UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO

POSGRADO EN CIENCIAS BIOLOGICAS
CENTRO DE INVESTIGACIONES EN ECOSISTEMAS

"EFECTOS DE LA HIBRIDACION DEL COMPLEJO QUERCUS CRASSIFOLIA X QUERCUS CRASSIPES SOBRE LAS COMUNIDADES DE INSECTOS FORMADORES DE AGALLAS."

TESIS

QUE PARA OBTENER EL GRADO ACADEMICO DE

DOCTOR EN CIENCIAS

PRESENTA

M. en C. EFRAIN TOVAR SANCHEZ

DIRECTOR DE TESIS: DR. ALBERTO KEN OYAMA NAKAGAWA

MEXICO, D. F. SEPTIEMBRE, 2004

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AGRADECIMIENTOS

Deseo hacer un fuerte agradecimiento a los miembros de mi comité tutoral Dres. Ken Oyama, Zenón Cano y Daniel Piñero por guiarme durante el desarrollo de la tesis. Así como a sus comentarios certeros y a su atenta disponibilidad en todo momento.

En particular quiero agradecer a los miembros del jurado, Dres. Ken Oyama, Zenón Cano, Daniel Piñero, Jorge Meave, Patricia Dávila, Dulce María Arias y Mauricio Quesada por la cuidadosa revisión de esta tesis.

Agradezco al Consejo Nacional de Ciencia y Tecnología (CONACyT), así como a la Dirección General de Estudios de Posgrado (DGEP) por otorgarme una beca de doctorado.

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Tovar-Sánchez, E. 2004. **Efecto de la hibridación del complejo *Quercus crassifolia* × *Q. crassipes* sobre las comunidades de insectos formadores de agallas.** Tesis de Doctorado. Posgrado en Ciencias Biológicas. Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México, México.

RESUMEN

México es considerado un centro de riqueza de especies del género *Quercus* ya que contiene 90% de las 150 especies que existen en el continente americano, de las cuales 86 son consideradas endémicas. *Quercus crassifolia* H. & B. y *Q. crassipes* H. & B. (Subg. *Erythrobalanus*) son dos elementos dominantes de los bosques templados de México y cuando estas especies se encuentran en simpatría se han detectado individuos con características morfológicas intermedias, los cuales han sido denominados como *Q. dysophylla* Benth. La integración de diversas herramientas en este estudio permitió: 1) evaluar si *Q. dysophylla* es el resultado de la hibridación entre *Q. crassifolia* y *Q. crassipes*, 2) reconocer si la proximidad geográfica de una planta híbrida a un sitio alopátrico de una especie parental incrementa su similitud morfológica y genética con su parental, 3) determinar la diversidad y la estructura genética de *Q. crassifolia*, *Q. crassipes* y poblaciones híbridas así como caracterizar la dinámica de la hibridación y la introgresión, 4) conocer la distribución de la diversidad de haplotipos a lo largo de su distribución geográfica para inferir los patrones históricos de dispersión y colonización, 5) analizar la estructura de la comunidad de insectos endófagos asociados al complejo para evaluar si los híbridos soportan menos fuerzas adaptativas para el origen de nuevas especies, y 6) evaluar la estructura de la comunidad de los artrópodos asociados al dosel. Se analizaron siete zonas mixtas con presencia de ambas especies distribuidas sobre el Eje Neovolcánico y la región más sureña de la Sierra Madre Oriental (SMOr) (10 árboles por taxon en cada zona), así como dos sitios alopátricos para cada especie parental (20 árboles por sitio). Para la evaluación de artrópodos del dosel se eligió otra zona híbrida (Canalejas, México) donde se fumigó el dosel de 30 árboles (10 árboles por taxon). Un total de 17 caracteres morfológicos fueron medidos en 8700 hojas provenientes de 290 árboles y también se analizó el ADN de 250 individuos con seis primers diagnósticos de RAPD. Los marcadores moleculares y los caracteres morfológicos indican que *Q. dysophylla* es un híbrido producido por *Q. crassifolia* y *Q. crassipes*; sin embargo, ambas especies se mantienen diferentes en las zonas de contacto. La cercanía de una planta híbrida a un sitio alopátrico de una especie parental incrementa la similitud morfológica y genética con su especie parental. Los genotipos de tres loci de microsatélites de cloroplasto sugieren que muchos alelos son compartidos entre las dos especies y que cada especie contiene alelos únicos. Un análisis bayesiano de la estructura genética en siete zonas mixtas identificó dos grupos genéticos altamente diferenciados que corresponden a *Q. crassifolia* y *Q. crassipes*. Los datos correspondientes a los tres loci fueron suficientes para detectar eventos de hibridación, retrocruza e introgresión. La introgresión es bidireccional y las poblaciones híbridas registraron los mayores valores de variación genética (H), diversidad genética (I) y número de haplotipos en comparación con sus parentales putativos. Además, las zonas híbridas mostraron altos niveles de diferenciación genética (F_{ST} y R_{ST}) y número de migrantes (Nm). Asimismo, se encontró que *Q. crassipes* se ha dispersado de la SMOr al Eje Neovolcánico, mientras que *Q. crassifolia* lo hizo de la Sierra Madre Occidental hacia el Eje Neovolcánico. En total, se reconocieron 35 taxa de insectos endófagos asociados al complejo *Q. crassifolia* × *Q. crassipes*. Las plantas híbridas presentan niveles intermedios de infestación, tanto de insectos formadores de agallas como de insectos minadores en relación con sus parentales. Los niveles de infestación de insectos minadores exhiben un creciente gradiente unidireccional hacia *Q. crassifolia*, mientras que los insectos agalleros muestran el mismo gradiente hacia *Q. crassipes*. En cada taxon se registró una correlación negativa y significativa entre la abundancia de los insectos minadores versus formadores de agallas. La proximidad geográfica de las plantas híbridas a un sitio alopátrico de una especie parental incrementa su similitud de insectos endófagos y el Eje Neovolcánico actúa como un corredor favoreciendo este patrón. En total, fueron reconocidas 352 taxa de artrópodos pertenecientes a 22 órdenes asociados al dosel del complejo. El estatus taxonómico de la planta hospedera mostró ser un importante factor en la estructuración de las comunidades de artrópodos. Sin embargo, la estacionalidad mostró no ser un factor que modifique la organización de la comunidad. Los resultados indican que los híbridos actúan como centros de diversidad, acumulando artrópodos de ambas especies parentales así como diferentes especies, incluyendo muchas especies raras.

ABSTRACT

Mexico is considered as a center of species richness for the *Quercus* genus, because it contains 90% of the 150 species that exist in the American Continent, out of which, 86 are considered endemic. *Q. crassifolia* H. & B. and *Q. crassipes* H. & B. (Subg. *Erythrobalanus*) are two dominant elements of temperate forests in Mexico, forming individuals with intermediate morphological characteristics when they occur in sympatry, (*Q. dysophylla*). Different methodological tools used for this study allowed us to: 1) evaluate if *Q. dysophylla* is the results of the hybridization process between *Q. crassifolia* and *Q. crassipes*, 2) if the geographical proximity of the hybrid plant to an allopatric site of a parental species, increases its genetic and morphological similarity with its parental, 3) determine the diversity and genetic structure of *Q. crassifolia, Q. crassipes* and of hybrid populations, as well as, to characterize introgression and hybridization dynamics, 4) know the distribution of haplotype diversity along their geographical distribution, in order to infer historic patterns of dispersal and colonization, 5) analyze the community structure of endophagous insects associated to the complex, to evaluate, if the hybrid supports less adaptive forces for the origin of new species, and 6) evaluate the community structure of canopy arthropods. Seven hybrid zones distributed on the Eje Neovolcánico and on the southest part of the Sierra Madre Oriental (10 trees per taxon in each zone) and two allopatric sites for each parental species (20 trees per site) were analyzed. Another hybrid zone (Canalejas, Mexico) for canopy arthropod evaluation was chosen, where the canopy of 30 trees was fogged (10 trees per taxon). A total of 17 morphological characters were measured of 8700 leaves belonging to 290 trees and the DNA of 250 individuals was analyzed with six diagnostics RAPD primers. Molecular and morphological markers indicated that *Q. dysophylla* is an hybrid produced by *Q. crassifolia* and *Q. crassipes* although both species remain distinct from the contact zones. The nearness of an hybrid plant to an allopatric site of a parental species increases the genetic and morphological similarity with its parental. Genotype of three cpSSR loci suggested that many alleles are shared between both species and that each species contain unique alleles. A bayesian analysis for the genetic structure on seven hybrid zones identify two genetic and highly differentiated clusters, that correspond to *Q. crassifolia* and *Q. crassipes*. Data corresponding to the three loci were sufficient to detect hybridization, backcrosses and introgression events. Introgression was bidirectional and hybrid populations registered the highest variation values (H), genetic diversity (I) and number of haplotypes in comparison with its putative parentals. Also, hybrid zones showed high levels of genetic differentiation (F_{TS} and R_{ST}), and migrant number (Nm). Also, we found that *Q. crassipes* has been dispersed from the Sierra Madre Oriental to the Eje Neovolcánico while *Q. crassifolia* has been dispersed from the Sierra Madre Occidental to the Eje Neovolcánico. In total, 35 endophagous insect taxa associated to the complex were recognized. Hybrid plants presented intermediate infestation levels of gall forming and mining insects, in relation to their parentals. Infestation levels of mining insects exhibited an increasing and unidirectional gradient towards *Q. crassifolia*. In each taxon a negative and significant correlation between mining insects abundance vs. gall forming insects was registered. The geographic proximity of hybrid plants to the allopatric site of a parental species increase its similarity of endophagous insects and the Eje Neovolcánico acts as a corridor favoring this pattern. In total 352 arthropod taxa, belonging to 22 orders associated to the complex were recognized. The taxonomic status of the host plant resulted to be an important factor on arthropod community structuring and the seasonality was not a modifying factor of the community organization. The results indicated that hybrid individuals act as centers of diversity by accumulating arthropods of both parental and different species including a considerable number of rare species.

I. INTRODUCCIÓN

Hibridación y zonas híbridas

La hibridación es un fenómeno natural que se presenta frecuentemente en plantas y raramente en animales (Harrison, 1993), y puede definirse como la cruza entre individuos de dos poblaciones, o grupos de poblaciones los cuales se pueden distinguir por uno o más caracteres heredados (Arnold, 1997). La hibridación puede tener un impacto en los procesos de evolución en al menos tres vías (Stebbins, 1959). Primero, debido a que la hibridación es una forma de recombinación genética, ésta puede generar genotipos nuevos que pueden exhibir heterosis, principalmente en hábitats nuevos que son generalmente los bordes de la distribución de las especies parentales y donde las zonas híbridas se forman. Segundo, la estabilidad de zonas híbridas (i. e., a través de poliploidía) puede resultar en la formación de nuevas especies biológicas. Finalmente, si los híbridos forman retrocruzas con especies parentales, entonces, el flujo génico estaría atravesando las barreras específicas de la especie (introgresión).

Es frecuente encontrar en la naturaleza *zonas híbridas*, las cuales se han definido como áreas de contacto donde poblaciones genéticamente distintas se encuentran, se cruzan y producen híbridos viables o por lo menos parcialmente fértiles (Barton y Hewitt, 1985; Ridley, 1996; Arnold, 1997). Las zonas híbridas han sido reconocidas mediante diferencias morfológicas (Rieseberg y Ellstrand, 1993; Kleinschmit *et al.*, 1995; Bacon y Spellenberg, 1996; Bruschi *et al.*, 2000; Hardig *et al.*, 2000; González *et al.*, 2004), anatómicas (Hillson, 1963; Webb y Carlquist, 1964; Stuessy, 1990), citogenéticas (Hunt y Selander, 1973; Moran *et al.*, 1980), palinológicas (Hauser y Morrison, 1964; Emboden, 1969; Graham y Tomb, 1974; Skvarla *et al.*, 1988), etológicas (Bull, 1979; Gartside *et al.*, 1979; Sage y Selander, 1979; Littlejohn y Watson, 1983; Gollmann, 1984; Burtlin y Hewitt, 1985), químicas (Zobel, 1951; Turner y

Alston, 1963; Smith y Levin, 1963; Levin, 1966, 1967; Orians y Fritz, 1995), con ayuda de herramientas moleculares como isoenzimas (Pasteur *et al.*, 1988; Murphy *et al.*, 1990; May, 1992; Kleinschmit *et al.*, 1995), RAPD-PCR (Amplificación azarosa de ADN polimórfico) (Arnold *et al.*, 1991; Arnold, 1993; Cruzan y Arnold, 1993; Crawford *et al.*, 1993; Marsolais *et al.*, 1993; Fritz *et al.*, 1994; Kleinschmit *et al.*, 1995; Smith *et al.*, 1996; Samuel, 1999; Hardig *et al.*, 2000; González *et al.*, 2004), PCR-RFLP (Reacción en cadena de la polimerasa para fragmentos de restricción polimórficos) (Behalhbib *et al.*, 2001; Ishida *et al.*, 2003) y microsatélites (Secuencias simples repetidas = SSRs) (Schaal *et al.*, 1991; Rieseberg y Soltis, 1991; Rieseberg, 1995; Bruschi *et al.*, 2000; Craft *et al.*, 2002). Sin embargo, el reconocimiento de las zonas híbridas ha tenido más éxito cuando se ha usado una combinación de estos caracteres.

Las zonas híbridas pueden ser vistas como etapas en un proceso de divergencia de poblaciones hasta el nivel de especie (Hewitt, 1988). Se dice que el proceso de especiación ha llegado a su fin cuando deja de haber flujo génico entre dos formas debido a barreras reproductivas (Mayr, 1942). El establecimiento de estas barreras es un proceso gradual que se debe a la acumulación de cambios genéticos (Szymura y Barton, 1986), y pueden ser clasificadas en *precigóticas* y *postcigóticas* (Futuyma, 1998). En las primeras se encuentra el aislamiento estacional, de hábitat, de temporalidad, de comportamiento, diferencia entre cortejos, así como la diferencia en las estructura genéticas. En las postcigóticas se encuentra la inviabilidad del embrión, del híbrido y la esterilidad híbrida (Mayr, 1963; Dobzhansky, 1970).

Introgresión en plantas

La introgresión es la incorporación de genes de una especie a otra por la retrocruza repetida de los híbridos con las especies parentales (Anderson y Hubricht, 1938; Rieseberg y Soltis, 1991), promoviendo un incremento en la diversidad genética y el desarrollo de nuevas adaptaciones, debido al número de combinaciones genéticas (Raven, 1976; Grant, 1981). Su papel en la evolución de las plantas ha sido tema de discusión desde la publicación de Anderson (1949) "introgressive hybridization", donde se establece que la transferencia interespecífica de genes es una potente fuerza evolutiva, y las variantes genéticas que se producen por introgresión deben exceder a las producidas por mutación. Por tanto, se esperaría una mayor variación genética en las poblaciones híbridas en comparación con sus parentales putativos.

La introgresión puede tener varias consecuencias (Arnold, 1992). Por un lado, puede causar unión o fusión de los individuos híbridos, por lo que algunas políticas conservacionistas no toman en cuenta este tipo de especies ya que se cree que pueden causar "desintegración genética" (O'Brien y Mayr, 1991). Por otro lado, la introgresión puede dar como resultado una selección de cruzas conespecíficas e incrementar el aislamiento reproductivo (Howard, 1986); además, también puede originar genotipos más adecuados, los cuales pueden ser capaces de colonizar nuevos hábitats (Lewontin y Birch, 1966).

La dinámica de la introgresión depende de la estructura genética de la especie, así como del ambiente al que está expuesta. La hibridación introgresiva puede ser bidireccional (simétrica) o unidireccional (asimétrica) y puede resultar en la formación de una nueva especie, la extinción de los híbridos o la extinción de alguna especie parental (Rieseberg *et al.*, 1996).

La importancia de los factores ambientales radica en que las diferencias ecológicas entre parentales putativos usualmente resultan en barreras de hibridación (Anderson, 1948). En contraste, si estas barreras se llegan a romper (p. ej., debido a un disturbio), la hibridación es más propensa a presentarse. En este sentido, se sugiere que el establecimiento de híbridos y su progenie se ven favorecidos en hábitats muy perturbados donde existe una menor competencia de los parentales, tal como puede ocurrir en el caso de la hibridación introgresiva (Arnold *et al.*, 1990; Klier *et al.*, 1991).

La hibridación en encinos

Dentro de la familia Fagaceae, el género *Quercus* (encino, encinas, robles) es el que presenta la más amplia distribución geográfica, la cual comprende las regiones templadas del hemisferio norte, agrupando aproximadamente 531 especies a escala mundial (Govaerts y Frodin, 1998). México es el sitio donde este género alcanza su mayor representatividad (Muller y McVaugh, 1972), por lo que es considerado un importante centro de diversificación de este grupo (Nixon, 1993). En México están presentes 90% de las 150 especies que existen en América, de las cuales 86 son consideradas endémicas (Nixon, 1993). Los encinos presentan una alta frecuencia de hibridación en condiciones naturales (Palmer, 1948; Muller, 1952; Tucker, 1961; McVaugh, 1974; Cottam *et al.*, 1982; Boecklen y Spellenberg, 1990; Whitemore y Schaal, 1991; Rushton, 1993; Preszler y Boecklen, 1994; Spellenberg, 1995; Spellenberg y Bacon, 1996; Howard *et al.*, 1997; González *et al.*, 2004), debido a sus incipientes barreras de flujo génico entre especies (Jensen y Eshbaugh, 1976; Whittemore y Schaal, 1991; Bacilieri *et al.*, 1995; Dumolin *et al.*, 1997), generando complejos patrones de variación morfológica y genética intra e interespecífica (Anderson, 1949; Van Valen, 1976; Rieseberg y Wendel, 1993). Las zonas híbridas son lugares de una alta actividad tanto ecológica como evolutiva, actuando como centros de interacción genética (Nixon, 1993). El dosel de los árboles en zonas híbridas, en particular, constituye un centro de diversidad de nichos debido a que éste puede ser colonizado por organismos que provengan de las especies parentales u otras muy ajenas a las que dieron origen a los híbridos.

Los encinares de México se encuentran en ambientes de climas templados, aunque también se les puede encontrar en zonas calientes, semiáridas y húmedas (Rzedowski, 1978). Están distribuidos desde el nivel del mar hasta 3500 m de altitud, aunque la mayoría se encuentran entre 1500 y 3000 m s.n.m. (Rzedowski, 1978). Flores *et al.* (1971) calculan que en México los encinares ocupaban originalmente 5.5% de la superficie del país y los bosques pino-encino 13.7%. Rzedowski (1978) ha descrito los bosques de encinos de México, según se expone a continuación. La altura de los encinares varía entre 5-30 m, alcanzando en ocasiones alturas mayores. La continuidad del dosel en los bosques de encinos va desde cerrado hasta muy abierto. Los bosques presentan una fenología que oscila desde totalmente caducifolios a totalmente perennifolios. Pueden formar bosques monoespecíficos pero es más frecuente que la dominancia se reparta entre varias especies del género y que compartan su hábitat con otras especies como pinos y oyameles.

En la naturaleza se ha observado que varios grupos de plantas han pasado por procesos de hibridación (Grant, 1981). De los 165 casos reportados de introgresión en plantas superiores, más de 90% corresponden a regiones templadas (Harrison, 1993). En México, las zonas templadas son consideradas como un centro de diversidad de pinos (*Pinus* spp.) y encinos (*Quercus* spp.), dos de los géneros más representativos, económicamente importantes y dominantes del dosel (Challenger, 1998).

Ecología del dosel

El dosel se define como la capa más alta de un bosque, el cual está constituido por hojas y ramas entrecruzadas de los árboles (Lowman y Wittman, 1996). Los estudios del dosel en comunidades de artrópodos incluyen a los organismos que están asociados a las plantas epífitas

(Palacios-Vargas, 1981; Murillo *et al.*, 1983), las ramas y el follaje (Basset *et al.*, 1992) y la materia orgánica muerta (Nadkarni y Longino, 1990).

El dosel de los bosques contiene el más alto porcentaje de organismos sobre la Tierra, así como la mayor biomasa y el mayor volumen fotosintéticamente activo de follaje (Lowman y Wittman, 1996). Para el estudio de las comunidades de artrópodos epífitos del dosel, los árboles conforman un sistema ideal, ya que puede definirse y delimitarse fácilmente (Moran y Southwood, 1982); además, su "apariencia" fomenta su colonización y por ello representan un recurso predecible (Southwood, 1978).

Los estudios sobre el dosel se han visto afectados por la complejidad espacial y temporal del hábitat (Lowman y Wittman, 1996), que incluyen: (a) el uso diferencial del espacio dentro de la copa de los árboles por los organismos del dosel, (b) la heterogeneidad del sustrato, (c) la variabilidad en las clases de edad de las hojas del dosel, (d) la variabilidad del microclima en la interfase atmósfera-dosel, y (e) la alta diversidad de organismos que provoca que muchas especies continúen sin nombrarse o descubrirse.

Estructura de las comunidades de artrópodos

Es importante estudiar los factores que expliquen la estructura de las comunidades, es decir, la variación de la riqueza y la abundancia relativa de los organismos. En la naturaleza ninguna comunidad es idéntica a otra; sin embargo, esto no quiere decir que no se puedan hacer comparaciones para revelar los procesos que se presentan en ellas (Roughgarden y Diamond, 1986). Elton (1927 citado en Diamond y Case, 1986) propuso como una posible explicación de la existencia de la estructura de la comunidad a la membresía limitada, esto es, la posibilidad de que ciertas especies sólo estén presentes en unas comunidades particulares y no en otras. Las causas de la membresía limitada son: (a) el ambiente físico, (b) las limitaciones en la dispersión y (c) la interacción entre especies (Roughgarden y Diamond, 1986).

