* T. P. Ramammoorthy, R. Bye, A. Lot, and J. Fa [eds.], Biological diversity of Mexico: origins and distribution, 447–458. Oxford University Press, New York, New York, USA.

NIXON, K. C. 1993b. Infrageneric classification of *Quercus* (Fagaceae) and typification of sectional names. *Annales des Sciences Forestieres* 50 (supplement 1): 255–345.

PALMER, E. J. 1948. Hybrid oaks of North America. *Journal of the Arnold Arboretum* 29: 1–48.

RAND, D. M., AND R. G. HARRISON. 1989. Ecological genetics of a mosaic hybrid zone: mitochondrial, nuclear and reproductive differentiation of crickets by soil type. *Evolution* 43: 432–449.

RIESEBERG, L. H., AND N. C. ELLSTRAND. 1993. What can molecular and morphological markers tell us about plant hybridization? *Critical Reviews in Plant Sciences* 12: 213–241.

RIESEBERG, L. H., AND J. F. WENDEL. 1993. Introgression and its consequences in plants. *In* R. G. Harrison [ed.], Hybrid zones and the evolutionary process, 70–109. Oxford University Press, New York, New York, USA.

SAMUEL, R., W. PINSKER, AND F. EHRENDORFER. 1995. Electrophoretic analysis of genetic variation within and between populations of *Quercus cerris*, *Q. pubescens*, *Q. petraea* and *Q. robur* (Fagaceae) from Eastern Austria. *Botanica Acta* 108: 290–299.

SHOEMAKER, D. D., K. G. ROSS, AND M. L. ARNOLD. 1996. Genetic structure and evolution of a fire ant hybrid zone. *Evolution* 50: 1958–1976.

SOKAL, R. R., AND F. J. ROHLF. 1995. Biometry: the principles and practice of statistics in biological research. Freeman and Company, New York, New York, USA.

TABACHNICK, B. G., AND L. S. FIDELL. 1989. Using multivariate statistics. Harper Collins, New York, New York, USA.

VALENCIA, S. 1994. Contribución a la delimitación taxonómica de tres especies del género *Quercus* subgénero *Erythrobalanus*. Tesis de Maestría, Facultad de Ciencias, Universidad Nacional Autónoma de México, México, D.F., México.

VAN VALEN, L. 1976. Ecological species, multispecies and oaks. *Taxon* 25: 233–239.

WHITTEMORE, A. T., AND B. A. SCHAAL. 1991. Interspecific gene flow in sympatric oaks. *Proceedings of the National Academy of Sciences, USA* 88: 2240–2544.

WU, C. I. 2001. The genic view of the process of speciation. *Journal of Evolutionary Biology* 14: 851–865.

IV.

Antonio González-Rodríguez, and Ken Oyama

Leaf morphometric variation in *Quercus affinis* and *Q. laurina* (Fagaceae), two hybridizing Mexican red oaks

Botanical Journal of the Linnean Society, *en prensa*.

Leaf morphometric variation in *Quercus affinis* and *Q. laurina* (Fagaceae), two hybridizing Mexican red oaks

ANTONIO GONZÁLEZ-RODRÍGUEZ* and KEN OYAMA

Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México, Antigua Carretera a Pátzcuaro No. 8701, Col. Ex Hacienda de San José de la Huerta, Morelia, 58190 Michoacán, México

RUNNING TITLE: LEAF VARIATION IN TWO MEXICAN OAKS

* Corresponding author:

Antonio González-Rodríguez

Centro de Investigaciones en Ecosistemas, UNAM

Antigua Carretera a Pátzcuaro No. 8701

Col. Ex–Hacienda de San José de la Huerta

Morelia, 58190 Michoacán

MÉXICO

e-mail: agrodrig@oikos.unam.mx

Fax: +52 55 56 23 27 19

Tel: +52 55 56 23 27 17

Leaf variation was examined in two hybridizing Mexican red oaks, *Quercus affinis* and *Q. laurina*. Data of nine traits were obtained for 10 randomly chosen leaves in each of 512 individuals from 16 populations sampled along a geographic gradient, including the distribution area of both species and a putative area of secondary contact and hybridization. A cluster analysis recognized two main groups of populations, which were congruent with the taxonomic assignment of the predominant morphological type within the populations and were thus labelled '*Q. affinis*-like' and '*Q. laurina*-like' population groups. A nested analysis of variance revealed that the largest proportion of the total variation was contained within populations, as among-tree variation (28–54%, depending on the trait), and as intra-individual variation (17–56%). However, differences between the two groups of populations (3–27%) and among populations within groups (5–21%) were also significant for the nine traits. A distinct pattern of change across populations was observed for each trait. Variation was particularly pronounced along the macrogeographic gradient for petiole length and leaf-margin teeth number, possibly implying selection on these two traits. Results suggest that phenotypic plasticity, gene flow, hybridization and natural selection have shaped foliar variation in this oak complex.

ADDITIONAL KEYWORDS: Foliar traits – geographic variation – hybrid zones – phenotypic plasticity – population differentiation

INTRODUCTION

Patterns of morphological variation in natural populations are created by gene flow, natural selection, phenotypic plasticity, genetic drift and historical causes (Mayr, 1963; Endler, 1977; 1986). Many studies have identified variation in phenotypic characters within plant species as a result of these factors (*e.g.* Wyatt & Antonovics, 1981; Hume & Cavers, 1982; Sokal *et al.*, 1986; Shaver *et al.*, 1986; Oyama, 1993; Domínguez *et al.*, 1998). The genus *Quercus* has been an interesting subject for studies of leaf morphology, because it is characterized by considerably high levels of variability. In various oak species, differentiation in foliar characters occurs among populations, among trees within populations, and among branches within a tree (Blue & Jensen, 1988; Bruschi *et al.*, 2003). Furthermore, significant seasonal (Blue & Jensen, 1988) and ontogenetic changes (Kleinschmit *et al.*, 1995) have also been described. These results demonstrate the dynamic nature of foliage and its capacity to adjust to environmental conditions through plasticity and/or adaptive differentiation.

The high frequency of hybridization in *Quercus* incorporates further variation to the intrinsic variability within species and may complicate the interpretation of taxonomic patterns (Tucker, 1961; Hardin, 1975; Aas, 1993; Rushton, 1993). For this reason, considerable efforts have been directed to understand phenotypic differentiation between species within particular complexes (Kleinschmit *et al.*, 1995; Bacilieri *et al.*, 1996; Bruschi *et al.*, 2000; Kremer *et al.*, 2002), and to assess the expression of characters in hybrid progenies with controlled parentage (Kleinschmit *et al.*, 1995; Bacilieri *et al.*, 1996). In natural populations, a continuum in leaf morphology has been observed in areas of range overlap between oak species that are well differentiated outside of the region of sympatry

(Jensen *et al.*, 1993; Bacon & Spellenberg, 1996; Howard *et al.*, 1997; González-Rodríguez *et al.*, 2004). These patterns of variation have been interpreted as supporting the view that such areas represent secondary hybrid zones, and imply that extensive hybridization and backcrossing have occurred.

In this study, we analyzed patterns of foliar variation in a complex consisting of two species of Mexican red oaks, *Quercus affinis* Scheidw., and *Q. laurina* Humb. & Bonpl. According to a preliminary phylogenetic analysis based on morphological characters, these two species are closely related taxa (S. Valencia, unpublished data). The two species have partially overlapping distributions and show morphological intergradation in the area of overlap (Valencia, 1994). However, outside of this area phenotypic differentiation is clear, and *Q. laurina* individuals usually have longer, wider leaves, with a longer petiole and fewer leaf-margin teeth, compared to *Q. affinis* individuals. Other architectural and microanatomical features of the leaves such as the venation pattern and the density of trichomes also show some degree of differentiation between morphologically representative populations of the two species (Valencia, 1994). Discriminant function analysis has been previously used to characterize foliar differentiation between reference populations of *Q. affinis* and *Q. laurina* from outside of the area of overlap, and then employed to assess the multivariate pattern of morphological change across a geographic gradient, which included the range of the two species and the area of intergradation (González-Rodríguez *et al.*, 2004). Foliar variation between the two species was found to be continuous across the gradient, although only a relatively small fraction of the individuals were morphologically intermediate. Within populations, the morphology of a single species predominated in most cases, that is, the majority of the individuals appeared to be similar to morphologically representative *Q. laurina*, or similar to morphologically representative *Q. affinis*. These

results, together with simultaneous genetic analyses, were interpreted as supporting the previous hypothesis of a secondary contact event for the origin of the area of overlap, and the occurrence of hybridization and introgression between the two oak species within this area (Valencia, 1994; González-Rodríguez *et al.*, 2004).

The specific questions addressed in this study were: What are the patterns of change of particular foliar traits in the *Q. affinis–Q. laurina* complex across the geographic gradient? What is the pattern of relationships among populations according to their morphological similarities? How is morphological variation partitioned among different hierarchical levels, including species, populations, trees and leaves? Are populations situated in the area of overlap morphologically more variable, as is expected for hybrid populations? Finally, we use the results to draw some inferences about the possible underlying evolutionary mechanisms that have produced patterns of morphological variation in these oaks.

MATERIALS AND METHODS

All specimens of the *Q. affinis–Q. laurina* complex deposited at the National Herbarium of Mexico (MEXU) were examined, and sixteen populations located throughout the geographic distribution of both species and the area of overlap, and considered to be representative of the range of morphological variation present in the complex (*i.e.* populations with typical individuals of the two species and populations with various degrees of morphological intergradation), were selected for sampling. As shown in Fig. 1, collection localities were situated in three main mountain ranges in Mexico: the Sierra Madre del Sur, the Trans-Mexican Volcanic Belt, and the Sierra Madre Oriental

(Ferrusquía-Villafranca, 1993). Morphologically representative populations of *Q. laurina* are mostly situated in the Sierra Madre del Sur and the western region of the volcanic belt. The area of morphological intergradation is in the eastern region of the volcanic belt, and morphologically representative populations of *Q. affinis* are along the Sierra Madre Oriental (Valencia, 1994). A number was assigned to each population that reflects its position in the geographic gradient defined by these patterns of geographical distribution. For this, we considered simultaneously the latitude and the longitude of each location, yielding a gradient from the west and south to the east and north (Fig. 1). Twenty to 45 trees were sampled per locality. In all cases, sampled individuals were randomly chosen adult trees separated at least 100 m from each other along a transect. Two branches from each individual situated within reach at no more than 5 m from the ground were chosen haphazardly without any perceived bias and pressed to prepare herbarium specimens.

Six measurements were made for ten randomly chosen leaves from each individual: total length (TL), lamina length (LL), petiole length (PL), maximal width (MW), distance from the base of the leaf to the point of maximal width (PMW), and leaf-margin tooth number (TN). The ratios of PL/TL, MW/LL, and PMW/LL were calculated, as ratios relate to shape rather than size variation, and thus may provide additional information (Frampton & Ward 1990). Before further analysis, the three ratios were arcsine square root transformed, and TN was log-transformed, to normalize the distribution of these variables (Sokal & Rohlf 1995).

To determine morphological relationships among populations, a matrix of pairwise Euclidean distances was calculated from the population means of the nine foliar characteristics, and a hierarchical cluster analysis based on this matrix was performed using the unweighted pair-group method with arithmetic average algorithm (UPGMA). A Mantel

permutation test (Mantel, 1967) was employed to investigate the relationship between the morphological similarities among populations and the linear geographic distance separating them using the zt software (Bonnet & Van de Peer, 2001).

Once the primary morphological subdivisions among populations were obtained through the cluster analysis, partitioning of the total variance in foliar morphology in terms of differences at various hierarchical levels was determined with a nested analysis of variance. The levels considered were groups of populations (*G*), populations within groups (*P*), trees within populations (*T*) and leaves within individuals, the latter level used as the error term (Sokal and Rohlf, 1995). In the model, *G* was treated as a fixed factor and *P* and *T* were treated as random effects. The variance components, the proportion of the total variation in a character that is accounted for by a particular hierarchical level (Sokal & Rohlf, 1995), were determined for each level. These analyses were performed using the JMP statistical package (SAS Institute, 1995).

As hybridization typically produces an increase in quantitative trait variance in resulting populations (Harrison, 1990), we estimated the coefficient of variation (CV) for each morphological character and population, to assess whether levels of trait variation change among *Q. affinis-Q. laurina* populations in different geographic areas (*i.e.* within and outside of the overlap area).

RESULTS

Figure 2A–D shows the mean values of the nine morphological variables measured in the 16 populations. All foliar traits varied significantly across the geographic gradient represented in this study. Two characters showed particularly clear patterns of change: from

southwest to northeast, mean petiole length decreased from approximately 1 cm to less than 0.4 cm, while the mean number of leaf-margin teeth increased from 0–1 to 4–6 (Fig. 2). The total length of the leaf and the lamina length also decrease in the same direction, although less markedly. In contrast, highest maximal widths were observed in populations situated at intermediate positions in the gradient (PR[8], RM[10], CN[12]), and a similar pattern was observed for the ratio of MW/LL, indicating that leaves in individuals from these populations are in fact wider, independently of the size. PMW first decreases, increases in populations RM[10], CN[12] and ZB[13], and then decreases again. The ratio of PL/TL is higher in more southwestern than in more northeastern populations. Finally, PMW/LL also seems to be higher in more southwestern populations, although the pattern of change of this character is less clear along the gradient (Fig. 2).

The UPGMA cluster analysis recognized two main groups of populations (Fig. 3). Five populations (ZC[16], ZX[15], JG[14], JC[9] and PA[11]), comprised the first group. Because these populations predominantly contain trees that have been classified as *Q. affinis* (Valencia 1994; A. González-Rodríguez pers. observ.), this group was labeled '*Q. affinis*-like'. The second group consisted of eleven populations, with a majority of '*Q. laurina*-like' individuals. In a few cases, populations that clustered together (*e.g.* OZ[6] and CA[3]; RM[10] and ZB[13]) are also in geographic proximity, but the Mantel test did not detect a significant general association between leaf similarity among populations and the linear geographical distances separating them ($r = 0.015$; $P = 0.43$).

Nested analysis of variance performed on all nine morphological variables revealed that all hierarchical levels considered (groups of populations, populations within groups, and individuals within populations), contributed significantly to foliar variation in the *Q. affinis-Q. laurina* complex, except MW/LL, which did not differ significantly between the

'*Q. affinis*-like' and '*Q. laurina*-like' population groups (Table 1). Differences between these two population groups explained only a small proportion of the total variation (3–5%) in other five characters (TL, LL, PL, MW and PL/TL). In contrast, the amount of variation between these two groups was greatest for PMW (9%), PMW/LL (10%) and TN (27%). Differences among populations within groups accounted for 5–21% of the variation. Characters that most strongly differentiated the two population groups (PMW, PMW/LL, AN) showed less variation among populations, and vice versa. For eight traits, the greatest proportion of variance (28–54%) was explained by differences among trees within populations. Finally, another substantial source of variation (17–56%) was accounted for by the error term, *i.e.*, differences among leaves within individuals.

The coefficients of variation for each foliar character and population are shown in Fig. 4. Coefficients of variation were generally lower in populations situated at both ends of the geographical gradient and higher in intermediate populations. Population ZC[16], situated at one geographical extreme, seems to be on average the morphologically more homogeneous, but population TQ[1], which constitutes the other end of the gradient, appears to be comparatively more variable. Among the geographically intermediate populations, none consistently had the highest CV for all characters, and for some traits (*e.g.* PL and MW/LL), the lowest value was found in geographically intermediate populations (Fig. 4).

DISCUSSION

There is considerable phenotypic variation within the red oak complex analyzed here. All measured foliar traits changed significantly across populations, and the pattern of change

was distinct for each trait. Although for most traits the patterns seemed to be continuous along the geographic gradient, a hierarchical cluster analysis recognized two main groups of populations, which were congruent with the taxonomic assignment of the predominant morphological type within the populations (Valencia 1994; A. González-Rodríguez pers. observ.), and were thus labeled '*Q. affinis*-like' and '*Q. laurina*-like' population groups. A nested ANOVA further revealed significant differences between the two population groups for all characters, but the magnitude of the differentiation was modest (3–5%) in the case of six characters, and in fact considerable (27%) only for leaf-margin tooth number. Variation among populations within these two groups was also significant for the nine traits, but relatively small in magnitude (5–21%). On the other hand, a remarkably high amount of the total variation was contained within populations, in the form of differences among individual trees (28–54%), as well as within-tree variation (17–56%).

The high intra individual variation observed can be the result of phenotypic plasticity and/or developmental instability. It is well known that, in trees, leaves from different parts of the crown often vary significantly in morphology (*e.g.* Niinemets *et al.*, 2004). In *Q. petraea*, the effect of the sun/shade dichotomy for branch position on 31 foliar characters was explicitly addressed (Bruschi *et al.*, 2003), and was found to account for a higher proportion of the total variance (2.3–58.3% depending on the character, mean = 23.5%) than differences among trees within populations (3.4–48.7%, mean = 16.5%), and differences among populations (5–36.5%, mean = 19.5%). The error term included the variation among leaves within branches and was also considerably high (11.7–50.8%, mean = 28.5%) (Bruschi *et al.*, 2003). Unfortunately, in our study, branches were haphazardly sampled with respect to position, and thus we cannot further subdivide the within-tree variation into deterministic (*i.e.* consistent differences depending on position) and random

(*i.e.* error) variation. However, while taking measurements, we observed both between-branch and within-branch differences among leaves, suggesting a role for both plasticity and developmental effects.

The high among-tree variation within the populations may be in part the expression of phenotypic plasticity due to the micro-environmental conditions experienced by each tree, but also the result of genotypic differences among individuals. Neutral genetic markers have usually revealed low population differentiation in oak species, suggestive of high amounts of nuclear gene flow (via pollen) among populations (Michaud *et al.*, 1995; Zanetto & Kremer, 1995; Le Corre *et al.*, 1997; Toumi & Lumaret, 1998; A. González-Rodríguez, unpublished data). This implies that a large proportion of the species' total genetic variation can be found within any population. For this reason, a wide range of genetically based phenotypic variation among individuals can be presumed, although morphological characters might certainly be subjected to developmental and environmental constraints not experienced by neutral markers, that may affect the amount and direction of the expressed variation. In the case of hybrid populations that originate through interspecific gene flow, an even more diverse array of genotypes is expected to occur, and an increase in quantitative trait variance may result from high heterozygosity and positive linkage disequilibria (Harrison, 1990). The coefficient of variation calculated for each trait in the 16 oak populations offered some support for this expectation, though the patterns were not completely consistent for all traits and populations.

In fact, the variation of foliar traits observed among populations suggests the action of other forces besides gene flow in determining morphological patterns in the *Q. affinis–Q. laurina* complex. If gene flow were the main factor, a higher phenotypic similarity could be expected for geographically more proximate populations. Indeed, a population genetic

study conducted for the same 16 populations analyzed here, revealed a significant correlation between the linear geographical distances separating the populations and genetic distances calculated from molecular markers (A. González-Rodríguez, unpublished data). Interestingly, the genetic similarities among populations seemed to be more related to the spatial distance separating them than to their morphological relationships. For example, one of the two main groups in a UPGMA dendrogram based on genetic distances was formed by eight populations from the eastern region of the volcanic belt and the Sierra Madre Oriental, and included four populations (PR[8], RM[10], CN[12], and ZB[13]) that belong to the '*Q. laurina*-like' group together with four '*Q. affinis*-like' populations (JC[9], JG[14], ZX[15], and ZC[16]) (A. González-Rodríguez, unpublished data). On the contrary, the results of the present study did not indicate a significant general relationship between overall phenotypic dissimilarity among populations, expressed as Euclidean distances, and spatial distances. It seems that '*Q. laurina*-like' and '*Q. affinis*-like' populations from the area of overlap between the two species, despite being in geographic proximity and having experienced considerable introgression of neutral genetic markers (González-Rodríguez *et al.*, 2004; A. González-Rodríguez, unpublished data), have remained morphologically different enough as to be classified into distinct groups in a hierarchical cluster analysis (Fig. 3).

Such incongruence between genetic and morphological patterns could result from the action of selection on hybrid individuals. This selection can take two forms, 'endogenous' and 'exogenous' (Barton & Hewitt, 1985, 1989; Harrison, 1990). The first is a consequence of the disruption of the coadapted gene complexes of the parental species in hybrids, leading to genetic and developmental malfunctioning in any situation, independently of external factors. In contrast, 'exogenous' selection implies that the

relative fitness of hybrid individuals is dependent on particular environmental conditions (Barton & Hewitt, 1985, 1989; Harrison, 1990). The two oak species are thought to have diverged in different geographic areas (*Q. affinis* in the Sierra Madre Oriental and *Q. laurina* in the Sierra Madre del Sur). During this process, adaptive differentiation of leaf morphology might have occurred. Petiole length and leaf-margin teeth number were the two characters most strongly differentiated between the two extremes of the geographic gradient, and both traits have a well-established functional significance. Petiole length influences leaf arrangement, affecting light interception efficiency under different circumstances (Pearcy & Yang, 1998; Niinemets *et al.*, 2004). Across tree species, petiole length is correlated with other traits such as leaf length and width and crown architecture, and these correlations change with latitude and elevation, temperate species having in general shorter petioles than tropical species (King, 1998; King & Maindonald, 1999). The proportion of tree species with lobed or toothed margins increases along the latitudinal gradient of decreasing mean annual temperature and more marked seasonality (Givnish, 1987). It has been shown that marginal leaf teeth and lobes represent adaptations for more rapid initiation of photosynthesis in flushing leaves in several temperate tree species, including three oak species (Baker-Brosh & Peet, 1997). The geographic variation in petiole length and leaf-margin teeth number observed in *Q. affinis–Q. laurina* populations could be related to environmental conditions resulting from the gradient of increasing rainfall that is observed in Mexico from west to east (Mosiño-Alemán & García, 1974), and from latitudinal changes in mean annual temperature, luminosity and seasonality. Adaptation to particular habitats may have also played a role: according to Valencia (1994), trees with *Q. affins*-like morphology are usually found at elevations between 1600–2225 m in protected and humid sites such as valleys and gorges, while trees with *Q. laurina*-like

morphology occur at higher elevations (2440–3065 m) in exposed and drier sites. These suggestions may be tested in future investigations on the functional significance of leaf morphological variation in these and other oaks.

From our results it can be further hypothesized that both exogenous and endogenous selection are acting in the hybrid zone between *Q. affinis* and *Q. laurina*. For example, petiole length changed steadily in a consistent direction along the geographic gradient, and the differentiation in this character between the '*Q. affinis*-like' and '*Q. laurina*-like' population groups was small (4%; Table 1). According to hybrid zone theory, the action of exogenous selection in secondary zones can result in an association between the patterns of change of particular traits and environmental gradients, which implies that intermediate character states are favored in intermediate environments (Barton & Hewitt, 1985, 1989; Harrison, 1990). In contrast, it is possible that endogenous selection is operating against intermediate states at the characters that were differentiated to a larger extent between the two population groups (PMW, TN and PMW/LL). For example, most leaves have the same number of leaf-margin teeth on each side of the blade: 0 or 1 pair in the case of *Q. laurina*, 2–3 pairs in the case of *Q. affinis*. The presence of asymmetrical leaves with unequal numbers of teeth on each side, particularly in the hybrid zone, could be an indication of developmental instability resulting from the disruption of parental gene complexes (Freeman *et al.*, 1995).

ACKNOWLEDGEMENTS

The authors thank E. Pedraza, R. Tapia and P. Cuevas-Reyes for field assistance, and S. Valencia for taxonomic advice. L. E. Eguiarte and M. Lara contributed throughout the

development of this work with useful suggestions. The comments by J. Nuñez-Farfán, D. Piñero, M. Olson and three anonymous reviewers on earlier versions of the manuscript are appreciated. Economic support was provided by CONACyT (grant 38550–V to K. O.) and DGAPA, UNAM (grant IN212999 to K. O.).

REFERENCES

Aas G. 1993. Taxonomical impact of morphological variation in *Quercus robur* and *Q. petraea*: a contribution to the hybrid controversy. *Annales des Sciences Forestières* **50** (Suppl. 1): 107s–113s.

Bacilieri R, Ducousso A, Kremer A. 1996. Comparison of morphological characters and molecular markers for the analysis of hybridization in sessile and pedunculate oak. *Annales des Sciences Forestières* **53**: 79–91.

Bacon JR, Spellenberg R. 1996. Hybridization in two distantly related Mexican black oaks *Quercus conzatti* and *Quercus eduardii* (Fagaceae: *Quercus*: Section *Lobatae*). *Sida* **17**: 17–41.

Baker-Brosh KF, Peet RK. 1997. The ecological significance of lobed and toothed leaves in temperate forest trees. *Ecology* **78**: 1250–1255.

Barton NH, Hewitt GM. 1985. Analysis of hybrid zones. *Annual Review of Ecology and Systematics* **16**: 113-148.