Las comunidades de artrópodos conforman un sistema complejo de estudio debido principalmente a su alta riqueza específica, su gran abundancia, su pequeño tamaño corporal y la falta de taxónomos especialistas para determinarlos. El Phylum Arthropoda es el más diverso del reino animal, ya que se calcula que este grupo contiene 65% de las 1.7 millones de especies vivas conocidas (Kim, 1993). Recientemente se ha calculado que el número de especies de insectos descritas es de 1'112,000 (Samways, 1994) y Erwin (1988) sugiere que el número de especies de insectos podría situarse en un punto desconocido entre los 30 y 50 millones.

Los artrópodos constituyen un elemento muy importante de los ecosistemas terrestres dado el papel ecológico que juegan, pudiendo actuar como depredadores, polinizadores, parásitos y recicladores de nutrientes y materia orgánica (Samways, 1994).

La estructura de la comunidad de artrópodos del dosel se ha analizado de tres maneras: (1) estudiando las interacciones (Morse *et al.*, 1988; Bassett y Kitching, 1991), (2) analizando la relación entre la riqueza de especies con el número de individuos y la talla corporal (Morse *et al.*, 1988; Bassett y Kitching, 1991), y (3) investigando la estructura de los gremios que constituyen a la comunidad (Stork, 1987).

Los factores que determinan la estructura de la comunidad de artrópodos epífitos son: (a) la variación temporal (Gut *et al.*, 1991; Recher *et al.*, 1996), (b) el origen de la especie vegetal (i. e., si es nativa, introducida o una planta híbrida) (Southwood *et al.*, 1982; Whitham, 1989; Boecklen y Spellenberg, 1990), (c) el tamaño del dosel de los árboles (Mac Arthur y Wilson, 1967; Janzen, 1973; Strong, 1974; Kuris *et al.*, 1980; Moran y Southwood, 1982), (d) la variación espacial en gradientes ambientales, que incluyen las condiciones de la localidad (i.e., altitud, latitud, relieve, etc.) (Elton, 1973; Ohmart *et al.*, 1983; Giller, 1984; Barbosa y Wagner, 1989; Reynolds y Crossley, 1997), (e) la edad del bosque (Ohmart *et al.*, 1983; Showalter, 1995), (f) la composición de especies vegetales (Ohmart *et al.*, 1983; Barbosa y Wagner, 1989), (g) el grado de disturbio (Showalter, 1985; Showalter y Crossley, 1987; Showalter, 1994) y (h) la amplitud de la distribución geográfica de los árboles (Lawton, 1982).

Insectos minadores de hojas y formadores de agallas asociados a los encinos

Los insectos endófagos constituyen un gremio importante que habita el dosel de los árboles; dentro de éste se encuentran los formadores de agallas y los minadores de hojas, los cuales se caracterizan por estar cubiertos por los tejidos de las hojas y alimentarse del tejido mesófilo de éstas (Cornell, 1990).

Los principales órdenes de insectos formadores de agallas son Diptera, Homoptera, Coleoptera e Hymenoptera (Miller y Howard, 1981; Barbosa y Wagner, 1989). Los himenópteros incluyen las avispas de las familias Chalcidae, Symphytae y Cynipidae (Quinlan y Evenhuis, 1980). Estas últimas son las más conocidas como formadoras de agallas por su alta especificidad hacia la planta hospedera (encinos) y el tejido atacado (Ananthakrishan, 1984), así como por el gran número de especies formadoras de agallas que se han reportado sobre encinos (600 especies), las cuales atacan alguna estructura en particular de la planta (Borror y DeLong, 1971).

Por su parte, los órdenes de insectos minadores más importantes son Lepidoptera, Diptera, Hymenoptera y Coleoptera (Claridge y Wilson, 1982), los cuales se caracterizan por la estrecha relación que mantienen con la planta hospedera. En el género *Quercus*, los insectos minadores de hoja del orden Lepidoptera se han caracterizado por ser el grupo dominante y por su grado de especialización (Cornell y Washburn, 1979), siendo las familias mejor representadas Nepticulidae y Gracilariidae (Godfray, 1984).

La especialización hacia un hospedero puede ser una táctica segura y eficiente de alimentación, en la cual la predicción de los recursos alimenticios es muy importante (Schowalter y Crossley, 1998). La estrategia especialista permite a los herbívoros utilizar de manera muy eficiente un recurso no disponible para otros, lo cual puede reducir la competencia (Crawley, 1983; Schowalter, 2000). Conforme se incrementa la relación entre la planta y el insecto, la tendencia hacia la especialización se incrementa. El máximo grado de especialización lo han alcanzado los insectos formadores de agallas, los cuales deben modificar el crecimiento de la planta y vivir dentro de los tejidos de ésta (Ananthakrishnan, 1984). Es claro que la formación de agallas es un paso evolutivo hacia una genética íntimamente más relacionada en la explotación de una especie vegetal (Ananthakrishnan, 1984).

Biología de la interacción agalla-planta

La formación de agallas en las plantas se debe al desarrollo anormal de algún tejido, ocasionado por hipertrofia y/o hiperplasia (Barbosa y Wagner, 1989; Hartley, 1998). La agalla se forma por estímulos químicos que producen los insectos al ovipositar o al alimentarse, ocasionando que la planta reaccione liberando sustancias (p. ej. ácido indolacético) que permiten la formación de ésta (Ananthakrishan, 1984). La interacción planta-fitófago se ha descrito como un "juego evolutivo" donde las plantas, al verse sometidas a una presión selectiva por parte del herbívoro, responden evolutivamente con adaptaciones que disminuyen sus efectos. Por su parte, los fitófagos presentan respuestas que contrarrestan las defensas desarrolladas por las plantas (Denno y McClure, 1983). Por lo anterior, el enorme grado de especialidad que presentan los insectos formadores de agallas hacia sus plantas hospederas, se pudo haber iniciado como un mecanismo de defensa de las plantas para "aislar" al parásito en tiempo y espacio y así obligarlo a desarrollarse dentro de la agalla (Ananthakrishan, 1984). Un posible contra ataque por parte de las plantas sería evitar el encapsulamiento del insecto no formando la agalla (Fernandes y Price, 1988).

El material utilizado para la formación de una agalla proviene totalmente de la planta hospedera, por lo que el herbívoro es el único organismo beneficiado con esta interacción. La agalla le proporciona al insecto alimento, protección contra depredadores, desecación y un sitio óptimo para la reproducción (Barbosa y Wagner, 1989; Harnett y Knapp, 1996).

Las agallas están formadas por varias capas celulares. La que se encuentra en contacto directo con la larva recibe el nombre de capa celular nutritiva, la cual contiene altos contenidos de azúcares y nitrógeno soluble, así como aminoácidos. La segunda capa está formada por células lignificadas. Por último, la capa de células corticales brinda resistencia externa a la agalla (Ananthakrishan, 1984; Barbosa y Wagner, 1989).

Los insectos formadores de agallas pueden modificar el crecimiento de su planta hospedera; esto se ha observado en avispas de la familia Cynipidae, las cuales pueden regular el crecimiento de su planta hospedera (Hartley, 1998), e incluso pueden controlar la composición química de los tejidos que forman la agalla (Fernandes y Price, 1988). Además, se ha reportado que la infestación de agallas puede provocar cambios o pérdidas de elementos químicos necesarios para el crecimiento de los encinos, ocasionando desórdenes en los tejidos del xilema y floema, así como la caída prematura de hojas (Barbosa y Wagner, 1989; Harnett y Knapp, 1996).

Cada forma de agalla representa a una especie diferente de insecto, siempre y cuando se encuentre localizada en un solo órgano de la planta hospedera (Ananthakrishan, 1984; Hartley, 1998). Por lo tanto, la formación de cada agalla representa diferencias en su anatomía y desarrollo morfológico por ser producidas por diferentes grupos de insectos (Bearsley, 1982). Sin embargo, hay insectos como las avispas de la familia Cynipidae que presentan heterogonia o alternancia de generaciones, desarrollando la fase unisexual en las raíces y la bisexual en las yemas de los encinos (*Quercus*), lo cual provoca la existencia de dos formas diferentes de agallas en una misma especie de hospedero (Ananthakrishan, 1984).

Selección de plantas hospederas en zonas híbridas

La abundancia, la riqueza y el alto grado de hibridación en condiciones naturales de los encinares en México, así como la gran riqueza de avispas formadoras de agallas (Cynipidae) e insectos minadores (Lepidoptera: Nepticulidae y Gracilariidae) especializados en algún órgano del dosel de los árboles ofrecen un escenario ideal para estudiar los mecanismos mediante los cuales estos insectos herbívoros adquieren la posibilidad de cambiar de hospedero (Floate y Whitham, 1993).

La probabilidad de que un fitófago especialista cambie de hospedero está determinado en parte por la disponibilidad de variación genética del insecto; para que el cambio ocurra el nuevo hospedero debe estar relacionado filogenéticamente con el hospedero actual (Denno y McClure, 1983). Por lo tanto, el nuevo hospedero debe tener una gama de compuestos nutricionales, secundarios, de defensa, fenológicos, etc. muy similares al del hospedero ancestral.

Recientemente, el cambio de hospedero ha sido explicado en términos de una preadaptación o mutación que requieren los herbívoros para realizar un "salto" y cambiar de una especie hospedera a otra (Floate y Whitham, 1993). En la hipótesis de "*preadaptación*" se sugiere que los herbívoros están preadaptados para cambiar a una nueva especie hospedera, pero no lo hacen debido a que el nuevo hospedero no está presente (Thomas *et al.*, 1987). En contraste, cuando los herbívoros no están preadaptados al hospedero debe ocurrir una o más mutaciones claves para que el herbívoro pueda reconocer un nuevo y mejor hospedero en él (Jermy, 1984).

Floate y Whitham (1993) proponen la hipótesis del "*puente híbrido*" que sugiere que la presencia de una planta híbrida intermediaria facilitaría el cambio de hospedero de los herbívoros de una especie vegetal a otra. Por ejemplo, Keim *et al.* (1989) encontraron que cuando una especie de planta presenta distribución alopátrica (con respecto a un segundo hospedero potencial), actúa como una barrera para el cambio de hospedero del herbívoro. La hipótesis de "*preadaptación*" sugiere que los herbívoros no cambiarán de especie hospedera a menos que la distribución de los hospederos sea simpátrica. Sin embargo, si dos especies sufren hibridación de tal manera que el espacio entre sus distribuciones constituye "*puentes espaciales*" formados por híbridos intermediarios, los herbívoros pueden cambiar a la nueva especie hospedera por medio de estas plantas intermediarias, aunque las especies de plantas parentales mantengan una distribución alopátrica.

El cambio de hospedero puede ser afectado por los patrones de hibridación de las plantas, los cuales determinan qué genotipo híbrido abre el espacio genético entre las especies vegetales. En este sentido, Floate y Whitham (1993) proponen cuatro sistemas hipotéticos. Primero, que no ocurra una hibridación entre las especie parentales. Segundo, si las especies parentales presentan hibridación y producen descendencia F_1 estéril, todos los individuos híbridos tendrán 50% del genoma de cada especie parental. Por lo tanto, la producción de los híbridos F_1 estériles debe facilitar el cambio de hospedero, pero dos grandes huecos genéticos permanecen entre el híbrido y cada una de las especies parentales. Tercero, si las especies parentales presentan hibridación y produce descendencia F_1 fértil, los híbridos presentarán retrocruza (introgresión) unidireccional, es decir, los híbridos se cruzarán sólo con una de las especies parentales, para producir un genotipo de híbridos continuo hacia este parental, pero un gran hueco genético permanece entre los F_1 y la otra especie parental. Este patrón debe facilitar la mitad del camino hacia el cambio de hospedero entre las dos especies, pero el hueco que permanece todavía representa una barrera para el cambio de hospedero en pasos graduales. El cuarto y último sistema explica que, la hibridación entre especies parentales producirá individuos híbridos F_1 fértiles, los cuales se retrocruzarán con las dos especies parentales produciendo un patrón de introgresión bidireccional que resultará en un continuo genético completo entre los híbridos y cada una de las especies parentales. Este continuo genético sin huecos debería facilitar el cambio de hospedero (Floate y Whitham, 1993).

Efecto de la hibridación sobre insectos monófagos

La respuesta de los insectos formadores de agallas y los minadores de hojas a hospederos híbridos ha mostrado resultados variables. Whitham (1989) encontró incrementos significativos en la incidencia y densidad de áfidos (*Pemphigus betae*) formadores de agallas, en hospederos híbridos del complejo *Populus fremontii* × *P. angustifolia*, donde sólo una especie parental sirve como hospedero. A partir de estos resultados Whitham propuso la hipótesis de híbridos como "*resumideros*", la cual establece que los híbridos hospederos son más susceptibles a insectos fitófagos que los hospederos parentales. Por su parte, Aguilar y Boecklen (1992), probaron la hipótesis de Whitham analizando la densidad de insectos minadores y avispas formadoras de agallas de la familia Cynipidae asociados al complejo de *Quercus grisea* x *Q. gambelii.* Sus resultados no apoyan la hipótesis propuesta por Whitham (1989), ya que los hospederos exhibieron un gradiente unidireccional de insectos endófagos, donde los hospederos híbridos soportaron niveles intermedios de fitófagos comparados con las especies parentales. En contraste, Boecklen y Spellenberg (1990), encontraron que las densidades promedio de insectos minadores (Nepticulidae y Gracilariidae) y formadores de agallas (Cynipidae) asociados a dos complejos de encinos (*Quercus coccolobifolia* × *Q. emoryi* y *Q. depressipes* × *Q. rugosa*) eran menores en los árboles híbridos en comparación con las especies parentales.

Los resultados antes mencionados sugieren que los patrones de herbivoría por insectos endófagos (minadores y agalleros) sobre plantas hospederas híbridas son un fenómeno muy complejo. En contraste, es clara la falta de estudios para entender el efecto de la hibridación sobre los mecanismos morfo-fisiológicos que intervienen en la formación de agallas. Además, no hay estudios sobre los cambios fisiológicos en la agalla que provoca la hibridación natural.

JUSTIFICACIÓN

A pesar de la extensa área que en México ocupan los bosques de encinos y de pino-encino, de la alta diversidad de especies del género *Quercus* en el país, de su alto grado de endemismo, del frecuente fenómeno de hibridación que ocurre en este género, de la amplia comunidad de artrópodos asociados al dosel de encinos, la existencia de un gran número de especies de avispas Cynipidae especialistas en la formación de agallas sobre especies del género *Quercus*, así como la controversia que *Q. dysophylla* ha generado sobre si merece o no el estatus de especie, no se han realizado estudios que aborden si *Q. dysophylla* es el resultado del fenómeno de hibridación, así como el efecto que tienen los individuos híbridos sobre la estructura de las comunidades de artrópodos asociados al dosel, por lo que en este trabajo se planteó el siguiente objetivo general.

Determinar el efecto de la hibridación del complejo *Quercus crassifolia* × *Q. crassipes* sobre la composición y la diversidad de los artrópodos asociados al dosel.

Asimismo, se buscó cubrir los siguientes objetivos particulares:

(1) Determinar si *Quercus dysophylla* es un híbrido derivado del flujo genético entre *Q. crassifolia* y *Q. crassipes* mediante caracteres morfológico foliares y marcadores moleculares (RAPDs).

(2) Determinar los niveles de hibridación a lo largo de la distribución geográfica de la zona híbrida con marcadores morfológicos (foliares) y moleculares (RAPDs).

(3) Examinar los niveles de variación genética dentro de *Q. crassifolia*, *Q. crassipes* y sus híbridos, y cuantificar los niveles de introgresión en las zonas híbridas con microsatélites de cloroplasto (cpSSR).

(4) Analizar la estructura genética y la distribución en la diversidad de haplotipos del complejo *Q. crassifolia* × *Q. crassipes* para inferir los patrones históricos de dispersión y colonización.

(5) Determinar la variación en la estructura de la comunidad de insectos endófagos (agalleros y minadores) del dosel entre taxa hospederos (*Q. crassipes*, *Q. crassifolia* e individuos considerados híbridos putativos).

(6) Determinar la variación en la estructura de la comunidad de insectos endófagos (agalleros y minadores) del dosel entre zonas híbridas.

(7) Conocer la variación en la estructura de la comunidad de artrópodos epífitos del dosel entre taxa hospederos.

(8) Evaluar la variación estacional en la estructura de la comunidad de artrópodos epífitos del dosel entre taxa hospederos.

HIPÓTESIS

Las hipótesis que se pusieron a prueba en este trabajo son las siguientes:

(1) Si *Quercus crassifolia* se ha dispersado desde la Sierra Madre Occidental hacia el centro de la República por el Eje Neovolcánico y *Q. crassipes* se ha dispersado desde la Sierra Madre Oriental hacia el Eje Neovolcánico se espera que mientras más cercana esté la planta híbrida de un sitio alopátrico de algún parental más similitud tendrá con éste y viceversa.

(2) Se espera que en temporada de lluvias la estructura de la comunidad de artrópodos epífitos sea más compleja por la formación de nuevo follaje y por los cambios estructurales que crean nuevos microhábitats en comparación con la temporada de sequía.

(3) Se espera que individuos pertenecientes al mismo taxon de encino (*Q. crassifolia*, *Q. crassipes* e individuos considerados híbridos) tengan una mayor semejanza en la estructura de la comunidad de artrópodos del dosel que entre individuos de taxa diferentes.

(4) Dada la estrecha relación entre los encinos y sus especies formadoras de agallas de la familia Cynipidae, se espera que la especie de insecto que ataque a individuos híbridos tengan más cercanía genética con la especie de insecto que ataca a una especie de encino parental que comparta más características genéticas con el híbrido.

(5) Dada la estrecha relación entre los insectos formadores de agallas y las especies de encinos hospederos se espera que exista una alta especificidad entre estos insectos y la especie de encino sobre la que se desarrollan, así como de la estructura vegetal que ataquen.

(6) Se espera que haya una estacionalidad marcada tanto en los niveles de infestación como en la composición de los gremios de estos insectos, lo cual estará estrechamente relacionada con la fenología foliar de los encinos.

SISTEMA DE ESTUDIO

Para este estudio se eligieron dos especies de encinos rojos (subg. *Erythrobalanus*), *Quercus crassifolia* H. & B. y *Q. crassipes* H. & B. Estas especies contienen notables diferencias en sus caracteres morfológico foliares (Romero, 1993) cuando se distribuyen en sitios alopátricos puros. Sin embargo, se observan con frecuencia árboles con características intermedias atípicas en la forma de la hoja cuando están en simpatría, lo que sugiere que el fenómeno de hibridación explica la variación observada. El estatus taxonómico de *Q. dysophylla* continua aún en discusión; algunos autores consideran a estos individuos con morfología intermedia como híbridos formados por *Q. crassifolia* y *Q. crassipes* (K. Nixon y S. Valencia, Universidad de Cornell y UNAM, respectivamente, comunicación personal), mientras que otros la consideran como una especie diferente (Romero, 1993; Zavala, 1995).

Quercus crassifolia y *Q. crassipes* son dos especies de encinos bien estudiados, desde el punto de vista taxonómico (Romero, 1993). Pueden diferenciarse fácilmente gracias a la forma angostamente-elíptica o lanceolada de la hoja, con borde entero y con aristas en el ápice de 3 mm de largo, el tamaño más pequeño y el envés con tomento denso blanco grisáceo en *Q. crassipes* (Romero, 1993). En contraste, *Q. crassifolia* presenta hojas con forma obovadas u oblongo-obovadas, con envés amarillo anaranjado o café, lanoso-tomentoso, el borde a veces entero, ondulado o dentado puede presentar de 1-10 aristas. *Q. crassifolia* presenta una amplia distribución geográfica que abarca desde la Sierra Madre Occidental, la Sierra Madre Oriental, la Sierra Madre del Sur y el Eje Neovolcánico, siendo este último donde se encuentra distribuida *Q. crassipes*, y a lo largo del cual existen numerosas localidades en las que se han registrado algunos individuos con morfología intermedia que han sido denominados como *Q. dysophylla* (Romero, 1993; Zavala, 1995).

De acuerdo con lo anterior, este estudio se realizó siguiendo cuatro líneas principales de investigación. Cada línea constituye un capítulo, como se describe a continuación:

Capítulo I. HIBRIDACIÓN DEL COMPLEJO *QUERCUS CRASSIFOLIA* × *Q. CRASSIPES.* El objetivo de esta sección fue evaluar con base a caracteres morfológicos de tipo foliar y los moleculares (RAPDs), si existe hibridación entre estas dos especies y si *Q. dysophylla* es el resultado de esta hibridación. Asimismo, conocer si se presentan hibridaciones, retrocruzas e introgresiones en siete zonas mixtas estudiadas. Cubriendo los objetivos 1 y 2.

Capítulo II. ESTRUCTURA GENÉTICA Y DISTRIBUCIÓN DE HAPLOTIPOS DEL COMPLEJO. El objetivo de esta sección fue estimar la diversidad y estructura genética, así como la distribución en la diversidad de haplotipos del complejo utilizando microsatélites de cloroplasto. Por último, conocer los niveles de introgresión e hibridación en las siete zonas estudiadas, asimismo estimar las rutas de migración que han tenido *Q. crassifolia* y *Q. crassipes*, utilizando como marcador molecular microsatélites de cloroplasto. Cubriendo el objetivo 3 y 4.

Capítulo III. ESTRUCTURA DE LA COMUNIDAD DE INSECTOS ENDÓFAGOS ASOCIADOS AL COMPLEJO. El objetivo de este capítulo fue evaluar la variación en la estructura de la comunidad de insectos endófagos (agalleros y minadores) del dosel entre taxa hospederos (*Q. crassipes*, *Q. crassifolia* e híbridos putativos) en siete zonas mixtas estudiadas durante cuatro estaciones. Cubriendo el objetivo 5 y 6.