Barton NH, Hewitt GM. 1989. Adaptation, speciation and hybrid zones. *Nature* **341**: 497-503.

Blue MP, Jensen RJ. 1988. Positional and seasonal variation in oak (*Quercus*: Fagaceae) leaf morphology. *American Journal of Botany* **75**: 939–947.

Bonnet E, Van de Peer Y. 2001. *zt: a software tool for simple and partial Mantel tests.* Belgium: Flanders Interuniversity Institute for Biotechnology, Ghent University.

Bruschi P, Grossoni P, Bussotti F. 2003. Within- and among tree variation in leaf morphology of *Quercus petraea* (Matt.) Liebl. natural populations. *Trees* **17**: 164–172.

Bruschi P, Vendramin GG, Bussotti F, Grossoni P. 2000. Morphological and molecular differentiation between *Quercus petraea* (Matt.) Liebl. and *Quercus pubescens* Willd. (Fagaceae) in Northern and Central Italy. *Annals of Botany* **85**: 325–333.

Domínguez CA, Eguiarte LE, Núñez-Farfán J, Dirzo R. 1998. Flower morphometry of *Rhizophora mangle* (Rhizophoraceae): geographical variation in Mexican populations. *American Journal of Botany* **85**: 637–643.

Endler JA. 1977. *Geographic variation, speciation, and clines*. Princeton New Jersey: Princeton University Press.

Endler JA. 1986. *Natural selection in the wild*. Princeton New Jersey: Princeton University Press.

Ferrusquía-Villafranca I. 1993. Geology of Mexico: a sinopsis. In: Ramammoorthy TP, Bye R, Lot A, Fa J, eds. *Biological diversity of Mexico: origins and distribution.* New York, New York: Oxford University Press, 3–107.

Frampton CM, Ward JM. 1990. The use of ratio variables in systematics. *Taxon* **39**: 586–592.

Freeman DC, Graham JH, Byrd DW, McArthur ED, Turner WA. 1995. Narrow hybrid zone between two subspecies of big sagebrush, *Artemisia tridentata* (Asteraceae). III. Developmental instability. *American Journal of Botany* **82**: 1144–1152.

Givnish TJ. 1987. Comparative studies of leaf form: assessing the roles of selective pressures and phylogenetic constraints. *New Phytologist* **106**: 131–160.

González-Rodríguez A, Arias DM, Valencia S, Oyama K. 2004. Morphological and RAPD analysis of hybridization between *Quercus affinis* and *Q. laurina* (Fagaceae), two Mexican red oaks. *American Journal of Botany* **91**: 401–409.

Hardin JW. 1975. Hybridization and introgression in *Quercus alba*. *Journal of the Arnold Arboretum* **56**: 336-363.

Harrison RG. 1990. Hybrid zones: windows on evolutionary process. In: Futuyma D, Antonovics J, eds. *Oxford Surveys in Evolutionary Biology 7*. 69-128.

Howard DJ, Preszler RW, Williams J, Fenchel S, Boecklen WJ. 1997. How discrete are oak species? Insights from a hybrid zone between *Quercus grisea* and *Quercus gambelli*. *Evolution* **51**: 747–755.

Hume L, Cavers PB. 1982. Geographic variation in a widespread perennial weed, *Rumex crispus*: the relative amounts of genetic and environmentally induced variation among populations. *Canadian Journal of Botany* **60**: 1928–1937.

Jensen RJ, Hokanson SC, Isebrands JG, Hancock JF. 1993. Morphometric variation in oaks of the Apostle Islands in Wisconsin: evidence of hybridization between *Quercus rubra* and *Q. ellipsoidalis* (Fagaceae). *American Journal of Botany* **80**: 1358–1366.

King DA, Maindonald JH. 1999. Tree architecture in relation to leaf dimensions and tree stature in temperate and tropical rain forests. *Journal of Ecology* **87**: 1012–1024.

King DA. 1998. Influence of leaf size on tree architecture: first branch height and crown dimensions in tropical rain forest trees. *Trees* **12**: 438–445.

Kleinschmit JRG, Bacilieri R, Kremer A, Roloff A. 1995. Comparison of morphological and genetic traits of pedunculate oak (*Q. robur* L.) and sessile oak (*Q. petraea* (Matt.) Liebl.). *Silvae Genetica* **44**: 256–268.

Kremer A, Dupouey JL, Deans JD, Cottrell J, Csaikl U, Finkeldey R, Espinel S, Jensen J, Kleinschmit J, Van Dam B, Ducousso A, Forrest I, de Heredia UL, Lowe AJ, Tutkova M, Munro RC, Steinhoff S, Badeau V. 2002. Leaf morphological differentiation between *Quercus robur* and *Quercus petraea* is stable across western European mixed stands. *Annals of Forest Science* **59**: 777–787.

Le Corre V, Dumolin-Lapègue S, Kremer A. 1997. Genetic variation at allozyme and RAPD loci in sessile oak *Quercus petraea* (Matt.) Liebl.: the role of history and geography. *Molecular Ecology* **6**: 519–529.

Mantel N. 1967. The detection of disease clustering and a generalized regression approach. *Cancer Research* **27**: 209–220.

Mayr E. 1963. *Populations, species, and evolution.* Cambridge, Massachusetts: The Belknap Press of Harvard University Press.

Michaud H, Toumi L, Lumaret R, Li TX, Romane F, Di Giusto F. 1995. Effect of geographical discontinuity on genetic variation in *Quercus ilex* L. (holm oak). Evidence from enzyme polymorphism. *Heredity* **74**: 590–606.

Mosiño-Alemán PA, García E. 1974. The climate of Mexico. In: Bryson RA, Hare FK, eds. *World survey of climatology Vol. 11. The climates of North America.* Amsterdam: Elsevier Scientific Publishing, 345–404.

Niinemets U, Afas NA, Cescatti A, Pellis A, Ceulmans R. 2004. Petiole length and biomass investment in support modify light-interception efficiency in dense poplar plantations. *Tree Physiology* **24**: 141–154.

Oyama K. 1993. Geographic differentiation among populations of *Arabis serrata* Fr. & Sav. (Brassicaceae). *Journal of Plant Research* **106**: 15–24.

Pearcy RW, Yang W. 1998. The functional morphology of light capture and carbon gain in the Redwood forest understorey plant *Adenocaulon bicolor* Hook. *Functional Ecology* **12**: 543–552.

Rushton BS. 1993. Natural hybridization within the genus *Quercus*. *Annales de Sciences Forestières* **50**: 73–91.

SAS Institute. 1995. *JMP. Statistics and graphics guide, Version 3.1.* Carey, North Carolina: SAS Institute Inc.

Shaver GR, Fecher N, Chapin FS III. 1986. Growth and flowering in *Eriophorum vaginatum*: annual and latitudinal variation. *Ecology* **67**: 1542–1535.

Sokal RR, Crovello TJ, Unnasch RS. 1986. Geographic variation of vegetative characters of *Populus deltoides*. *Systematic Botany* **11**: 141–155.

Sokal RR, Rohlf FJ. 1995. *Biometry*, 3rd edn. New York: Freeman.

Toumi L, Lumaret R. 1998. Allozyme variation in cork oak (*Quercus suber* L.): the role of phylogeography and genetic introgression by other Mediterranean oak species and human activities. *Theoretical and Applied Genetics* **97**: 647–656.

Tucker JM. 1961. Studies in the *Quercus undulata* complex. I. A preliminary statement. *American Journal of Botany* **48**: 202–208.

Valencia S. 1994. Contribución a la delimitación taxonómica de tres especies del género *Quercus* subgénero *Erythrobalanus*. Tesis de Maestría, Facultad de Ciencias, Universidad Nacional Autónoma de México, México D. F., México.

Wyatt R, Antonovics J. 1981. Butterfly weed revisited: spatial and temporal patterns of leaf shape variation in *Asclepias tuberosa*. *Evolution* **35**: 529–542.

Zanetto A, Kremer A. 1995. Geographical structure of gene diversity in *Quercus petraea* (Matt.) Liebl. I. Monolocus patterns of variation. *Heredity* **75**: 506–517.

Figure captions

Figure 1. Map illustrating the geographical distribution of the collection sites for populations of *Q. affinis–Q. laurina*. Numbers in brackets indicate the position of the populations in the macrogeographic gradient. See text for details.

Figure 2A–D. Mean and standard error for nine foliar characters in 16 populations of *Quercus affinis–Q. laurina* from Mexico. A, Total leaf length (TL) (cm); B, Lamina length (LL) (cm); C, Petiole length (PL) (cm); D, Maximal width (MW) (cm); E, Distance from base of the leaf to point of maximal width (PMW) (cm); F, Teeth number (TN); G, MW/LL; H, PL/TL; I, PMW/LL.

Figure 3. UPGMA phenogram of 16 populations of *Quercus affins–Q. laurina* based on Euclidean distances of foliar morphology. See text for details.

Figure 4. Coefficients of variation of nine foliar morphological traits in 16 populations of the *Q. affinis–Q. laurina* complex.

Table1. Partitioning of variance by hierarchical level for nine foliar morphological traits in the *Q. affinis–Q. laurina* complex. Variance components and significance levels were determined with a nested ANOVA.

Trait	Level			
	Group	Population [Group]	Individual [Population, Group]	Leaves [Group, Population, Individual]
Total length (TL)	0.03*	0.09***	0.54***	0.34
Lamina length (LL)	0.03*	0.08***	0.54***	0.35
Petiole length (PL)	0.04*	0.14***	0.48***	0.34
Maximal width (MW)	0.05*	0.12***	0.54***	0.29
Distance from base to point of maximal width (PMW)	0.09**	0.05***	0.44***	0.42
Teeth number (TN)	0.27***	0.09***	0.47***	0.17
MW/LL	0.03 n.s.	0.21***	0.39***	0.37
PL/TL	0.04*	0.15***	0.41***	0.40
PMW/LL	0.10**	0.06***	0.28***	0.56

* $P < 0.05$; ** $P < 0.001$; *** $P < 0.0001$; n.s. = non significant

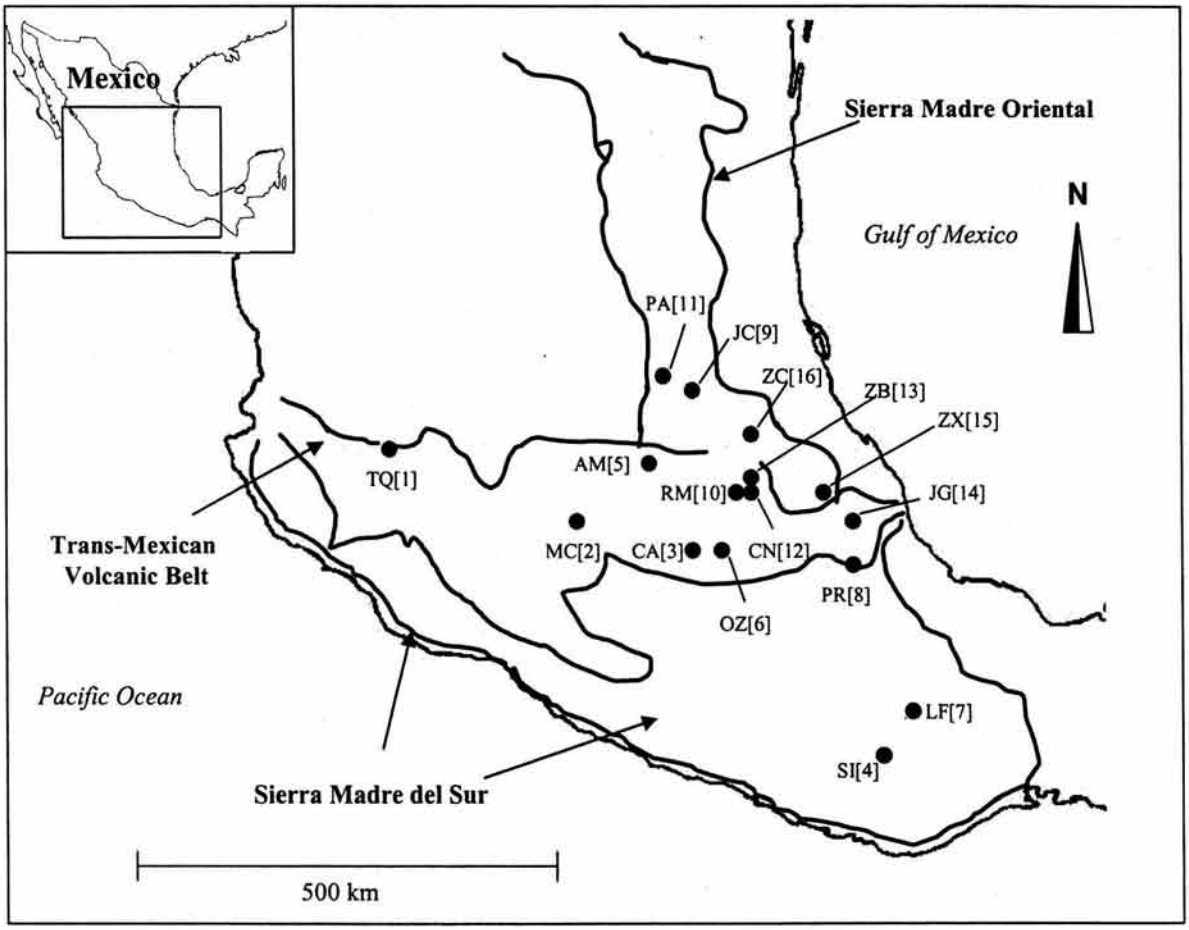

Mexico
Sierra Madre Oriental
N
Gulf of Mexico
PA[11]
JC[9]
ZC[16]
ZB[13]
ZX[15]
TQ[1]
AM[5]
RM[10]
JG[14]
Trans-Mexican
Volcanic Belt
MC[2]
CA[3]
CN[12]
PR[8]
OZ[6]
Pacific Ocean
LF[7]
SI[4]
Sierra Madre del Sur
500 km

Figure 1

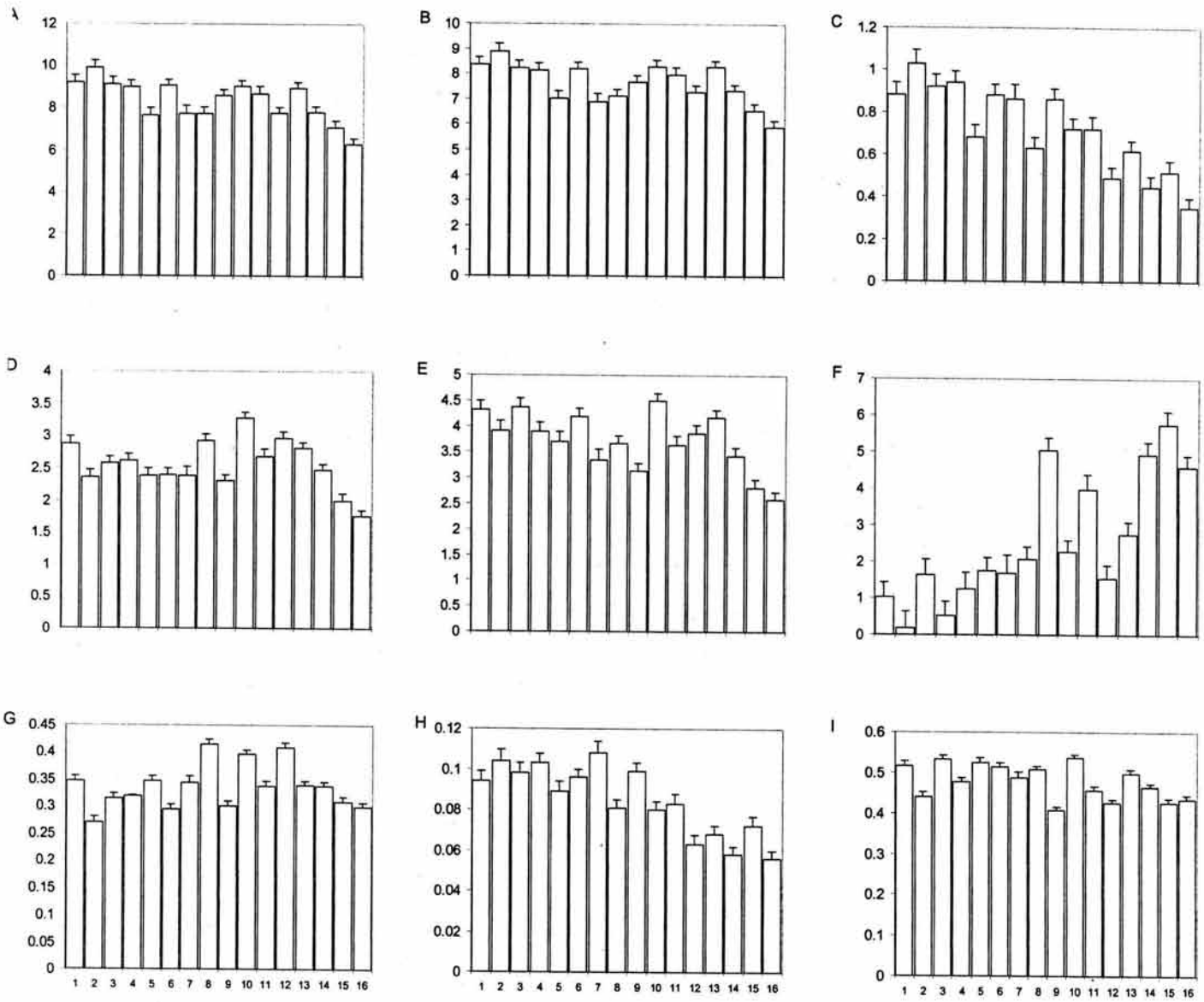


Figure 2

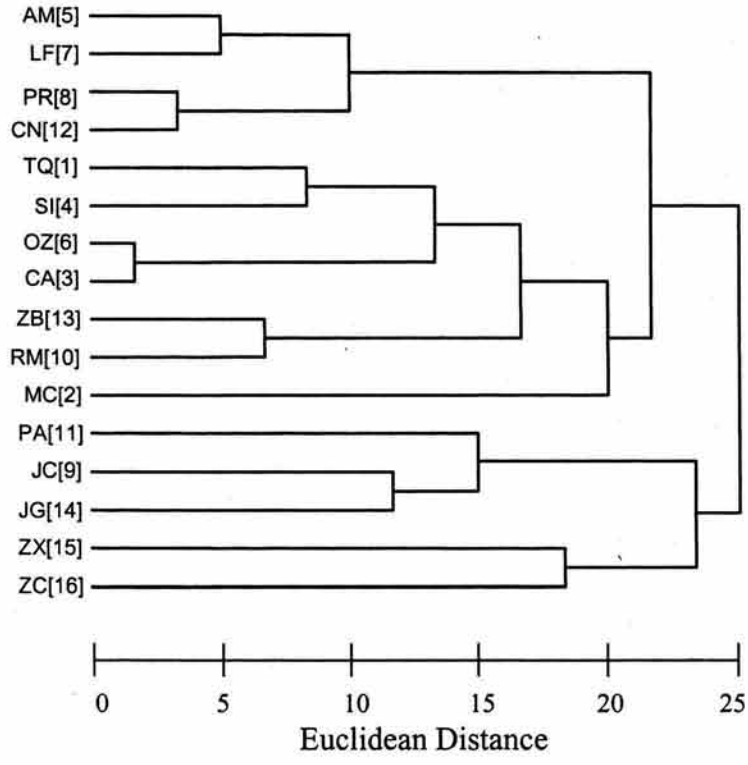

AM[5]
LF[7]
PR[8]
CN[12]
TQ[1]
SI[4]
OZ[6]
CA[3]
ZB[13]
RM[10]
MC[2]
PA[11]
JC[9]
JG[14]
ZX[15]
ZC[16]
0
5
10
15
20
25
Euclidean Distance

Figure 3

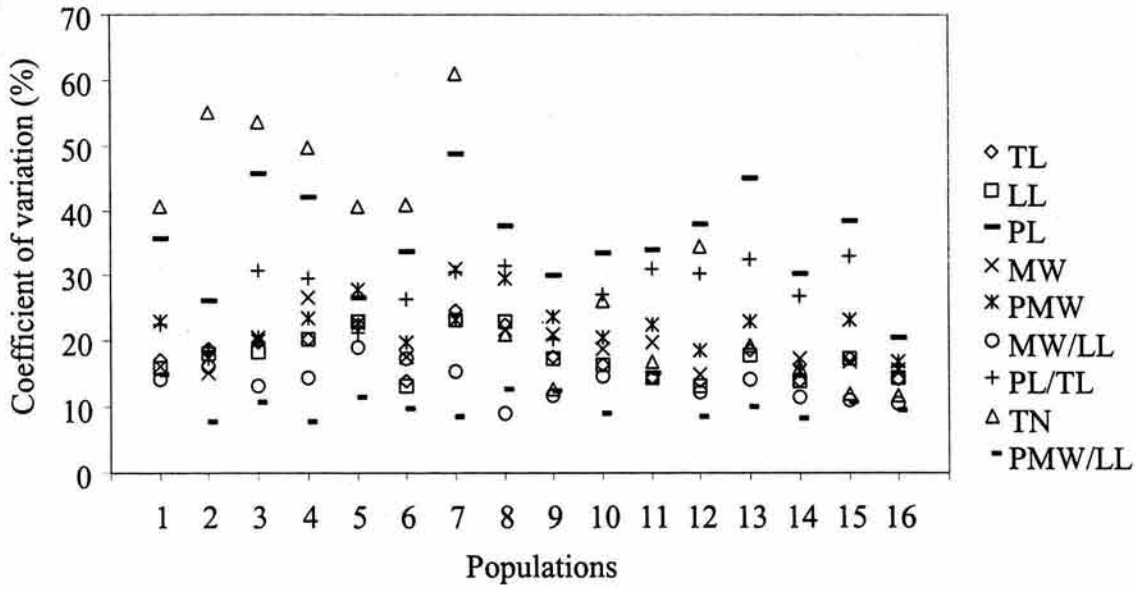

Coefficient of variation (%)
70
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
Populations
TL
LL
PL
MW
PMW
MW/LL
PL/TL
TN
PMW/LL

Figure 4

V.

Antonio González-Rodríguez, Dulce M. Arias, and Ken Oyama

Genetic variation and differentiation of populations within the *Quercus affinis–Q. laurina* (Fagaceae) complex analyzed with RAPD markers

Canadian Journal of Botany, *en prensa.*

Genetic variation and differentiation of populations within the *Quercus affinis-Q. laurina* (Fagaceae) complex analyzed with RAPD markers

Antonio González-Rodríguez, Dulce M. Arias, and Ken Oyama

Addresses of the authors:

A. González-Rodríguez, and Ken Oyama. Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México, Antigua Carretera a Pátzcuaro No. 8701, Col. Ex Hacienda de San José de la Huerta, Morelia, 58190, Michoacán, México. E-mail addresses: agrodrig@oikos.unam.mx; akoyama@oikos.unam.mx

D. M. Arias. Centro de Educación Ambiental e Investigación Sierra de Huautla, Universidad Autónoma del Estado de Morelos, Av. Universidad 1001, Col. Chamilpa, Cuernavaca, 62210, Morelos, México. E-mail: dulce@buzon.uaem.mx

Corresponding author:

Antonio González-Rodríguez

Centro de Investigaciones en Ecosistemas, UNAM

Antigua Carretera a Pátzcuaro No. 8701

Col. Ex–Hacienda de San José de la Huerta

Morelia, 58190 Michoacán

MÉXICO

Fax: +52 55 56 23 27 19

E-mail: agrodrig@oikos.unam.mx

Abstract: The population genetics of two hybridizing Mexican red oaks, *Quercus affinis* and *Q. laurina*, was investigated with 54 randomly amplified polymorphic DNA (RAPD) markers scored in 415 individuals from 16 populations representing the distribution area of the two species and a probable secondary hybrid zone. Genetic relationships among populations, depicted in a unweighted pair group method with arithmetic averaging (UPGMA) dendrogram, were largely incongruent with the morphological classification of populations as *Quercus affinis*-like or *Q. laurina*-like obtained in previous studies. In contrast, the two main population clusters in the UPGMA dendrogram corresponded to the location of populations in two distinct geographical areas, 'south-western' and 'north-eastern'. A Mantel test confirmed a significant association between geographic and genetic distances among populations. Analyses of molecular variance (AMOVA) indicated that most genetic variation is contained within populations (84%), while 10.5% ($P < 0.0001$) is among populations, and 5.1% ($P = 0.007$) is between the two morphological groups. Differentiation between the 'south-western' and 'north-eastern' geographical groups (as recognized by the UPGMA), was 7.8% ($P < 0.0001$). The incongruence between genetic and phenotypic patterns suggests that introgression of neutral markers has been considerable between the two species in the hybrid zone, while morphological differentiation has remained comparatively stable.

Key words: hybridization, population genetics, *Quercus*, RAPD markers

Introduction

Population genetics studies of species in genus *Quercus* L. (the oaks) using neutral markers of nuclear origin have shown that, as with other highly outcrossing, wind pollinated and long-lived forest tree species, oaks generally have high levels of within-population genetic variation and low differentiation among populations (Hokanson et al. 1993; Michaud et al. 1995; Samuel et al. 1995; Zanetto and Kremer 1995; Le Corre et al. 1997; 1998; Montalvo et al 1997; Mayes et al. 1998; Toumi and Lumaret 1998, 2001; Gömöry et al. 2001). Also, simultaneous genetic surveys of related oak species have usually revealed very low levels of interspecific differentiation (Guttman and Weigt 1989; Hokanson et al. 1993; Samuel et al. 1995; Bodénès et al. 1997a; Howard et al. 1997; Bruschi et al. 2000; Gömöry et al. 2001; Coart et al. 2002; Dodd and Kashani 2003). Sharing of a large proportion of neutral genetic variation among oak species has most often been explained by a probable high frequency of genetic exchange between the involved taxa through hybridization and introgression, although other mechanisms may also be significant, such as the incomplete sorting of polymorphisms during relatively rapid and recent speciation processes (Bruschi et al. 2000).

Significant geographical patterns in the distribution of intra- and interspecific genetic variation have been detected in several range-wide studies of oak species. Variation in within-population diversity levels among regions, and longitudinal clinal frequency changes in some markers have been described, patterns which seemingly reflect the postglacial recolonization dynamics of oak populations, and isolation by distance (Zanetto and Kremer 1995; Le Corre et al. 1997; 1998). In some species there is a correspondence between geographical discontinuities and the patterns of genetic subdivision among

population groups (Michaud et al. 1995; Toumi and Lumaret 1998). The extent of interspecific genetic differentiation between sympatric populations of closely related, hybridizing species can also be heterogeneous among geographic locations (Bodénès et al. 1997b). The inference of the processes underlying such patterns is essential to understand the evolutionary biology of oak species. For example, it may be relevant to distinguish if genetic subdivisions among populations reflect current gene flow as determined by distance and other geographic factors, or are due to the persistence of nonequilibrium patterns of differentiation resulting from the action of historical causes (Le Corre et al. 1997).

Mexico has a very high number of *Quercus* species, ranging between 135 and 150 (Nixon 1993). The considerable proportion of endemics (approximately 86) indicates that secondary radiation of the genus occurred within this area. Genetic diversity and structure of populations in oaks has been almost exclusively investigated in North America or Europe, although initial studies of Mexican red oak complexes have been recently performed using morphological and molecular characters (González-Rodríguez et al. 2004a, b; Tovar-Sánchez and Oyama 2004). For this study, we conducted a survey of genetic diversity in populations of a complex formed by two Mexican red oaks, *Quercus affinis* Scheidw. and *Q. laurina* Humb. & Bonpl. This complex was first described from a taxonomic standpoint (Valencia 1994). A subsequent analysis assessed the patterns of variation of nine semi-diagnostic RAPD (Randomly Amplified Polymorphic DNA) markers and several foliar traits in 16 localities across a macrogeographic gradient, including populations from a region of overlap in distribution in which morphological intergradation occurs, and phenotypically 'pure' representative populations of each species situated outside of this area (González-Rodríguez et al. 2004a). A shift in the genetic composition of populations from one species to the other along the macrogeographic

gradient was observed, with genetically intermediate populations situated in the area of overlap (González-Rodríguez et al. 2004a). Foliar variation was also continuous between the two species, but only a comparatively small fraction of the individuals was intermediate, and a particular morphology predominated in most populations (i.e. *Q. affinis*-like or *Q. laurina*-like individuals). The observed patterns were interpreted as consistent with the originally proposed hypothesis (Valencia 1994) of an origin for the area of intergradation through secondary contact between the two oak species and subsequent hybridization and introgression.

The previously described results have provided insight into the probable origin and structure of the hybrid zone between *Q. affinis* and *Q. laurina*. In this study, we used a larger, random sample of molecular markers constituted by 54 RAPD bands ('loci') to estimate genetic diversity in these oaks and to analyze genetic differentiation among populations, and between morphologically defined groups of populations representing the two species (i.e. *Q. affinis*-like and *Q. laurina*-like populations). We also examined the pattern of genetic relationships among populations and determined how this pattern is related to the morphological classification of populations and/or to the geographic distances separating them.

Materials and methods

Plant material

Sixteen populations of the *Q. affinis-Q. laurina* complex were sampled throughout the geographic distribution of both species. Figure 1 illustrates the location of the sampled populations and the three main mountain ranges where oaks of this complex are distributed.

Morphologically representative populations of *Q. affinis* are distributed along the Sierra Madre Oriental, while morphologically representative populations of *Q. laurina* occur in the Sierra Madre del Sur and the western region of the Trans-Mexican Volcanic Belt (Valencia 1994). The area of overlap is situated in the eastern region of the volcanic belt. At each site, young, intact leaves were collected from randomly chosen adult trees separated from each other by at least 100 m, to avoid sampling related individuals. The leaves were immediately frozen in liquid nitrogen and then transferred to a – 80 °C freezer, until further analyses were performed. Sample size per location varied between 20 and 30 trees (Table 1). Each population was classified as '*Q. affinis*-like' or '*Q. laurina*-like' according to the predominant morphological type in the locality, determined by morphological analyses (González-Rodríguez et al. 2004a; González-Rodríguez and Oyama in press).

DNA extraction and PCR conditions

Approximately 100 mg of frozen leaf tissue were used for DNA extraction following the protocol of Lefort and Douglas (1999) with only minor modifications. The concentration of DNA in solution was estimated using a DNA fluorometer (Hoefer Pharmacia Biotech., San Francisco, Calif.).

All amplification reactions contained 10 ng of template DNA, 1 X PCR buffer, 2 mM $MgCl_2$, 0.1 mM each dNTP (Fermentas, Hanover, Md.), 0.2 μM of a single 10-mer oligonucleotide (Operon Technologies, Alameda, Calif.), 5 μg bovine serum albumin (BSA), and 1 U *Taq* DNA polymerase (Invitrogen, San Diego, Calif.), in a total volume of 25 μL. The thermal cycling program was run on a MJ Research (Waterton Mass.) thermal cycler. The program was as follows: 1 cycle of 2 min at 94 °C; followed by 45 cycles of 1

min at 94 °C, 1 min at 36 °C and 2 min at 72 °C. A final extension step at 72 °C for 7 min was included.

PCR products were electrophoresed at 200 V for 2 h in 1.5% agarose gels with 0.5 X TBE buffer, and photographed under UV light after staining with ethidium bromide. A 123 –bp ladder (Invitrogen, San Diego, Calif.) was included in all gels as reference to estimate the size of the amplified fragments.

To assess the reproducibility of PCR products, first the DNA from 38 individuals was amplified independently three times with each primer. A total of 54 bands (loci) appeared consistently in the three assays, and were later scored in all individuals. These 54 fragments included the nine semi-diagnostic markers (produced by seven primers, OP-A05, OP-A07, OP-A08, OP-A10, OPB-17, OP-C09, and OP-I07) of the two species used in the previous study (González-Rodríguez et al. 2004a), plus other 45 bands amplified simultaneously by the same seven primers, and two additional primers (OP-D10, OP-G19). Only a few of the fragments showed an association with chloroplast DNA variation assessed in the same individuals (González-Rodríguez et al. 2004b), and the association was in all cases very weak. Thus, all the fragments were assumed to be of nuclear origin.

Data analysis

Amplified fragments were recorded as absent (0) or present (1) in all individuals. For each fragment, these two possible states were considered as the molecular phenotypes resulting from the expression of two alleles at a single locus, one dominant and one recessive, the dominant being the one that determines the presence of the band. Although the frequency of the two alleles at each locus can be inferred from the frequency of presence and absence of the band (e.g. Lynch and Milligan 1994), analyses that do not rely

on knowing these frequencies were preferred in this study, to avoid the uncertain assumption of Hardy-Weinberg equilibrium. Molecular diversity within each population was assessed by calculating the percentage of polymorphic fragments (%P), and with the Shannon diversity index (S_S), using POPGENE ver. 1.31 (Yeh et al. 1999). The Shannon index was also calculated at the level of the total population (S_T), and from this value and the average of S_S over all populations, the proportion of the total genetic variation found among populations was calculated as 1 – (average S_S / S_T) (Martín and Hernández-Bermejo 2000). In order to identify possible differences in population levels of genetic diversity between morphological groups and geographic areas, values of %P and S_S were compared with a Wilcoxon test (Sokal and Rohlf 1995).

To determine genetic relationships among populations, a matrix of pairwise Manhattan distances (this distance is based on the frequency of absence or presence of bands and does not assume allelic frequencies, Swofford et al. 1996) was generated with the RAPDDIST program (Black 1995), and a dendrogram based on this matrix was constructed with the NEIGHBOR procedure in the PHYLIP3.5 C package (Felsenstein 1993), using the unweighted pair group method with arithmetic averaging (UPGMA).

The influence of spatial separation on the degree of differentiation among populations was investigated with a Mantel permutation test (Mantel 1967). Because in our case a spurious correlation between geographic and genetic distances could potentially arise from the fact that populations from the same morphological group are expected to be genetically more similar and also tend to occur in similar geographical areas, the partial version of the Mantel test was used (Smouse et al. 1986). In this version, the goal is to test the correlation between two matrices while controlling the effect of a third matrix, to remove possibly confounding effects (Smouse et al. 1986). This test was performed

between the matrices of pairwise Manhattan distances and the corresponding geographical distances, controlling the effect of a third matrix indicating the morphological classification of the populations. The elements of this third matrix were 1 for pairs of populations belonging to the same morphological group, or 2 for pairs of populations belonging to interspecific groups.

Analysis of molecular variance (AMOVA) was used to investigate the partitioning of genetic variation among groups of populations, among populations within groups and within populations. The significances of the different variance components were estimated from distributions generated from 10,000 random permutations. These analyses were carried out using ARLEQUIN version 2000 (Schneider et al. 2000).

Results

Fifty-one (94.44%) out of 54 amplified fragments were polymorphic within or between populations (Table 1). In single populations, the percentage of polymorphic fragments (%*P*) ranged from 42.59 to 64.81 with an average of 54.98. The Shannon diversity index within populations (S_S) varied between 0.195 and 0.293, with an average (SD) of 0.244 (0.031). The overall value of the Shannon index (S_T), when considering the whole sample as a single population, was 0.299 (Table 1). From these values, the proportion of the total genetic variation residing among populations [1 – (average S_S / S_T)] was 0.184.

The UPGMA dendrogram generated from the matrix of pairwise Manhattan distances is shown in Fig. 2. Two main groups of populations were defined in this dendrogram, which clearly corresponded to two distinct geographical areas; one group was

formed by populations situated in the Sierra Madre del Sur and the western region of the volcanic belt ('south-western' group), while the other clustered populations from the eastern region of the volcanic belt and the Sierra Madre Oriental ('north-eastern' group). Within these two main groups there was also some tendency for geographically proximate populations to cluster together (Figs. 1 and 2). This pattern was confirmed by the partial Mantel test, which detected a significant correlation between the matrix of pairwise genetic distances and the matrix of corresponding geographical distances, even when controlling for the taxonomic grouping of populations ($r = 0.341$; $P = 0.01$). In contrast, the genetic relationships among populations, as depicted in the UPGMA dendrogram, were largely non congruent with their morphological classification. For example, the 'south-western' group included seven *Q. laurina*-like populations and one *Q. affinis*-like population, and in the 'north-eastern' cluster there were four populations from each morphological group (Fig. 2, Table 1). Nevertheless, it might be significant that three of the four *Q. laurina*-like populations included into the 'north-eastern' group (Real del Monte, Cerro Navajas and Puerto Aire) clustered together and separated from the other five populations of this group (Fig. 2).

The AMOVA performed over all 16 populations for partitioning of RAPD variation between the two main morphological groups ('*Q. affinis*-like' and '*Q. laurina*-like' groups), among populations within these two groups, and among individuals within populations, revealed that most of the variation (84.4%) is found within populations. Differences between the two population groups (5.07%; $P = 0.007$), and among populations within groups (10.49%; $P < 0.0001$), accounted for comparatively smaller amounts of the total variance, although both effects were significant. The overall differentiation among populations (Φ_{ST}) was 0.16. A second AMOVA analysis, conducted for populations

arranged according to the two main groups defined in the UPGMA dendrogram which are concordant with geographic location (i.e. 'south-western' and 'north-eastern' population groups) indicated that 7.85% of the variation was distributed between the two groups ($P < 0.0001$), that 8.85% of the variation was among populations within groups ($P < 0.0001$), and that 83.3% was among individuals within populations. Within the 'north-eastern' group, the differentiation between the cluster formed by the three populations Real del Monte, Puerto Aire and Cerro navajas, and the other five populations was 2.88% ($P = 0.017$).

A one-tailed Wilcoxon two-sample test was used to compare levels of within-population genetic diversity between the '*Q. affinis*-like' and '*Q. laurina*-like' groups of populations, as well as between the 'south-western' and 'north-eastern' groups. The comparisons were significant for both the proportion of polymorphic loci ($Z = -2.635$, $P = 0.008$) and the Shannon index ($Z = -2.626$, $P = 0.009$) in the case of the geographic groups, being the populations of the 'north-eastern' group the ones characterized by higher genetic diversity (Table 1). In contrast, genetic diversity levels are equivalent between the morphological groups ($Z = -0.114$, $P = 0.913$ for the proportion of polymorphic loci; $Z = -0.397$, $P = 0.743$ for the Shannon index).

Discussion

The distribution patterns of putatively neutral nuclear genetic variation within and between species in the *Quercus affinis-Q. laurina* complex are similar to those described for other oak species. A large proportion of the total genetic variation is found within populations of these two Mexican red oaks. Although most of the previous studies reporting

this result in oak species as well as in other trees with similar life history traits (Hamrick and Godt 1996) have been conducted using allozymes, the fewer analyses using RAPD markers have found that both methods usually provide comparable values of population subdivision (Le Corre et al. 1997; Nybom and Bartish 2000). The two estimates of overall genetic differentiation among populations of the *Q. affinis–Q. laurina* complex obtained in this study with different analytical methods were [1 – (average S_S / S_T)] = 0.18 (using the Shannon diversity index), and Φ_{ST} = 0.16 (from AMOVA). The AMOVA further revealed that this Φ_{ST} can be significantly partitioned into a component of differentiation between *Q. affinis*-like and *Q. laurina*-like populations (0.05), and a component of average differentiation among populations within these two groups (0.105). Although it is necessary to be careful when comparing results across studies, because differences in sampling strategies, geographic scale considered, markers employed and analytical procedures are customary, the values of interspecific differentiation and population differentiation within *Q. affinis* and *Q. laurina* obtained in this study seem to fit within the general literature on oak population genetics. For example, the overall interspecific differentiation among the four California species of red oak estimated with AMOVA from AFLP data (with dominant expression as RAPD markers), was 0.087 (Dodd and Kashani 2003). Coart et al. (2002) estimated allelic frequencies at AFLP loci and obtained a F_{ST} value of 0.073 for the differentiation between *Q. robur* and *Q. petraea*. In another study on these two species, which used codominant allozyme markers and considered a very different geographic area, an interespecific F_{ST} of 0.021 was estimated (Gömöry et al. 2001). Intraspecific population differentiation in oak species has been estimated to be on average 0.11 (Hamrick et al. 1992, based on data from 28 studies) and 0.07 (Kremer and Petit 1993, based on data from 25 studies), although it should be noticed that these values come almost exclusively from

allozyme markers. Variation around these means is ample among studies, from population differentiation as low as 0.018 in *Q. chrysolepis* (Montalvo et al. 1997) and 0.02–0.03 in *Q. petraea* and *Q. robur* (Zanetto and Kremer 1995; Le Corre et al. 1997; Coart et al. 2002), to the remarkably high values recently reported for three of the four species of California red oak, *Q. wislizeni* (0.18), *Q. kelloggii* (0.34) and *Q. agrifolia* (0.24), although the authors of this study cautioned about the reliability of the estimates for the last two species because of limited sampling (Dodd and Kashani 2003).

Our results also suggest that the subdivision of genetic variation in the *Q. affinis–Q. laurina* complex may be more strongly related to geography than to the morphologically defined classification of populations. In a UPGMA dendrogram based on Manhattan distances (Fig. 2), the genetic relationships among our sampled populations appeared to be more associated with geographic location than to taxonomic grouping. This correspondence between geography and the distribution of genetic variation was further indicated by the partial Mantel test, which detected a significant correlation between geographic and genetic distances, independently of the morphological grouping of populations. According to a second AMOVA, the degree of differentiation between the two main population groups defined in the dendrogram (i.e. 'south-western' and 'north- eastern' population groups) was 0.078. This value is higher than the amount of genetic variation that differentiated the morphologically defined *Q. affinis*-like and *Q. laurina*-like population groups (0.05), although this comparison should be taken with caution because the two values are not completely independent since there is some degree of overlap between the geographic and the morphological groups analyzed in the two AMOVAs. Another observation supporting that the 'south-western' and 'north-eastern' groups represent an actual genetic subdivision between populations was the detection of significant differences between the two groups in

within population diversity levels, as measured by the proportion of polymorphic loci (%*P*) and the Shannon diversity index (S_S). In contrast, no differences in genetic variation were found between the *Q. affinis*-like and *Q. laurina*-like groups.

On the other hand, there is also some evidence of the taxonomic effect in the clustering pattern in the UPGMA dendrogram. This is particularly illustrated by the three *Q. laurina*-like populations (Real del Monte, Cerro Navajas and Puerto Aire) that belong to the 'north-eastern' group, but formed a clearly separated sub-cluster, indicating genetic affinity among them and a degree of differentiation from the other five populations within this group.

In conclusion, although the two factors are difficult to separate in some analyses, it appears that the distribution of nuclear genetic variation among populations in the *Q. affinis-Q. laurina* complex is firstly a function of geography, and in second place, but also significantly, a reflection of the morphologically-based taxonomic subdivision of populations. This result implies gene flow and isolation by distance as predominant forces shaping the population structure of neutral, nuclear genetic variation within this complex. According to previous work (Valencia 1994; González-Rodríguez et al. 2004a), a hybrid zone between previously diverged *Q. affinis* and *Q. laurina* putatively formed after secondary contact in the eastern region of the volcanic belt. As was originally proposed by Valencia (1994), *Q. laurina* populations probably migrated along the volcanic belt in an eastward direction, and established within the area already occupied by populations of *Q. affinis*. After this happened, the weak reproductive barriers between the two oak species permitted interspecific gene flow that resulted in the bi-directional genetic introgression of both species in this area. The increased levels of genetic diversity in populations of the 'north-eastern' group are consistent with this scenario. During the formation of the hybrid

zone, geographical distances, according to a pattern of isolation-by-distance, largely influenced genetic relationships among populations. However, the existence of the weaker taxonomic effect suggests that the amount of interspecific genetic exchange may be constrained to some extent in comparison to intraspecific gene flow, or that time since secondary contact has been insufficient to erode genetic differentiation completely and homogenize populations.

The incongruence between genetic and morphological relationships among populations suggests that interspecific genetic exchange has affected morphological differentiation between *Q. affinis* and *Q. laurina* in a lesser extent. In contrast to what was observed with genetic distances, morphological similarities among populations did not show any association with the geographical distances separating them (González-Rodríguez and Oyama in press). Although there are several possible explanations for this incongruence between molecular and phenotypic patterns (Rieseberg and Ellstrand 1993), it seems likely that if foliar morphology has experienced restricted introgression despite interspecific gene flow and exchange of neutral markers, it is probably because selective factors are operating against the recombination of genomic regions controlling adaptively relevant traits, indicating a 'semipermeable' species barrier (Arnold 1992).

Acknowledgements

The authors thank Euler Pedraza, Rosalinda Tapia, and Pablo Cuevas-Reyes for help with field collections, and Nidia Pérez-Nasser, Rolando Ramírez and Esaú Leyva for laboratory assistance. The taxonomic advice of Susana Valencia was essential for this work. Luis E. Eguiarte and Miguel Lara contributed with useful suggestions throughout the

development of the study. We also appreciate the constructive comments by two anonymous reviewers. Funding from DGAPA, UNAM (grant IN229803 to K. O.) and CONACyT (grant 38550-V to K. O.) is gratefully acknowledged.

References

Arnold, M.L. 1992. Natural hybridization as an evolutionary process. Ann. Rev. Ecol. Syst. **23**: 237-261.

Black, W.C. 1995. FORTRAN programs for the analysis of RAPD PCR markers in populations. Colorado State University, Ft. Collins.

Bodénès, C., Joandet, S., Laigret, F., and Kremer, A. 1997a. Detection of genomic regions differentiating two closely related oak species *Quercus petraea* (Matt.) Liebl. and *Quercus robur* L. Heredity, **78**: 433–441.

Bodénès, C., Labbé, T., Pradère, S., and Kremer, A. 1997b. General vs. local differentiation between two closely related white oak species. Mol. Ecol. **6**: 713–724.

Bruschi, P., Vendramin, G.G., Bussotti, F., and Grossoni, P. 2000. Morphological and molecular differentiation between *Quercus petraea* (Matt.) Liebl. and *Quercus pubescens* Willd. (Fagaceae) in Northern and Central Italy. Ann. Bot. **85**: 325–333.

Coart, E., Lamote, V., De Loose, M., Van Bockstaele, E., Lootens, P., and Roldán-Ruiz, I. 2002. AFLP markers demonstrate local genetic differentiation between two indigenous oak species [*Quercus robur* L. and *Quercus petraea* (Matt.) Liebl.] in Flemish populations. Theor. Appl. Genet. **105**: 431–439.

Dodd, R.S., and Kashani, N. 2003. Molecular differentiation and diversity among the California red oaks (Fagaceae; *Quercus* section Lobatae). Theor. Appl. Genet. **107**: 884–892.

Felsenstein, J. 1993. PHYLIP (Phylogeny Inference Package) v. 3.5c. Department of Genetics, The University of Washington. Seattle, WA.

Gömöry, D., Yakovlev, I., Zhelev, P., Jedináková, J., and Paule, L. 2001. Genetic differentiation of oak populations within the *Quercus robur* / *Quercus petraea* complex in Central and Eastern Europe. Heredity, **86**: 557–563.

González-Rodríguez, A., and Oyama, K. In press. Leaf morphometric variation in *Quercus affinis* and *Q. laurina* (Fagaceae), two hybridizing Mexican red oaks. Bot. J. Linn. Soc. In press.

González-Rodríguez, A., Arias, D. M., Valencia, S., and Oyama, K. 2004a. Morphological and RAPD analysis of hybridization between *Quercus affinis* and *Q. laurina* (Fagaceae), two Mexican red oaks. Am. J. Bot. **91**: 401–409.

González-Rodríguez, A., Bain, J.F., Golden, J.L., and Oyama, K. 2004b. Chloroplast DNA variation in the *Quercus affinis–Q. laurina* complex in Mexico: geographic structure and associations with nuclear and morphological variation. Mol. Ecol. **13**: 3467–3476.

Guttman, S.I., and Weigt, L.A. 1989. Electrophoretic evidence of relationships among *Quercus* (oaks) of eastern Noth America. Can J. Bot. **67**: 339–351.

Hamrick, J.L., Godt, M.J.W., and Sherman-Broyles, S.L. 1992. Factors influencing levels of genetic diversity in woody plant species. *In* Population genetics of forest trees. *Edited by* W.T. Adams, S.H. Strauss, D.L. Copes, and A.R. Griffin. Kluwer Academic Publishers, Dordrecht, The Netherlands. pp. 95–124.

Hokanson, S.C., Isebrands, J.G., Jensen, R.J., and Hancock, J.F. 1993. Isozyme variation in oaks of the Apostle Islands in Wisconsin: genetic structure and levels of inbreeding in *Quercus rubra* and *Q. ellipsoidalis* (Fagaceae). Am. J. Bot. **80**: 1349–1357.

Howard, D.J., Preszler, R.W., Williams, J., Fenchel, S., and Boecklen, W.J. 1997. How discrete are oak species? Insights from a hybrid zone between *Quercus grisea* and *Quercus gambelli*. Evolution, **51**: 747–755.

Kremer, A., and Petit, R.J. 1993. Genetic diversity in natural populations of oak species. Ann. For. Sci. **50** (Suppl. 1): 186s–202s.

Le Corre, V., Dumolin-Lapègue, S., and Kremer, A. 1997. Genetic variation at allozyme and RAPD loci in sessile oak *Quercus petraea* (Matt.) Liebl.: the role of history and geography. Mol. Ecol. **6**: 519–529.