Capítulo IV. ESTRUCTURA DE LA COMUNIDAD DE ARTRÓPODOS ASOCIADOS AL DOSEL DEL COMPLEJO. El objetivo de esta sección fue explorar la variación en la estructura de la comunidad de artrópodos epífitos del dosel entre taxa hospederos y estaciones (lluvias y sequía). Cubriendo el objetivo 7 y 8.

Capítulo I.

HIBRIDACIÓN DEL COMPLEJO *QUERCUS CRASSIFOLIA* × *Q. CRASSIPES*

American Journal of Botany 91(9): 000–000. 2004.

NATURAL HYBRIDIZATION AND HYBRID ZONES BETWEEN *QUERCUS CRASSIFOLIA* AND *QUERCUS CRASSIPES* (FAGACEAE) IN MEXICO: MORPHOLOGICAL AND MOLECULAR EVIDENCE[1]

EFRAÍN TOVAR-SÁNCHEZ AND KEN OYAMA[2]

Centro de Investigaciones en Ecosistemas, UNAM, Campus Morelia, Antigua Carretera a Pátzcuaro No. 8701, Col. San José de la Huerta, C.P. 58190, Morelia, Michoacán, México

Hybrid zones provide interesting systems to study genetic and ecological interaction between different species. The correct identification of hybrids is necessary to understand the evolutionary process involved in hybridization. An oak species complex occurring in Mexico formed by two parental species, *Quercus crassifolia* H. & B. and *Q. crassipes* H. & B., and their putative hybrid species, *Q. dysophylla*, was analyzed with molecular markers (random amplified polymorphic DNA [RAPDs]) and morphological tools in seven hybrid zones (10 trees per taxa in each hybrid zone) and two pure sites for each parental species (20 trees per site). We tested whether geographic proximity of hybrid plants to the allopatric site of a parental species increases its morphological and genetic similarity with its parent. Seventeen morphological traits were measured in 8700 leaves from 290 trees. Total DNA of 250 individuals was analyzed with six diagnostic RAPD primers. *Quercus crassifolia* differed significantly from *Q. crassipes* in all the examined characters. Molecular markers and morphological characters were highly coincident and support the hypothesis of hybridization in this complex, although both species remain distinct in mixed stands. Clusters and a hybrid index (for molecular and morphological data) showed that individuals from the same parental species were more similar among themselves than to putative hybrids, indicating occasional hybridization with segregation in hybrid types or backcrossing to parents. Evidence does not indicate a unidirectional pattern of gene flow.

Key words: Fagaceae; hybrid zones; hybridization; leaf morphology; Mexico; *Quercus*; RAPDs.

Hybridization is a natural phenomenon that occurs frequently in plants and animals (Harrison, 1993). This process produces new genetic combinations by the introduction of semicompatible genes into another genotype, upon which interaction of environment and genetic variation can isolate a novel taxon from parental types. Hybrids may be defined as species, subspecies, variants, or races, depending on the degree of divergence (Futuyma, 1998). Interspecific gene transfer is an important evolutionary force, because the genetic material introduced by introgression exceeds that which is produced directly by mutation (Anderson, 1949).

The genus *Quercus* (Fagaceae) is one of the most diversified groups of temperate trees with more than 500 species distributed worldwide (Nixon, 1993). Hybridization and hybrid zones are common among oaks (Trelease, 1924; Palmer, 1948; Muller, 1952; Tucker, 1961; Cottam et al., 1982; Jensen et al., 1993; Spellenberg, 1995; Howard et al., 1997; Ishida et al., 2003). However, despite the perception that hybrid zones are well documented among oaks, few comparative analyses of oak hybrid zones have used both morphological characters and genetic markers (Howard et al., 1997).

Hybridization in oaks was initially detected based on morphological characters (Stebbins et al., 1947; Barlett, 1951; Tucker, 1961; Benson et al., 1967; Hardin, 1975; Cottam et al., 1982; Rushton, 1993). L eaf morphology in particular has been useful to demonstrate hybridization (Bacon and Spellenberg, 1996). However, in some cases morphological characters alone do not confirm unequivocally the existence of hybridization (Bacilieri et al., 1995; Manos et al., 1999; Mayol and Rosselló, 2001) requiring other methods such as DNA markers (Crawford et al., 1993; Rieseberg and Ellstrand, 1993). Random amplified polymorphic DNA (RAPD) markers have been particularly successful in the detection of interspecific hybridization and introgression in plants (Arnold et al., 1991; Arnold, 1993; Crawford et al., 1993; Cruzan and Arnold, 1993; Marsolais et al., 1993; Fritz et al., 1994; Smith et al., 1996; Samuel, 1999).

Mexico is considered one of the centers of diversification of the genus *Quercus* (oaks) (Muller and McVaugh, 1972; Rzedowski, 1978; González, 1993; Nixon, 1993), with 135–150 species that include 86 endemics (Nixon, 1993). However, hybridization has only recently been reported for some species of Mexican oaks (McVaugh, 1974; Boecklen and Spellenberg, 1990; Spellenberg, 1992, 1995; Spellenberg and Bacon, 1996). The taxonomy and evolutionary relationships of Mexican oaks are currently being studied, and some species complexes formed by potential parental species and putative hybrids have been recently detected by oak specialists. We chose two red oak species (subg. *Erythrobalanus*) for this study, *Q. crassifolia* H. & B. and *Q. crassipes* H. & B. These species have noticeable differences in several morphological characters (Romero, 1993) when they form allopatric pure stands. However, intermediate trees with atypical leaf shapes are observed when both species occur in sympatry, suggesting that hybridization may explain the observed variations. It is important to indicate that other oak species that can be considered as reasonable putative parents as judged from the morphological fea-

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[1] Manuscript received 10 July 2003; revision accepted 20 May 2004.

The authors thank Richard Spellenberg and one anonymous reviewer whose comments improved previous versions of this manuscript. We also thank Antonio González, Susana Valencia, Patricia Mussali-Galante, Rocío Esteban-Jiménez, Marco Romero, and Nidia Pérez- Nasser for technical assistance. This research was supported by grants from CONACYT- Mexico (38550-V) and DGAPA, UNAM (IN212999) to K. O., a PAEP-UNAM grant and a CONACYT scholarship to E. T. S.

[2] E-mail: akoyama@oikos.unam.mx.

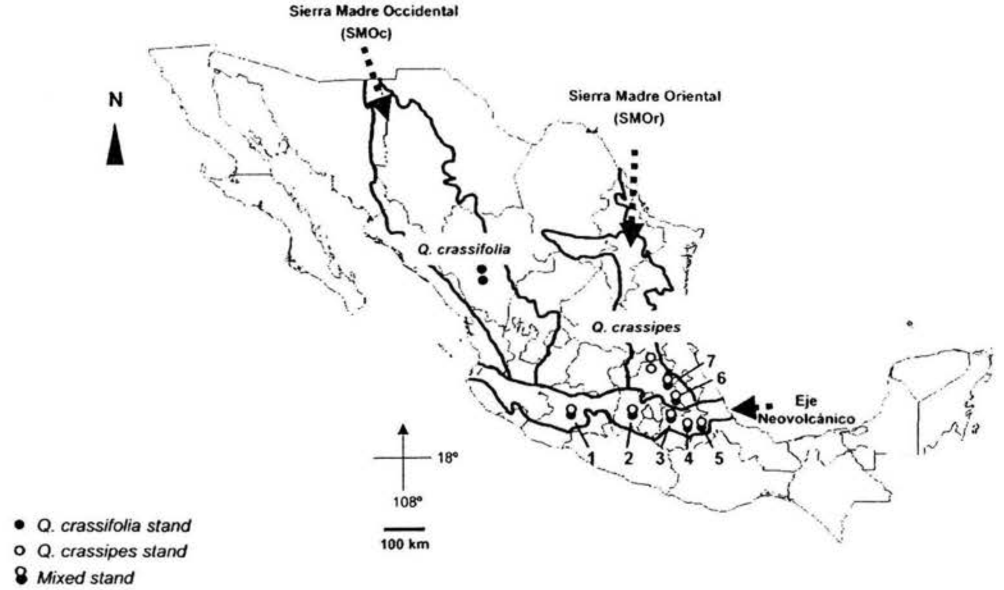

Fig. 1. Map of sampled populations of *Quercus crassifolia* × *Q. crassipes* complex. The mixed stands are represented by numbers, 1 = Cantera, 2 = Canalejas, 3 = Tlaxco, 4 = Acajete, 5 = Esperanza (located at the Eje Neovolcánico), 6 = Agua Blanca, 7 = Palo Bendito (located at the Sierra Madre Oriental).

tures of *Q. dysophylla* do not occur in the area. The taxonomic status of *Q. dysophylla* is still under discussion; some authors consider this species as a hybrid formed by *Q. crassifolia* × *Q. crassipes* (K. Nixon and S. Valencia, Cornell University and UNAM, respectively, personal communication), while others recognize it as a different species (Romero, 1993; Zavala Chávez, 1995).

In this paper, we describe and compare the patterns of morphological and genetic variation of the *Q. crassifolia* × *Q. crassipes* complex, document the structure of overlapping zones and hybridization in the Eje Neovolcánico and Central Mexico, and assess the taxonomic distinctness of the two species and the putative hybrid.

MATERIALS AND METHODS

Study sites—*Quercus crassifolia* is distributed throughout the Sierra Madre Occidental (SMOc, northwest Mexico), whereas *Q. crassipes* is distributed along the Sierra Madre Oriental (SMOr) on the opposite side of the country. The distribution of these species overlaps along the Eje Neovolcánico, a range of volcanic mountains that traverses central Mexico in an east–west direction, where individuals with intermediate leaf morphology are present (Romero, 1993). Seven sympatric zones were chosen in the Eje Neovolcánico and Sierra Madre Oriental, where *Q. crassifolia* and *Q. crassipes* overlap geographically: Cantera, Canalejas, Tlaxco, Acajete, Esperanza, Agua Blanca, and Palo Bendito. These localities were chosen because they contain the highest numbers of intermediate individuals of this complex. Two localities were also chosen for each parental species, where they are dominant and no hybrids were observed (Fig. 1). In general, the hybrids occur in a very low frequency sporadically interspersed and near to the putative parents. Intermediate trees occur in more disturbed habitats but we cannot assess the preference of hybrids for some type of habitats (e.g., type of soils, forest gaps). It is rare to find intermediate trees outside of the contact zones. No evidence exists on another oak species hybridizing with trees of the species studied. When other oak species were found, they were white oaks that cannot hybridize with red oak species.

Morphological data—Seventeen morphological characters of leaves were measured (Table 1) in 290 trees. A total of 210 trees were sampled in the seven mixed stands. 10 trees for each taxa: *Q. crassifolia*, *Q. crassipes*, and the hybrid. Twenty trees were sampled in each pure site (two sites for each parental species). Thirty mature leaves, without any apparent damage, were randomly sampled in each tree. Seventeen characters were measured in a total of 8700 leaves.

Molecular data—We collected undamaged young leaves from 250 trees of *Q. crassifolia* ($n = 90$), *Q. crassipes* ($n = 90$), and hybrids ($n = 70$), distributed in seven mixed stands and 40 trees in two pure zones for each parental species. Total DNA was extracted using a DNAeasy Plant Mini Kit (Qiagen, Valencia, California, USA). A genetic analysis was performed using RAPD (Welsh and McClelland, 1990; Williams et al., 1990), and the polymerase chain reaction (PCR). Forty-eight 10-base pair (bp) primers of random sequence (Kits A, B, C: Operon Technologies) were tested to find specific, diagnostic markers for each parental species. Diagnostic markers are those that are present in all individuals of one species and absent in a second species, while species-specific markers are those unique to one species but not necessarily present in all individuals within the species (Howard et al., 1997). Lastly, 250 individuals were tested using six primers (four species-specific markers [two for *Q. crassifolia*, OPA-09 and OPA-13, and two for *Q. crassipes*, OPB-01 and OPC-06], and two diagnostic markers [*Q. crassifolia*, OPA-14, and *Q. crassipes*, OPB-18]). DNA fragments were separated by electrophoresis on 1.5% agarose gel, stained with ethidium bromide, and developed on a UV table. The molecular mass of the DNA fragments was estimated with a 1-kilo base (kb) DNA ladder.

Statistical analysis—Nested variance analyses were conducted (ANOVA) to determine the effects of oak species, locality, and individual (tree) on the morphological leaf variability of each of the 17 studied characters (Table 1). Hybrids were not included in this analysis. Trees were considered as a random factor nested within species, because they were representative of each population. Percentage data were corrected as $X = \arcsin (\%)^{1/2}$, and discontinuous data were transformed as $X = (x)^{1/2} + 0.5$ (Zar, 1999).

To quantify variation in leaf morphology among oak species and hybrids, we randomly selected 30 leaves from vouchered specimens, and all the 17 characters were measured. All morphological characters were used for discriminant analysis to assess the most useful character for taxonomic discrimination between *Q. crassifolia* and *Q. crassipes* and to determine how leaf

TABLE 1. List of the leaf morphological characters examined for *Q. crassifolia* × *Q. crassipes* complex in Mexico.

Abbreviation	Description
Macromorphological characters	
LP	Length of petiole
LL	Length of lamina
TLL	Total leaf length (LL + LP)
MWL	Maximal width of lamina
HMW	Height of maximal width (length of lamina from base to widest part)
PD	Petiole diameter
MD	Midvein diameter
NV	Number of veins
LWB	Leaf width at basal 1/3 of leaf
LWA	Leaf width at apical 1/3 of leaf
NA	Number of aristae
LLW	Length of lamina from base to widest part (LL − HMW)
Combinations of characters	
P%	Length of petiole × 100/total leaf length
HW%	Height of maximal width × 100/total leaf length
DW%	Length of lamina from base to widest part × 100/total leaf length
LL/MWL	Length of lamina/maximal width of lamina
LLW/MWL	Length of lamina from base to widest part/maximal width of lamina

morphological characters separate individuals into groups. Seventeen characters were measured for the character count procedure to determine hybrids with intermediate leaf morphology of the *Q. crassifolia* × *Q. crassipes* complex following the procedure of Wilson (1992). Variation in size and shape due to shape alone was quantified using the ratios of the sums of eigen values from the discriminant analysis (Darroch and Mosimann, 1985).

In the seven mixed stands, the Anderson hybrid index (Anderson, 1949, 1953) was used to identify intermediate individuals and possible backcrosses. A histogram for each mixed stand was obtained. The Anderson hybrid index was calculated using the 17 morphological characters because they demonstrated differences between parental species. The representative characters of *Q. crassipes* received a rank of 2; *Q. crassifolia* characters were assigned a rank of 0; while intermediate characters were assigned a rank of 1 (Wilson, 1992).

A general cluster diagram for all zones was obtained, including pure and mixed stands. STATISTICA 6.0 for Windows was used for all the statistical analyses (Statsoft, 1998).

The maximum likelihood (ML) hybrid index score from RAPD analysis was calculated using Hardig-Hybrid software (Hardig et al., 2000). This index is useful to identify intermediate individuals, showing backcrosses as well as the structures of hybrid swarms. The results were represented in a frequency histogram for each mixed stand and for the pure sites. A Mantel Z-test matrix and Tools for Population Genetic Analyses (TFPGA, version 1.3) were used to test isolation by distance, as well as to create a general cluster diagram for the mixed stands and the four pure zones (Miller, 1997).

RESULTS

Morphological analysis for parental and hybrid plants—All of the examined characters in *Quercus crassifolia* differ significantly from those of *Q. crassipes*. Locality and tree also had highly significant effects on each of the measured leaf characters (Table 2). Three characters (NA, LL/MWL, and LLW/MWL) were clearly separated without overlap between the two species in the pure zones as well as in the mixed stands and can be considered as species-specific characters. Hybrids

TABLE 2. Mean ± standard error and nested ANOVA results (*F* statistics) for all characters of *Quercus crassifolia* and *Q. crassipes* in Mexico. *F* significant at $P < 0.001$ (***); $P < 0.01$ (**); and $P < 0.05$ (*).

Character	Units	*Q. crassifolia*	*Q. crassipes*	Locality *F* (df = 6)	Species *F* (df = 1)	Tree *F* (df = 90)
Macromorphological characters						
LP	cm	1.26 ± 0.019	0.53 ± 0.006	52.32***	1233.57***	5.51***
LL	cm	10.10 ± 0.060	5.54 ± 0.032	30.90***	7579.13***	19.52***
TLL	cm	11.64 ± 0.066	6.07 ± 0.030	23.64***	23792.13***	18.22***
MWL	cm	5.94 ± 0.046	1.63 ± 0.010	14.06***	16810.99***	19.36***
HMW	cm	6.16 ± 0.064	2.68 ± 0.020	49.15**	8089.17**	16.58**
PD	cm	0.23 ± 0.002	0.09 ± 0.001	121.28**	5345.47**	16.86**
MD	cm	0.16 ± 0.002	0.07 ± 0.001	127.87**	2643.26**	40.75**
NV	no.	14.48 ± 0.086	25.25 ± 0.217	121.34**	2156.06**	4.96**
LWB	cm	4.55 ± 0.031	1.44 ± 0.008	13.42***	14684.32***	21.21***
LWA	cm	5.30 ± 0.037	1.41 ± 0.008	22.63***	16552.18***	19.93***
NA	no.	3.15 ± 0.064	1.22 ± 0.000	119.62**	49800.28**	16.14**
LLW	cm	3.94 ± 0.029	2.86 ± 0.018	14.32***	1109.32***	10.98***
Combinations of characters						
P	%	0.11 ± 0.001	0.08 ± 0.001	143.82**	143.07**	9.00**
HW	%	0.54 ± 0.002	0.44 ± 0.002	56.05**	1594.82**	5.99**
DW	%	0.52 ± 0.002	0.27 ± 0.001	37.32***	14809.43***	8.54***
LL/MWL		1.72 ± 0.005	3.47 ± 0.016	242.20**	269715.9**	8.50***
LLW/MWL		0.68 ± 0.004	1.80 ± 0.012	84.50**	255214.3**	4.50**

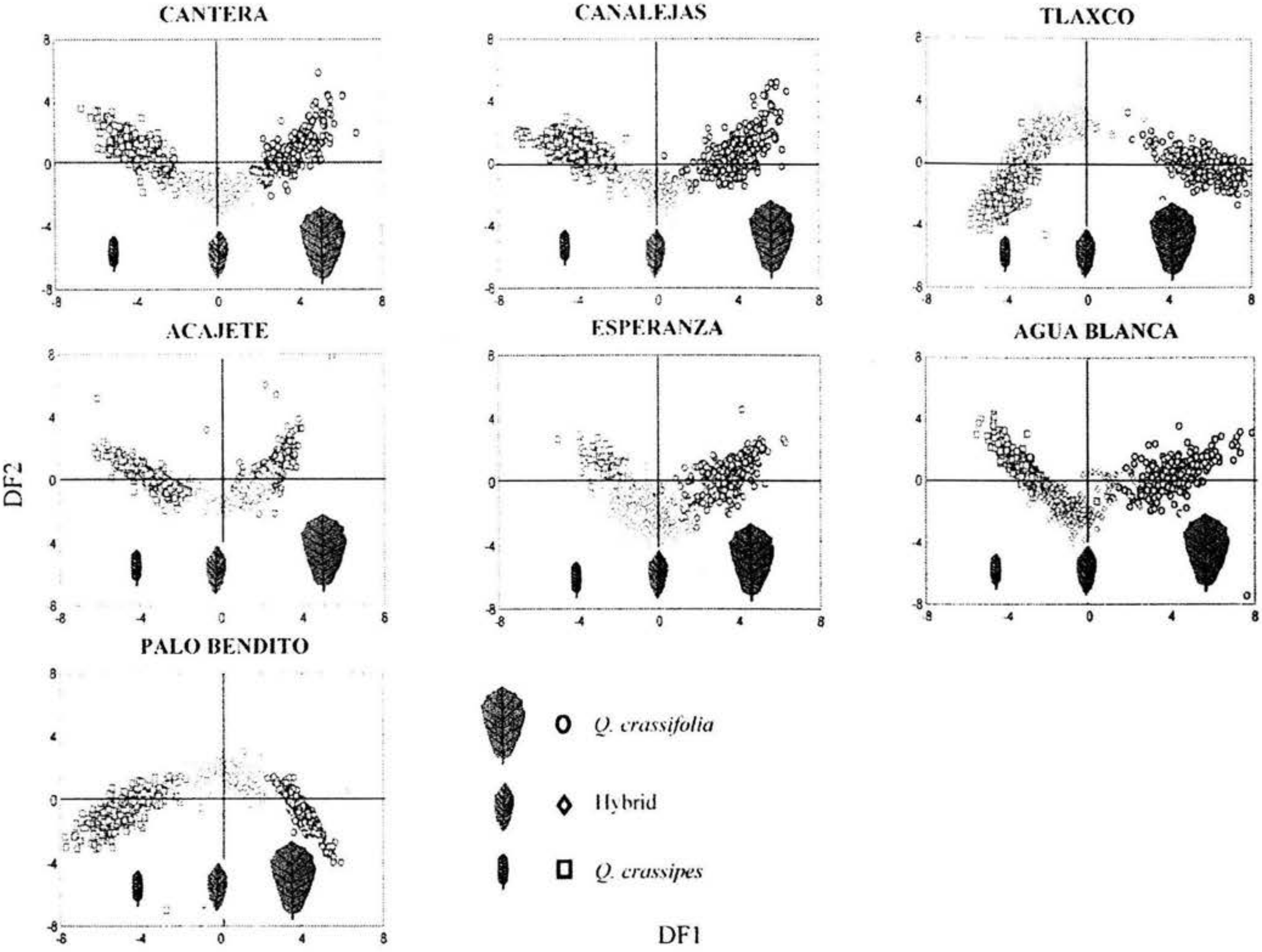

Fig. 2. Discriminant function analysis for leaf morphology variation in *Quercus crassifolia* × *Q. crassipes* complex (17 measured characters) in seven hybrids zones in Mexico. See abbreviations in Table 1.

presented intermediate morphological leaf traits between *Q. crassifolia* and *Q. crassipes*. The binomial sign analysis showed that the deviation was highly significant ($P < 0.001$), accepting the hybridization hypothesis. Intermediate characters were found in the 17 examined characters of the samples from Palo Bendito ; 16 of 17 examined characters in the samples from Cantera and Esperanza; and 15 of 17 examined characters in the samples from Canalejas, Tlaxco, Agua Blanca, and Acajete (Table 3).