Le Corre, V., Roussel, G., Zanetto, A., and Kremer, A. 1998. Geographical structure of gene diversity in *Quercus petraea* (Matt.) Liebl. III. Patterns of variation identified by geostatistical analyses. Heredity, **80**: 464–473.

Lefort, F., and Douglas, G.C. 1999. An efficient micro-method of DNA isolation from mature leaves of four hardwood tree species *Acer*, *Fraxinus*, *Prunus* and *Quercus*. Ann. For. Sci. **56**: 259–263.

Lynch, M., and Milligan, B.G. 1994. Analysis of population genetic structure with RAPD markers. Mol. Ecol. **3**: 91–99.

Mantel, N.A. 1967. The detection of disease clustering and a generalized regression approach. Can. Res. **27**: 209–220.

Martín, J.P., Hernández-Bermejo, J.E. 2000. Genetic variation in the endemic and endangered *Rosmarinus tomentosus* Huber-Morath & Maire (Labiatae) using RAPD markers. Heredity **85**: 434–443.

Mayes, S.G., McGinley, M.A., and Werth, C.R. 1998. Clonal population structure and genetic variation in sand-shinnery oak, *Quercus havardii* (Fagaceae). Am. J. Bot. **85**: 1609–1617.

Michaud, H., Toumi, L., Lumaret, R., Li, T.X., Romane, F., and Di Giusto, F. 1995. Effect of geographical discontinuity on genetic variation in *Quercus ilex* L. (holm oak). Evidence from enzyme polymorphism. Heredity, **74**: 590–606.

Montalvo, A.M., Conard, S.G., Conkle, M.T., and Hodgskiss P.D. 1997. Population structure, genetic diversity, and clone formation in *Quercus chrysolepis* (Fagaceae). Am. J. Bot. **84**: 1553–1564.

Nixon, K.C. 1993. The genus *Quercus* in Mexico. *In* Biological diversity of Mexico: origins and distribution. *Edited by* T.P. Ramamoorthy, R. Bye, A. Lot, and J. Fa. Oxford University Press, New York, USA. pp. 397–420.

Nybom, H., and Bartish, I. V. 2000. Effects of life history traits and sampling strategies on genetic diversity estimates obtained with RAPD markers in plants. Pers. Plant. Ecol. Evol. Syst. **3**: 93–114.

Rieseberg, L.H., and Ellstrand, N.C. 1993. What can molecular and morphological markers tell us about plant hybridization? Crit. Rev. Plant. Sci. **12**: 213–241.

Samuel, R., Pinsker, W., and Ehrendorfer, F. 1995. Electrophoretic analysis of genetic variation within and between populations of *Quercus cerris*, *Q. pubescens*, *Q. petraea* and *Q. robur* (Fagaceae) from Eastern Austria. Bot. Acta **108**: 290–299.

Schneider, S., Roessli, D., and Excoffier, L. (2000) Arlequin ver. 2000. A software for population genetics data analysis. Genetics and Biometry Laboratory, Department of Ecology and Anthropology, University of Geneva, Switzerland.

Smouse, P. E., Long, J.C., and Sokal, R. 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. Syst. Zool. **35**: 627–632.

Sokal, R.R., and Rohlf, F.J. 1995. Biometry, the Principles and Practice of Statistics in Biological Research. Freeman and Company, New York, NY, USA.

Swofford, D.L., Olsen, G.J., Waddell, P.J., and Hillis D.M. 1996. Phylogenetic inference. *In* Molecular Systematics. *Edited by* Sinauer Associates Inc., Sunderland, MA. pp. 407–514.

Toumi, L., and Lumaret, R. 1998. Allozyme variation in cork oak (*Quercus suber* L.): the role of phylogeography and genetic introgression by other Mediterranean oak species and human activities. Theor. Appl. Genet. **97**: 647–656.

Toumi, L., and Lumaret, R. 2001. Allozyme characterization of four Mediterranean oak species. Biochem. Syst. Ecol. **29**: 799–817.

Tovar-Sánchez, E., and Oyama, K. 2004. Natural hybridization and hybrid zones between *Quercus crassifolia* and *Quercus crassipes* (Fagaceae) in Mexico: morphological and molecular evidence. Am. J. Bot. **91**: 1352–1363.

Valencia, S. 1994. Contribución a la delimitación taxonómica de tres especies del género *Quercus* subgénero *Erythrobalanus*. M. Sc. thesis, Facultad de Ciencias, Universidad Nacional Autónoma de México, México D.F., México.

Yeh, F.C., Yang, R.C., and Boyle, T.B.J. 1999. POPGENE Version 1.31, microsoft window-based free ware for population genetic analysis. University of Alberta and Centre for International Forestry Research, Alberta, Canada.

Zanetto, A., and Kremer, A. 1995. Geographical structure of gene diversity in *Quercus petraea* (Matt.) Liebl. I. Monolocus patterns of variation. Heredity, **75**: 506–517.

Table 1. Name, geographic coordinates, morphological group (A = *Quercus affinis*-like; L = *Quercus laurina*-like), sample size (*N*), percentage of polymorphic loci (%*P*) and Shannon diversity index (S_S) for 16 populations within the *Q. affinis-Q. laurina* complex.

Population number and name	Latitude, N/ Longitude, W	Morphological group	*N*	%*P*	S_S
1 Tequila	20°50'/103°48'	L	23	48.15	0.218
2 Mil Cumbres	19°40'/100°55'	L	25	51.85	0.239
3 Cuernavaca	19°05'/99°15'	L	24	50.00	0.195
4 Santa Inés	17°03'/96°55'	L	24	50.00	0.223
5 Amealco	20°10'/100°20'	L	27	53.70	0.244
6 Ozumba	19°05'/98°42'	L	29	57.41	0.252
7 Llano de Flores	17°30'/96°30'	L	20	42.59	0.219
8 Pinal de Amoles	21°01'/99°40'	A	22	46.30	0.204
9 Puerto Aire	18°45'/97°30'	L	30	64.81	0.284
10 Jacala	20°50'/99°05'	A	27	59.26	0.255
11 Real del Monte	20°05'/98°40'	L	28	57.41	0.229
12 Cerro Navajas	20°12'/98°30'	L	30	64.81	0.285
13 Zembo	20°15'/98°32'	L	29	62.96	0.277
14 Jalacingo	19°30'/97°15'	A	28	61.11	0.293
15 Zacapoaxtla	19°50'/97°40'	A	20	46.30	0.221
16 Zacualtipán	20°39'/98°40'	A	29	62.96	0.273

Figure captions

Fig. 1. Map illustrating the collection sites of oak populations. Squares represent *Quercus affinis*-like populations and circles represent *Quercus laurina*-like populations. Numbers next to each symbol correspond to those given in Table 1 with the name of the localities.

Fig. 2. Unweighted pair group method with arithmetic averaging (UPGMA) dendrogram based on genetic distances among *Quercus affinis-Quercus laurina* populations. **L** = *Q. laurina*-like populations; **A** = *Q. affinis*-like populations. See Table 1 for details about populations.

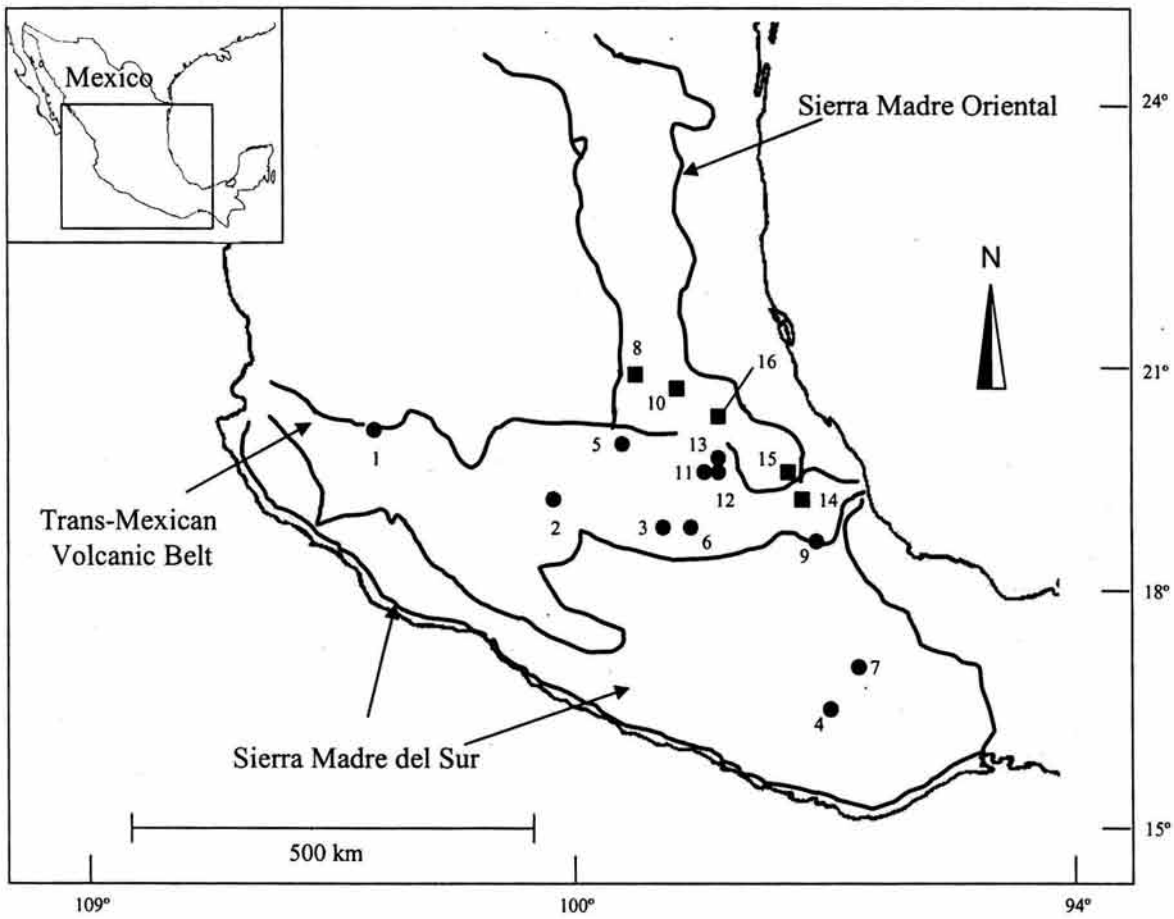

Mexico
Sierra Madre Oriental
N
24°
21°
18°
15°
Trans-Mexican
Volcanic Belt
Sierra Madre del Sur
500 km
109°
100°
94°
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

Figure 1

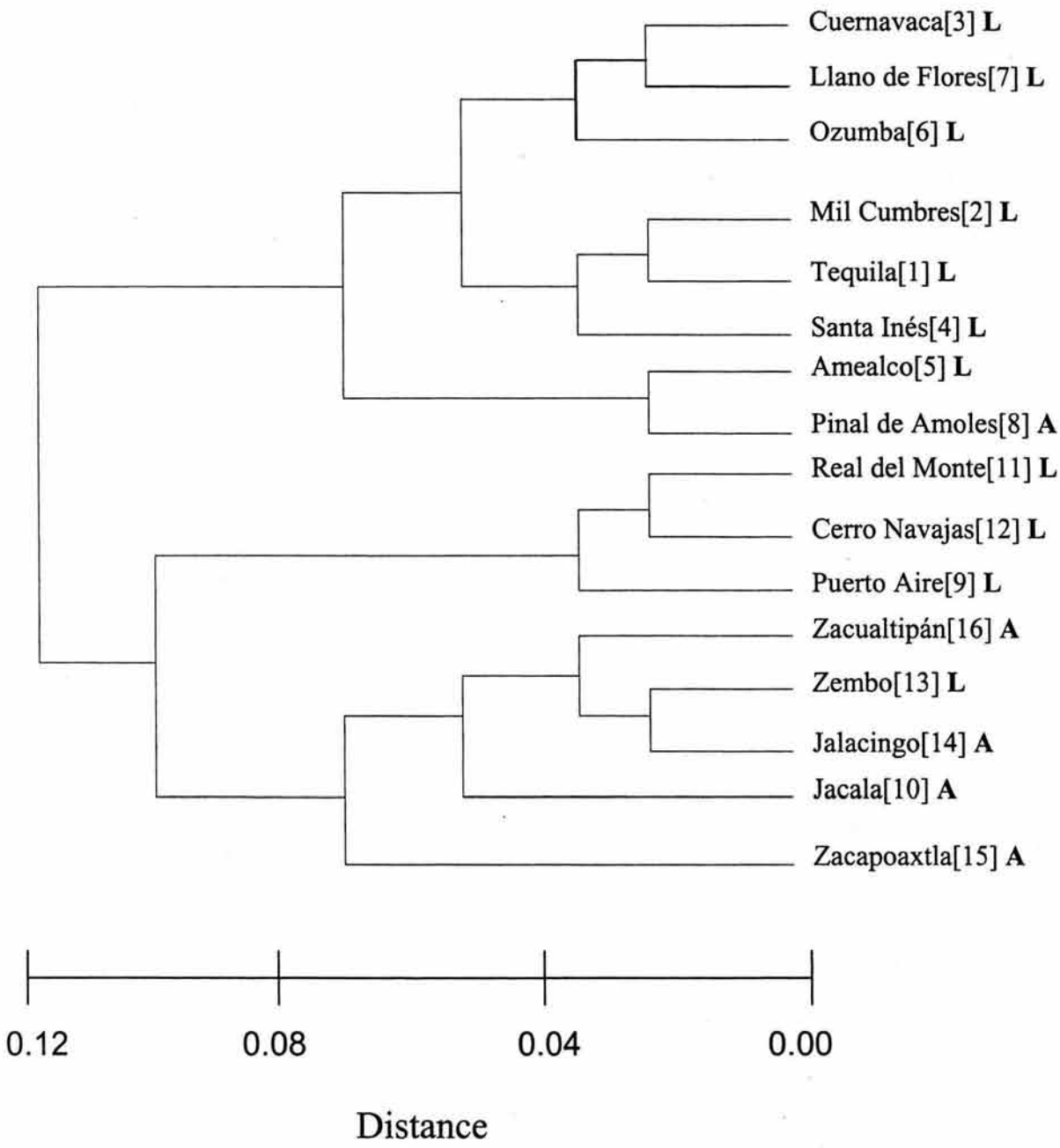

Cuernavaca[3] L
Llano de Flores[7] L
Ozumba[6] L
Mil Cumbres[2] L
Tequila[1] L
Santa Inés[4] L
Amealco[5] L
Pinal de Amoles[8] A
Real del Monte[11] L
Cerro Navajas[12] L
Puerto Aire[9] L
Zacualtipán[16] A
Zembo[13] L
Jalacingo[14] A
Jacala[10] A
Zacapoaxtla[15] A
0.12
0.08
0.04
0.00
Distance

Figure 2

VI.

Antonio González-Rodríguez, John F. Bain, Joanne L. Golden, and Ken Oyama

Chloroplast DNA variation in the *Quercus affinis-Q. laurina* complex in Mexico: geographical structure and associations with nuclear and morphological variation.

Molecular Ecology, 13: 3467–3476. 2004.

Molecular Ecology (2004) 13, 3467–3476 doi: 10.1111/j.1365-294X.2004.02344.x

Chloroplast DNA variation in the *Quercus affinis–Q. laurina* complex in Mexico: geographical structure and associations with nuclear and morphological variation

A. GONZÁLEZ-RODRÍGUEZ,* J. F. BAIN,† J. L. GOLDEN† and K. OYAMA*
Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México, Antigua Carretera a Pátzcuaro no. 8701, Col. Ex–Hacienda de San José de la Huerta, Morelia, 58190 Michoacán, México, †Department of Biological Sciences, University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4

Abstract

The geographical distribution of chloroplast DNA (cpDNA) variation in 39 populations of two hybridizing Mexican red oaks, *Quercus affinis* and *Q. laurina*, was investigated using polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP). Six haplotypes were identified. Of these, two (H1 and H4), separated by four mutations, had high frequencies (58 and 23% of the individuals, respectively) and were present across the whole geographical range of both species, often co occurring in the same populations. The other four haplotypes were rare, geographically restricted, and are probably derived from the two frequent haplotypes. Latitudinal or other clinal patterns in diversity levels or haplotype composition of populations were not apparent. The pattern of haplotype distribution was characterized by some mosaicism, with contrasting populations often situated in proximity. Average within-population diversity (h_S = 0.299) and population differentiation (G_{ST} = 0.499) were, respectively, higher and lower than values reported in previous studies of oak species. There was evidence for phylogeographical structure, as indicated by N_{ST} (0.566) being significantly higher than G_{ST}. Haplotypic variation was largely species-independent, although some very weak associations were detected between haplotypes H1 and H4 and morphological and nuclear molecular variation correspondingly characterizing *Q. affinis* and *Q. laurina*. These oaks probably did not experience a marked restriction to one or a few particular subregions of their present range during the last glacial cycle. It is more likely that substantial populations persisted throughout several episodes of climatic change, but experienced recurrent latitudinal and altitudinal migrations which may have caused the widespread distribution of haplotypes H1 and H4 and frequent intermixing of populations.

Keywords: chloroplast DNA, geographical structure, hybridization, Mexico, population history, *Quercus*

Received 21 April 2004; revision received 14 July 2004; accepted 9 August 2004

Introduction

Analysis of chloroplast DNA (cpDNA) variation within plant species is a useful tool to reconstruct historical events such as population expansions and contractions, migration and colonization (McCauley 1995; Ennos *et al.* 1999), as well as to detect ancient and contemporary hybridization (Rieseberg & Soltis 1991; Rieseberg *et al.* 1996). In a number of angiosperm species, particularly from northern latitudes, high differentiation among populations and clear-cut geographical distributions of cpDNA haplotypes have indicated restricted seed dispersal, and seemingly reflect drastic isolation of populations into refugia during the last glaciation, postglacial migration and range expansions (Sewell *et al.* 1996; Soltis *et al.* 1997; Lumaret *et al.* 2002; Palmé & Vendramin 2002; Cavers *et al.* 2003; Petit *et al.* 2003; Rendell & Ennos 2003). However, there are some species that appear to have been affected less severely by climatic changes. For example, *Salix caprea* was probably not confined to small and isolated refugia in southern

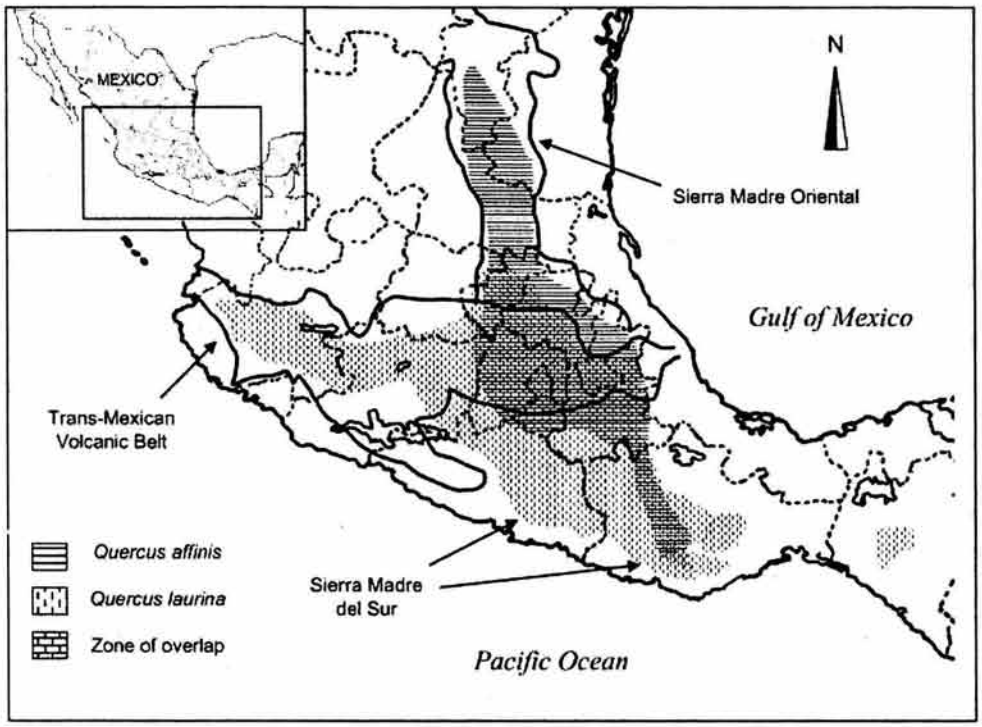
Correspondence: Antonio González-Rodríguez. Fax: + 52 55 56 23 27 19; E-mail: agrodrig@oikos.unam.mx

Europe during the last glacial maximum, but persisted in intermediate latitude refugia with large population sizes which, when combined with high dispersal ability, have resulted in the absence of geographical structure of cpDNA variation (Palmé *et al.* 2003). At lower latitudes it could have been more common for plant species to experience such comparatively moderate population size changes and range shifts, because recent climatic fluctuations were probably less extreme in these areas (Flenley 1998). Consequently, the spatial distribution of cpDNA variation in these cases is in general expected to reflect less markedly recent climatic fluctuations and, instead, patterns considerably more ancient than the last glacial cycle may be present in some species (Cannon & Manos 2003). However, few plant phylogeographical studies have been conducted so far in these areas.

The genus *Quercus* is represented in Mexico by 135–150 species, with approximately 86 of them endemic, which indicates that a major secondary radiation of this group occurred within this area (Nixon 1993; Manos *et al.* 1999). All the major mountain ranges in Mexico are rich in species of *Quercus* (Nixon 1993) and approximately 5.5% of the country is covered by oak forests and woodlands, and 13% by pine–oak forests. In all these communities the presence of several intermixed oak species is common (Rzedowski 1981). Some oaks also occur in xeric areas, cloud forests and tropical forests (Rzedowski 1981; Nixon 1993). However, the evolution of oaks in Mexico is poorly understood. Fossil pollen belonging to this genus has been identified in Mio–Pliocene formations situated in Guatemala and Panama (Graham 1999), which suggests a minimum late Miocene age for the arrival of oaks into what is now Mexico. Diverse topography, climate and habitat may have been very important in the processes of radiation and maintenance of *Quercus* diversity in this region. The impact of changes in temperature and precipitation regimes during the late Tertiary and the Quaternary on the populations of particular oak species is unknown, but it is probable that recurrent latitudinal and elevational displacements occurred, affecting patterns of intra- and interspecific gene flow dynamics and to an important extent shaping the geographical distribution and variation among species that we observe today. Human influence during the last few thousand years has almost certainly also caused some changes in the genetic diversity and structure of Mexican oak populations (Butzer & Butzer 1997; Conserva & Byrne 2002), but these have probably not been of great extent. In many regions oaks are used intensively at a local scale, mainly as a source of timber and firewood, and forests have been cleared and fragmented to create land for agriculture and grazing of cattle, but large-scale exploitation has been kept low (Rzedowski 1981). The establishment of populations through plantation and artificial seed transfer has been relatively infrequent.

In previous cpDNA phylogeographical studies conducted on *Quercus* species, most notably involving the European white oaks, extensive documentation of variation at a continental scale has revealed strong geographical structuring of cpDNA lineages, mainly along a longitudinal gradient (Petit *et al.* 2002a,b). This information, complemented with the examination of fossil pollen data, has been used to obtain a precise delineation of the location of the refugia where oaks survived the last glacial period and to assess the postglacial recolonization dynamics of these species (Brewer *et al.* 2002; Petit *et al.* 2002b). Together with the clear-cut geographical patterns revealed by these results, an overall picture of nearly species-independent sharing of cpDNA variation in *Quercus* has emerged, which implies systematic cpDNA exchanges between sympatric oak species through hybridization and introgression (Whittemore & Schaal 1991; Dumolin-Lapègue *et al.* 1999; Belahbib *et al.* 2001; Petit *et al.* 2002a,b). This makes it difficult to identify unambiguously the origin of shared cpDNA haplotypes, but nevertheless some initial associations between some white oak species and particular haplotype lineages have been suggested (Petit *et al.* 2002a).

Quercus affinis Scheidweiler and *Q. laurina* Humboldt et Bonpland are closely related Mexican red oaks. Phenotypic and genetic patterns of variation in this complex suggest that ancient events of secondary contact between the two species have resulted in frequent hybridization and introgression, leading to extensive morphological and genetic intergradation (Valencia 1994; González-Rodríguez *et al.* 2004). Hybridization probably also occurs between *Q. laurina* and at least four other red oak species (*Q. crassifolia*, *Q. crassipes*, *Q. mexicana* and *Q. rubramenta*), although with much lower frequency (Valencia 1994). Intergradation between *Q. affinis* and *Q. laurina* occurs mainly within a wide region of overlap in the distribution of the two oaks, whereas interspecific populations outside this area show clear genetic and phenotypic differentiation (González-Rodríguez *et al.* 2004). The area of overlap is situated in the eastern portion of the Trans-Mexican Volcanic Belt and northern Oaxaca, while representative populations of *Q. laurina* are distributed at altitudes between 2440 and 3065 m along the Sierra Madre del Sur and the western region of the volcanic belt, and representative populations of *Q. affinis* occur with an altitudinal range of 1600–2800 m in the Sierra Madre Oriental (Fig. 1). From these patterns of geographical variation, it has been suggested (Valencia 1994) that the divergence between the two species occurred in isolation (*Q. laurina* in the Sierra Madre del Sur and *Q. affinis* in the Sierra Madre Oriental). The time when this could have happened is not clear, but it was possibly during the mid- or late Pliocene (Valencia 1994). Subsequently, secondary contact probably took place in the volcanic belt and northern Oaxaca during episodes of range expansion favoured by climatic conditions. The glacial and interglacial



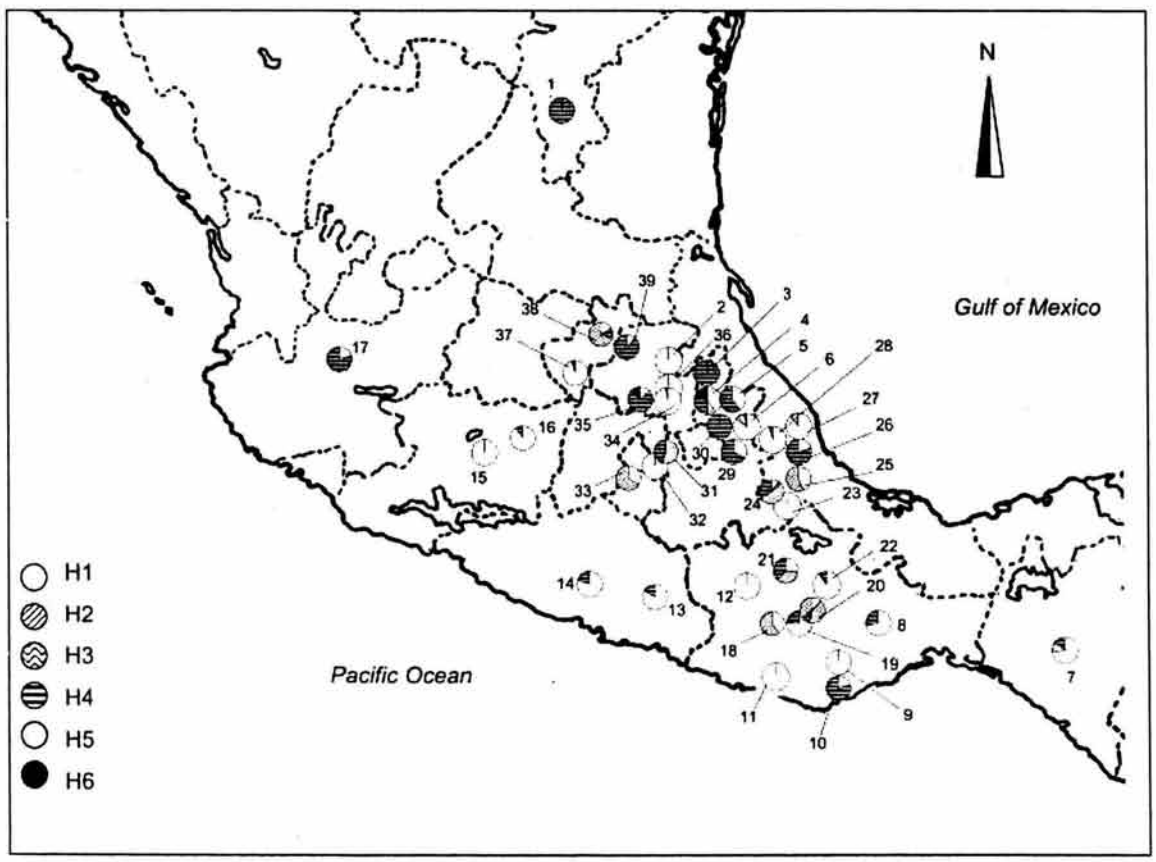
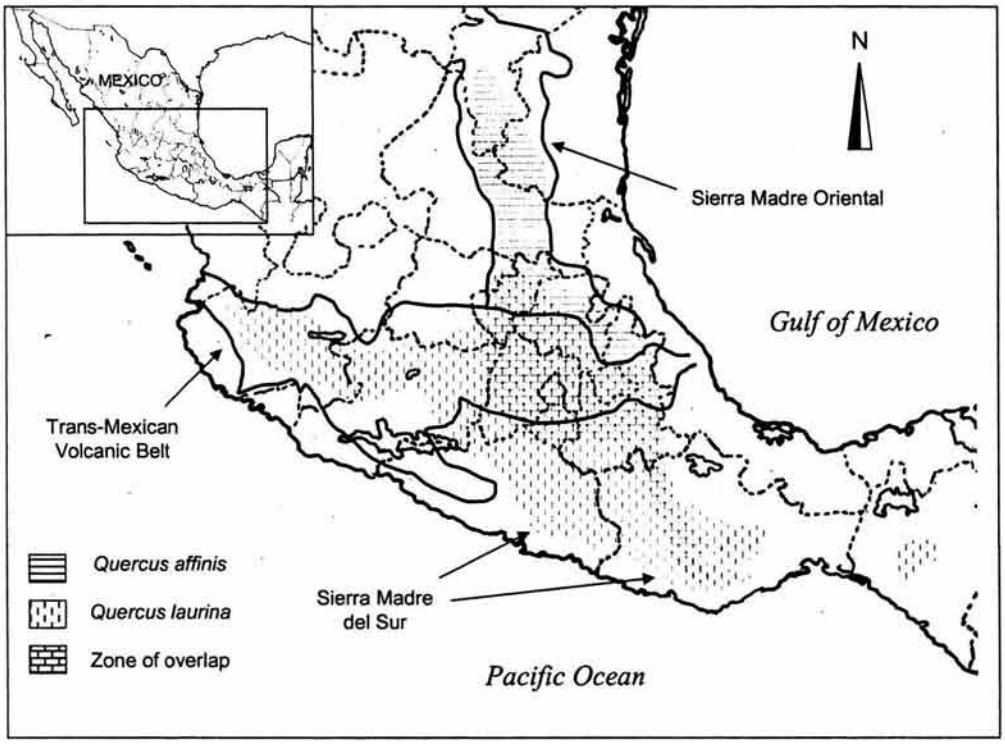
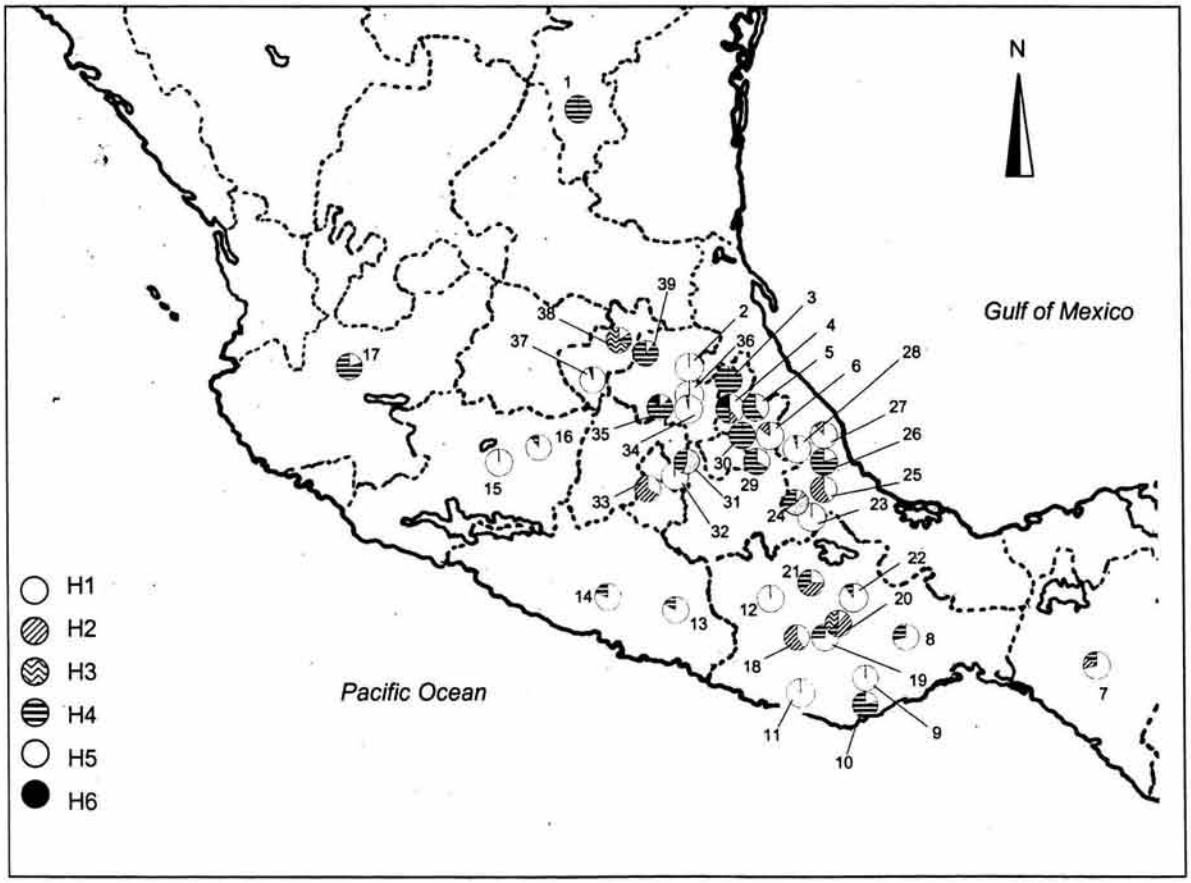


Fig. 1 Top panel, map of the geographical distribution of *Quercus affinis* and *Q. laurina*. The mountain ranges mentioned in text are sketched. Note the overlap area situated in the eastern portion of the volcanic belt and northern Oaxaca. Bottom panel, map of the populations and the geographical distribution of chloroplast PCR–RFLP haplotypes.

Figure 1


MEXICO
N
Sierra Madre Oriental
Gulf of Mexico
Trans-Mexican
Volcanic Belt
Quercus affinis
Quercus laurina
Zone of overlap
Sierra Madre
del Sur
Pacific Ocean


N
Gulf of Mexico
Pacific Ocean
H1
H2
H3
H4
H5
H6
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39

periods of the Pleistocene probably determined further episodic changes in the geographical distribution and, thus, in the intra- and interspecific gene flow dynamics of these oaks (Valencia 1994).

In this study we performed restriction enzyme analysis of polymerase chain reaction (PCR)-amplified regions [polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP)] to examine cpDNA variation in the *Q. affinis*–*Q. laurina* complex. First, we try to make some inferences about the spatio-temporal dynamics of these species, as suggested by the current distribution of cpDNA variants. We also wish to assess if cpDNA variation further supports that intergradation between *Q. affinis* and *Q. laurina* is the product of hybridization between previously allopatric species. If so, we expect the intermixing of rather divergent haplotypes in putative hybrid populations (Beckstrom-Sternberg *et al.* 1991; Holman *et al.* 2003). Finally, and if this occurs, we examine the degree of association between haplotypic variation and the morphological and nuclear molecular variation used previously to discriminate between both species (González-Rodríguez *et al.* 2004).

Materials and methods

Sampling

A total of 537 individuals of *Q. affinis* and *Q. laurina* from 39 localities across the whole distribution range of both species were included in the present study (Fig. 1 and Table 1). Of these, 355 individuals from 16 populations (Table 1) had been included previously in an analysis of hybridization between the two species using random amplified poymmorphic DNA (RAPD) markers and morphological variables (González-Rodríguez *et al.* 2004). At each site, young, undamaged leaves were collected from randomly chosen adult trees and frozen in liquid nitrogen, and then stored in a −80 °C freezer. In all cases, sampled individuals within a locality were separated by at least 100 m. A voucher specimen from each tree was also collected. Each individual was classified as *Q. affinis*, *Q. laurina* or intermediate (Table 1) using a previously obtained canonical discriminant function based on nine foliar traits (González-Rodríguez *et al.* 2004). Other sympatric oak species were observed in most localities, particularly *Q. rugosa* (a white oak) and *Q. candicans*, *Q. crassifolia* and *Q. crassipes* (red oaks). Individuals suspected to have resulted from hybridization of *Q. affinis* or *Q. laurina* with any other species were avoided in the present sampling.

PCR–RFLP

Genomic DNA was extracted from 100 mg of frozen leaf tissue using the protocol of Lefort & Douglas (1999) with minor modifications. PCR conditions were optimized for the amplification of six cpDNA regions (AS, CD, DT, HK, TF, ST), using universal primer pairs (Taberlet *et al.* 1991; Démesure *et al.* 1995). The thermal cycling programme was as follows for fragments CD, DT, HK, TF and ST: one cycle of 2 min at 94 °C; 30 cycles of 1 min at 94 °C, 1 min at 55 °C and 1 min 30 s at 72 °C. For fragment AS, the programme differed in that 35 cycles were used; the annealing temperature was 60 °C, and the extension time 2 min 30 s. A final step of 7 min at 72 °C was included in both programmes. All amplification reactions were carried out in 25 μL volumes, containing 10 ng template DNA; 1 × PCR buffer (Invitrogen); 2 mM $MgCl_2$; 0.1 mM each dNTP (Fermentas), 0.2 μM each primer; 5 μg bovine serum albumin (BSA); and 1.5 U *Taq* polymerase (Invitrogen).

The six amplified regions were screened for variation with nine restriction enzymes (Fermentas): *Alu*I, *Dra*I, *Mbo*I, *Hae*III, *Hha*I, *Hin*fI, *Msp*I, *Taq*I and *Vsp*I, using 20 individuals from 10 geographically separated populations. The restriction reactions were performed by adding 10 μL of PCR product to a mix containing 17.5 μL H_2O, 2 μL 10 × enzyme buffer (Fermentas), 5 U of the enzyme and 0.5 μL BSA if required. Reactions with all enzymes were incubated for 3 h at 37 °C, except *Taq*I, which was incubated at 65 °C. The restriction fragments were electrophoresed on 8% polyacrylamide gels using 1 × Tris-Borate-EDTA (TBE) buffer at 300 V for 3 h. The gels were stained with ethidium bromide and photographed under UV light.

Data analysis

Haplotype frequencies were calculated and graphed for each population. Genealogical relationships among haplotypes were depicted in a minimum-spanning network generated using the ARLEQUIN version 2000 program (Schneider *et al.* 2000). This software was also used to obtain haplotype diversity values (h_S) within each population according to the method of Nei (1987), which is based only on haplotype frequencies (unordered alleles). Average within-population haplotype diversity, total diversity (h_T) and the coefficient of population differentiation (G_{ST}) were obtained with the program PERMUT (http://www.pierroton.inra.fr/genetics/labo/Software/). This same program was used to estimate *v*-type parameters (Pons & Petit 1996), which take into account the genealogical relationships among haplotypes (ordered alleles). The permutation test implemented in PERMUT (Burban *et al.* 1999) was employed to compare parameters of population differentiation with unordered and ordered alleles (G_{ST} and N_{ST}, respectively). A value of N_{ST} significantly higher than the G_{ST} value indicates that genealogically more closely related haplotypes tend to occur together within populations, signalling a phylogeographical structure in the distribution of haplotypes (Pons & Petit 1996). The influence of spatial separation on the degree of differentiation among populations was

Table 1 Details of the geographical location of collection sites and number of *Quercus affinis*, *Q. laurina* and intermediate individuals sampled, frequency of PCR–RFLP haplotypes in each population and estimated within population diversity (h_S)

				Number of individuals			Frequency of haplotypes						
No.	Population name	Lat N/Long W	Geographic region	*Q. affinis*	*Q. laurina*	Intermediate	H1	H2	H3	H4	H5	H6	h_S (SE)
1	Cerro Potosí	24°55′/100°15′	A	8	0	1	0	0	0	9	0	0	0.000 (0.000)
2	Zacualtipán*	20°39′/98°40′	A	13	0	0	13	0	0	0	0	0	0.000 (0.000)
3	Huauchinango	20°05′/98°07′	A	7	0	4	0	0	0	11	0	0	0.000 (0.000)
4	Zacatlán	20°12′/98°03′	A	9	0	1	4	1	0	3	0	2	0.779 (0.091)
5	Teteles	19°50′/97°28′	A	4	0	1	2	0	0	3	0	0	0.600 (0.175)
6	Zacapoaxtla*	19°50′/97°40′	A	34	0	6	34	5	0	1	0	0	0.268 (0.084)
7	San Cristobal	16°50′/92°45′	L	0	7	0	5	1	0	1	0	0	0.524 (0.209)
8	Ayutla	17°01′/96°03′	L	0	7	0	5	0	0	2	0	0	0.476 (0.171)
9	Suchiltepec I	16°12′/96°30′	L	0	8	0	8	0	0	0	0	0	0.000 (0.000)
10	Suchiltepec II	16°10′/96°31′	L	0	5	0	1	0	0	4	0	0	0.400 (0.237)
11	Lachao	16°15′/97°10′	L	0	6	0	6	0	0	0	0	0	0.000 (0.000)
12	Shini Yucu	17°15′/97°45′	L	0	9	0	9	0	0	0	0	0	0.000 (0.000)
13	Tlapa	17°30′/98°55′	L	0	6	0	5	0	0	1	0	0	0.333 (0.215)
14	Filo de Caballo	17°35′/99°50′	L	0	5	0	4	0	0	1	0	0	0.400 (0.237)
15	Cerro Burro	19°25′/101°30′	L	0	6	0	6	0	0	0	0	0	0.000 (0.000)
16	Mil Cumbres*	19°40′/100°55′	L	0	7	3	9	0	0	1	0	0	0.200 (0.154)
17	Tequila*	20°50′/103°48′	L	0	20	0	4	0	0	16	0	0	0.337 (0.110)
18	Santa Inés*	17°03′/96°55′	O	0	15	5	8	12	0	0	0	0	0.505 (0.056)
19	Corral de Piedra	17°05′/96°32′	O	0	6	2	6	0	0	2	0	0	0.476 (0.171)
20	Llano de Flores*	17°30′/96°30′	O	5	10	2	2	7	6	2	0	0	0.721 (0.068)
21	Pápalo	17°50′/96°49′	O	3	3	2	2	3	0	3	0	0	0.750 (0.096)
22	Comaltepec	17°43′/96°30′	O	0	5	5	9	0	0	1	0	0	0.200 (0.154)
23	Zoquitlán	18°17′/97°03′	O	0	10	2	12	0	0	0	0	0	0.000 (0.000)
24	Puerto Aire*	18°45′/97°30′	O	2	22	5	3	1	0	10	15	0	0.623 (0.059)
25	Orizaba	18°45′/97°05′	O	2	8	4	6	8	0	0	0	0	0.527 (0.064)
26	Perote	19°35′/97°10′	O	0	4	1	1	0	0	4	0	0	0.400 (0.237)
27	Tonayan	19°51′/96°55′	O	3	0	6	8	1	0	0	0	0	0.222 (0.166)
28	Jalacingo*	19°45′/97°15′	O	8	2	12	21	0	0	1	0	0	0.091 (0.081)
29	Tetela	19°45′/97°56′	O	1	2	3	2	0	0	4	0	0	0.533 (0.172)
30	Chignahuapan	20°30′/98°32′	O	1	1	3	0	0	0	5	0	0	0.000 (0.000)
31	Río Frío	19°20′/98°37′	O	0	6	3	0	0	0	4	5	0	0.556 (0.090)
32	Ozumba*	19°05′/98°42′	O	2	16	10	28	0	0	0	0	0	0.000 (0.000)
33	Cuernavaca*	19°05′/99°15′	O	0	14	4	6	12	0	0	0	0	0.471 (0.082)
34	Cerro Navajas*	20°12′/98°30′	O	4	16	9	28	0	0	1	0	0	0.069 (0.063)
35	El Chico*	20°05′/98°40′	O	0	24	7	4	0	0	23	0	4	0.453 (0.099)
36	El Zembo*	20°15′/98°32′	O	4	14	7	25	0	0	0	0	0	0.000 (0.000)
37	Amealco*	20°10′/100°20′	O	1	17	3	20	0	0	0	0	1	0.095 (0.084)
38	Pinal de Amoles*	21°01′/99°40′	O	6	11	8	4	0	17	4	0	0	0.507 (0.099)
39	Jacala*	20°50′/99°05′	O	2	1	6	1	0	0	8	0	0	0.222 (0.166)
Total				109	294	134	311	51	23	125	20	7	

*The 16 populations previously analysed for morphological and RAPD variation (González-Rodríguez *et al.* 2004).
Geographic region: A = distribution area of *Q. affinis*; L = distribution area of *Q. laurina*; O = overlap area between the two species.

investigated by calculating pairwise G_{ST} and N_{ST} values with the program DISTON (http://www.pierroton.inra.fr/genetics/labo/Software/), and plotting means of these parameters against geographical distance classes.

To test for associations of cpDNA variation with morphological variation and nuclear markers differentiating the two species, we employed the data sets of nine foliar variables and nine semidiagnostic RAPD markers obtained previously for 355 of the same individuals analysed here (González-Rodríguez *et al.* 2004). The morphological data set was complemented with new measurements obtained for the rest of the individuals. Because two haplotypes were largely predominant, the rare haplotypes were excluded, and *t*-tests were used to compare the nine morphological variables between the two groups of individuals defined by the possession of each haplotype. The two groups were also compared for the frequency of the nine RAPD markers with Fisher's exact test (Sokal & Rohlf 1995).

Results

All individuals were screened using the five polymorphic fragment/enzyme combinations that were identified, AS-*Taq*I, DT-*Hha*I, HK-*Hha*I, HK-*Taq*I and TF-*Dra*I. These combinations revealed three insertion/deletion mutations (indels) and three restriction site mutations, constituting six haplotypes (Fig. 2, Table 2). Haplotype H1 was the most frequent and geographically widespread (present in 35 populations and 58% of the individuals), followed by haplotype H4, which was found in 26 populations and 23% of the individuals. These two more frequent haplotypes were separated from each other by four mutations. Haplotype H2 was distinguished from haplotype H1 by only one change and appeared in 10 populations and 9.6% of the individuals. Haplotypes H3, H5 and H6 differed from haplotype H4 by one mutation each. These three haplotypes were rare (4.3%, 3.7% and 1.3% of the individuals, respectively) and each restricted to two or three populations, that were in relative geographical proximity in the case of haplotypes H5 and H6 and quite distant in the case of haplotype H3 (Fig. 1, Table 1).

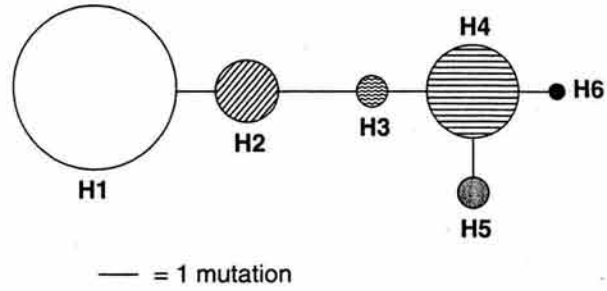


Fig. 2 Minimum-spanning network of the six haplotypes identified in populations of *Quercus affinis* and *Q. laurina*. One unit of branch length represents a single mutation. The sizes of the circles represent the frequency of each haplotype in the total sample set.