Discriminant function analysis for size and shape variation showed that in all of the analyzed hybrid zones the hybrids had intermediate characters between their parents. The discriminant axis described a gradient of leaf shapes from elliptic narrow (*Q. crassipes*) to the hybrids and the obovate leaves of *Q. crassifolia* (Fig. 2).

For the *Q. crassifolia* × *Q. crassipes* comparison, one highly significant ($P < 0.001$) discriminant axis accounted from 81.23% (Esperanza) to 94.78% (Acajete) of the variation between taxa. Discriminant function analysis for leaf morphology variation showed that in general the MWL contributed with the highest value to the ordination model for the first axis in Cantera, Canalejas, and Agua Blanca while in Tlaxco and Palo Bendito it was LL, and in Esperanza HW% and in Acajete it was LLW. On the other hand, the character with the highest value for the second axis was LL in Cantera, Canalejas, Agua Blanca, Esperanza, and Acajete, LL/MWL in Palo Bendito, and TLL in Tlaxco (Table 4).

The shape variable produced two significant discriminant axes, and the ratio of the sums of eigenvalues of the Cantera hybrid zone indicated that approximately 4.48/7.16 = 62.57% of shape alone was accountable for the total variation in size and shape. In the other hybrid zones, less than 50% of the total variation in size and shape was attributable to the shape alone: in Agua Blanca, 1.65/6.44 = 25.62 %; Canalejas, 2.48/8.14 = 30.47%; Palo Bendito, 2.88/8.52 = 33.80%; Tlaxco, 3.55/9.48 = 37.45%; Acajete, 2.28/5.17 = 44.10%; and Esperanza, 3.34/7.94 = 42.06%.

In a global cluster analysis based on morphological leaf traits for the seven mixed stands and four pure populations (two for *Q. crassifolia* and two for *Q. crassipes*), the populations having the same parental species were more similar to each other. F or the five hybrid zones located on the Eje Neovolcánico (mixed stands from 1 to 5), nearness of the hybrid zones to the allopatric putative parent correlated with increas-

TABLE 3. Character count procedure from the seven hybrid zones of the *Quercus crassifolia* × *Q. crassipes* complex in Mexico. Abbreviations of characters are described in Table 1; + = the hybrid is intermediate between the parental species, − = the hybrid is not intermediate. SD = standard deviation.

Character	*Q. crassifolia* (mean ± SD)	Hybrid (mean ± SD)	*Q. crassipes* (mean ± SD)	The hybrid is different from (Tukey, $P < 0.05$)	The hybrid is intermediate?
CANTERA					
LP	1.25 ± 0.62	0.71 ± 0.55	0.41 ± 0.27	both	+
LL	10.89 ± 2.02	9.43 ± 2.08	6.30 ± 1.31	both	+
TLL	12.14 ± 2.19	10.13 ± 2.36	6.71 ± 1.39	both	+
MWL	6.35 ± 1.41	3.70 ± 0.98	1.58 ± 0.40	both	+
HMW	6.83 ± 1.55	4.98 ± 1.32	3.23 ± 0.89	both	+
PD	0.20 ± 0.04	0.14 ± 0.27	0.10 ± 0.02	both	+
MD	0.13 ± 0.03	0.08 ± 0.02	0.07 ± 0.09	*Q. crassifolia*	+
NV	14.85 ± 4.23	21.16 ± 2.77	28.30 ± 6.15	both	+
LWB	4.72 ± 1.15	3.15 ± 0.83	1.32 ± 0.29	both	+
LWA	5.92 ± 1.42	3.12 ± 0.86	1.33 ± 0.32	both	+
NA	3.36 ± 0.43	1.29 ± 0.25	1.22 ± 0.00	both	+
LLW	4.06 ± 1.13	4.44 ± 1.05	3.07 ± 0.73	both	−
P	18.30 ± 4.49	14.83 ± 2.97	14.43 ± 3.04	*Q. crassifolia*	+
HW	48.63 ± 4.65	44.44 ± 3.44	43.76 ± 4.42	*Q. crassifolia*	+
DW	46.38 ± 4.06	37.12 ± 2.74	29.18 ± 3.52	both	+
LL/MWL	1.74 ± 0.25	2.59 ± 0.34	4.07 ± 0.68	both	+
LLW/MWL	0.65 ± 0.16	1.23 ± 0.25	1.99 ± 0.46	both	+
Scores of intermediate characters					16:01
CANALEJAS					
LP	1.62 ± 0.70	0.99 ± 0.51	0.63 ± 0.36	both	+
LL	10.02 ± 2.14	8.40 ± 1.88	5.52 ± 1.33	both	+
TLL	11.64 ± 2.40	9.39 ± 2.08	6.16 ± 1.42	both	+
MWL	6.08 ± 1.47	3.20 ± 0.84	1.58 ± 0.36	both	+
HMW	5.98 ± 1.58	4.02 ± 1.21	2.46 ± 0.76	both	+
PD	0.19 ± 0.05	0.12 ± 0.07	0.08 ± 0.02	both	+
MD	0.13 ± 0.05	0.08 ± 0.01	0.07 ± 0.01	both	+
NV	11.28 ± 2.93	19.71 ± 2.48	26.17 ± 4.36	both	+
LWB	5.06 ± 1.28	2.83 ± 0.67	1.43 ± 0.29	both	+
LWA	5.41 ± 1.41	2.64 ± 0.68	1.39 ± 0.30	both	+
NA	2.92 ± 0.44	1.23 ± 0.05	1.22 ± 0.00	*Q. crassifolia*	+
LLW	4.04 ± 1.34	4.38 ± 1.14	3.06 ± 0.84	both	−
P	21.67 ± 3.86	18.42 ± 4.72	18.94 ± 3.59	*Q. crassifolia*	−
HW	45.84 ± 5.40	40.73 ± 4.15	39.04 ± 4.85	both	+
DW	46.43 ± 5.07	35.76 ± 3.93	30.64 ± 3.11	both	+
LL/MWL	1.68 ± 0.25	2.70 ± 0.55	3.53 ± 0.59	both	+
LLW/MWL	0.68 ± 0.22	1.42 ± 0.42	1.96 ± 0.45	both	+
Scores of intermediate characters					15:02
ESPERANZA					
LP	1.49 ± 0.64	0.73 ± 0.44	0.52 ± 0.12	*Q. crassifolia*	+
LL	10.59 ± 2.35	7.77 ± 2.12	5.14 ± 0.83	both	+
TLL	12.08 ± 2.54	8.50 ± 2.22	5.66 ± 0.85	both	+
MWL	6.10 ± 1.82	3.34 ± 0.92	1.85 ± 0.31	both	+
HMW	6.97 ± 1.80	3.82 ± 1.19	2.33 ± 0.50	both	+
PD	0.20 ± 0.07	0.14 ± 0.03	0.11 ± 0.02	both	+
MD	0.14 ± 0.04	0.09 ± 0.02	0.08 ± 0.02	*Q. crassifolia*	+
LV	15.43 ± 3.63	20.99 ± 3.28	15.60 ± 2.01	both	+
LWB	4.61 ± 0.90	2.93 ± 0.82	1.70 ± 0.30	both	+
LWA	5.66 ± 1.37	2.71 ± 0.76	1.59 ± 0.24	both	+
NA	2.91 ± 0.36	1.24 ± 0.12	1.22 ± 0.00	*Q. crassifolia*	+
LLW	3.62 ± 1.22	3.95 ± 1.18	2.80 ± 0.54	both	+
P	20.37 ± 4.13	16.98 ± 3.55	17.65 ± 4.13	*Q. crassifolia*	−
HW	49.42 ± 4.60	41.90 ± 3.39	39.87 ± 3.18	*Q. crassifolia*	+
DW	44.93 ± 3.43	39.06 ± 4.27	34.93 ± 1.92	both	+
LL/MWL	1.77 ± 0.23	2.36 ± 0.41	2.78 ± 0.28	both	+
LLW/MWL	0.60 ± 0.17	1.20 ± 0.25	1.52 ± 0.20	both	+
Scores of intermediate characters					16:01
AGUA BLANCA					
LP	0.71 ± 0.44	0.61 ± 0.21	0.41 ± 0.26	both	+
LL	9.01 ± 2.29	8.57 ± 2.09	6.08 ± 1.57	both	+
TLL	8.35 ± 2.03	7.96 ± 2.00	5.67 ± 1.46	both	+
MWL	4.59 ± 1.34	3.46 ± 1.93	1.95 ± 0.43	both	+
HMW	5.13 ± 1.35	4.37 ± 1.28	2.95 ± 1.09	both	+
PD	0.12 ± 0.02	0.22 ± 1.00	0.08 ± 0.01	*Q. crassipes*	−

TABLE 3. Continued.

Character	*Q. crassifolia* (mean ± SD)	Hybrid (mean ± SD)	*Q. crassipes* (mean ± SD)	The hybrid is different from (Tukey, $P < 0.05$)	The hybrid is intermediate?
MD	0.09 ± 0.02	0.07 ± 0.02	0.05 ± 0.01	both	+
NV	15.32 ± 2.63	18.95 ± 3.86	21.18 ± 2.94	both	+
LWB	3.54 ± 1.03	2.85 ± 0.64	1.71 ± 0.39	both	+
LWA	4.05 ± 1.29	2.85 ± 0.74	1.65 ± 0.39	both	+
NA	2.64 ± 0.64	0.85 ± 0.39	0.71 ± 0.00	both	+
LLW	3.99 ± 2.07	4.49 ± 2.19	3.72 ± 1.13	*Q. crassipes*	–
P	17.03 ± 7.01	16.15 ± 2.79	15.34 ± 4.30	both	+
HW	51.63 ± 3.74	47.55 ± 3.25	45.73 ± 5.43	both	+
DW	47.86 ± 4.92	40.76 ± 3.80	36.2 ± 2.92	both	+
LL/MWL	1.86 ± 0.30	2.37 ± 0.29	2.91 ± 0.04	both	+
LLW/MWL	0.92 ± 1.24	1.37 ± 0.28	1.91 ± 0.40	both	+
Scores of intermediate characters					15:02
TLAXCO					
LP	1.11 ± 1.23	0.49 ± 0.20	0.56 ± 0.19	*Q. crassifolia*	–
LL	11.26 ± 3.30	7.85 ± 2.17	5.04 ± 1.16	both	+
TLL	12.37 ± 3.76	8.33 ± 2.30	5.61 ± 1.26	both	+
MWL	6.56 ± 2.01	2.72 ± 0.97	1.42 ± 0.34	both	+
HMW	7.07 ± 2.45	3.99 ± 1.37	2.39 ± 0.78	both	+
PD	0.25 ± 0.05	0.11 ± 0.03	0.08 ± 0.02	both	+
MD	0.16 ± 0.04	0.08 ± 0.02	0.06 ± 0.01	both	+
NV	14.01 ± 3.41	21.32 ± 4.07	26.73 ± 5.41	both	+
LWB	4.74 ± 1.72	2.36 ± 0.74	1.29 ± 0.32	both	+
LWA	5.72 ± 2.02	2.37 ± 0.86	1.22 ± 0.31	both	+
NA	3.29 ± 0.45	1.23 ± 0.08	1.22 ± 0.00	*Q. crassifolia*	+
LLW	4.18 ± 1.38	3.86 ± 0.98	2.65 ± 0.79	both	+
P	16.55 ± 4.51	13.85 ± 1.95	18.37 ± 2.86	both	–
HW	49.13 ± 4.96	43.40 ± 3.53	40.67 ± 5.97	both	+
DW	47.12 ± 4.39	34.78 ± 3.32	30.43 ± 3.06	both	+
LL/MWL	1.73 ± 0.21	2.96 ± 0.50	3.59 ± 0.62	both	+
LLW/MWL	0.64 ± 0.15	1.48 ± 0.34	1.90 ± 0.56	both	+
Scores of intermediate characters					15:02
ACAJETE					
LP	0.84 ± 0.68	0.58 ± 0.34	0.56 ± 0.34	*Q. crassifolia*	+
LL	9.66 ± 2.21	6.98 ± 1.76	5.89 ± 1.29	both	+
TLL	10.50 ± 2.16	7.57 ± 1.94	6.44 ± 1.37	both	+
MWL	5.47 ± 1.26	2.62 ± 0.71	1.84 ± 0.42	both	+
HMW	5.52 ± 1.45	3.63 ± 1.17	3.09 ± 0.94	both	+
PD	0.30 ± 0.15	0.12 ± 0.19	0.10 ± 0.05	both	+
MD	0.25 ± 0.19	0.08 ± 0.02	0.06 ± 0.01	*Q. crassifolia*	+
NV	16.97 ± 2.39	20.21 ± 3.84	28.33 ± 4.83	both	+
LWB	4.41 ± 1.21	2.25 ± 0.60	1.57 ± 0.34	both	+
LWA	4.84 ± 1.16	2.22 ± 0.62	1.62 ± 0.37	both	+
NA	3.19 ± 0.28	1.25 ± 0.14	1.22 ± 0.00	*Q. crassifolia*	+
LLW	4.14 ± 1.33	3.35 ± 0.95	2.80 ± 0.93	both	+
P	16.65 ± 4.47	15.96 ± 2.66	16.76 ± 3.60	*Q. crassifolia*	–
HW	46.39 ± 4.28	43.59 ± 4.14	43.58 ± 4.94	*Q. crassifolia*	–
DW	46.33 ± 3.87	36.20 ± 3.72	32.45 ± 3.32	both	+
LL/MWL	1.78 ± 0.21	2.71 ± 0.41	3.25 ± 0.53	both	+
LLW/MWL	0.76 ± 0.19	1.31 ± 0.30	1.55 ± 0.47	both	+
Scores of intermediate characters					15:02
PALO BENDITO					
LP	1.20 ± 1.03	0.43 ± 0.60	0.32 ± 0.16	both	+
LL	12.56 ± 2.94	8.59 ± 1.92	6.11 ± 1.43	both	+
TLL	11.36 ± 2.75	8.16 ± 1.77	5.79 ± 1.36	both	+
MWL	6.71 ± 1.89	3.80 ± 2.63	1.88 ± 1.23	both	+
HMW	7.05 ± 2.04	4.40 ± 1.13	2.89 ± 0.92	both	+
PD	0.20 ± 0.08	0.13 ± 0.03	0.07 ± 0.01	both	+
MD	0.17 ± 0.09	0.09 ± 0.02	0.06 ± 0.03	*Q. crassifolia*	+
NV	16.65 ± 2.40	20.21 ± 6.86	24.02 ± 4.35	both	+
LWB	5.13 ± 1.32	3.12 ± 0.93	1.60 ± 0.32	both	+
LWA	6.19 ± 3.31	3.13 ± 1.01	1.61 ± 0.33	both	+
NA	2.93 ± 0.56	1.21 ± 0.25	0.71 ± 0.00	both	+
LLW	4.33 ± 1.26	3.76 ± 0.87	2.89 ± 0.72	both	+
P	18.14 ± 4.53	13.74 ± 2.63	13.49 ± 3.07	*Q. crassifolia*	+
HW	51.52 ± 4.71	47.18 ± 3.27	44.65 ± 4.80	both	+
DW	49.79 ± 5.03	49.47 ± 3.57	34.33 ± 2.87	both	+
LL/MWL	1.73 ± 0.28	2.31 ± 0.35	3.19 ± 0.48	both	+
LLW/MWL	0.67 ± 0.19	1.07 ± 0.22	1.61 ± 0.34	both	+
Scores of intermediate characters					17:00

TABLE 4. Discriminant function analysis for leaf morphology variation from seven hybrid zones of the *Quercus crassifolia* × *Q. crassipes* complex in Mexico. Abbreviations of characters are described in Table 1.

Variable	DF1	DF2
CANTERA		
LL/MWL	−0.48	1.33
LWA	0.76	0.57
LL	0.00	−2.25
PD	0.31	0.44
NV	−0.41	−0.02
HMW	1.24	1.14
MWL	−1.41	1.05
DW%	0.76	0.21
HW%	−0.33	−0.49
LWB	0.09	−0.43
LLW/MWL	0.24	−0.29
LLW	0.00	−0.06
P%	0.06	0.01
Constant	−0.82	−1.83
Eigenvalue	8.97	1.03
% Variation	86.32	13.68
Significance	<0.001	<0.001
CANALEJAS		
MWL	−0.85	1.98
NV	−0.65	0.07
LL	0.66	−2.61
LL/MWL	−0.31	0.90
LWA	0.28	0.55
DW%	0.43	−0.79
PD	0.18	0.14
LWB	0.32	0.15
MD	0.09	0.19
HMW	0.14	0.19
HW%	−0.24	−0.07
LLW/MWL	−0.24	−0.08
P%	0.00	−0.12
Constant	−0.82	−1.83
Eigenvalue	9.63	1.03
% Variation	89.96	10.04
Significance	<0.001	<0.001
TLAXCO		
NA	0.68	−0.38
DW%	1.15	0.29
TLL	1.80	3.75
MWL	−0.91	−1.61
P%	0.00	−1.11
NV	−0.39	−0.30
LL/MWL	−0.03	−0.73
HWM	0.68	−0.98
LL	−1.21	−0.94
LLW/MWL	0.50	0.09
PD	0.05	−0.19
MD	0.12	0.08
HW%	0.24	0.25
Constant	−1.66	10.20
Eigenvalue	17.53	1.93
% Variation	89.06	10.94
Significance	<0.001	<0.001
ACAJETE		
MWL	0.30	0.85
LL/MWL	0.24	1.03
LL	0.81	−2.36
NV	0.28	0.51
LWA	−0.73	0.69
PD	−0.21	0.09
LWB	−0.50	0.41
DW%	−0.47	0.23
P%	−0.31	1.49
LP	−0.12	−0.99
MD	−0.12	−0.11
HW%	−0.82	1.18
LLW	−0.83	1.04
LLW/MWL	−0.45	0.43
Constant	0.56	0.52
Eigenvalue	6.69	0.52
% Variation	94.78	5.22
Significance	<0.001	<0.001
ESPERANZA		
LLW/MWL	1.20	1.46
NV	−0.29	−0.80
LL	1.83	−2.27
HMW	−1.00	1.28
MWL	−0.86	1.49
DW%	0.39	−1.11
LP	0.87	0.03
HW%	1.92	0.71
LL/MWL	−1.56	−0.93
MD	0.00	0.17
NA	1.83	0.80
P%	−0.72	0.70
LL	0.06	−0.91
PD	1.73	2.75
Constant	−4.98	−1.46
Eigenvalue	7.79	1.54
% Variation	81.23	18.77
Significance	<0.001	<0.001
AGUA BLANCA		
NA	0.81	0.51
LWA	0.37	0.05
LL/MWL	−0.42	1.21
LL	0.83	−4.15
MWL	−0.89	1.67
NV	−0.39	0.26
HW%	0.31	−0.78
MD	0.21	−0.06
HWL	−0.26	2.04
PD	−0.05	−0.21
LWB	0.48	0.29
P%	0.29	−0.19
LP	−0.19	0.21
DW%	0.14	−0.16
Constant	5.13	−2.07
Eigenvalue	9.86	1.32
% Variation	92.57	7.43
Significance	<0.001	<0.001
PALO BENDITO		
NA	−0.81	−0.41
LL/MWL	0.7	3.01
HW%	−0.43	−0.26
LL	−1.47	−1.79
MWL	1.32	−1.81
NV	0.26	−0.04
LP	−0.15	0.08
PD	−0.19	−0.05
LWA	−0.32	0.19
DW%	−0.70	1.04
HWL	0.68	−1.78
P%	−0.07	−0.18
LLW/MWL	−0.39	0.53
LLW	−0.17	−0.55
Constant	5.86	−4.45
Eigenvalue	13.07	0.25
% Variation	88.11	11.89
Significance	<0.001	<0.001

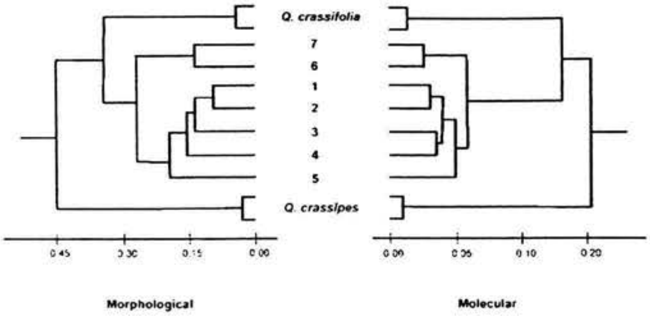

Fig. 3. Phenogram of similarity based on leaf morphology and RAPD data in seven hybrids zones of the *Quercus crassifolia* × *Q. crassipes* complex in Mexico. The mixed stands are represented by numbers. 1 = Cantera. 2 = Canalejas. 3 = Tlaxco. 4 = Acajete. 5 = Esperanza (located at the Eje Neovolcánico). 6 = Agua Blanca. 7 = Palo Bendito (located at the Sierra Madre Oriental).

ing similarity of the complex to the parental species. Lastly, mixed stands 6 and 7, located on the Sierra Madre Oriental, were more similar to *Q. crassifolia* (Fig. 3, left).