A large proportion of the populations (28; 76%) was polymorphic, with a weighted mean number of haplotypes in these populations of 2.55. Sample sizes within locations were not correlated with the levels of haplotype diversity detected ($r = -0.04$; $P = 0.787$). The spatial distribution of cpDNA variation did not suggest patterns of clinal change or clear differences in diversity levels between geographical areas (Fig. 1). The average within-population diversity, total diversity and differentiation estimates for the analysis with unordered alleles were $h_S = 0.299$, $h_T = 0.597$ and $G_{ST} = 0.499$; and for ordered alleles $v_S = 0.260$, $v_T = 0.598$ and $N_{ST} = 0.566$. The permutation test indicated a significantly higher value for the N_{ST} estimate than for the G_{ST} estimate ($P = 0.006$), indicating an association between the genealogical relationships among haplotypes and their geographical distribution. Specifically, haplotype H2 always occurred in populations in which haplotype H1 was also present, and haplotypes H3, H4 and H5 were found together with haplotype H4 in all but one occasion (Population Amealco, no. 34). Nonetheless, there were several populations in which mixing of haplotypes differing by four or five mutations occurred. In particular, the two more common haplotypes, H1 and H4 (differing by four mutations), were present together in 22 populations. These populations are situated along the distribution area of both species and not only in the region of overlap. Three

Table 2 Description of the cpDNA haplotypes identified in *Q. affinis–Q. laurina* populations by PCR–RFLP

	Polymorphic fragment/enzyme combinations					
Haplotype	AS-*Taq*I indel, band 1	AS-*Taq*I indel, band 2	DT-*Hha*I site	HK-*Hha*I site	HK-*Taq*I indel	TF-*Dra*I site
H1	1	1	0	1	1	1
H2	2	1	0	1	1	1
H3	2	1	1	0	1	1
H4	2	1	1	0	2	1
H5	2	1	1	0	2	0
H6	2	2	1	0	2	1

Table 3 Comparison of nine morphological variables between groups of individuals with haplotypes H1 and H4

Morphological variable	Mean values of morphological variables (standard error) H1	H4	*t*†	*P*	R^2
Total leaf length	7.94 (0.102)	8.66 (0.172)	−3.59	0.0004	0.037
Lamina length	7.32 (0.092)	7.93 (0.155)	−3.39	0.0008	0.033
Petiole length	0.64 (0.019)	0.75 (0.032)	−2.67	0.0079	0.021
Maximal width	2.41 (0.037)	2.71 (0.063)	−4.11	< 0.0001	0.048
Distance from base to point of maximal width	3.73 (0.064)	3.87 (0.124)	−1.25	0.2139	0.005
Aristae number	3.02 (0.281)	2.49 (0.160)	−2.41	0.016	0.017
Petiole length/total length	0.08 (0.002)	0.08 (0.003)	−1.56	0.1193	0.007
Maximal width/lamina length	0.33 (0.004)	0.34 (0.006)	−1.74	0.0835	0.009
Distance from base to point of maximal width/lamina length	0.49 (0.004)	0.48 (0.007)	0.43	0.6707	0.001
Combined probability				< 0.0001	

†*t*-test statistic; d.f. = 536.

R^2, proportion of the total variance in each trait related to the possession of either haplotype.

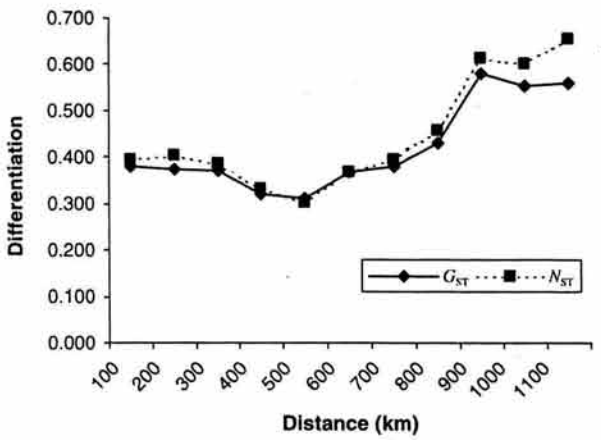


Fig. 3 Relationship of average pairwise G_{ST} and N_{ST} values with the geographical distances separating populations.

Table 4 Comparisons of the frequencies of nine semidiagnostic RAPD markers between groups of individuals with haplotypes H1 and H4

RAPD marker	Frequency of presence of the RAPD marker H1	H4	Fisher's exact test *P*
A05–07	0.54	0.41	0.058
A07–09	0.39	0.52	0.068
A08–01	0.82	0.81	0.168
A10–03	0.94	0.88	0.132
B17–04	0.59	0.40	0.016
B17–06	0.56	0.52	0.386
C09–03	0.20	0.12	0.100
C09–05	0.23	0.29	0.250
I07–02	0.33	0.32	0.472
Combined probability			0.004

populations had particularly high levels of haplotype diversity (Table 1): Llano de Flores (no. 16, with haplotypes H1, H2, H3 and H4), Zacatlán (no. 27, with haplotypes H1, H2, H4 and H6) and Puerto Aire (no. 36, with haplotypes H1, H2, H4 and H5).

The plot of mean pairwise differentiation measures vs. geographical distances (Fig. 3) indicated that, up to a distance of about 700 km, the difference between G_{ST} and N_{ST} is small, and both measures do not show a clear increase from the initial values. Above this distance class there is an increase in differentiation, and the difference between G_{ST} and N_{ST} becomes larger.

The means of nine morphological variables and the frequency of nine RAPD markers that have been shown previously to be discriminatory between *Q. affinis* and *Q. laurina* (González-Rodríguez *et al.* 2004) were compared between individuals possessing the two more frequent haplotypes, H1 and H4. Results are given in Tables 3 and 4. The *t*-tests indicated that five of the morphological traits differed significantly between the two groups of individuals (Table 3), but these differences were weak, because only a very small proportion of the total variation in these five traits was related to the possession of one haplotype or the other (R^2 = 0.017–0.048). An overall test of these morphological differences between individuals with the two haplotypes was also performed with a meta-analysis in which the probabilities from the individual significance tests for the nine morphological variables were combined (Sokal & Rohlf 1995), and the result was highly significant (Table 3). In general, there was some tendency for leaves of trees with haplotype H4 to be longer, wider, with a longer petiole and fewer aristae, compared to those with haplotype H1 (Table 3). This is the same direction of the differentiation in these five traits between typical individuals of *Q. laurina*

and *Q. affinis* (González-Rodríguez *et al.* 2004). These results suggest a weak but still detectable association of haplotype H4 with the morphology of *Q. laurina*, and of haplotype H1 with the morphology of *Q. affinis*. In the case of RAPD markers, the frequency of only one (B17–4) differed significantly between individuals with haplotypes H1 and H4 according to Fisher's exact test (Table 4). However, from these tests probabilities close to significance were obtained for several of the other RAPD markers, and the overall combined probability obtained with the meta-analysis was significant (Table 4). For all markers, the frequency differences between individuals with the two haplotypes were in the direction of associating haplotype H1 with *Q. affinis* and H4 with *Q. laurina*. For example, the estimated frequencies of marker B17–4 were 0.844 and 0.000 in representative populations of *Q. affinis* and *Q. laurina*, respectively (González-Rodríguez *et al.* 2004).

Discussion

Oaks species have seeds with low dispersal potential, and are characterized in general by low population cpDNA diversity, relatively high levels of overall diversity and high population differentiation ($h_S = 0.025–0.183$; $h_T = 0.635–0.847$; $G_{ST} = 0.781–0.961$ for eight European white oaks, Petit *et al.* 2002b). In this study we found comparatively higher levels of average within population variation ($h_S = 0.299$) and lower, but still considerable, among-population variation ($G_{ST} = 0.499$). Furthermore, the geographical distribution of haplotypes was not as clearly defined as in previous studies involving species in genus *Quercus* (Belahbib *et al.* 2001; Lumaret *et al.* 2002; Petit *et al.* 2002a,b). The pattern of spatial cpDNA variation in the *Q. affinis*–*Q. laurina* complex is characterized by the presence of two distantly related haplotypes (H1 and H4) at high frequency throughout the species' range, distributed in a rather mosaic fashion and co-occurring frequently, and at least four less common, most probably derived haplotypes that are geographically more restricted. Some phylogeographical structuring of the haplotype distributions was indicated because N_{ST} (0.566) was significantly higher than G_{ST}. There is no reason to think that dispersal in *Q. affinis* and *Q. laurina* may be more efficient than in any other oak species, so these patterns imply that processes allowing the two probably ancestral haplotypes H1 and H4 to reach a widespread distribution and causing extensive mixing of populations have been important in the historical dynamics of these species. Available palaeoenvironmental reconstructions (Metcalfe *et al.* 2000) suggest that the climatic changes during the late Pleistocene were not as drastic in Mexico as to reduce oak species to small populations isolated into a few refugia. These reconstructions indicate significant oscillations between cold–moist, cold–dry, cool–moist and warm–dry conditions in Northern and Central Mexico, although these changes were smaller in magnitude than in other parts of the northern hemisphere tropics and subtropics (Metcalfe *et al.* 2000). More significantly, important amounts of *Quercus* pollen are almost constantly present, with some fluctuations, in palynological records from several locations in Mexico extending back to 44 000 years BP (Lozano-García & Xelhuantzi-López 1997; Metcalfe *et al.* 2000). As a result of the climatic changes, oak species presumably experienced geographical displacements according to their particular ecological requirements, but forests in general have been probably present and widespread for a long time. The continued persistence of *Q. affinis* and *Q. laurina* populations is plausible, although with several latitudinal and elevational displacements and range fragmentations and expansions, which combined could have produced the observed extensive distribution of the probably ancestral haplotypes H1 and H4, as well as their frequent intermixing. The other four haplotypes, but particularly haplotypes H5 and H6, are probably more recently derived variants that have had comparatively less time to disperse. However, the disjunct distribution of haplotype H3 in two localities separated by 500 km (Fig. 1) seems difficult to explain as a result of seed dispersal. It is possible that the mutation characterizing this haplotype occurred independently in the two populations. Another tentative explanation is that this haplotype was acquired through hybridization with another related species.

The lack of a latitudinal or other obvious clinal pattern in diversity levels or haplotype composition of populations indicates further that *Q. affinis* and *Q. laurina* did not experience a recent restriction to one or a few particular subregions of their present range that later acted as sources of population expansions. South–north partitioning of haplotypic composition, as well as a northwards decrease in diversity levels within populations, has been observed in several temperate plant species (Démesure *et al.* 1996; Sewell *et al.* 1996; Soltis *et al.* 1997; King & Ferris 1998; Petit *et al.* 2002a,b). These patterns imply the existence of two different areas of glacial refugia situated at different latitudes in the case of some species (Sewell *et al.* 1996; Soltis *et al.* 1997), or more commonly a leading-edge effect (Hewitt 2001) during recolonization from southern refugia. This model predicts a successive decrease in the genetic diversity of populations along the direction of recolonization due to consecutive founder effects (Hewitt 2001). In general the pattern of geographical distribution of cpDNA variation in *Q. affinis* and *Q. laurina* is probably the accumulated result of several episodes of change possibly covering the whole Pleistocene, rather than predominantly the product of the last glacial cycle.

The geographical distribution of cpDNA variation in *Q. affinis* and *Q. laurina* also suggests that, in addition to recurrent migration, limited gene flow between populations, drift and founder effects have played a significant role. The

mosaic structure of haplotype distribution is evident, and contiguous populations often have very different genetic compositions and levels of variation (Fig. 1). This structure is reflected in the high average G_{ST} (0.381) between population pairs separated by a maximum of 100 km (Fig. 3), which are expected to be the genetically more similar. Furthermore, there is no clear increasing trend for average G_{ST} values until the 700–800 km distance class. The mosaic distribution of haplotypes at these comparatively shorter geographical distances seems to largely reflect the interplay of stochastic events such as drift and founder effects, produced by a general low average cpDNA exchange among populations combined with rare events of long distance dispersal. Isolation by distance and phylogeographical structuring become more evident between populations pairs situated at larger distance classes (Fig. 3).

Although haplotype variation in the studied populations appears to be almost completely independent of the morphological and nuclear genetic variation that demarcates the two oak species, there are some weak indications of an association of haplotype H1 with *Q. affinis*, and of haplotype H4 with *Q. laurina*. This result is in agreement with previous studies showing that haplotype sharing in many combinations is the rule rather than the exception among oak species, even when they are not closely related (Whittemore & Schaal 1991; Dumolin-Lapègue *et al.* 1999; Belahbib *et al.* 2001; Petit *et al.* 2002a,b). A scenario of hybridization and cytoplasmic introgression would explain haplotype sharing in the *Q. affinis–Q. laurina* complex although, unfortunately, we cannot evaluate the degree to which incomplete lineage sorting could be also responsible for haplotype sharing in these species, because with the present data it is impossible to infer the original extent of cpDNA differentiation between the two oaks. The divergence between H1 and H4 could have occurred coupled to the separation of *Q. affinis* and *Q. laurina*, or they could represent more ancient polymorphisms, each haplotype being initially only associated in part with a species. These issues will probably be clarified to some extent by analysing a larger sample of species and locations, so we can understand better the taxonomic and geographical distribution of cpDNA variation in Mexican red oaks and its past evolutionary dynamics.

Acknowledgements

Financial support for this study was provided by DGAPA, UNAM (grant IN212999 to K. Oyama) and CONACyT (grant 38550-V to K. Oyama). The authors thank J. L. Ibarra and L. Valencia for help with field collections and Nidia Pérez-Nasser for laboratory assistance. We appreciate the useful comments by L. E. Eguiarte and M. Lara throughout the development of this work, and the constructive contributions of three anonymous reviewers to the first version of the manuscript. A. González-Rodríguez was supported by a doctoral scholarship from CONACyT.

References

Beckstrom-Sternberg SM, Rieseberg LH, Doan K (1991) Gene lineage analysis in populations of *Helianthus niveus* and *H. petiolaris* (Asteraceae). *Plant Systematics and Evolution*, **175**, 125–138.

Belahbib N, Pemonge MH, Ouassou A, Sbay H, Kremer A, Petit RJ (2001) Frequent cytoplasmic exchanges between oak species that are not closely related: *Quercus suber* and *Q. ilex* in Morocco. *Molecular Ecology*, **10**, 2003–2012.

Brewer S, Cheddadi R, de Beaulieu JL, Reille M (2002) The spread of deciduous *Quercus* throughout Europe since the last glacial period. *Forest Ecology and Management*, **156**, 27–48.

Burban C, Petit RJ, Carcreff E, Jactel H (1999) Rangewide variation of the maritime pine bast scale *Matsucoccus feytaudii* Duc. (Homoptera: Matsucoccidae) in relation to the genetic structure of its host. *Molecular Ecology*, **8**, 1593–1602.

Butzer KW, Butzer EK (1997) The 'natural' vegetation of the Mexican Bajío: archival documentation of a 16th-century savanna environment. *Quaternary International*, **43**, 161–172.

Cannon CH, Manos PS (2003) Phylogeography of the Southeast Asian stone oaks (*Lithocarpus*). *Journal of Biogeography*, **30**, 211–226.

Cavers S, Navarro C, Lowe AJ (2003) Chloroplast DNA phylogeography reveals colonization history of a Neotropical tree, *Cedrela odorata* L., in Mesoamerica. *Molecular Ecology*, **12**, 1451–1560.

Conserva ME, Byrne R (2002) Late Holocene vegetation change in the Sierra Madre Oriental of Central Mexico. *Quaternary Research*, **58**, 122–129.

Démesure B, Comps B, Petit RJ (1996) Chloroplast DNA phylogeography of the common beech (*Fagus sylvatica* L.) in Europe. *Evolution*, **50**, 2515–2520.

Démesure B, Sodzi N, Petit RJ (1995) A set of universal primers for amplification of polymorphic non-coding regions of mitochondrial and chloroplast DNA in plants. *Molecular Ecology*, **6**, 393–397.

Dumolin-Lapègue S, Kremer A, Petit RJ (1999) Are chloroplast and mitochondrial DNA variation species independent in oaks? *Evolution*, **53**, 1406–1413.

Ennos RA, Sinclair WT, Hu X-S, Langdon A (1999) Using organelle markers to elucidate the history, ecology and evolution of plant populations. In: *Molecular Systematics and Plant Evolution* (eds Hollingsworth PM, Bateman RM, Gornall RJ), pp. 1–19. Taylor & Francis Ltd, London.

Flenley JR (1998) Tropical forests under the climates of the last 30 000 years. *Climatic Change*, **39**, 177–197.

González-Rodríguez A, Arias DM, Valencia S, Oyama K (2004) Morphological and RAPD analysis of hybridization between *Quercus affinis* and *Q. laurina* (Fagaceae), two Mexican red oaks. *American Journal of Botany*, **91**, 401–409.

Graham A (1999) The Tertiary history of the northern temperate element in the northern Latin America biota. *American Journal of Botany*, **86**, 32–38.

Hewitt GM (2001) Speciation, hybrid zones and phylogeography — or seeing genes in space and time. *Molecular Ecology*, **10**, 537–549.

Holman JE, Hughes JM, Fensham RJ (2003) A morphological cline in *Eucalyptus*: a genetic perspective. *Molecular Ecology*, **12**, 3013–3025.

King RA, Ferris C (1998) Chloroplast DNA phylogeography of *Alnus glutinosa* (L.) Gaertn. *Molecular Ecology*, **7**, 1151–1161.

Lefort F, Douglas GC (1999) An efficient micro-method of DNA isolation from mature leaves of four hardwood tree species *Acer*, *Fraxinus*, *Prunus* and *Quercus*. *Annals of Forest Science*, **56**, 259–263.

Lozano-García MS, Xelhuantzi-López MS (1997) Some problems in the late Quaternary pollen records of Central Mexico: basins of Mexico and Zacapu. *Quaternary International*, **43**, 117–123.

Lumaret R, Mir C, Michaud H, Raynal V (2002) Phylogeographical variation of chloroplast DNA in holm oak (*Quercus ilex* L.). *Molecular Ecology*, **11**, 2327–2336.

Manos PS, Doyle JJ, Nixon KC (1999) Phylogeny, biogeography, and processes of molecular differentiation in *Quercus* subgenus *Quercus* (Fagaceae). *Molecular Phylogenetics and Evolution*, **12**, 333–349.

McCauley DE (1995) The use of chloroplast DNA polymorphism in studies of gene flow in plants. *Trends in Ecology and Evolution*, **10**, 198–202.

Metcalfe SE, O'Hara SL, Caballero M, Davis SJ (2000) Records of Late Pleistocene–Holocene climatic change in Mexico — a review. *Quaternary Science Reviews*, **19**, 699–721.

Nei M (1987) *Molecular Evolutionary Genetics*. Columbia University Press, New York.

Nixon KC (1993) The genus *Quercus* in Mexico. In: *Biological Diversity of Mexico: Origins and Distribution* (eds Ramamoorthy TP, Bye R, Lot A, Fa J), pp. 397–420. Oxford University Press, New York.

Palmé AE, Semerikov V, Lascoux M (2003) Absence of geographical structure of chloroplast DNA variation in sallow, *Salix caprea* L. *Heredity*, **91**, 465–474.

Palmé AE, Vendramin GG (2002) Chloroplast DNA variation, postglacial recolonization and hybridization in hazel, *Corylus avellana*. *Molecular Ecology*, **11**, 1769–1779.

Petit RJ, Aguinagalde I, de Beaulieu J-L *et al.* (2003) Glacial refugia: hotspots but not melting pots of genetic diversity. *Science*, **300**, 1563–1565.

Petit RJ, Brewer S, Bordács S *et al.* (2002a) Identification of refugia and post-glacial colonization routes of European white oaks based on chloroplast DNA and fossil pollen evidence. *Forest Ecology and Management*, **156**, 49–74.

Petit RJ, Csaikl UM, Bordács S *et al.* (2002b) Chloroplast DNA variation in European white oaks: phylogeography and patterns of diversity based on data from over 2600 populations. *Forest Ecology and Management*, **156**, 5–26.

Pons O, Petit RJ (1996) Measuring and testing genetic differentiation with ordered versus unordered alleles. *Genetics*, **144**, 1237–1245.

Rendell S, Ennos RA (2003) Chloroplast DNA diversity of the dioecious European tree *Ilex aquifolium* L. (English holly). *Molecular Ecology*, **12**, 2681–2688.

Rieseberg LH, Soltis DE (1991) Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants*, **5**, 65–84.

Rieseberg LH, Whitton J, Randal Linder C (1996) Molecular marker incongruence in plant hybrid zones and phylogenetic trees. *Acta Botanica Neerlandica*, **45**, 243–262.

Rzedowski J (1981) *Vegetación de México*. Limusa, México DF, México.

Schneider S, Roessli D, Excoffier L (2000) *ARLEQUIN* Version 2000: *a Software for Population Genetics Data Analysis*. Genetics and Biometry Laboratory, Department of Ecology and Anthropology, University of Geneva, Switzerland.

Sewell MM, Parks CR, Chase MW (1996) Intraspecific DNA variation and biogeography of North American *Liriodendron* L. (Magnoliaceae). *Evolution*, **50**, 1147–1154.

Sokal RR, Rohlf FJ (1995) *Biometry, the Principles and Practice of Statistics in Biology Research*. Freeman, New York.

Soltis DE, Gitzendanner MA, Strenge DD, Soltis PS (1997) Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. *Plant Systematics and Evolution*, **206**, 353–373.

Taberlet P, Gielly L, Pautou G, Bouvet J (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology*, **17**, 1105–1109.

Valencia S (1994) *Contribución a la delimitación taxonómica de tres especies del género* Quercus *subgénero* Erythrobalanus. Tesis de Maestría, Facultad de Ciencias, Universidad Nacional Autónoma de México.

Whittemore AT, Schaal BA (1991) Interespecific gene flow in sympatric oaks. *Proceedings of the National Academy of Sciences, USA*, **88**, 2240–2544.

This study is part of the Ph.D. thesis of A. González-Rodríguez on the population genetics, hybridization processes and phylogeography of *Quecus affinis* and *Q. laurina*. J. F. Bain and J. L. Golden are interested in plant taxonomy, molecular systematics and phylogeography. The research of K. Oyama has focused on plant population ecology and genetics and plant–insect interactions.

VII.

DISCUSIÓN GENERAL

Conceptos de especie en el género Quercus y consideraciones sobre el complejo Q. affins–Q. laurina

Durante mucho tiempo ha estado claro que en el género *Quercus* existen mucho mayores dificultades para delimitar a las especies utilizando como criterio el aislamiento reproductivo o genético que en la mayoría de las plantas y los animales (Burger, 1975; Van Valen, 1976). Diversos autores han entonces enfatizado los aspectos morfológicos, funcionales y adaptativos que permiten diferenciar a las especies de encinos. Por ejemplo según Burger (1975), definir a las especies de *Quercus* en términos morfológicos permite identificar unidades biológicas más significativas que el tratar de delimitar grupos aislados genéticamente. Según Van Valen (1976), estas unidades biológicas (*i.e.* especies) en los encinos podrían permanecer distintas a pesar del flujo génico entre ellas por el hecho de ocupar distintas "zonas adaptativas", idea que dio origen al "concepto ecológico" de especie (Van Valen, 1976). En una elaboración posterior, Whittemore y Schaal (1991), consideraron que "un modelo [de especie] mas apropiado para los encinos sería considerar a las especies como picos adaptativos, en el que la tendencia de las especies a fusionarse por el flujo génico introgresivo se contrarresta debido a la selección sobre grupos de alelos coadaptados". En general, la mayor parte de los autores actuales considera a las especies en el género *Quercus* bajo estos conceptos: esencialmente como grupos de individuos caracterizados por alelos coadaptados y caracteres fenotípicos correlacionados (Zoldos *et al.*, 1999).

El género *Quercus* ilustra particularmente bien lo que en años recientes se ha denominado la "visión génica" de la especiación (Wu, 2001; Wu y Ting, 2004). Dentro de esta perspectiva, se critica al concepto biológico de especie por su énfasis en el aislamiento e independencia de las especies al nivel de todo el genoma y se plantea que podría ser más

adecuado considerar tal aislamiento al nivel de genes individuales o grupos de genes. Dos especies pueden mantener su identidad aunque experimenten una cantidad importante de flujo génico entre ellas si permanecen efectivamente aisladas para aquellos genes que determinan la base funcional de su divergencia adaptativa. Por lo tanto el genoma puede ser un mosaico de regiones con diferentes grados de diferenciación interespecífica (Wu, 2001; Wu y Ting, 2004).

¿Cómo puede entonces describirse el caso de *Q. affinis* y *Q. laurina*? Con los resultados presentados en esta tesis, quedó establecido que en este complejo la congruencia entre la variación morfológica, nuclear y del ADNcp, tanto al nivel individual (Cáps. III y VI), como al nivel de las poblaciones y grupos de poblaciones (Cáps. IV y V), es por lo general débil. La preponderante falta de congruencia entre los diferentes marcadores puede resumirse como se ilustra en la Fig. 1 para el caso de la variación morfológica y nuclear. De acuerdo con los diferentes análisis presentados en este trabajo, los marcadores neutros de núcleo y cloroplasto sugieren un grado considerable de introgresión entre *Q. affinis* y *Q. laurina*. Sin embargo, la variación morfológica mostró niveles menores de recombinación interespecífica. La tenue distinción observada entre *Q. affinis* y *Q. laurina*, así como la débil congruencia entre los distintos tipos de marcadores en estas especies no son resultados inesperados ni excepcionales en comparación con lo que ha sido observado en otros complejos de especies de encinos cercanamente relacionados (ver referencias en la Introducción y Capítulos III–VI). En todos estos casos, las incongruencias entre diferentes marcadores se han interpretado como una indicación de que los distintos taxa no están bien diferenciados debido a que su divergencia ocurrió rápida y recientemente, a frecuentes eventos de hibridación e introgresión, o a ambos factores.

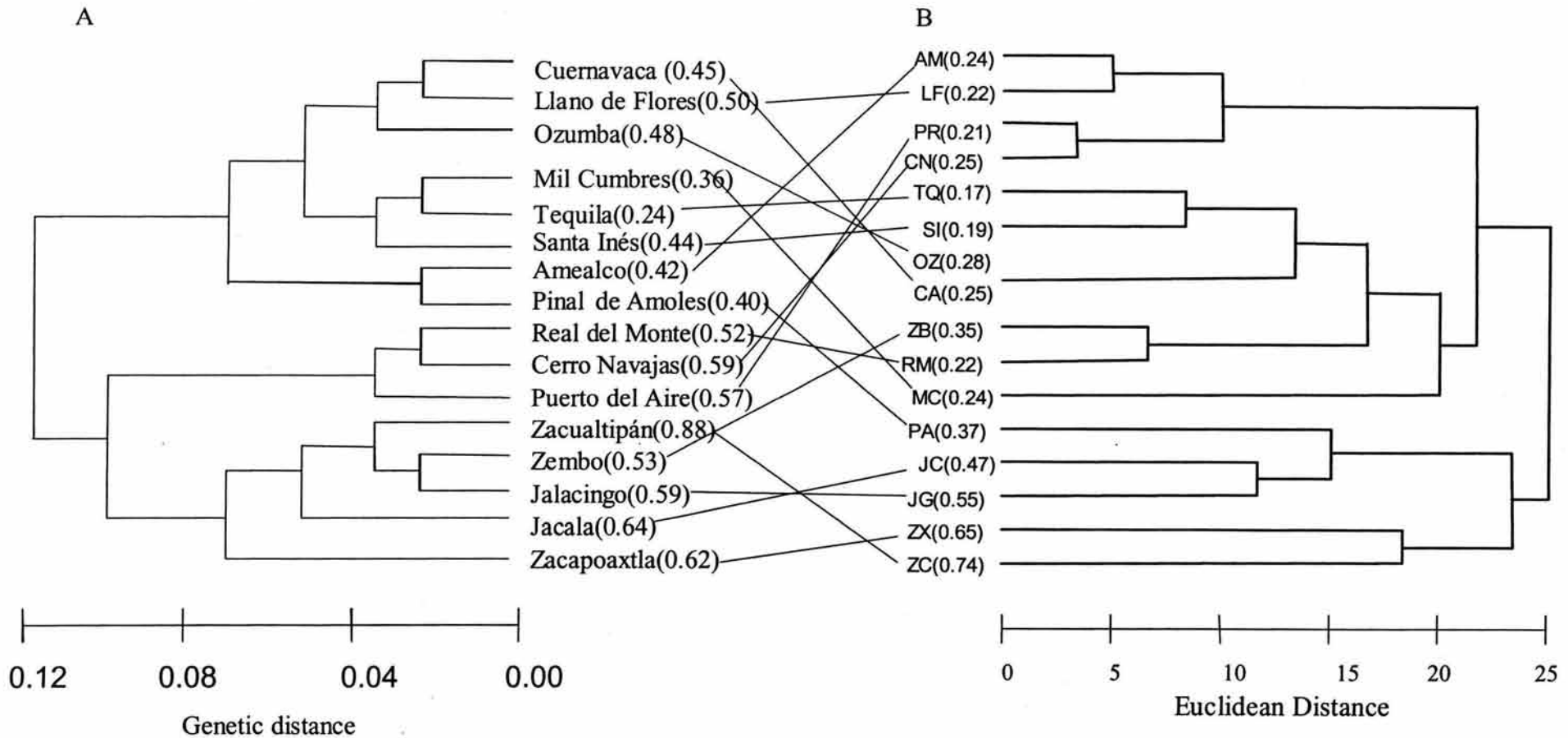


Figura 1. Comparación de los patrones de similitud entre las poblaciones del complejo *Quercus affinis–Q. laurina* obtenidas a partir de una matriz de distancias genéticas basada en 54 fragmentos de RAPDs (A) y una matriz de distancias euclidianas obtenida a partir de nueve caracteres morfológicos (B). Los números entre paréntesis al lado del nombre de cada población corresponden a los promedios del índice de hibridación de máxima verosimilitud (A) y el score discriminante morfológico (B, ver el Capítulo III).

Un aspecto de originalidad de este trabajo es que se investigó de manera explícita el origen de la zona de hibridación entre *Q. affinis* y *Q. laurina* y, como resultado, se encontraron en gran medida los patrones de variación esperados para una zona de hibridación secundaria (ver también más adelante). Hasta donde se puede afirmar, este es el primer trabajo en que este tipo de análisis se realiza para un caso de hibridación entre encinos. El punto es sumamente relevante para discutir la identidad como especies de *Q. affinis* y *Q. laurina*, pues el escenario de contacto secundario implica diferenciación previa en condiciones preponderantemente alopátricas. Durante tal proceso, debió ocurrir divergencia funcional en algunos loci adaptativos y diferenciación estocástica (*i.e.* por deriva génica) para los loci neutros. Una vez establecido el contacto secundario, el flujo génico entre los dos grupos de poblaciones ha sido considerable para los loci neutros y probablemente más restringido para los loci adaptativos. Si nuevamente se usa como referencia el marco conceptual de la visión génica de la especiación, puede considerarse que *Q. affinis* y *Q. laurina* probablemente alcanzaron la "Fase II" de diferenciación durante su divergencia alopátrica (Wu, 2001). Se considera que en esta fase es cuando aparecen conjuntos de alelos coadaptados que constituyen la base de la diferenciación funcional de las poblaciones en divergencia y, como resultado, la hibridación produce algunos genotipos con menor adecuación intrínseca o extrínseca. Sin embargo, la mayor parte del genoma puede experimentar introgresión sin que esto afecte la adecuación de los individuos. Si ocurre contacto secundario durante esta fase, las poblaciones pueden mantener su identidad y continuar su divergencia a pesar del flujo génico interespecífico o bien, fusionarse y dar origen a una nueva forma híbrida. El resultado dependerá en gran medida de situaciones ecológicas y factores selectivos decisivos (Wu, 2001).

Por lo tanto, la delimitación entre *Q. affinis* y *Q. laurina* con mayor significado biológico de que se dispone en este momento es la basada en caracteres morfológicos, puesto que estos caracteres puede suponerse que reflejan la divergencia adaptativa y funcional entre ambas especies producto de diferenciación alopátrica histórica, pero hasta cierto punto mantenida actualmente en simpatría mediante selección endógena en contra de la recombinación de algunos caracteres, así como por selección ecológica resultado de adaptación a diferentes "nichos", como se sugirió en el capítulo IV. Es probable que entre ambas especies también exista diferenciación en caracteres fisiológicos, lo que constituye sin duda un tema interesante de investigación futura. En última instancia, el análisis de la base genética de los caracteres morfológicos y fisiológicos que difieren entre *Q. affinis* y *Q. laurina* y la cuantificación de los niveles de intercambio de estos genes a través de la hibridación proporcionará la mejor caracterización de su identidad como especies y permitirá prever el posible resultado futuro del proceso de hibridación entre ambas.

Algunas de las ideas que acabamos de plantear se discuten con más detalle a continuación.

Origen y dinámica de la zona de hibridación

Los patrones de variación encontrados para los caracteres morfológicos y los marcadores moleculares en las poblaciones estudiadas de *Q. affinis* y *Q. laurina* en general resultan consistentes con la hipótesis de que estos encinos se han hibridado de manera frecuente en una extensa área de simpatría formada por contacto secundario entre ambas especies. Es claro también que los eventos históricos y los procesos evolutivos que han producido tales patrones de variación han sido complejos.

Muchas veces no es trivial distinguir el origen, primario o secundario, de los patrones de variación en las zonas de hibridación, y se pueden requerir análisis detallados utilizando marcadores genéticos (Beckstrom-Sternberg *et al.*, 1991; Villani *et al.*, 1999; Holman *et al.*, 2003). Tres evidencias tomadas en conjunto apoyan fuertemente la conclusión de que el área de intergradación morfológica y genética entre *Q. affinis* y *Q. laurina* es de origen secundario. La primera es la variación espacial significativamente concordante que se observó en general en los marcadores moleculares y los caracteres morfológicos (capítulo III). La segunda es la mayor variación genética existente en las poblaciones situadas en la probable zona de hibridación. La tercera es la presencia de dos haplotipos relativamente divergentes en el complejo.

Es muy poco probable que la variación concordante entre diferentes marcadores como la detectada en este estudio sea el resultado de diferenciación primaria a lo largo de un gradiente de selección direccional (Hewitt & Barton, 1980; Hewitt, 1989; Harrison, 1990). Sin embargo, el que la concordancia entre algunos marcadores haya sido comparativamente débil con respecto a lo que se ha observado en algunas zonas de hibridación bien estudiadas en plantas (dePamphilis & Wyatt, 1990; Villani *et al.*, 1999) y animales (Szymura & Barton, 1986; Shoemaker *et al.*, 1996), requiere una explicación. El escenario sugerido en el capítulo III es que la introgresión ha sido diferencial entre distintos fragmentos de RAPDs (presumiblemente localizados en regiones separadas del genoma) y también entre estos y los caracteres morfológicos. Esta explicación se basa en evidencias que indican que cuando ocurre hibridación, algunas porciones del genoma cruzan fácilmente la barrera específica, mientras que otras lo hacen en grado mínimo (Rieseberg *et al.*, 1996; Martinsen *et al.*, 2001). Para que esto ocurra, es necesario que la selección en contra de los híbridos sea lo suficientemente relajada como para permitir la producción de

una variedad de híbridos (en el sentido amplio, ver introducción) que combinen en distintas formas los genomas de las especies progenitoras. Además, para que la introgresión diferencial aparezca en poblaciones naturales, se requiere que las interacciones genéticas entre los taxa transcurran por un período prolongado, de forma que los efectos de la selección tengan tiempo de manifestarse. El complejo *Q. affinis–Q. laurina* aparentemente reúne las dos condiciones. De acuerdo con este estudio, la variación genética y morfológica entre ambas especies es prácticamente continua, lo que indica la presencia de numerosas poblaciones híbridas segregantes ("enjambres híbridos") en este complejo. Por su parte, los elementos paleobotánicos y biogeográficos reunidos por Valencia (1994) sugieren que los procesos de hibridación entre *Q. affinis* y *Q. laurina* se pudieron haber iniciado a principios del Pleistoceno, hace aproximadamente 1.6 millones de años.

A pesar de que en el capítulo III se mostró que algunos RAPDs individuales (*i.e* A05-1, B17-4) variaron de manera acentuadamente concordante con ciertos rasgos morfológicos (*i.e* longitud del pecíolo, número de aristas), una conclusión clara de los capítulos III, IV y V es que el patrón de relaciones generales de similitud morfológica entre las poblaciones es incongruente con el patrón de similitudes genéticas (ver Fig. 1). La incongruencia es particularmente evidente en las poblaciones situadas en la zona de hibridación, en la región del este del Eje Neovolcánico. En esta área existen cuatro poblaciones (Puerto Aire, Cerro Navajas, Real del Monte y El Zembo) en las que predomina el tipo morfológico de *Q. laurina*, pero que muestran mayor similitud genética con las poblaciones de *Q. affinis* geográficamente cercanas que con otras poblaciones de *Q. laurina.* Esta incongruencia podría estar ilustrando también el fenómeno de introgresión diferencial recién mencionado: se puede proponer que la introgresión y recombinación heteroespecífica de las regiones genómicas que controlan los caracteres morfológicos puede

resultar la mayoría de las ocasiones selectivamente desventajosa, pero en cambio el intercambio de regiones neutras puede ocurrir con mayor facilidad. Esto parecen sugerirlo también los resultados del capítulo III, en el que los marcadores semidiagnósticos indicaron una proporción mucho mayor de individuos genéticamente intermedios que la proporción de individuos intermedios que se encontró en los análisis morfológicos. El que las relaciones de similitud genética entre las poblaciones de *Q. affinis–Q. laurina* tuvieran correlación con la distancia geográfica (capítulo V), mientras que las relaciones morfológicas no (capítulo IV), parece que puede traducirse como un signo de conflicto entre los patrones de flujo génico y la selección natural. Este mismo tipo de conflicto parece estar operando en otras zonas de hibridación, como la reportada recientemente entre *Pinus pumila* y *P. parviflora* var. *pentaphylla* (Watano *et al.*, 2004). Otro caso es el de una población de híbridos entre *Helianthus annuus* y *H. bolanderi* en la que se encontró completa incongruencia entre la morfología y la composición genética de los individuos (Carney *et al.*, 2000). Particularmente, se observaron numerosos individuos del tipo morfológico de *H. annuus* que tuvieron una alta proporción (hasta 20%) de los marcadores propios de la otra especie. Para este caso, se sugirió que los híbridos experimentan selección ecológica a favor de los caracteres morfológicos de *H. annuus* (Carney *et al.*, 2000).

D. J. Howard (com. pers.) sugirió una explicación mucho más simple para la incongruencia de los patrones morfológicos y genéticos observada en el complejo *Q. affinis-Q. laurina* en este estudio, según la cual, podría ser resultado de que solo se utilizó una población de cada especie (Zacualtipán y Tequila, respectivamente) como referencia para identificar los marcadores semi-diagnósticos utilizados. Por lo tanto, es posible que entre otras poblaciones morfológicamente representativas de las especies no se observen las

mismas diferencias en la frecuencia de algunos de estos marcadores. En un estudio de la hibridación entre *Quercus grisea* y *Q. gambelli*, Howard *et al.* (1997) obtuvieron un conjunto altamente optimizado de marcadores (RAPDs) diagnósticos y especie-específicos identificados en tres poblaciones de referencia para cada especie. Posteriormente se encontró en una población híbrida, una correspondencia muy alta entre la morfología de los individuos y su composición genética (Howard *et al.*, 1997). Esta objeción al presente estudio es posiblemente válida. En este momento se está desarrollando un estudio (L. Valencia *et al.*, datos no publicados) que permitirá evaluar la robustez de los marcadores semi-diagnósticos utilizados y de las conclusiones presentadas en este trabajo.

Sin embargo, este punto puede discutirse más extensamente. En la población Zacualtipán, representativa de *Q. affinis*, se identificó la presencia de siete marcadores semi-diagnósticos, mientras que en Tequila, población representativa de *Q. laurina*, se identificaron solo dos, A7-9 y C9-5, los cuales tuvieron frecuencia intermedia (0.619 y 0.5, ver Tabla 2 en el capítulo III). En el capítulo IV se mostró que la población Zacualtipán es morfológicamente homogénea en comparación con todas las demás poblaciones estudiadas, mientras que en Tequila la variación es mucho mayor. Finalmente, en el capítulo VI se encontró únicamente el haplotipo H1 en Zacualtipán, mientras que en Tequila estuvieron presentes los haplotipos H1 y H4 (que están separados por cuatro mutaciones). Todo esto indica que en efecto Zacualtipán es una población "pura" de *Q. affinis*, mientras que Tequila parece haber tenido un origen mixto. Esto es hasta cierto punto sorprendente, dada la lejanía geográfica de esta población de la zona de intergradación morfológica con *Q. affinis*. Tequila, aunque muestra considerable variación fenotípica, contiene exclusivamente individuos "representativos" de *Q. laurina*. La hipótesis a adelantar en este momento es que, más que reflejar un cierto descuido al realizar la elección de las poblaciones de

referencia, esto muestra que es probablemente difícil que existan poblaciones "puras" de *Q. laurina*. Como se mencionó en los antecedentes, *Q. laurina* es una especie que se hibrida por lo menos con otros cuatro encinos, además de *Q. affinis* (*Q. crassifolia*, *Q. crassipes*, *Q. rubramenta* y *Q. mexicana*), aunque con menor frecuencia (Valencia, 1994). El gran espectro de variación morfológica de *Q. laurina*, así como las dificultades taxonómicas que presenta (por ejemplo, se han descrito 25 taxa que se consideran sinónimos de esta especie) podrían ser manifestaciones de que se trata de un linaje heterogéneo que a través de su historia evolutiva se ha comportado como una "compiloespecie" (Harlan & de Wet, 1963; Aguilar *et al.*, 1999). Este término se propuso para designar a aquellas especies genéticamente agresivas que capturan porciones del genoma de otras especies mediante hibridación extensiva (Harlan & de Wet, 1963; Aguilar *et al.*, 1999).

Estructura geográfica de la zona de hibridación

Los resultados de este estudio no permiten concluir con suficiente rigor sobre la estructura geográfica de la zona de hibridación entre *Q. affinis* y *Q. laurina*. Como se mencionó en el capítulo III, el problema consiste fundamentalmente en que el muestreo se realizó preferentemente en aquellas poblaciones en las que previamente se identificó intergradación morfológica entre ambas especies. A pesar de estas limitaciones, los resultados sugieren que en la región este del Eje Neovolcánico existen poblaciones en las que predomina el tipo morfológico de *Q. laurina* entremezcladas con poblaciones con morfología predominantemente de tipo *Q. affinis*. Aunque en un grado mucho menor, también subsiste cierta diferenciación genética entre ambos tipos de poblaciones. Estos patrones indican una estructura de tipo mosaico para esta zona de hibridación.

Para caracterizar adecuadamente la estructura geográfica de una zona de hibridación, generalmente el muestreo de poblaciones se lleva a cabo a lo largo de un transecto, sin elegir previamente las poblaciones de acuerdo a algún criterio (Arnold, 1997). Un estudio con estas características se está llevando a cabo en la zona de hibridación entre *Q. affinis* y *Q. laurina* (L. Valencia *et al.*, datos no publicados). Cabe enfatizar que documentar la estructura geográfica de las zonas de hibridación es relevante porque constituye un reflejo de su dinámica interna. Por ejemplo, el modelo de mosaico le asigna gran importancia a la selección exógena en la estructuración de la distribución espacial de las especies progenitoras y sus híbridos (Harrison y Rand, 1989). Por su parte, el modelo de zona de tensión asume que las zonas de hibridación clinales se forman exclusivamente a causa de selección endógena en contra de los híbridos (Barton y Hewitt, 1989).

Filogeografía del complejo

El análisis de la variación en el ADN de cloroplasto (ADNcp) en *Q. affinis* y *Q. laurina* mostró que dos haplotipos separados por cuatro mutaciones (H1 y H4) se encuentran en la mayoría de los individuos (81%) de estas especies. Considerando que el método empleado para detectar la variación fue PCR-RFLP, puede considerarse que la distancia entre los dos haplotipos indica un nivel de divergencia medianamente distante (Ennos *et al.*, 1999), lo cual es congruente con la idea de que el complejo *Q. affinis-Q. laurina* se formó por contacto secundario entre dos linajes diferentes. La distribución espacial de los dos haplotipos frecuentes, así como de otros cuatro menos frecuentes, no mostró los patrones típicos que se han encontrado en muchas plantas de latitudes medias y altas, producto de la reducción de las áreas de distribución y los tamaños poblacionales de estas especies durante la última glaciación y su expansión post-glacial (McCauley 1995; Ennos *et al.*, 1999). Por

lo tanto, una conclusión importante del capítulo VI es que tal dinámica histórica no ocurrió en el caso de las poblaciones de *Q. affinis-Q. laurina*. Es evidente que debieron haber sucedido migraciones latitudinales y altitudinales, así como fragmentaciones y expansiones de las áreas de distribución, pero probablemente los efectos de la última glaciación fueron relativamente moderados en estos encinos debido a su localización a menores latitudes (Hewitt, 2004). Entonces, los patrones detectados en el estudio pueden ser el resultado acumulado de varios periodos de cambio climático. Otro aspecto que merece destacarse es que la distribución de los haplotipos no mostró la distribución geográfica esperada de acuerdo con el escenario histórico propuesto por Valencia (1994) para el origen del complejo *Q. affinis-Q. laurina*. Según este escenario, se debió haber observado un haplotipo (o linaje) asociado con *Q. affinis* distribuido en la Sierra Madre Oriental, otro haplotipo o linaje asociado con *Q. laurina* en la Sierra Madre del Sur y en el oeste del Eje Neovolcánico, y finalmente, la mezcla de ambos haplotipos o linajes en la zona de hibridación, en el este del Eje Neovolcánico. En contraste, los haplotipos H1 y H4 se encontraron entremezclados a todo lo largo del área de distribución de ambas especies. Aún así, esto no constituye evidencia contundente en contra de la hipótesis de Valencia (1994). Es posible que la huella genética de los eventos históricos postulados en esta hipótesis haya quedado enmascarada por sucesos posteriores. Si se considera que durante el Pleistoceno (aproximadamente los últimos 1.8 millones de años) los periodos glaciales se alternaron con periodos interglaciales entre 18 y 20 veces (Jansen *et al.*, 2000), se hace evidente que una historia extremadamente compleja ha contribuido a dar forma a los patrones de variación contemporáneos. Sin embargo y como lo ilustra el capítulo VI, es muy limitado el grado en el que es posible inferir con detalle la historia de especies particulares,

especialmente cuando se carece de evidencia independiente a la de lo marcadores moleculares.

Recientemente, Lascoux *et al.* (2004) argumentaron que es muy difícil hacer cualquier inferencia filogeográfica cuando se carece de un registro fósil que sirva de apoyo a los patrones genéticos. En cambio, la fortaleza de las conclusiones cuando se dispone de ambos tipos de datos está particularmente ilustrada por los recientes estudios realizados en varias especies de árboles y arbustos europeos (Petit *et al.*, 2003a; Lascoux *et al.*, 2004). Es claro que en México el conocimiento del registro fósil es muy limitado para ser de ayuda para los estudios filogeográficos de especies particulares de encinos y otros grupos de plantas. El polen constituye por lo general la principal fuente de información paleobotánica. Sin embargo, muchas veces los granos de polen no son identificados por debajo del nivel de género. Si consideramos que en México existen más de 100 especies de encinos, es fácil darse cuenta de que, aun si se multiplicaran los esfuerzos, difícilmente se logrará el nivel requerido de resolución para que esta información sea relevante para casos de especies particulares. En situaciones como ésta, es probable que se requiera profundizar en el conocimiento de los requerimientos ecológicos de las especies, para con la ayuda de modelos bioclimáticos analizar su distribución hipotética bajo las probables condiciones de temperatura y precipitación que imperaron durante distintos episodios. Esta información podrá contrastarse con las inferencias históricas derivadas de la distribución actual de los marcadores genéticos. La acumulación de estudios filogeográficos de caso permitirá además identificar en los patrones tanto aspectos comunes como discordantes y analizar sus posibles causas, como ya se ha hecho para otras regiones del mundo (Taberlet *et al.*, 1998).

Consideraciones finales

Para concluir este trabajo, cabe plantear dos preguntas. La primera es por qué la hibridación ocurre con frecuencia excepcionalmente alta en el género *Quercus*. Como se mencionó en la introducción y en el capítulo V, existe una gran cantidad de estudios que mediante el uso de marcadores genéticos de origen nuclear, han reportado niveles muy bajos de diferenciación entre especies de encinos que se hibridan. Sin embargo, es difícil distinguir si esta baja diferenciación es una causa o una consecuencia de la hibridación. Este problema se puede abordar mediante estudios que evalúen de forma comparativa la diferenciación genética entre especies con distintos niveles de relación taxonómica, utilizando de preferencia marcadores nucleares distribuidos aleatoriamente en todo el genoma, como los RAPDs o los AFLPs. Simultáneamente, se deberá analizar en que grado varía la diferenciación entre especies cuando se encuentran en cercanía o lejanía geográfica. De esta forma se podrá separar la diferenciación genética entre las especies de encinos en un componente taxonómico y un componente geográfico, lo cual a su vez permitirá detectar la influencia de la hibridación sobre los patrones observados.

Las diferencias cromosómicas muchas veces son igualmente o más importantes que las génicas en limitar la hibridación e introgresión (Rieseberg *et al.*, 1995). Es posible que en el caso de los encinos estos procesos ocurran con una alta frecuencia debido a la baja diferenciación entre especies al nivel génico (Santaigne *et al.*, 2004). De manera muy significativa, algunos estudios recientes han revelado que el número y estructura de los cromosomas es notablemente similar en varias especies de *Quercus* (Zoldos *et al.*, 1999, 2001). Por ejemplo, el número cromosómico de todas las especies estudiadas hasta la fecha es n = 12 (Nixon, 1989) y los cromosomas son pequeños y de forma parecida. Diversos análisis, en los que se han incluido hasta 11 especies de encinos geográfica y

taxonómicamente distantes, han mostrado un grado sorprendente de similitud interespecífica en el tamaño y organización del genoma. En particular, el número y la posición de las regiones cromosómicas que contienen heterocromatina y genes ribosomales mostraron un alto grado de conservación (Zoldos *et al.*, 1999). Por lo tanto, es posible que una evolución cromosómica particularmente conservadora explique en una buena proporción la facilidad con la que las especies de este género se hibridan. Sin embargo, aún no se han reportado en encinos resultados sobre interacciones a escala cromosómica producto de hibridación e introgresión.