We used 17 morphological characters to estimate the status of *Q. crassifolia* and *Q. crassipes* plants marked previously as "pure" or "hybrid" plants. The results for the Anderson hybrid index support the field identification of 250 plants (Fig. 4). In general, the frequency histogram (Anderson hybrid index) had a pattern similar to the global cluster analysis. In Eje Neovolcánico, the mixed stands (Cantera and Canalejas) nearer to SMOc presented unidirectional introgression towards *Q. crassifolia*. Subsequently, the site closest to central Mexico (Tlaxco) was the only site that showed bidirectional introgression, but the two mixed stands nearest to SMOr (Acajete and Esperanza) registered unidirectional introgression towards *Q. crassipes*. Finally, the two hybrid zones localized north of Tlaxco (Agua Blanca and Palo Bendito) on SMOr presented unidirectional introgression towards *Q. crassifolia* (Fig. 4).

Individuals A10 (Cantera), A7, A8 (Tlaxco), and A9 (Agua Blanca) were classified as *Q. crassifolia* in the field, but the index showed that they were backcrosses towards *Q. crassifolia*. Individuals B4 and B8 (Tlaxco), B2 (Acajete), and B2 (Esperanza) were originally marked as *Q. crassipes*, but the index analysis confirmed that they were backcrosses toward *Q. crassipes*. Individuals AB1 and AB9 (Agua Blanca), as well as AB2 (Acajete), and AB2 and AB10 (Esperanza), were marked as hybrids, but the index showed that they were individuals of *Q. crassifolia* and *Q. crassipes*, respectively. Also, the individuals AB8, AB9, and AB10 (Cantera); AB1 and AB10 (Canalejas); AB8 and AB10 (Tlaxco); AB6 and AB10 (Agua Blanca); and AB3 and AB5 (Palo Bendito) were classified as hybrid plants, but the index analysis indicated that they were backcrosses toward *Q. crassifolia*. Finally, AB3 (Tlaxco), AB3, AB9, and AB10 (Acajete), and AB3, AB5, and AB9 (Esperanza) were marked as hybrids, but the data showed that they were backcrosses toward *Q. crassipes* (Fig. 4).

Genetic analysis (RAPDs) of parental and hybrid plants—We used six primers to estimate the genetic status of *Q. crassifolia* and *Q. crassipes* plants morphologically identified as "pure" or "hybrid" plants. These primers yielded 49 distinct markers (bands). The RAPD analysis proved to be a powerful tool for characterizing hybrid individuals between *Q. crassifolia* and *Q. crassipes*. The analysis of the ML hybrid index using six RAPD markers supported the field identification of 250 plants (Fig. 5). Individuals A10 (Cantera); A1, A7, and A8 (Tlaxco); and A5 and A6 (Palo Bendito) were classified as *Q. crassifolia* in the field, but the RAPD showed that they were backcrosses toward *Q. crassifolia*. Individuals B4 and B8 (Tlaxco) and B2 (Esperanza) were originally marked as *Q. crassipes*, but RAPDs analysis confirmed that they were backcrosses toward *Q. crassipes*. The individuals AB9 (Agua Blanca) and AB7 (Tlaxco) were marked as hybrids, but RAPDs and subsequent field examinations showed that they were individuals of *Q. crassifolia* and *Q. crassipes*, respectively. Individuals AB10 (Cantera), AB1 (Agua Blanca), and AB3, AB5, and AB7 (Palo Bendito) were classified as hybrid plants, but the molecular analysis indicated that they were backcrosses toward *Q. crassifolia*. Lastly, AB3, AB6, and AB9 (Tlaxco) ; AB4, AB5, AB8, AB9, and AB10 (Acajete); and AB2 and AB10 (Esperanza) were identified as hybrids, but RAPD data showed that they were backcrosses toward *Q. crassipes* (Fig. 5).

Few plants had perfect marker additivity as may be expected in F_1 (12 plants), but 54 individuals were interpreted as F_1, 26 deviated by only one character (0.437–0.562), and 16 plants deviated by only two characters (0.375–0.625). Twelve plants were interpreted as backcrosses toward *Q. crassipes* (0.250–0.312) and 11 as backcrosses toward *Q. crassifolia* (0.687–0.750) (Fig. 5).

The mean ML hybrid index for *Q. crassifolia* individuals was 0.85 (SD 0.07), for *Q. crassipes* was 0.08 (SD 0.06), and for the hybrids, 0.48 (SD 0.09).

The Hardig hybrid index showed the same general pattern as the Anderson hybrid index (see earlier). No introgression was registered only in Canalejas (Fig. 5).

The cluster analysis for genetic (RAPDs) and morphological data showed that these are very similar. The allopatric zones (two zones for *Q. crassifolia* and two for *Q. crassipes*) were located at the edges, while the seven hybrid zones were located between them (Fig. 3).

The Mantel *Z*-test matrix showed that no correlation exists between the geographic distances and the genetic distances for any of the species (*Q. crassifolia*, $r = 0.37$, $P > 0.05$; and *Q. crassipes*, $r = 0.14$, $P > 0.05$).

DISCUSSION

Oaks frequently present complex patterns of variation leading to taxonomic problems in differentiating species (Burger, 1975). Interspecific hybridization and shared ancestral polymorphisms are two of the most common explanations for the observed pattern of variation (Jensen et al., 1993; Kleinschmitt et al., 1995; Bruschi et al., 2000). *Quercus crassifolia* and *Q. crassipes* form hybrids, but they remain morphologically distinct in their allopatric and sympatric distributions. All the examined morphological leaf characters in these species differed significantly by localities, populations, and individuals. Relatively few diagnostic molecular markers differentiated between *Q. crassifolia* and *Q. crassipes*. However, these markers demonstrated geographic consistency in support of the morphological evidence, indicating that each species is distinct and that each has some degree of genetic cohesiveness.

Furthermore, the character count procedure has allowed us to confirm statistically that *Q. dysophylla* is the result of hybridization between *Q. crassifolia* and *Q. crassipes* in the seven hybrid zones. The ordination analysis also demonstrated

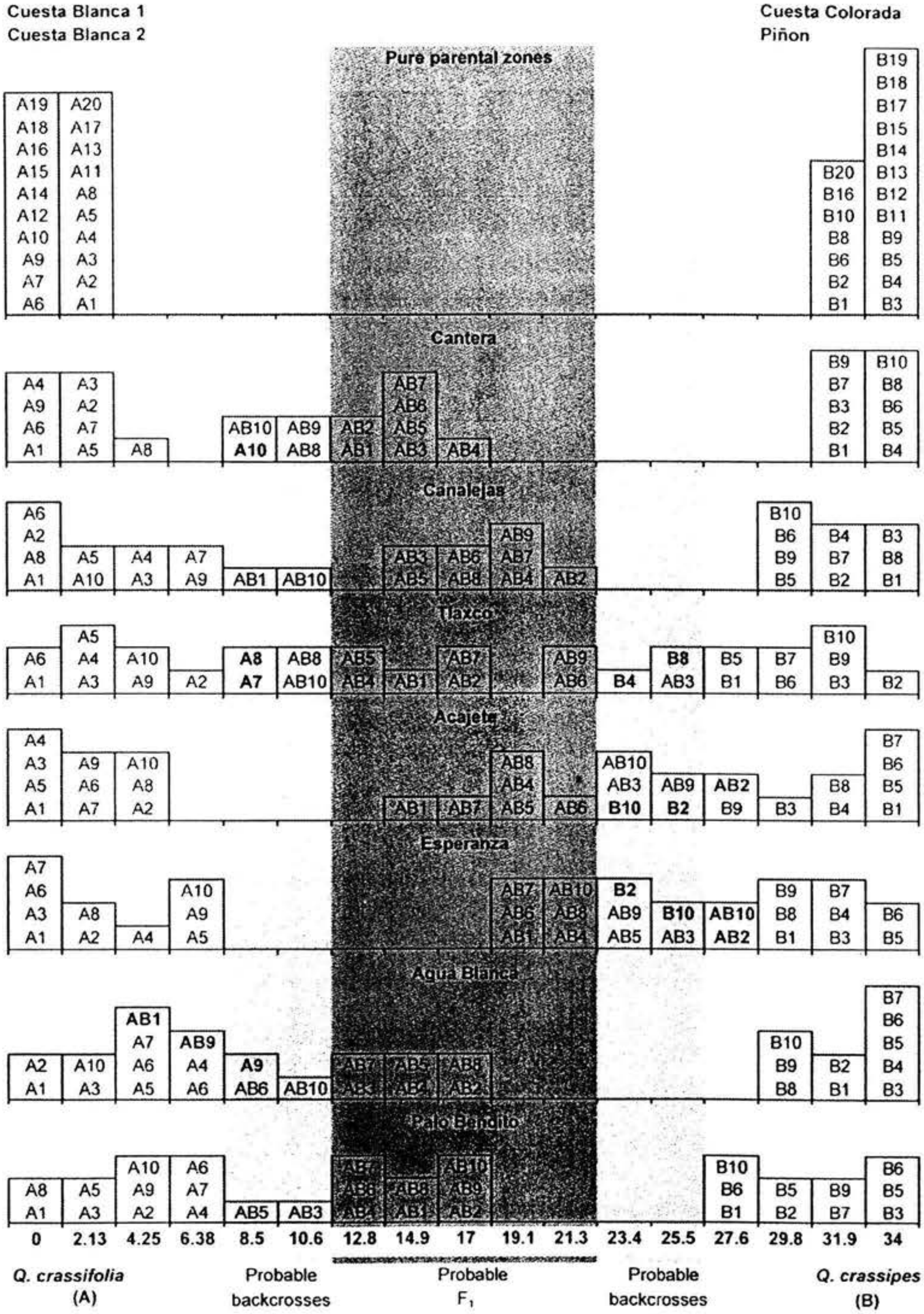

Fig. 4. Frequency distribution of individuals vs. the Anderson hybrid index derived from 17 morphological characters in two pure and seven hybrid zones. The number of each plant evaluated is represented. The figure shows the cluster results of the pure or nearly pure parentals in four zones (index = 0 or 34), a cluster for probable F_1 hybrids (index = 12.75–25.5), probable backcrosses towards *Q. crassifolia* (index = 8.5–10.63), and finally probable backcrosses toward *Q. crassipes* (index = 23.38–25.5). The plants misidentified in the field are indicated in boldface type.

that hybrids presented intermediate morphology between the parental species and that leaf shape explains a major percentage of variation.

Quercus crassifolia ranges from the Sierra Madre Occidental (SMOc) to the center of Mexico along the Eje Neovolcánico, whereas *Q. crassipes* ranges from the Sierra Madre Oriental (SMOr) to the Eje Neovolcánico, where both species overlap producing hybrid zones. The Eje Neovolcánico, an orographic system that traverses the central part of the country in an east–west direction, is considered geologically the youngest mountain range in Mexico and contains valleys higher than 2000 m in altitude and the tallest mountains in Mexico

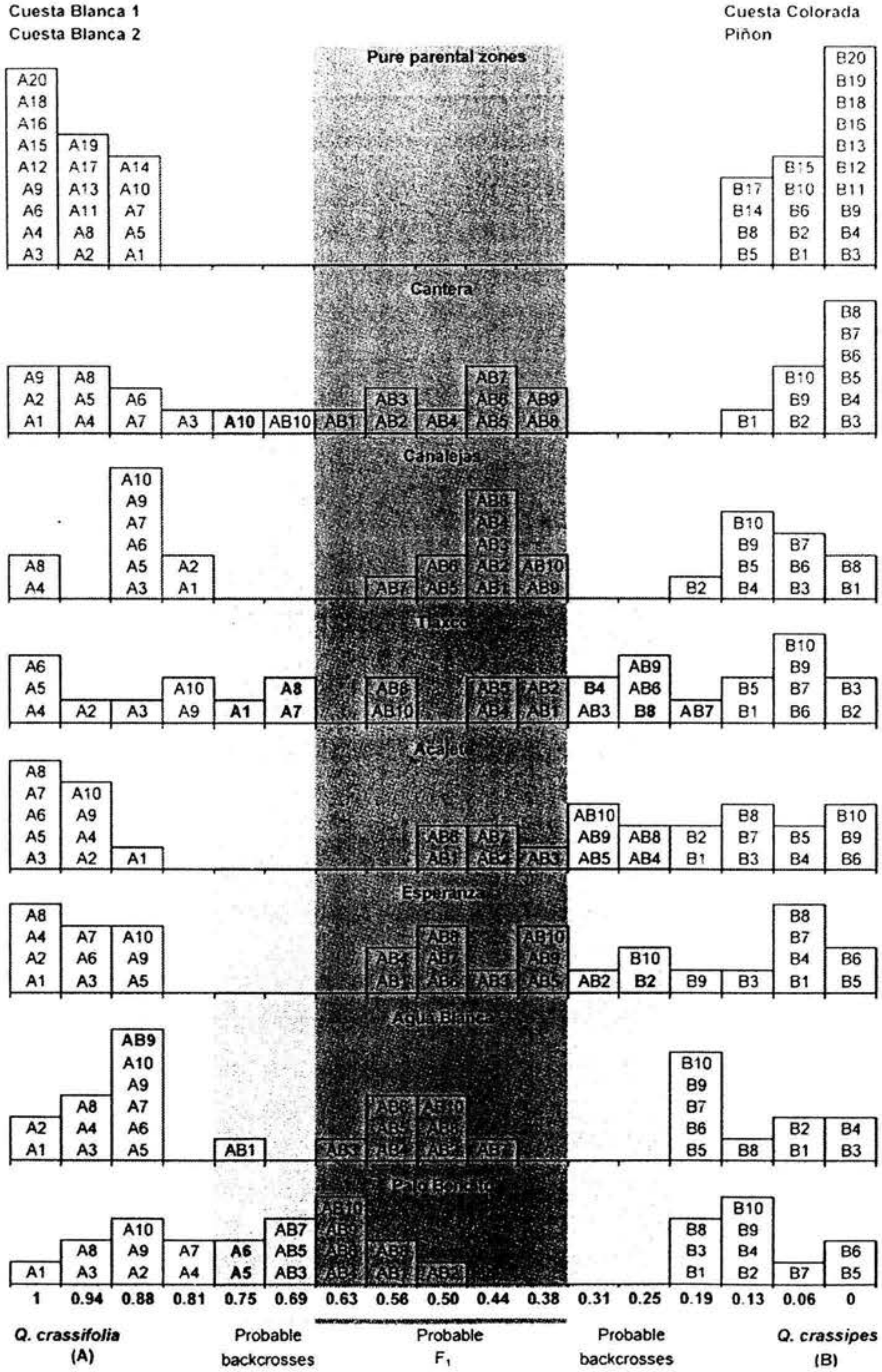

Fig. 5. Frequency distribution of individuals vs. the Hardig hybrid index derived from the RAPD band data using six primers. The number of each plant evaluated is represented. The figure shows cluster results of the pure or nearly pure parentals in four zones (index = 0 or 1), a cluster of probable F_1 hybrids (index = 0.437–0.562), probable backcrosses toward *Q. crassifolia* (index = 0.687–0.750), and finally probable backcrosses towards *Q. crassipes* (index = 0.250–0.312). The plants misidentified in the field are indicated in boldface type.

(Ferrusquia-Villafranca, 1993). Phylogeographic studies are in progress to understand the process of migration and the colonization routes of this oak complex, as has been done for other oak species (e.g., Dumolin-Lapègue et al., 1997).

The genetic results indicate that the introgression process is present in both species, but the direction changes depending on the localization of the hybrid zone. The hybrid zones closest to the SMOc (Cantera and Canalejas) registered unidirectional introgression towards *Q. crassifolia*, while the hybrids from the intermediate locality between the two mountain ranges (Tlaxco) showed bidirectional introgression, and the hybrids from the two closest localities to the SMOr registered unidirectional introgression towards *Q. crassipes*. These findings suggest that the closeness of hybrids to an allopatric site of either parental species is directly related to their similarity and vice versa. Thus, the Eje Neovolcánico acts as a corridor where the proximity to an allopatric site favors the introgression of the hybrid towards the parental species, increasing its variation from the species with which it is maintaining a genetic exchange, diluting the limits with the parental species in the allopatric site. Lastly, the two hybrid zones located north of Tlaxco (SMOr) showed unidirectional introgression towards *Q. crassifolia*. These results confirm that patterns of variation in oaks do not follow simple monotonic clines (e.g., Barton and Hewit, 1985) but form complex mosaic zones characterized by patches of pure populations and mixed populations scattered across a zone of overlap (Howard et al., 1997). A bidirectional hybrid zone was detected for *Q. crassifolia* and *Q. crassipes*.

In the seven hybrid zones studied along the Eje Neovolcánico, where intermediate plants are mixed with their parental species, hybrids are rare and they are in a narrow contact zone between well-differentiated taxa. The presence of hybrid individuals in the hybrid zones was very low (between 10 and 17 trees), requiring an extensive field search. Oak hybrids are produced in an isolated and sporadic manner and they may introgress with parental species (Bacon and Spellenberg, 1996). Hybrid zones with high levels of disturbance (i.e., Canalejas, Acajete, and Esperanza) were the ones with the highest number of hybrid individuals (mostly juveniles). Disturbances produced by human activities such as logging, deforestation, fires, and agriculture, may enhance the establishment of hybrids as they modify reproductive barriers (Arnold et al., 1990; Klier et al., 1991).

Our results suggest that the sympatric zones of *Q. crassifolia* and *Q. crassipes* are mosaic hybrid zones as proposed by Howard (1982, 1986) and Harrison (1986, 1990), because of the patchy distribution pattern of the parental species in sympatric and allopatric sites and the lack of a gradual transition from *Q. crassipes* to *Q. crassifolia*. It is important to mention that hybrid plants were less frequent than putative parents in the mixed stands and that *Q. crassipes* prefers drier habitats and lower sites than *Q. crassifolia*. Ecological divergence rather than genetic incompatibility may maintain hybrid zones (Jiggins and Mallet, 2000) by causing local adaptations to different environmental conditions (e.g., Howard et al., 1997).

In summary, we found that molecular markers (RAPD) and morphological leaf traits are highly coincident and support the phenomenon of hybridization between *Q. crassifolia* and *Q. crassipes* complex (Fig. 3). Inasmuch as hybridization was evident, both species remain distinct in mixed stands. We also observed that the Eje Neovolcánico acts as a corridor where proximity to an allopatric site favors the introgression of the hybrid with its parental species, increasing its divergence from the species with which it maintains a genetic exchange, and thus diluting the limits with parental species in the allopatric site. Hybrid plants constitute a heterogeneous group in which many individuals were F_1 and others appeared as backcrosses of *Q. crassifolia* or *Q. crassipes*, depending on the locality.

Our data and field observations suggest that the sympatric zones of *Q. crassifolia* and *Q. crassipes* must be considered as mosaic hybrid zones (e.g., Howard, 1982, 1986; Harrison, 1986, 1990), because of the patchy distribution pattern of the parental species in sympatric and allopatric sites, and there is not a gradual transition from *Q. crassipes* to *Q. crassifolia*. Finally, we suggest that *Q. dysophylla* does not deserve the status of species but it must be recognized as an entity of potential evolutionary importance, named as *Quercus* × *dysophylla* Benth. pro sp.

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Capítulo II.

ESTRUCTURA GENÉTICA Y DISTRIBUCIÓN DE HAPLOTIPOS DEL COMPLEJO

Molecular Ecology

Chloroplast DNA polymorphism reveals geographic structure and introgression in *Quercus crassifolia* × *Q. crassipes* complex in Mexico

E. TOVAR-SÁNCHEZ[a], C. SÁNCHEZ-HERNÁNDEZ[b], R. ESTEBAN-JIMÉNEZ[b], D. PIÑERO[b] and K. OYAMA[a].

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

[b] *Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, México D.F. 04510, México*

Author for correspondence: E. Tovar-Sánchez. Fax: 52 (55) 56232719; E-mail: efratosa@yahoo.com

Manuscript received________________; revision accepted_________________.

The authors thank Susana Valencia, Patricia Mussali, Marco Romero, Oscar Rodríguez, Mauricio Mora, and Maribel Paniagua for technical assistance. This research was supported by a PAEP-UNAM grant and a CONACYT scholarship to Efraín Tovar Sánchez.

Abstract

Quercus crassifolia H. & B. and *Q. crassipes* H. & B. (Subg. *Erythrobalanus*) are two important species of temperate forests in Mexico and form hybrids when they occur in sympatry (*Quercus* × *dysophylla* Benth pro sp.) at the Eje Neovolcánico and in the southest part of the Sierra Madre Oriental (SMOr) in central Mexico. In this study, we used chloroplast microsatellites to infer phylogeographic patterns on genetic structure of populations of both parental species and characterize hybridization and introgression between them. The distribution of haplotype diversity of 11 populations in *Q. crassifolia*, *Q. crassipes* and hybrids, were sampled from seven hybrid zones and four allopatric sites. A bayesian analysis of genetic structure in seven stands identified two highly differentiated genetic clusters, corresponding to *Q. crassifolia* and *Q. crassipes*. Data from three loci were sufficient to assign all individual trees to one of the two species. The results showed introgressive hybridization events, resulting in the incorporation of cytoplasmic material in a bidirectional way. In total, of the 210 trees sampled in seven hybrid zones, 21 (10.0%) trees registered introgression and 4 (1.9%) backcrosses towards *Q. crassipes*, 54 (25.7%) trees were identified as putative hybrids, 16 (7.6%) trees showed introgression and 11 (5.2%) backcrosses towards *Q. crassifolia*. Hybrid populations registered the highest levels of genetic variation (*H*), Shannon diversity index (*I*), and haplotype number (*nh*) in comparison with their putative parentals. Also, hybrid zones showed the highest values of genetic differentiation F_{ST}, R_{ST} and migrant estimates (*Nm*).