La segunda pregunta es cuál es el impacto que han tenido la hibridación y la introgresión en la evolución de los encinos. En las plantas en general, se ha documentado una serie de posibles consecuencias de estos procesos. En un extremo, se encuentra el debilitamiento de las barreras reproductivas y la fusión entre taxa. En el otro extremo se encuentra la especiación por el reforzamiento de las barreras reproductivas precigóticas (Rieseberg & Wendel, 1993). Otras posibles consecuencias son la transferencia de adaptaciones, el incremento de la variación genética de los taxa, el origen de nuevas combinaciones genéticas sobre las que la selección puede actuar y la aparición y establecimiento de nuevos taxa (Arnold, 1997). En los tratamientos recientes sobre hibridación, se ha hecho énfasis especialmente en este último aspecto (Arnold, 1997; Rieseberg, 1997). Mucha de la evidencia disponible para *Quercus* (incluido este trabajo) sugiere que una situación que ocurre frecuentemente es que los dos taxa progenitores se mantienen distintos (*i.e.* no se fusionan ni dan origen a nuevos taxa), pero aun así mantienen un cierto nivel constante de flujo génico "evolutivamente ajustado". En otras palabras, las consideraciones más recientes apuntan a que la introgresión diferencial regulada por selección exógena es una consecuencia común de la hibridación entre encinos

(Petit *et al.*, 2003b; Dodd & Afzal-Rafii, 2004). En zonas de hibridación entre especies de otros grupos de plantas, como *Populus*, también se han observado marcados patrones de introgresión diferencial, lo que se ha sugerido podría tener valor adaptativo (Martinsen *et al.*, 2001).

Finalmente, es posible que la hibridación represente un mecanismo de dispersión para algunas especies de encinos (Petit *et al.*, 2003b), lo cual ha sido previamente propuesto también para *Eucalyptus* (Potts & Reid, 1988). La idea es esencialmente que una especie determinada puede colonizar un área exclusivamente mediante la dispersión de polen (sin necesidad de dispersión de semillas), si en tal área existe previamente una segunda especie relacionada con la cual la primera pueda hibridarse. De esta forma, la segunda especie sufre una inundación del polen ("pollen swamping") de la primera, lo que provoca sucesivas generaciones de hibridación y retrocruza que gradualmente transforman a la población en individuos de la primera especie. Un aspecto interesante de este modelo es que permite explicar el alto grado con el que se comparten entre especies de encinos los haplotipos del ADNcp (Whittemore & Schaal, 1991; Petit *et al.*, 2002a, b, este estudio). Si existen distintos haplotipos en ambas especies, tras la "inundación por polen", se obtendrá una población de la primera especie que poseerá el ADNcp de la segunda, puesto que no ha ocurrido movimiento de semillas (Petit *et al.*, 2003b).

REFERENCIAS GENERALES

Aguilar, J.F., J.A. Rosello, y G.N. Feliner. 1999. Molecular evidence for the compilospecies model of reticulate evolution in *Armeria* (Plumbaginaceae). *Systematic Biology* 48: 735–754.

Anderson, E. 1948. Hybridization of the habitat. *Evolution* 2:1-9.

Anderson, E. 1949. *Introgressive hybridization*. Wiley, NY.

APG II. 2003. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants. *Botanical Journal of the Linnean Society* 141: 399–436.

Arnold, J. 1993. Cytonuclear disequilibria in hybrid zones. *Annual Review of Ecology and Systematics* 24: 521-554.

Arnold, M.L. 1992. Natural hybridization as an evolutionary process. *Annual Review of Ecology and Systematics* 23: 237-261.

Arnold, M.L. 1997. Natural Hibridization and Evolution. Oxford University Press, New York.

Asmussen, M.A., J. Arnold, y J.C. Avise. 1987. Definition and properties of disequilibrium statistics for associations between nuclear and cytoplasmic genotypes. *Genetics* 115: 755-768.

Axelrod, D.L. 1983. Biogeography of oaks in the Arcto-Tertiary province. *Annals of the Missouri Botanical Garden* 70: 629-657.

Bacilieri, R., A. Ducousso, R. J. Petit, y A. Kremer. 1996. Mating system and asymmetric hybridization in a mixed stand of european oaks. *Evolution* 50: 900-908.

Bain, J.F. y R.K. Jansen. 1996. Numerous chloroplast DNA polymorphisms are shared among different populations and species in the aureoid *Senecio* (*Packera*) complex. *Canadian Journal of Botany* 74: 1719–1728.

Barton, N.H. 2001. The role of hybridization in evolution. *Molecular Ecology* 10: 551–568.

Barton, N.H. y K.S. Gale. 1993. Genetic analysis of hybrid zones. *En*: R.G. Harrison, ed. *Hybrid zones and the Evolutionary Process*. Oxford University Press.

Barton, N.H., y G.M. Hewitt. 1985. Analysis of hybrid zones. *Annual Review of Ecology and Systematics* 16: 113-148

Barton, N.H., y G.M. Hewitt. 1989. Adaptation, speciation and hybrid zones. *Nature* 341: 497-503.

Beckstrom-Sternberg, S.M., L.H. Rieseberg, y K. Doan. 1991. Gene lineage analysis in populations of *Helianthus niveus* and *H. petiolaris* (Asteraceae). *Plant Systematics and Evolution* 175: 125-138.

Belahbib, N., M.H. Pemonge, A. Ouassou, H. Sbay, A. Kremer y R.J. Petit. 2001. Frequent cytoplasmic exchanges between oak species that are not frequently related: *Quercus suber* and *Q. ilex* in Morocco. *Molecular Ecology* 10: 2003–2012.

Borgardt, S.J., y K.B. Pigg. 1999. Anatomical and developmental study of petrified *Quercus* (Fagaceae) fruits from the Middle Miocene, Yakima Canyon, Washington, USA. *American Journal of Botany* 86: 307-325.

Bullini, L. 1994. Origin and evolution of animal hybrid species. *Trends in Ecology and Evolution* 7: 422-426.

Burger, W.C. 1975. The species concept in *Quercus*. *Taxon* 24: 45-50.

Burke, J.M., M.R. Bulger, R. A. Wesselingh, y M.L. Arnold. 2000. Frequency and spatial pattering of clonal reproduction in Louisiana iris hybrid populations. *Evolution* 54: 137-144.

Byrne, M., B. Macdonald y D. Coates. 2002. Phylogeographical patterns in chlorplast DNA variation within the *Acacia acuminata* (Leguminosae: Mimosoideae) complex in Western Australia. *Journal of Evolutionary Biology* 15: 576–587.

Cannon, C.H. y P.S. Manos. 2003. Phylogeography of the Southeast Asian stone oaks (*Lithocarpus*). *Journal of Biogeography* 30: 211–226.

Carney, S.E., K.A. Gardner, y L.H. Rieseberg. 2000. Evolutionary changes over the fifty-year history of a hybrid population of sunflowers (Helianthus). *Evolution* 54: 462–474.

Cruzan, M.B., y M.L. Arnold. 1993. Ecological and genetic associations in an *Iris* hybrid zone. *Evolution* 47: 1432-1445.

Cruzan, M.B., y M.L. Arnold. 1994. Assortative mating and natural selection in an *Iris* hybrid zone. *Evolution* 48: 1946-1958.

Daghlian, C.P., y W.L. Crepet. 1983. Oak catkins, leaves and fruits from the Oligocene Catahoula Formation and their evolutionary significance. *American Journal of Botany* 70: 639-649.

Darwin, C. 1859. On the origin of species by means of natural selection. 1st ed. Murray, London.

dePamphilis. C.W. y R. Wyatt. 1990. Electrophoretic confirmation of interespecific hybridization in *Aesculus* (Hippocastanaceae) and the genetic structure of a broad hybrid zone. *Evolution* 44: 1295-1317.

Dobzhansky, T. 1940. Speciation as a stage in evolutionary divergence. *American Naturalist* 74: 312-321.

Dodd, R.S., y Z. Afzal-Rafii. 2004. Selection and dispersal in a multispecies oak hybrid zone. *Evolution* 58: 261–269.

Dorado, O., L.H. Rieseberg, y D.M. Arias. 1992. Chloroplast DNA introgression in southern California sunflowers. *Evolution* 46: 566-572.

Dowling, T.E., y C.L. Secor. 1997. The role of hybridization and introgression in the diversification of animals. *Annual Review of Ecology and Systematics* 28: 593-619.

Dumolin-Lapègue, S., A. Kremer, y R.J. Petit. 1999. Are chloroplast and mitochondrial DNA variation species independent in oaks? *Evolution* 53: 1406-1413.

Endler, J.A. 1977. *Geographic variation, speciation and clines*. Princeton University Press, NJ.

Ennos, R.A., W.T. Sinclair, X.-S. Hu y A. Langdon. 1999. Using organelle markers to elucidate the history, ecology and evolution of plant populations. En: P.M. Hollingsworth, R.M. Bateman y R.J. Gornall, eds. *Molecular Systematics and Plant Evolution*. Taylor & Francis Ltd., Londres.

Freeman, D.C., J.H. Graham, D.W. Byrd, E.D. McArthur, y W.A. Turner. 1995. Narrow hybrid zone between two subspecies of big sagebrush, *Artemisa tridentata* (Asteraceae). III. Developmental instability. *American Journal of Botany* 82: 114-1152.

Golden, J.L. y J.F. Bain. 2000. Phylogeographic patterns and high levels of chloroplast DNA diversity in four Packera (Asteraceae) species in southwestern Alberta. *Evolution* 54: 1566–1579.

González-Rivera, R. 1993. La diversidad de los encinos mexicanos. *Revista de la Sociedad Mexicana de Historia Natural* Vol. Esp. (XLIV): 125-142.

Graham, A. 1999. The Tertiary history of the northern temperate element in the northern Latin America biota. *American Journal of Botany* 86: 32–38.

Graham, J.H., D.C. Freeman, y E.D. McArthur. 1995. Narrow hybrid zone between two subspecies of big sagebrush (*Artemisia tridentata*: Asteraceae).II. Selection gradients and hybrid fitness. *Americam Journal of Botany* 82: 709-716.

Grant, V. 1981. *Plant speciation*. Columbia University Press, NY.

Hardig, T.M., S.J. Brunsfeld, R.S. Fritz, M.Morgan, y M. Orians. 2000. Morphological evidence for hybridization and introgression in a willow (*Salix*) hybrid zone. *Molecular Ecology* 9: 9-24.

Hardin, J.W. 1975. Hybridization and introgression in *Quercus alba*. *Journal of the Arnold Arboretum* 56: 336-363.

Harlan, J., y J.M.J. de Wet. 1963. The compilospecies concept. *Evolution* 17: 497–501.

Harrison, R.G. 1990. Hybrid zones: windows on evolutionary process. *En*: D. Futuyma y J. Antonovics, eds. *Oxford Surveys in Evolutionary Biology* 7: 69-128.

Harrison, R.G. 1993. Hybrids and hybrid zones: Historical Perspective. *En*: R.G. Harrison, ed. *Hybrid zones and the Evolutionary Process*. Oxford University Press.

Harrison, R.G., y D.M. Rand. 1989. Mosaic hybrid zones and the nature of species boundaries. *En*: D. Otte y J. Endler, eds. *Speciation and its consequences*. Sinauer Associates, Sunderland, Massachusetts.

Heiser, C.B. 1973. Introgression re-examined. *The Botanical Review* 39: 347-366.

Hewitt, G.M. 1988. Hybrid zones –natural laboratories for evolutionary studies. *Trends in Ecology and Evolution* 3: 158-167.

Hewitt, G.M. 1989. The subdivision of species by hybrid zones. *En*: D. Otte y J. Endler, eds. *Speciation and its consequences*. Sinauer Associates, Sunderland, Massachusetts.

Hewitt, G.M. 2004. Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London series B* 359: 183–195.

Hewitt, G.M., y N.H. Barton. 1980. The structure and maintenance of hybrid zones as exemplified by *Podisma pedestris*. *En*: R. Blackman, G.M. Hewitt, y M. Ashburner, eds. *Insect cytogenetics* (Royal Entomological Society of London Symposia 10). Blackwell, Oxford.

Hewitt, G.M., y N.H. Barton. 1980. The structure and maintenance of hybrid zones as exemplified by *Podisma pedestris*. *En*: R. Blackman, G.M. Hewitt, y M. Ashburner (eds.). *Insect cytogenetics* (Royal Entomological Society of London Symposia 10). Blackwell, Oxford.

Heywood, J.S. 1986. Clinal variation associated with edaphic ecotones in hybrid populations of *Gaillardia pulchella*. *Evolution* 40: 1132-1140.

Holman, J.E., J.M. Hughes y R.J. Fensham. 2003. A morphological cline in *Eucalyptus*: a genetic perspective. *Molecular Ecology* 12: 3013–3025.

Howard, D.J. 1993. Reinforcement: origin, dynamics and fate of an evolutionary hypothesis. *En*: R.G. Harrison, ed. *Hybrid zones and the Evolutionary Process*. Oxford University Press.

Howard, D.J., R.W. Preszler, J. Williams, S. Fenchel y W. J. Boecklen. 1997. How discrete are oak species? Insights from a hybrid zone between *Quercus grisea* and *Quercus gambelli*. *Evolution* 51: 747-755.

Humboldt, A., y A. Bonpland. 1809. Voyage de Humboldt et Bonpland. Sixieme Partie. Botanique. Pl. Aequinoct 2: 24-58.

Jansen, E., T. Fronval, F. Rack y J.E.T. Channell. 2000. Pliocene-Pleistocene ice rafting history and cyclicity in the Nordic Seas during the last 3.5 Myr. *Paleocenagraphy* 15: 709-721.

Jones, J.H. 1986. Evolution of the Fagaceae: the implications of foliar features. *Annals of the Missouri Botanical Garden* 73: 228-275.

Kaul, R.B. 1985. Reproductive morphology of *Quercus* (Fagaceae). *American Journal of Botany* 72: 1962-1977.

Kaul, R.B. 1986. Evolution and reproductive biology of inflorescences in *Lithocarpus*, *Castanopsis*, *Castanea*, and *Quercus* (Fagaceae). *Annals of the Missouri Botanical Garden* 73: 284-296.

Key, K.H.L. 1968. The concept of stasipatric speciation. *Systematic Zoology* 17: 14-22.

Lascoux, M., A.E. Palmé, R. Cheddadi, y R.G. Latta. 2004. Impact of ice ages on the genetic structure of trees and shrubs. *Philosophical Transactions of the Royal Society of London series B* 359: 197–207.

Manos, P.S. y A.M. Stanford. 2001. The biogeography of Fagaceae: tracking the Tertiary history of temperate and subtropical forests of the Northern Hemisphere. *International Journal of Plant Sciences* 162(suppl): S77–S93.

Manos, P.S., J.J. Doyle, y K.C. Nixon. 1999. Phylogeny, biogeography, and processes of molecular differentiation in *Quercus* subgenus *Quercus* (Fagaceae). *Molecular Phylogenetics and Evolution* 12: 333-349.

Manos, P.S., Z.-K. Zhou y C.H. Cannon. 2001. Systematics of Fagaceae: phylogenetic tests of reproductive trait evolution. *International Journal of Plant Sciences* 162: 1361–1379.

Martin, P.S., y B.E. Harrell. 1957. The Pleistocene history of temperate biotas in Mexico and eastern United States. *Ecology* 38: 468-480.

Martínez-Hernández, E. 1992. La vegetación y el clima de México. *Ciencia* 43: 69-72.

Martinsen, G.D., T.G. Whitham, R.J. Turek y P. Keim. 2001. Hybrid populations selectively filter gene introgression between species. *Evolution* 55: 1325–1335.

Mayr, E. 1942. *Systematics and the Origin of Species*. Columbia University Press, New York.

Mayr, E. 1963. *Animal Species and Evolution*. Belknap Press, Cambridge, MA.

McCauley, D.E. 1995. The use of chloroplast DNA polymorphism in studies of gene flow in plants. *Trends in Ecology and Evolution* 10: 198–202.

McKinnon, G.E., G.J. Jordan, R.E. Vaillancourt, D.A. Steane y B.M. Potts. 2004. Glacial refugia and reticulate evolution: the case of the Tasmanian eucalypts. *Philosophical Transactions of the Royal Society of London series B* 359: 275–284.

McKinnon, G.E., R.E. Vaillancourt, H.D. Jackson y B.M. Potts. 2001. Chloroplast sharing in the Tasmanian Eucalypts. *Evolution* 55: 703–711.

Moore, W.S. 1977. An evaluation of narrow hybrid zones in vertebrates. *Quaterly Review of Biology* 52: 263-277.

Muller, C.H. 1952. Ecological control of hybridization in *Quercus*: A factor in the mechanism of evolution. *Evolution* 6: 147-161.

Nixon, K.C. 1989. Origins of Fagaceae. *En*: P.R. Crane y S. Blackmore (eds.) *Evolution, Systematics, and Fossil History of the Hamamelidae. Vol. 2: "Higher" Hamamelidae*, pp. 23-44. Clarendon, Oxford.

Nixon, K.C. 1993. Infrageneric classification of *Quercus* (Fagaceae) and typification of sectional names. *Annales des Sciences Forestieres* 50 (supplement 1): 255-345.

Nixon, K.C. 1997. Fagaceae. *En*: Flora of North America Editorial Committee (ed.), *Flora of North America north of Mexico* 3: 436-506. Oxford University Press, New York, NY.

Nixon, K.C., y Q.D. Wheeler. 1990. An amplification of the phylogenetic species concept. *Cladistics* 6: 211-223.

Paige, K.N., W.C. Capman, y P. Jenneten. 1991. Mitochondrial inheritance across a cottonwood hybrid zone: citonuclear disequilibria and hybrid zone dynamics. *Evolution* 45: 1360-1369.

Petit, R.J., C. Bodénès, A. Ducousso, G. Roussel, y A. Kremer. 2003b. Hybridization as a mechanism of invasion in oaks. *New Phytologist* 161: 151–164.

Petit, R.J., I. Aguinagalde, J.-L. de Beaulieu, *et al.* 2003a. Glacial refugia: hotspots but not melting pots of genetic diversity. *Science* 300: 1563–1565.

Petit, R.J., S. Brewer, S. Bordács, *et al.* 2002a. Identification of refugia and post-glacial colonization routes of European white oaks based on chloroplast DNA and fossil pollen evidence. *Forest Ecology and Management* 156: 49–74.

Petit, R.J., U.M. Csaikl, S. Bordács, et al., 2002b. Chloroplast DNA variation in European white oaks: phylogeography and patterns of diversity based on data from over 2600 populations. *Forest Ecology and Management* 156: 5–26.

Potts, B.M., y J.B. Reid. 1988. Hybridization as a dispersal mechanism. *Evolution* 42: 1245–1255.

Rand, D.M., y R.G. Harrison. 1989. Ecological genetics of a mosaic hybrid zone: mitochondrial, nuclear, and reproductive differentiation of crickets by soil type. *Evolution* 43: 432-449.

Rieseberg, L.H. 1991. Homoploid reticulate evolution in *Helianthus* (Asteraceae): evidence from ribosomal genes. *American Journal of Botany* 78: 1218-1237.

Rieseberg, L.H. 1995. The role of hybridization in evolution: old wine in new skins. *American Journal of Botany* 82: 944-953.

Rieseberg, L.H. 1997. Hybrid origins of plant species. *Annual Review of Ecology and Systematics* 28: 359-389.

Rieseberg, L.H. y J.F. Wendel. 1993. Introgression and its consequences in plants. *En*: R.G. Harrison, ed. *Hybrid zones and the Evolutionary Process*. Oxford University Press.

Rieseberg, L.H., C.R. Linder, y G.J. Seiler. 1995. Chromosomal and genic barriers to introgression in *Helianthus*. *Genetics* 141: 1163–1171.

Rieseberg, L.H., J. Whitton, y C.R. Linder. 1996. Molecular marker incongruence in plant hybrid zones and phylogenetic trees. *Acta Botanica of the Netherlands* 45: 243-262.

Rieseberg, L.H., S. J.E. Baird, y A.M. Desrochers. 1998. Patterns of mating in wild sunflowers hybrid zones. *Evolution* 52: 713-726.

Rieseberg, L.H., y D.E. Soltis. 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evolutionary Trends in Plants* 5:65-84.

Rieseberg, L.H., y N.C. Ellstrand. 1993. What can molecular and morphological markers tell us about plant hybridization? *Critical Reviews in Plant Sciences* 12: 213-241.

Saintagne C., C. Bodénès, T. Barreneche, D. Pot, C. Plomion, y A. Kremer. 2004. Distribution of genomic regions differentiating oak species assessed by QTL detection. *Heredity* 92: 20–30.

Sang, T., D.J. Crawford y T.F. Stuessy. 1995. Documentation of reticulate evolution in peonies (*Paeonia*) using internal transcribed spacer sequences of nuclear ribosomal DNA: Implications for biogeography and concerted evolution. *Proceedings of the National Academy of Sciences USA* 92: 6813-6817.

Scheidweiler, M. 1837. Chênes de Mexique. L' Horticulteur Belge. Bruselas, Bélgica. 4: 321.

Shoemaker, D., K.G. Ross, y M.L. Arnold. 1996. Genetic structure and evolution of a fire ant hybrid zone. *Evolution* 50:1958-1976.

Stebbins, G.L. 1950. *Variation and evolution in plants*. Columbia University Press, NY.

Szymura, J.M., y N.H. Barton. 1986. Genetic analysis of a hybrid zone between the fire-bellied toads, *Bombina bombina* and *Bombina variegata*, near Cracow in southern Poland. *Evolution* 40: 1141-1159.

Taberlet, P., L. Fumagalli, A.G. Wust-Saucy, J.-F. Cossons. 1998. Comparative phylogeography and postglacial colonization routes in Europe. *Molecular Ecology* 7: 453–464.

Trelease, W. 1924. The American Oaks. *Memories of the National Academy of Sciences* 20: 1-255.

Valencia, S. 1994. Contribución a la delimitación taxonómica de tres especies del género *Quercus* subgénero *Erythrobalanus*. Tesis de Maestría, Facultad de Ciencias, UNAM.

Van Valen, L. 1976. Ecological species, multispecies and oaks. *Taxon* 25: 233-239.

Villani, F., A. Sansotta, M. Cherubini, D. Cesaron, y V. Sbordoni. 1999. Genetic structure of natural populations of *Castanea sativa*: evidence of a hybrid zone. *Journal of Evolutionary Biology* 12:233-244.

Wang, H., E.D. McArthur, S.C. Sanderson, J.H. Graham, y D.C. Freeman. 1997. Narrow hybrid zone between two subspecies of big sagebrush (*Artemisia tridentata*: Asteraceae).IV. Reciprocal transplant experiments. *Evolution* 51: 95-102.

Watano, Y., A. Kanai, y N. Tani. 2004. Genetic structure of hybrid zones between *Pinus pumila* and *P. parviflora* var. *pentaphylla* (Pinaceae) revealed by molecular hybrid index analysis. *American Journal of Botany* 91: 65–72.

Wendel, J.F., Stewart, J. McD., y J.H. Rettig. 1991. Molecular evidence for homoploid reticulate evolution among Australian species of *Gossuypium*. *Evolution* 45: 694-711.

White, J.M., T.A. Ager, D.P. Adam, E.B. Leopold, G. Liu, H. Jetté y C.E. Schweger. 1997. An 18 million year record of vegetation and climate change in northwestern Canada and Alaska: tectonic and global climatic correlates. *Paleogeography, Paleoclimatology, Paleoecology* 130: 293–306.

Whittemore, A.T. y B.A. Schaal. 1991. Interespecific gene flow in sympatric oaks. *Proceedings of the National Academy of Sciences USA* 88: 2540-2544.

Wu, C–I. 2001. The genic view of the process of speciation. *Journal of Evolutionary Biology* 14: 851–865.

Wu, C–I., y C–T. Ting. 2004. Genes and speciation. *Nature Reviews* (Genetics) 5: 114–122.

Young, N.D. 1996. Concordance and discordance: A tale of two hybrid zones in the Pacific Coast Irises (Iridaceae). *American Journal of Botany* 83: 1623-1629.

Zavala-Chávez, F. 1998. Observaciones sobre la distribución de encinos en México. *Polibotánica* 8: 47-64.

Zoldos V., S. Siljak-Yakovlev, D. Papes, A. Sarr, y O. Panaud. 2001. Representational difference analysis reveals genomic differences between *Q. robur* and *Q. suber*: implications for the study of genome evolution in the genus *Quercus*. *Molecular Genetics and Genomics* 265: 234–241.

Zoldos, V., D. Papes, M. Cerbah, O. Panaud, V. Besendorfer, y S. Siljak-Yakovlev. 1999. Molecular-cytogenetics studies of ribosomal genes and heterochromatin reveal conserved genome organization among 11 *Quercus* species. *Theoretical and Applied Genetics* 99: 969–977.