Keywords: cpSSRs, haplotypes, hybridization, Quercus, phylogeography, genetic structure.

Introduction

The genus *Quercus* L. (the oaks; Fagaceae) includes 531 species (Govaerts and Frodin, 1998) distributed mainly across the warmer temperate regions of the northern hemisphere, and Mexico contains 135-150 species (Nixon, 1993). Oaks forests cover around 4.29% (84 622 km^2) of Mexican territory (Flores-Villela and Gerez, 1994). Fossil records of *Quercus* (Daghlian and Cerped, 1982) suggest that North American flora spread into Mexico during Oligocene (approximately 35 Mys ago), throughout the Sierra Madre Oriental, coming from the Apalaches mountains (Martin and Harrell, 1957). So, it is possible to suggest that after the migration of *Quercus* to Mexico, this group could have colonized some regions originated from an intensive volcanic activity, characteristic of many Mexican mountains (Miocene) (Graham, 1993). One example, is the contact between oaks species that were isolated in the Sierra Madre Oriental (SMOr) and in the Sierra Madre Occidental (SMOc), by the Eje Neovolcánico mountains.

Oaks have high frequency of hybridization in nature (Trelease, 1924; Palmer, 1948; Muller, 1952; Tucker, 1961; Cottam et al., 1982; Jensen et al., 1993; Spellenberg, 1995; Howard et al., 1997; Ishida et al., 2003; González-Rodríguez et al., 2004; Tovar-Sánchez and Oyama, 2004) due to its poor development of sterility barriers between species (Jensen and Eshbaugh, 1976; Grant, 1981; Whittemore and Schaal, 1991; Rushton, 1993; Bacilieri et al., 1995; Dumolin et al., 1997). This group presents complex patterns of variation, which leads to problems on species identification (Burger, 1975). *Quercus crassifolia* and *Q. crassipes* (Subg. *Erythrobalanus*) are two important elements of the temperate forests in Mexico that form hybrids when they occur in sympatry (*Quercus* × *dysophylla* Benth pro sp.). This interfertile pair of species show extensive ecological overlap forming hybrid zones over areas of sympatry at the Eje Neovolcánico, and in the southeast part of the Sierra Madre Oriental in central Mexico and remain distinct despite occasional hybridization (Tovar-Sánchez and Oyama, 2004).

Microsatellites or simple sequence repeats (SSRs) have become a popular tool for genetic identification of closely related taxa in plants (Bruschi et al. 2000). Microsatellites markers have high mutation rates and high levels of heterozygosity and polymorphism and their molecular structure and evolution are relatively well understood (Jarne and Lagoda 1996; Estoup and Cornuet 1999; Xu et al. 2000), making them suitable for studies of hybridization and introgression (Muir et al. 2000). Recently, chloroplast SSRs have been used in many species of *Quercus* to study diversity, chloroplast lineages and evolutionary history of species (Petit et al. 1993, 2002; Bacilieri et al. 1996; Dumolin-Lapègue et al. 1998; 1999).

Organellar DNA is a key element for tracing the long-term effects of hybridization. Because of their uniparental and asexual nature of inheritance, groups of associated restriction sites are not separated by recombination, so this makes them particularly suited for historical information and phylogeographic studies (Whittemore and Schaal, 1991). Cytoplasmic genomes (chloroplast and mitochondrial) variants also show high rates of introgression among plant species (Rieseberg and Soltis, 1991). In oaks, cpDNA is maternally inherited therefore is transmitted by seeds only (Dumolin et al. 1995), therefore, chloroplast based markers are more suited than nuclear ones for the study of seed dispersal and the geographic structure of genetic diversity (Petit et al. 1997). Dumolin-Lapègue et al. (1997) showed that intraspecific geographical structuring of cpDNA in oaks and related haplotypes often have similar distribution. Because of the intrinsic historical information contained in these phylogenies, the spatial-temporal dynamics of the maternal lineage can be covered by first studying the phylogenetic relationships of cpDNA variant and then mapping their geographic distribution. Recent studies on oak phylogeography that included several species, demonstrated extensive sharing of cytoplasmic variant among species (Petit et al. 2002).

In addition to conventional analysis to identify genetic structure in mixed populations a bayesian analysis has the advantage of assig individuals to populations according to likelihood based on allele frequencies and to identify individuals whose genetic makeup may be drawn from two populations (hybrids).

Thus, in this study we examine the level of cpDNA variation within *Q. crassifolia*, *Q. crassipes* and putative hybrids, and to quantify the level of introgression in seven hybrid zones. Also, we determine the genetic structure of each taxa analyzing the distribution of haplotypic diversity and genetic relationships between taxa.

Materials and methods

Sample collection, DNA extraction, PCR and cpSSR markers

Samples were collected from 250 individuals representing 11 populations. Two allopatric populations in Sierra Madre Occidental (SMOc) for *Q. crassifolia*, two allopatric populations in Sierra Madre Oriental (SMOr) for *Q. crassipes*. In the Eje Neovolcánico five mixed populations and other two mixed populations in the southeast part of the Sierra Madre Oriental (Figure 1). In each mixed population we sampled ten individuals per each taxa, and in each allopatric population 20 individuals. Fresh leaf material were collected in the field from permanently tagged specimens and returned to the laboratory for DNA extraction. Total DNA was extracted and purified using a CTAB-based method and light solvents extraction, combined with anion-exchange membrane purification, and this protocol is a modification to DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) to remove proteins and other secondary compounds. The DNA concentration of each sample was obtained by fluorometric analysis, and the DNA quality was estimated by comparing the intensity of bands with known standards of lambda DNA on an agarose gel at 0.8%. Finally, we diluted all DNA samples to a final concentration of 10 ng/μl.

Chloroplast microsatellites primers were obtained from Weising and Gardner (1999) and we selected three loci for the genetic analysis (Ccmp3, Ccmp4, and Ccmp41). The amplification mixture for each sample contained 20 mM Tris-HCl, 50 mM KCl, 2 mM $MgCl_2$, 0.13 mM of each dNTP, primer 25 μM, 15 ng of chloroplast DNA and 0.8 units of Taq polymerase, in a final volume of 25 μl. The polymerase chain reactions (PCR) were carried out on a PTC-100 Programmable Thermal Controller (MJ Research Inc.) using the following program: 5 min at 95°C, followed by 30 cycles of 1 min at 94°C, 1 min at 50°C, 30 sec at 72°C, and a final extension of 8 min at 72°C. The annealing temperature was changed to different microsatellite primers: 50°C for Ccmp3, 48°C for Ccmp4; and 55°C for Ccmp41, and all annealing cycles remaining 1 minute. Products were resolved on 6% denaturing polyacrylamide gels (7 M urea) at 60 W constant power for 3 h.

Polymorphic fragments were labelled by decreasing order of fragment length as visualized in the polyacrylamide geles. Haplotypes were defined according to different combinations of length variants.

Statistical analysis

We registered the genetic variation of *Q. crassifolia*, *Q. crassipes* and putative hybrids using the allele frequencies per locus in each population. Also, we registered in each taxon the haplotypic variation, which one was estimated using the unbiased genetic diversity (*H*, Nei 1987), and the global expected heterozygosity or gene diversity for the total number of individuals (H_T), number of haplotypes (*nh*), and Shannon diversity index (*I*). Theta (θ) was estimated indirectly based on heterozygosity using both mutational models (stepwise and infinite allele) (to an haploid marker, $\theta = 2Nu$, were N is the population size and u is the mutation rate; Schneider et al. 2000). The analyses were performed with ARLEQUIN version 2000 (Schneider et al. 2000).

Population structure for *Q. crassifolia*, *Q. crassipes* and putative hybrids was estimated using an analysis of molecular variance (AMOVA; Excoffier et al. 1992), to test how sequence variation is partitioned within and among populations in all sites.

The genetic distance between pair of haplotypes was estimated using both the infinite alleles (F_{ST}) and the stepwise mutation models (R_{ST}) (Kimura and Crow 1964). The null distribution of pairwise F_{ST} and R_{ST} values under the hypothesis of panmixia was obtained with 1000 random permutations of haplotypes between populations. R_{ST} and F_{ST} are index of genetic differentiation, typically applied to population subdivision within species, these statistics we used here to assess genetic differentiation between the two species. Also average number of migrants (*Nm*) was estimated for all loci in both species (Schneider et al. 2000).

Genetic distance among populations was determined using D_a, for both mutational models (stepwise and infinite allele) (Nei et al. 1983). The statistical significance between D_a and geographical distance was tested with a Mantel test (Mantel, 1967).

UPMGA tree was constructed with genetic distance of haplotypes for each genetic entity and was validated with bootstrap in a set of 2000 iterations (POPGEN, Ver. 1.31).

We selected the haplotypes that registered synapomorphies to elaborate a map of geographic distribution of haplotypes (absolute frequency superior two) throughout the seven hybrid zones, and allopatric sites of *Q. crassifolia* and *Q. crassipes*.

We applied a bayesian clustering approach to assign individual trees to species on basis of their genotypes alone. This allowed us to determine how well the genetic structure in the entire data set corresponded to phenotypic assignment to species. This approach, implemented using the program STRUCTURE (Pritchard et al. 2000), uses a model-based clustering method for inferring population structure from multilocus genotype data and to simultaneously assign individuals and estimates the fraction of their alleles that are derived from each species.

We determined introgression, backcrosses, and hybridization levels with a multivariate Bayesian analysis with all haplotypes in seven hybrid zones (STRUCTURE; Pritchard et al. 2000). The criteria for statistic selection to determine hybrid individuals was that they must share proportions ranging from 0.4 to 0.6 of parental alleles, for backcrosses an interval of > 0.6 to < 0.9 and for introgression > 0.9 (Pritchard et al. 2000).

An analysis of shared alleles by maximal verosimilitude was conducted because the oak cpDNA presents high rates of inversions and translocations (Palmer et al. 1985; Soltis et al. 1992; Hong et al. 1993) which alters the mutation rates in microsatellite sequences, making them unusually high, and when an exclusive allele analysis is conducted, it permits to verify the parental allele associations in hybrid individuals by the sharing allele index obtained by maximal verosimilitude, also, it verifies total ancestral and actual allelic patterns in populations.

Results

Genetic variation

Three (Ccmp3, Ccmp4, and Ccmp41) cpSSR loci were polymorphic in 250 individuals of *Q. crassifolia*, *Q. crassipes* and derived hybrid; a total of 16 alleles (having either five or six alleles per locus) and 126 haplotypes were found. Eighteen haplotypes registered absolute frequency above two. *Quercus crassifolia* and *Q. crassipes* shared alleles at all loci but each harbored private alleles not found in the other species. The test for homogeneity of allele distribution was highly significant ($P < 0.001$) for all three loci. Considering the frequencies of all 126 haplotypes for the analysis, we found that genetic diversity varies as the following patterns: hybrids populations > *Q. crassifolia* > *Q. crassipes* (Table 1). Hybrid populations registered the highest levels of genetic variation (H), Shannon diversity index (I), and haplotype number (nh) in comparison with their putative parentals. Also, hybrid zones showed the highest values of genetic differentiation F_{ST}, R_{ST} and migrant estimates (*Nm*; Table 1).

Since θ is obtained from heterozygosity data in each population, both models [stepwise (SMM) and infinite allele (IAM)] follow the same pattern. In all cases θ estimates were higher using the stepwise mutation model. Also, θ can be interpreted as a relative estimate of population size. The highest θ estimates were found in the allopatric site Cuesta Colorada for *Q. crassipes* (SMM; θ = 391.50, and IAM θ = 25.27), followed by the allopatric site Durango 1 for *Q. crassifolia* (SMM; θ = 112.00, and IAM θ = 12.50), while the smallest estimate corresponds to Tlaxco (SMM; θ = 8.88, and IAM θ = 2.55) (Table 1).

Genetic differentiation among populations

Molecular variance analysis with the infinite allele model showed that the fixation index was statistically significant for each taxa (*Q. crassifolia* = 0.59, *Q. crassipes* = 0.45, and hybrids = 0.40). The highest variation was registered within hybrid populations (60.19%) and the remaining 39.81% among them. *Q. crassipes* (42.76) and *Q. crassifolia* (34.52) presented the highest variation among populations (Table 2).

On the other hand, molecular variance analysis with the stepwise mutation model showed that the fixation index was statistically significant for each taxa (*Q. crassifolia* = 0.33, *Q. crassipes* = 0.31, and hybrids = 0.39). *Quercus crassifolia*, *Q. crassipes* and hybrids showed the highest variation within populations (Table 3).

Geographic variation of haplotypes

The geographic distribution of the 18 cpDNA haplotypes is clearly not random (Fig. 2). The frequency of the different haplotypes varies between the two species and among regions. The hybrids contain haplotypes of both parental species. The haplotypes **A**, **B**, **C** and **D** are characteristic of *Q. crassifolia*'s allopatric sites (Durango 1 y 2) localized at the SMOc. **A** and **B** haplotypes are common among *Q. crassifolia* populations, localized at the five hybrid zones of the Eje Neovolcánico where haplotype **A** is passing to hybrid individuals of Esperanza. Haplotype **C** is common in *Q. crassifolia* from Palo Bendito (SMOr), and hybrids and *Q. crassipes,* It was also the dominant haplotype for *Q. crassipes* in Tlaxco. Haplotype D was registered in Durango 1 and Agua Blanca (SMOr) for *Q. crassifolia* (Fig. 2).

On the other hand, haplotypes **F**, **G**, **H**, **I**, **J**, **K**, **L** and **N** are characteristic of *Q. crassipes's* allopatric sites (Piñon and Cuesta Colorada) localized at the SMOr . Haplotype **N** was common among *Q. crassipes* populations in Agua Blanca and Esperanza and hybrid individuals in Palo Bendito, Agua Blanca and Tlaxco. Haplotype **K** is also common in hybrids from Palo Bendito and for *Q. crassipes* of Esperanza. Acajete and Canalejas and Esperanza presented haplotype **L**. In the Eje Neovolcánico, haplotype **E** is characteristic of *Q. crassifolia* and hybrid individuals from Esperanza (Fig. 2).

Haplotype **O** is in *Q. crassipes* of Agua Blanca, Palo Bendito, Esperanza, Canalejas and Cantera, and Cantera hybrids. Haplotype **Q** is in *Q. crassifolia*, hybrids and *Q. crassipes* from Palo Bendito and also hybrids from Agua Blanca. *Q. crassipes* from Esperanza and Acajete and hybrids from Tlaxco and Cantera registered haplotype **M**. Haplotype **P** in *Q. crassifolia* population from Palo Bendito as well as hybrid populations from Agua Blanca, Esperanza, Acajete and Canalejas was registered. Finally, haplotype **R** is present in populations of *Q. crassifolia* from Acajete, Canalejas and hybrids from Canalejas (Fig. 2).

For both mutation models (stepwise (SMM) and infinite allele (IAM)), we found a significant association between genetic and geographic distances to *Q. crassifolia* (Mantel 1967;

with SMM $r = 0.72$; $P = 0.002$ and IAM $r = 0.32$; $P = 0.04$) and *Q. crassipes* (Mantel 1967; with SMM $r = 0.37$; $P = 0.03$ and IAM $r = 0.51$; $P = 0.003$).

Introgression, hybridization and backcross

For the Bayesian analysis, we first used STRUCTURE to cluster our data without using information regarding species assignment of individual trees. The two genetically distinct clusters found in the analysis corresponded well to our assignment of individuals to *Quercus crassifolia* and *Q. crassipes*. Of 70 *Q. crassifolia* genotyped, 68 (97%) had inferred ancestry in a "*Q. crassifolia*" cluster with probability >0.90, and 100% (70) of *Q. crassipes* had >0.90 inferred ancestry in a "*Q. crassipes*" cluster (Fig. 3). Of the 210 sampled trees in seven hybrid zones, 21 (10.0%) trees registered introgression and four (1.9%) backcrosses towards *Q. crassipes*, 54 (25.7%) trees were assigned as putative hybrids, finally, 16 (7.6%) trees showed introgression and 11 (5.2%) backcrosses towards *Q. crassifolia* (Fig. 3). These last results suggest bidirectional introgression (Figs. 3 and 4). Bayesian analysis showed that in the seven hybrid zones hybridization, backcrosses and introgression phenomena exists (Fig. 4).

Discussion

Quercus crassifolia and *Q. crassipes* (subg. *Erytrobalanus*) are related oak species that have broad areas of sympatry in Eje Neovolcánico and in the southeast part of the Sierra Madre Oriental in central Mexico and it has been reported that they hybridize in nature (Tovar-Sánchez and Oyama, 2004), showing occasional individuals with intermediate morphologies. An important factor that can favor hybridization between these two species is the overlap in flowering time. *Q. crassifolia* registers its flowering peak in April and *Q. crassipes* in May (Romero, 1993), but we observed in the mixed stands flowering overlaps in the last days of April.

The distribution of chloroplast genotypes in *Q. crassifolia* and *Q. crassipes* indicate that in sympatric sites both species do not represent fully isolation gene pools, but are actively exchanging chloroplast genes. Despite *Q. crassifolia* and *Q. crassipes* are hybridizing in nature, they remain morphologically, genetically, and ecologically distinct as do other sibling pairs of *Quercus* (Kleinschmit et al., 1995; Howard et al., 1997; Bruschi et al., 2000; Tomlinson et al., 2000; Ishida et al., 2003). In fact, most pairs of *Quercus* species that remain distinct despite hybridization is because they are maintaining more effectively by ecological divergence between parental species than by their genetic incompatibility (Kleinschmit et al., 1995; Howard et al., 1997; Bruschi et al., 2000; Jiggins and Mallet 2000; Tomlinson et al., 2000; Williams et al. 2001). Furthermore, this may be explained by "isolation by distance" phenomenon, as our data suggest through the significant correlation of genetic (with infinite allele model and stepwise mutation model) and geographical distance to both species. However, this strong geographical isolation is not surprising, because seeds are less mobile than pollen (Stanley and Linskens, 1974). For example, Dow and Ashley (1996) studied American oaks using microsatellite nuclear markers and found high levels of long distance pollination and more limited acorn dispersal.

The estimated number of migrant chloroplast per generation (*Nm*), suggest that a moderate genetic interchange between both species exists (*Q. crassifolia* = 0.841 and *Q. crassipes* = 1.062). The migrant estimates increase between species at the same locality (*Nm* = 1.358), corresponding to an *Nm* of 2.716 from diploid nuclear locus (Birsky et al. 1989), is higher that rates of gene flow between populations within many plant species (Govindaraju, 1988). The above, suggest that populations of *Q. crassifolia* and *Q. crassipes* experiment a high level of gene flow in the same geographical area very much greater that the level of gene flow from distant conspecific populations. The high level of interspecific gene flow is reasonable, considering the high characterization of morphologically recognizable hybrids in the field. Despite the high level of interspecific gene flow shown in our study, *Q. crassifolia* and *Q. crassipes* are still differentiated.

Our results also showed that phenotypically intermediate trees between *Q. crassifolia* and *Q. crassipes* are probably the result of hybridization. All trees with intermediate morphology showed evidence of mixed ancestry. Also, the bidirectional genetic introgression was registered in all hybrid zones, in contrast with previous populations studies of this genus (Dumolin-Lapègue et al. 1999; Belahbib et al. 2001; Petit et al. 2001; Petit et al., 2003) observing unidirectional introgression. In this study, both estimates of genetic differentiation (F_{ST} and R_{ST}) were significant different between *Q. crassifolia* and *Q. crassipes*. Over all loci, the estimate of R_{ST} was higher than $F_{ST,}$ indicating that the two species did not just differed allele frequencies distribution, but also in allele size. Such shifts likely occur over longer divergence times than changes in frequencies (Slatkin, 1995), suggesting historically low levels of introgression. This explains why the species remains distinct despite occasional hybridization.

The highest estimate of genetic structure was seen between hybrid populations (R_{ST} = 0.614 and F_{ST} = 0.388) in comparison with their putative parentals. Also, the presence of unique haplotypes in these populations suggests that introgressive hybridization has occurred constantly trough various generations and it is now being reflected when observing new variants as well as variants of hybrid individuals developing in these environments.

The level of differentiation (F_{ST}) among populations in *Q. crassifolia* (0.353) and *Q. crassipes* (0.302) are relatively similar to *Q. ilex* populations Morocco (0.33, Belahbib et al. 2001), and the coefficient of differentiation was relatively lower than other European Fagaceae studies (0.856 for *Q. petrea*; 0.902 for *Q. pubescens*; 0.781 for *Q. robur*; 0.832 for *Q. frainetto*; 0.946 for *Q. faginea*; 0.961 for *Q. pyrenaica* (Petit et al. 2002), and 0.84 for *Q. suber* from Morocco (Belahbib et al. 2001)). Another comparative study on cytoplasmic diversity reported that the mean estimate of population subdivision measured for 97 plant species was $G_{ST} = 0.70$; in particular the G_{ST} value was 0.73 for angiosperm tree species. The low level of cpDNA differentiation among *Q. crassifolia* populations and among *Q. crassipes* populations should be re-evaluated, given the low level of cpDNA diversity for this species in Mexico and the more limited sample size; the occasional cases of introgression between them, may also contribute to produce this unusually low estimate when comparing with other oak species (Petit et al. 2001).

Our results suggest that these hybrid zones are promoting an increase in the genetic diversity levels (*H*) and Shannon's index (*I*) in relation to parental species indicating that there is introgression in the mixed stands (Raven, 1976; Grant, 1981). Hybrid populations on the Eje Neovolcánico show an unidirectional gradient of genetic diversity and Shannon index in an east-west direction. The Eje Neovolcánico, an orographic system that traverses the central part of the country in an east–west direction, is considered geologically the youngest mountain range in Mexico. It is hypothesized that its evolutionary process began during the Mid-Terciary with the occidental portion formation followed by the development of the central and oriental portion during the Quaternary-Pliocene. (Ferrusquia-Villafranca, 1993), and it has been considerate as a center where new plant species arise due to introgressive hybridization (Rzedowski, 1978). Divergence values (θ) suggest that *Q. crassipes* is older than *Q. crassifolia*, and both species are older than all the hybrid populations localized at the Eje Neovolcánico and at the southest part of the SMOr. We suggest that *Q. crassifolia* distributes from the SMOc to the center of Mexico through the Eje Neovolcánico, and *Q. crassipes* distribution is from the SMOr to the Eje Neovolcánico, where both species overlap producing hybrid zones. North American flora spread into Mexico during Oligocene (approximately 35 Mys ago), throughout the Sierra Madre Oriental, coming from the Apalaches mountains (Martin and Harrell, 1957), supported by fossil records of *Quercus* (Daghlian and Creped, 1982). It is possible to suggest that after the migration of *Quercus* to Mexico, the oaks could colonize the volcanic remains originated from an intensive volcanic activity, characteristic of many Mexican mountains (Miocene) (Graham, 1993), this last scenario, may facilitate the contact between species that were isolated in the SMOr and in the SMOc, by the Eje Neovolcánico mountains. In consequence, both populations could have meet and diversify.

Cytoplasmic exchanges in plants have been described in a large number of cases (Rieseberg y Soltis, 1991), particularly in the genus *Quercus* (Belahbib et al. 2001; Petit et al., 2003). Our study suggest a bidirectional cytoplasmic introgression between *Q. crassifolia* and *Q. crassipes* (subgen *Erytrobalanus*), in contrast with previous populations studies in this genus (Dumolin-Lapègue et al. 1999; Belahbib et al. 2001; Petit et al. 2001; Petit et al., 2003) where there is unidirectional introgression.

Clearly, the Eje Neovolcánico was confirmed as a hot spot for diversity, where different maternal lineages merged which also exhibited high levels of diversity. These results need to be compared with diversity estimates of other traits, in order to draw wide range scale conclusions on conservation strategies. Despite the differences noted in the way species partition cpDNA diversity, they share extensively the same haplotypes, even at the local scale. This finding confirms the results of previous studies in France between *Q. robur* and *Q. petrea* (Petit et al. 1997) or between *Q. robur*, *Q. petrea*, and *Q. pubescens* (Dumolin-Lapègue et al. 1999). It supports the view that introgression was not restricted to rare episodes during long periods of sympatry.

The high level of differentiation between *Q. crassifolia* and *Q. crassipes* for microsatellite loci suggests that these markers may provide improved resolution for studying hybridization, introgression and historic dynamics in oaks.

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Figure legends

Fig. 1. Map of sampled populations of *Quercus crassifolia* × *Q. crassipes* complex. The mixed stands are represented by numbers, 1 = Cantera, 2 = Canalejas, 3 = Tlaxco, 4 = Acajete, 5 = Esperanza (located at the Eje Neovolcánico), 6 = Agua Blanca, 7 = Palo Bendito (located at the Sierra Madre Oriental).

Fig. 2. Geographic distribution of 18 cpDNA haplotypes identified in *Quercus crassifolia, Q. crassipes* and putative hybrids in Mexico.

Fig. 3. Upper histogram shows the mean values of $q_1^{(i)}$ (the proportion of ancestry in population 1, the "*crassipes*" cluster) over a single run of the Gibbs sampler in *structure* program for trees identified in the field as *Quercus crassipes*. Middle histogram shows $q_1^{(i)}$ for trees identified in the field as *Q. crassifolia*. Lower histogram shows $q_1^{(i)}$ for trees identified as putative hybrids based on intermediate morphology.

Fig. 4. Introgression backcrosses, and hybridization levels with a multivariate bayesian analysis with all haplotypes in seven hybrid zones of the *Quercus crassifolia* × *Q. crassipes* complex in Mexico. The criteria for statistic selection to determine hybrid individuals was: They must share proportions raging from 0.4 to 0.6 of parental alleles, for backcrosses an interval of > 0.6 to < 0.9 and for introgression > 0.9 (Pritchard et al. 2000).

Table 1 Genetic diversity and genetic structure at cpDNA markers in 11 Mexican population of *Q. crassifolia* × *Q. crassipes* complex

Taxa						SMM	IAM			SMM	IAM
	N	*nh*	No. loci	*H*	*I*	($\theta = 2Nu$)	($\theta = 2Nu$)	Nm	K ($N_{e\text{-}0}$)	R_{ST}	F_{ST}
Q. crassifolia											
Durango 1	20	7	3	0.507 ± 0.042	0.765 ± 0.163	112.00	12.498 ± 13.521	0.536	0.487 ± 0.149		
Durango 2	20	6	3	0.407 ± 0.192	0.639 ± 0.290	40.00	6.772 ± 5.690	0.644	0.450 ± 0.168		
mean								**0.841**	**0.332 ± 0.286**	**0.501 ***	**0.353 ***
Hybrids											
Cantera	30	14	3	0.644 ± 0.010	1.097 ± 0.312	14.77	3.454 ± 2.342	1.342	0.384 ± 0.474		
Canalejas	30	15	3	0.616 ± 0.132	1.096 ± 0.084	88.00	8.88 ± 3.689	1.287	0.377 ± 0.223		
Tlaxco	30	11	3	0.601 ± 0.142	1.094 ± 0.380	8.88	2.549 ± 1.923	1.045	0.499 ± 0.212		
Acajete	30	12	3	0.579 ± 0.121	1.042 ± 0.278	10.74	3.110 ± 2.575	2.24	0.507 ± 0.227		
Esperanza	30	16	3	0.508 ± 0.080	0.770 ± 0.202	66.26	8.775 ± 6.272	1.53	0.459 ± 0.426		
Agua Blanca	30	11	3	0.582 ± 0.073	0.962 ± 0.250	26.54	4.170 ± 3.912	1.09	0.459 ± 0.280		
Palo Bendito	30	10	3	0.592 ± 0.095	1.023 ± 0.251	9.03	2.601 ± 1.439	0.972	0.425 ± 0.181		
mean								**1.358**	**0.444 ± 0.289**	**0.614 ***	**0.388 ***
Q. crassipes											
Piñón	20	6	3	0.427 ± 0.221	0.676 ± 0.354	40.00	6.772 ± 6.865	1.579	0.843 ± 0.380		
Cuesta Colorada	20	7	3	0.200 ± 0.211	0.312 ± 0.213	391.50	25.272 ± 9.763	1.893	0.938 ± 0.413		
mean								**1.062**	**0.553 ± 0.446**	**0.398 ***	**0.302 ***

nh = number of haplotypes, *H* = genetic diversity, *I* = Shannon index, N = number of individuals
θ estimates correspond to the stepwise mutation model (SMM) and the infinite allele model (IAM)
Nm = migrant estimate, R_{ST} and F_{ST} = genetic diferentiation, *K* = population size

Table 2 Molecular analysis of variance (AMOVA) with infinite allele model (F_{ST}) for *Quercus crassifolia* × *Q. crassipes* complex in Mexico.

Source of variation	Percentage of variation		
	Q. crassifolia	hybrids	*Q. crassip*es
Among populations	59.37	39.81	44.95
Within populations	40.63	60.19	55.05
F_{ST}	0.59	0.40	0.45

Table 3 Molecular analysis of variance (AMOVA) with stepwise mutation model (R_{ST}) for *Quercus crassifolia* × *Q. crassipes* complex in Mexico.

Source of variation	Percentage of variation		
	Q. crassifolia	hybrids	*Q. crassipes*
Among populations	32.37	16.07	31.28
Within populations	67.29	83.93	68.72
R_{ST}	0.33	0.39	0.31

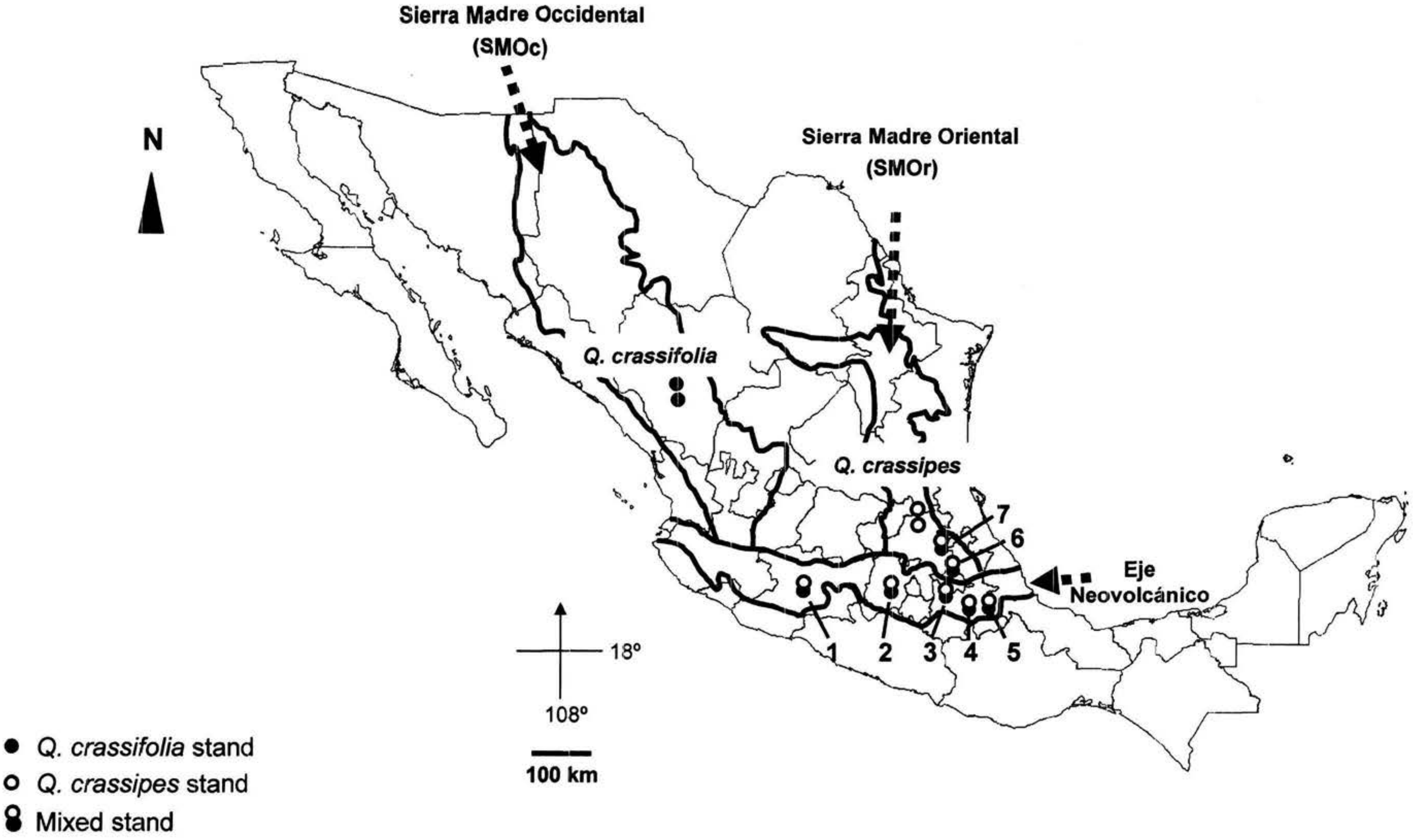

Sierra Madre Occidental
(SMOc)
N
Sierra Madre Oriental
(SMOr)
Q. crassifolia
Q. crassipes
7
6
Eje
Neovolcánico
1
2
3
4
5
18°
108°
100 km
● Q. crassifolia stand
○ Q. crassipes stand
Mixed stand

Fig. 1

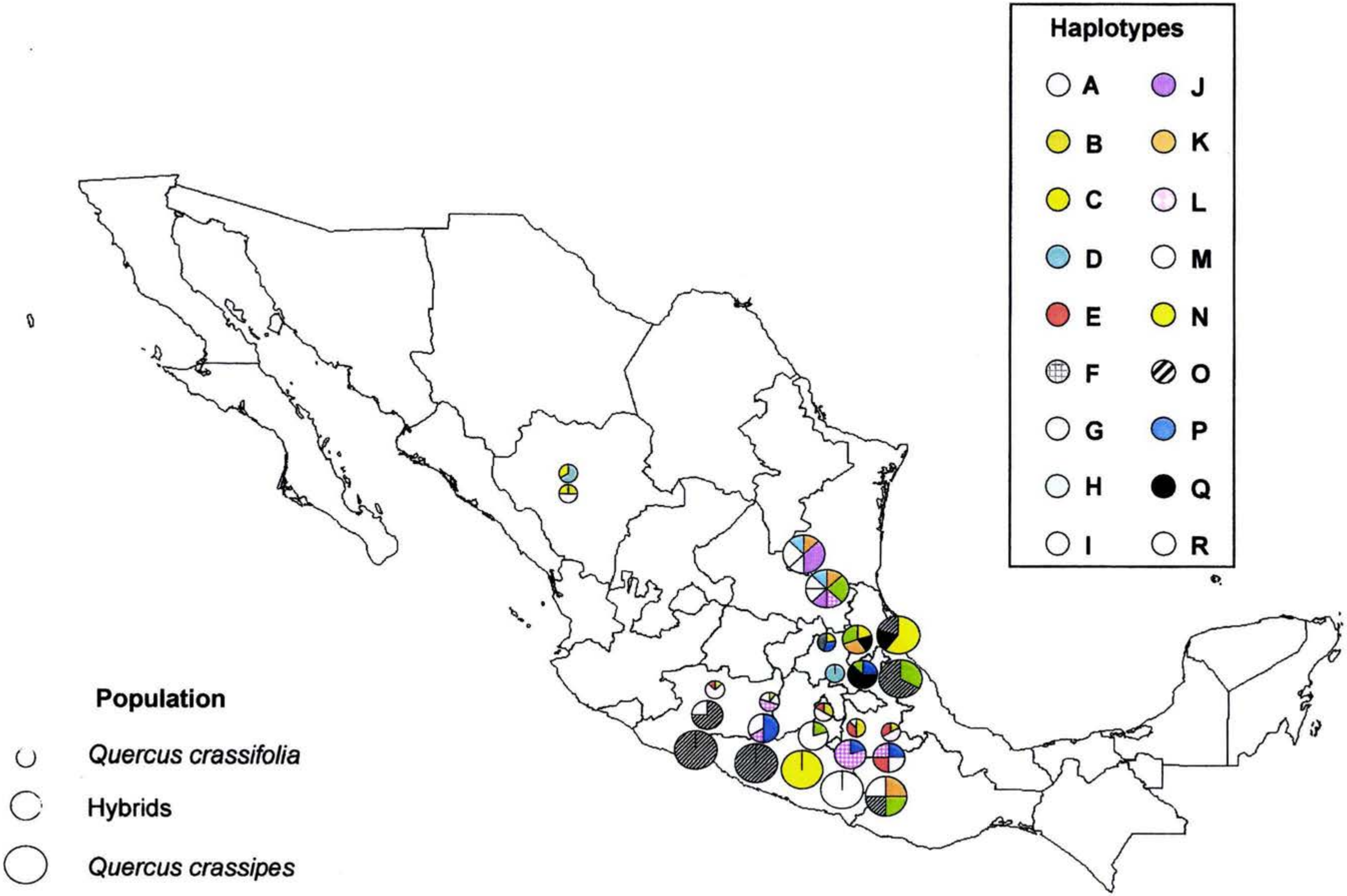
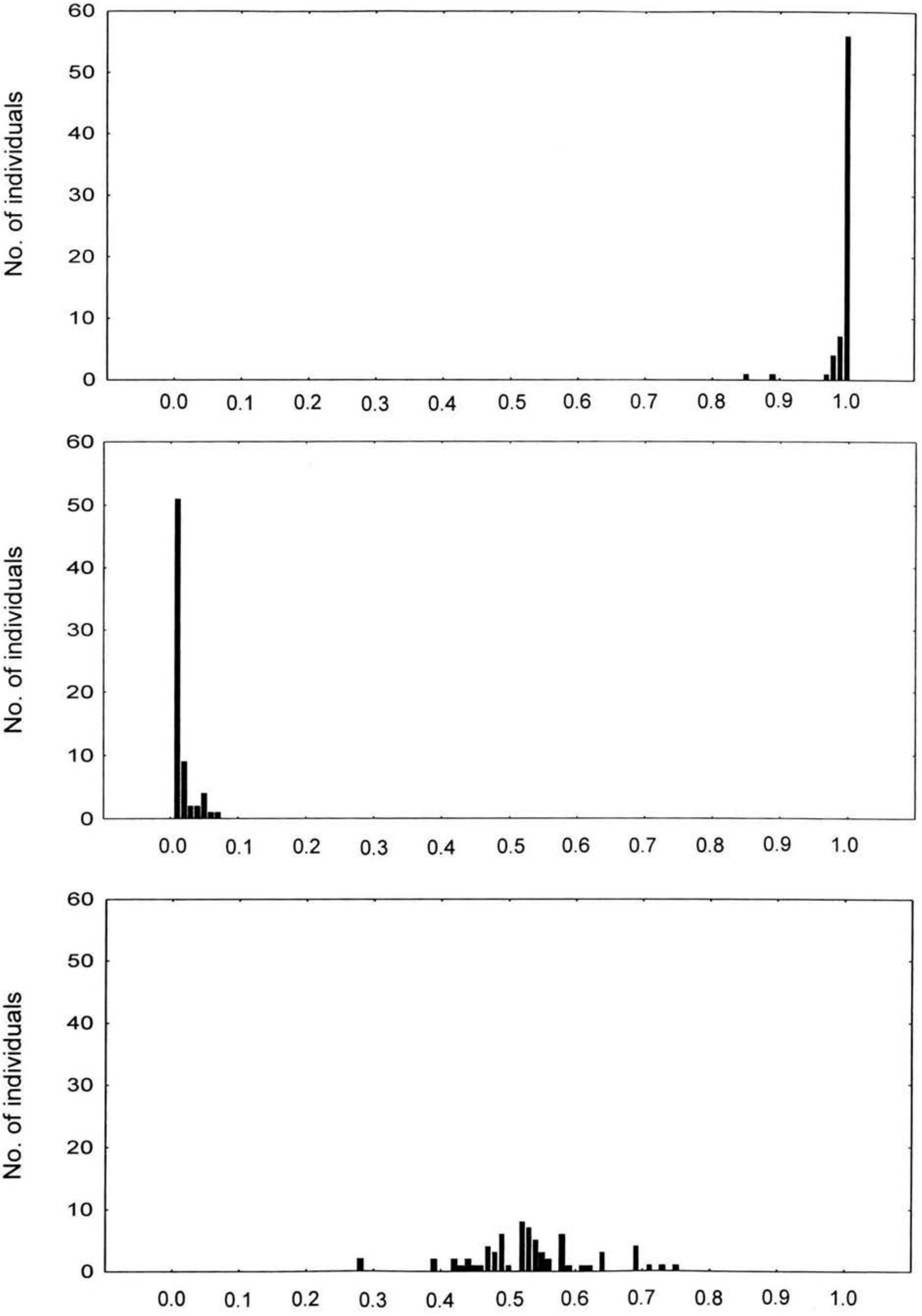

Haplotypes
A
B
C
D
E
F
G
H
I
J
K
L
M
N
O
P
Q
R
Population
Quercus crassifolia
Hybrids
Quercus crassipes

Fig. 2



Proportion of inferred ancestry in *Quercus crassipes* top, *Q. crassifolia* middle, and putative hybrids bottom.

Fig. 3

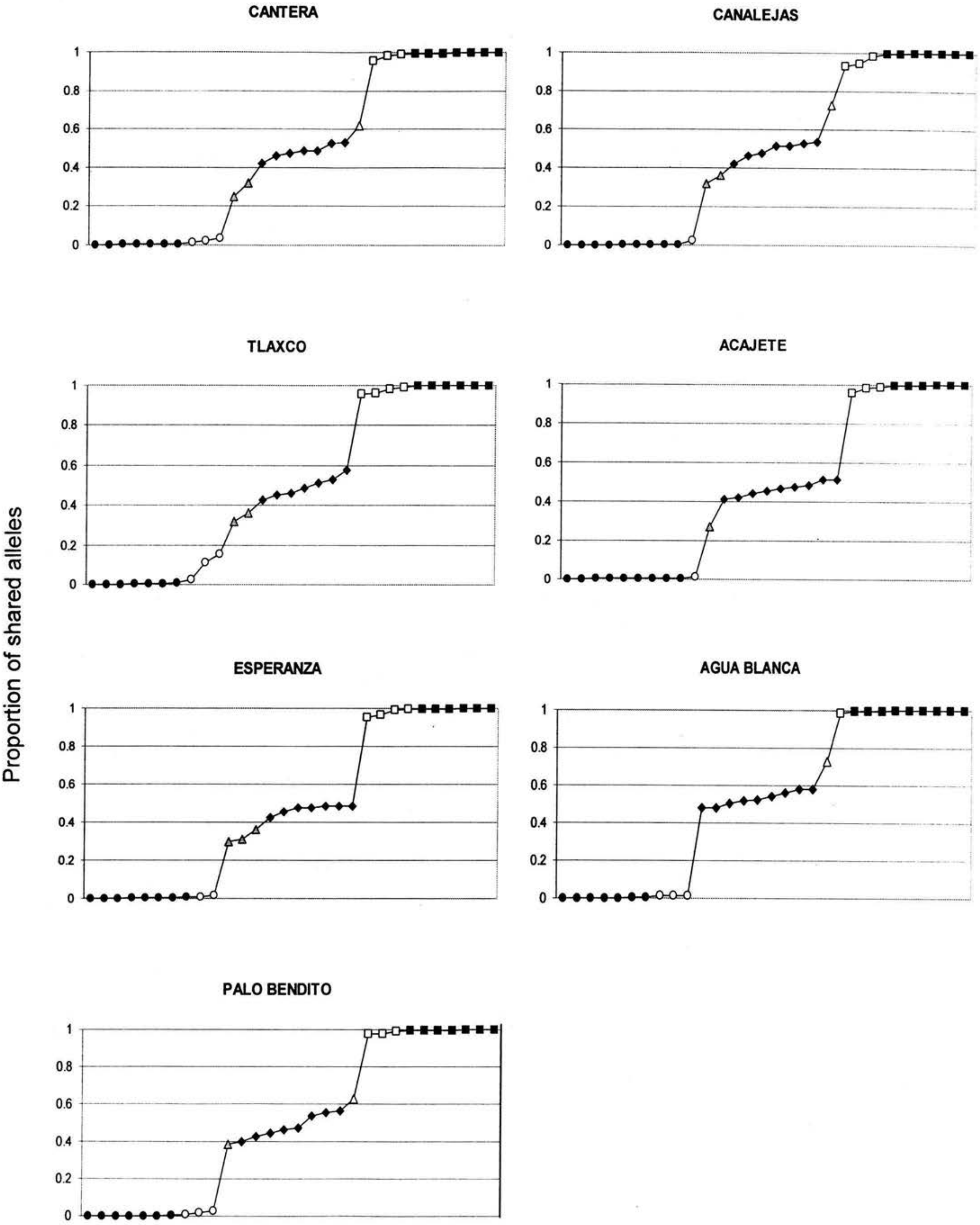

Proportion of shared alleles
CANTERA
CANALEJAS
TLAXCO
ACAJETE
ESPERANZA
AGUA BLANCA
PALO BENDITO
0
0.2
0.4
0.6
0.8
1

Fig. 4

Capítulo III.

ESTRUCTURA DE LA COMUNIDAD DE INSECTOS ENDÓFAGOS ASOCIADOS AL COMPLEJO

Oecologia

Effect of hybridization of the *Quercus crassifolia* × *Quercus crassipes* complex on the community structure of endophagous insects.

Efraín Tovar-Sánchez and Ken Oyama

Centro de Investigaciones en Ecosistemas, UNAM. Campus Morelia. Antigua Carretera a Pátzcuaro No. 8701. Col. San José de la Huerta. Morelia, 58190, Michoacán, México.

Author for correspondence: E. Tovar-Sánchez. Fax: 52 (55) 56232719; E-mail: efratosa@yahoo.com

Manuscript received________________; revision accepted__________________.

The authors thank Susana Valencia, Patricia Mussali, Mauricio Mora, and Maribel Paniagua for technical assistance. This research was supported by a PAEP-UNAM grant and a CONACYT scholarship to Efraín Tovar Sánchez.

Abstract

In Mexico, *Quercus* has reached the highest diversity along with its canopy endophagous insects. In a previous study, we showed that the geographic proximity of hybrid plants to the allopatric areas of parental species increases its morphological and genetic similarity with them. In the present work, we explored if the endophagous fauna of hybrid plants follows the same pattern. We studied the canopy species richness, diversity and composition of leaf-mining moths (Lepidoptera) and gall-forming wasps (Hymenoptera: Cynipidae) associated with two species of red oaks (*Quercus crassifolia* H. & B. and *Q. crassipes* H. & B.) and their interspecific hybrid (*Quercus* × *dysophylla* Benth. pro sp.) in seven hybrid zones through the Eje Neovolcánico and the southest part of the Sierra Madre Oriental in central Mexico, during four seasons in two years. The study was conducted on 194 oak trees with known genetic status (identified by leaf morphology and molecular markers [RAPDs]), the evidence indicate a bidirectional pattern of gene flow. Hybrid plants supported intermediate levels of infestation of gall-forming and leaf-mining insects compared to their putative parentals. The infestation level of leaf mining insects varied significantly following the next pattern: *Q. crassifolia* > hybrids > *Q. crassipes* whereas, the gall forming insects showed an inverse pattern. A negative and significant relationship was found between these two types of insect guilds in each taxa, when the infestation percentage is evaluated. The 35.5% of the gall insects were specific to *Q. crassipes*, 26.6% to *Q. crassifolia*, and 3.2% of hybrid individuals. The 21.87% of gall-forming wasps supported the "hybrid bridge hypothesis". The geographic proximity of hybrid plants to the allopatric site of a parental species increase its similarity of gall forming insects and the Eje Neovolcánico acts as a corridor favoring this pattern.

Key words. Hybridization, gall-forming, leaf-mining, *Quercus*, community structure.

Introduction

Hybridization between plants is considered a prevalent phenomenon in nature (Anderson 1949), which may have originated between 50% and 70% of the total number of species (Stance 1987; Wendel et al. 1991). This phenomenon contributes to genetic diversity and plant speciation (Grant 1981; Rieseberg and Brunsfeld 1992; Rieseberg and Wendel; 1993). Hybridization between species is found in localized areas (Hardin 1975), and this hybrid zones are places of high ecological and evolutionary activity, providing spots of biodiversity forming new habitats for associate fauna. *Quercus* species (Fagaceae) present scarce sterility barriers, promoting a high level of hybridization among species that are morphologically and physiologically very different from each other (Jensen and Eshbaugh 1976; Guttman and Weight 1989; Whittemore and Schaal 1991; Bacilieri et al. 1995; Dumolin et al. 1997; Bruschi et al. 2000).

The greatest richness of *Quercus* species is found in Mexico, where 90% of the approximately 150 oak species of North America, including 86 endemics, are distributed (Nixon 1993). Oak distribution and patterns of species richness have played a major role in the distribution and species richness of oak gall wasps (Hymenoptera: Cynipidae) (Stone et al. 2002), Mexico has the greatest richness of oak gall wasps, with approximately 700 species included in 29 genera (Weld 1960). Studies of cynipid fauna in oak hybrid zones have shown that gall wasps are highly sensitive to levels of introgression between host species (Boecklen and Spellenberg 1990; Moorehead et al. 1993). Valuable insights into these processes can be gained through investigation of distribution, abundance and diversity of canopy insects in response to host hybrid plants related to their parentals (e. g., Boecklen and Larson 1994; Fritz et al. 1994; Whitham et al. 1994; Morrow et al. 1994; Fritz et al. 1996). It has been found that different herbivore species respond in a variety of ways to hybrids vs. their putative parentals (Boecklen and Larson 1994; Fritz et al. 1994; Morrow et al. 1994; Whitham et al. 1994). Recent studies indicate that host hybrid plants can support low, intermediate or high densities of herbivores in relation to their parentals (Boecklen and Spellenberg 1990; Aguilar and Boecklen 1992; Fritz et al. 1994; Gange 1995; Hjältén 1997). This variability has been explained by age and geographical range of hybrid zones, environmental gradients, microsite differences, and genetic status of hybrids (F_1 or backcrosses) (Boecklen and Spellenberg 1990; Floate and Whitham 1993; Hanhimäki et al. 1994; Martinsen and Whitham 1994; Strauss 1994). Also, Floate and Whitham (1993) propose the hybrid bridge hypothesis in which they suggest that intermediate hybrid plants favor the endophagous insect probability of changing from one host species to another. Host shift may be affected by plant hybridization patterns, therefore, four hypothetic systems are proposed: 1) there is no hybridization, 2) there is hybridization, producing infertile F1 offspring, where hybrid individuals will have 50% of each parental genome, 3) there is hybridization, producing fertile F_1 offspring where hybrids will have unidirectional introgression and 4) there is hybridization, producing fertile F_1 offspring where hybrids will have bidirectional introgression

Quercus crassifolia and *Q. crassipes* are two red oak species (Subg. *Erythrobalanus*) that overlap at the Eje Neovolcánico, and in the southest part of the Sierra Madre Oriental in central Mexico, where they meet to form hybrids (*Quercus* × *dysophylla* Benth pro sp.) (Tovar-Sánchez and Oyama 2004). In a previous study, each of these species was well characterized by leaf morphology and genetic markers (RAPDs), reporting that the geographic proximity of hybrid plants to the allopatric site of a parental species increases its morphological and genetic similarity with its parental (Tovar-Sánchez and Oyama 2004). If the parental species have species-specific parasite insects (gall-forming and leaf-mining) and if attractants to these parasites present codominant or intermediate inheritance, hybrid host trees may harbor parasites of both parental species. In such case, the endophagous insect species associated to the hybrid plant will increase its similarity with the closest parental species.

Questions that have not been addressed about an oak hybrid system is: does geographic proximity of hybrid plants to the allopatric area of a parental species increases the similarity of gall forming insects in relation with is parental? and does a bidirectional introgression support the hybrid bridge hypothesis?. We compared endophagous insects among trees of *Q. crassipes*, *Q. crassifolia* and derived hybrids in seven hybrid zones to know how hybrid trees modify the patterns of species richness, diversity, and composition of insects in time and space. Also, we determined the effect of hybridization on patterns of infestation of gall-forming and leaf-mining guilds and we evaluated if each taxa (*Q. crassifolia*, *Q. crassipes* and hybrids) has specific endophagous insects and if they vary among localities.

Materials and methods

The species

Q. crassifolia include large trees up to 23 m in height with a trunk diameter of 1 m. Leaves are deciduous, with aristate and coriaceous and ovate, obovate or elliptic shape, coriaceous surface and the lower surface is yellow-tomentose, orange or brown. The flowering season is in April (Romero 1993). This species presents a broad range of geographical distribution in Mexico, occupying the major mountain ranges (Sierra Madre Oriental, Sierra Madre Occidental, Sierra Madre del Sur and Eje Neovolcánico). *Quercus crassipes* include trees up to 17 m tall and 0.40 to 1 m in trunk diameter. Leaves are deciduos, coriaceous, narrowly-elliptic and lanceolate, their surface is barely lustrous and the lower surface is tomentose, withe-greiysh. The flowering season is in May. It distributes at the southeast part of the Sierra Madre Oriental and at the Eje Neovolcánico, where they meet and overlap with *Q. crassifolia* forming hybrid individuals with intermediate characteristics. The flowering phenology may explain the hybridization occurrence between these two species, overlapping at the last days of April and the first days of May (Tovar-Sánchez and Oyama 2004).

The parental and hybrid plants used for this study had been morphologically (leaf characters) and genetically (RAPD's) determined (Tovar-Sánchez and Oyama 2004). Also, all individuals were in adult stage and without any apparent damage. Plants that were found to be backcrosses were excluded for this study.

Study sites

We found seven zones in which hybridization occurs naturally between these two species, through the Eje Neovolcánico and the southest part of the Sierra Madre Oriental in central Mexico. Cantera, Canalejas, Tlaxco, Acajete, La Esperanza, Agua Blanca, and Palo Bendito (Fig. 1). In each locality the parental species were dominant while the hybrid individuals were infrequent (between 10 and 17 trees), requiring an extensive field search. The altitude of hybrid zones oscillates between 1,790 m (Agua Blanca) to 2,772 m (Tlaxco). Hybrid zones with high levels of disturbance (i.e., Canalejas, Acajete, Esperanza; and Agua Blanca) were the ones with the highest number of hybrid individuals.

A total of 194 trees were sampled in the seven hybrid zones, 10 trees for each parental species (*Q. crassifolia* and *Q. crassipes*), and a variable number of trees of the hybrid (*Quercus* × *dysophylla* Benth pro sp.) (Cantera: *n*=9; Canalejas: *n*=10; Tlaxco: *n*=6; Acajete: *n*=5; Esperanza: *n*=9; Agua Blanca: *n*=8; and Palo Bendito: *n*=7) per each hybrid zone. Leaf-mining moths (Lepidoptera) and gall forming wasps (Hymenoptera: Cynipidae) associated to the trees were collected and separated by morphospecies, thereafter, they were transported to the lab, and kept at room temperature. The individuals that emerged were placed in alcohol at 100% and determined to family (Goulet and Huber 1993).

Infestation by leaf-mining and gall-forming insects associated in each host tree was estimated using four randomly selected branches and 200 leaves (50 leaves per branch). For each insect species average infestation value was estimated (number of galls or miners)/200 leaves * 100) over the four branches. A total of 155 200 leaves in seven hybrid zones and for four seasons were sampled. The number and type of galls and mines were registered for each host tree in the seven hybrid zones.

Analysis

Nested analysis of variance (Model I fixed effects; Zar 1999) was used to test differences in gall-forming and leaf-mining infestation percentage among taxa (*Q. crassifolia*, *Q. crassipes* and hybrid), seasons for all hybrid zones and within each particular hybrid zone. Trees were considered as a random factor nested within species, because they were representative of each population. Percentage data were corrected as $X = \arcsin (\%)^{½}$ (Zar 1999).

A Tukey analysis was conducted to determinate differences in mean infestation (%) between hybrid plants and parental species for all hybrid zones and within each hybrid zone. The correlation analysis was used to test the relationship between leaf-mining and gall-forming infestation associated to each taxa (*Q. crassifolia*, *Q. crassipes* and the hybrid).

A cluster analysis was performed to group individual taxa in each hybrid zone according to taxonomical similarity of gall-forming insects (morphospecies).

Abundance of gall-forming insects was classified in contingency tables and differences among taxa and hybrid zones were tested with a chi-square test. Gall-forming species diversity was estimated using Shannon index (*H'*). The leaf-mining insects were excluded from all diversity and similarity analysis, because three morphospecies was only registered.

Results

Infestation of gall-forming and leaf-mining associated with *Q. crassifolia* × *Q. crassipes* complex.

The mean percentage of infestation of leaf mining insects was 3.03 ± 0.09 and for gall forming insects was 2.34±0.07. The percentage of infestation for leaf mining ($F_{2,840}=46.02$, $P<0.001$) and gall forming ($F_{2,840}=43.52$, $P<0.001$) insects varied significantly among taxa, hybrid zones (leaf-mining: $F_{6,840}=3.17$, $P<0.01$; gall-forming: $F_{6,840}=9.54$, $P<0.001$), trees (leaf-mining: $F_{189,840}=2.76$, $P<0.001$; gall-forming: $F_{189,840}=3.60$, $P<0.001$) and between hybrid zones×tree (leaf-mining: $F_{12,840}=1.82$, $P<0.05$; gall-forming: $F_{12,840}=2.21$, $P<0.001$). Also, significant differ among seasons for all seven hybrid zones were obtained; for leaf mining (Cantera: $F_{4,145}=9.97$, $P<0.001$; Canalejas: $F_{4,145}=3.71$, $P<0.01$; Tlaxco: $F_{4,145}=2.95$, $P<0.05$; Acajete: $F_{4,145}=5.05$, $P<0.001$; Esperanza: $F_{4,145}=5.62$, $P<0.001$; Agua Blanca: $F_{4,145}=3.63$, $P<0.01$; Palo Bendito: $F_{4,145}=4.12$, $P<0.01$) but not for gall forming insects.

Infestation levels of endophagous insects varied significantly among taxa, in general, *Q. crassifolia* registered the highest percentages of infestation by leaf mining (4.16±0.19), followed by the hybrids (2.95±0.15), and *Q. crassipes* (2.10±0.12). In contrast, infestation levels for gall forming insects showed an inverse pattern: *Q. crassipes* (2.96±0.14) registered the highest infestation, followed by the hybrid hosts (2.53±0.12), and *Q. crassifolia* (1.53±0.07) (Fig. 2).

Leaf-mining infestation values ranged between 1.57±0.19 (mean±S.E.) (*Q. crassipes* at Canalejas) to 5.45±0.58 infestation percentage (*Q. crassifolia* at Tlaxco). *Q. crassifolia* registered the highest infestation percentage, followed by the hybrid hosts, and the lowest density was in *Q. crassipes* (Table 1). Gall-forming infestation values ranged between 1.08±0.16 (*Q. crassifolia* at Tlaxco) to 4.76±0.66 infestation percentage (*Q. crassipes* at Acajete). *Quercus crassipes* registered the highest infestation percentage, followed by the hybrid hosts (F_1s), and the lowest infestation was in *Q. crassifolia* (Table 1).

At Cantera and Canalejas infestation levels of leaf mining in hybrid hosts were intermediate compared to their parentals. At Tlaxco, Acajete, Esperanza and Palo Bendito, hybrids did not differ from *Q. crassipes* who showed the lowest infestation values. Finally, at Agua Blanca hybrids did not differ from *Q. crassifolia* who registered the highest infestation values (Fig. 3). On the other hand, infestation values for gall forming insects in hybrid hosts at Cantera, Tlaxco, Acajete and Esperanza did not differ from *Q. crassipes* who registered the highest infestation estimates. At Canalejas and Agua Blanca, *Q. crassifolia* and hybrids did not differed significantly, showing the lowest values. Finally, at Palo Bendito, no significant differences were registered on infestation values for the complex.

Negative and significant relationships were found between the log infestation by galls vs. log infestation by mines for *Q. crassifolia* ($r = -0.49$, $F = 107.90$, $P < 0.001$), hybrids ($r = -0.55$, $F = 152.83$, $P < 0.001$), and for *Q. crassipes* ($r = -0.51$, $F = 122.03$, $P < 0.001$) (Fig. 4).

Composition of gall-forming insects associated to *Q. crassifolia* × *Q. crassipes* complex in seven hybrid zones

In general, no significant differences in gall forming insect composition (e.g. morphospecies) for each taxa among seasons were found, except for the hybrid hosts at Agua Blanca ($X^2 = 111.42$, $P < 0.001$) (Table 2). Gall forming insect composition varied significantly between taxa, in each season for the seven hybrid zones (Table 3).

Diversity

A total of 32 morphospecies of gall forming (Hymenoptera: Cynipidae) and three morphospecies for leaf mining (Lepidoptera) insects were found. In four hybrid zones, *Quercus crassipes* had higher species diversity followed by hybrids and *Q. crassifolia.* In three hybrid zones, hybrids had higher species diversity than *Q. crassipes* and *Q. crassifolia.* In all cases, *Q. crassifolia* had the lowest diversity values (Table 4).

In general, species richness showed an unidirectional gradient in all hybrid zones except Esperanza, *Q. crassipes* presented the highest values, followed by hybrids and the lowest values obtained were for *Q. crassifolia*. In Esperanza, the hybrid individuals showed the highest value, followed by *Q. crassipes* and *Q. crassifolia* who showed the lowest species richness.

Specificity of host plant

The 34.37% of gall forming insects are exclusive of *Q. crassipes* out of which 45.5% have already colonized hybrid individuals. From the 25.0% of insects that infest *Q. crassifolia,* 25.0% have already colonized hybrid trees. Only 3.12% of gall insects are hybrid specific, 6.25% are generalist insects that infest all taxa in all localities, 3.12% are associated to all the complex in the Eje Neovolcánico, 3.12% to the complex of Esperanza and Palo Bendito, another 3.12% to the complex of Palo Bendito and the remaining 21.87% does not present a specific infestation pattern.

Similarity between taxa and among hybrid zones

Gall forming and leaf-miner insect composition group oak species by their taxonomical affinity and not by geographical position of hybrid zones in the Eje Neovolcánico and in the southest part of the Sierra Madre Oriental in central Mexico, excepting for Tlaxco that is localized in the central region of the Eje Neovolcánico. The similarity of insects associated to hybrid hosts in comparison to their parental species, changes between hybrid zones (Fig. 5). The endophagous insect composition associated to hybrids in Cantera and Canalejas showed more similarity to *Q. crassifolia* from Cantera, Canalejas and Acajete. The hybrids from Agua Blanca and Palo Bendito were more similar to *Q. crassifolia* from Esperanza, Palo Bendito and Agua Blanca. On the other hand, the insects associated to hybrids from Esperanza and Acajete were more similar to *Q. crassipes* from Acajete, Canalejas, Esperanza, Tlaxco, Palo Bendito and Agua Blanca. Finally, the insects associated to the hybrids in Tlaxco were more similar to their putative parentals (Fig. 5).

Discussion

The complex *Q. crassifolia* × *Q. crassipes* shows how 35 insect morphospecies grouped into two guilds (leaf-mining and gall-forming) of 194 trees, of a known genetic status, reveal that genetic taxa and locality variation affect significantly the community structure of canopy endophagous insects. Five gall-forming wasps specific of *Q. crassipes* and two wasps specific of *Q. crassifolia* have already colonized hybrid individuals, favoring a future host species shift. Our findings support the "hybrid bridge hypothesis" which suggests that the presence of a hybrid intermediary plant may favor host herbivore shift from one plant species to another (Floate and Whitham 1993). Fifty nine percent of gall forming insects are associated to a particular host parental species (*Q. crassifolia* or *Q. crassipes*), and then they shift to the hybrid plant. This complex presents a bidirectional introgression (Tovar-Sánchez and Oyama 2004), that results in a complete morphological and genetic continuum of hybrids with no gaps that may facilitate host shift (Floate and Whitham 1993). Also, intermediate inherence of leaf morphology and genetic traits had been showed in hybrid plants of *Q. crassifolia* × *Q. crassipes* (Tovar-Sánchez and Oyama 2004). Therefore, the new hybrid host should have very similar characteristics (e.g., leaf morphology, defense, phenologic and secondary compounds) to both parental species, which facilitates the change of the endophagous insects from the parental species to the hybrid individual. Currently, mithochondrial DNA analysis of gall forming wasps are being conducted in order to know the in which direction the host shift took place, also the chemical analysis of the leaf complex to know the variation in secondary metabolites.

In general, the hybrid hosts support intermediate percentages of infestation in relation to their parentals (when analyzing all hybrid zones together), showing an increasing and unidirectional gradient toward *Q. crassifolia* for leaf mining and toward *Q. crassipes* for gall forming insects. Our study supports the additive hypothesis (Fritz et al. 1996), which predicts that hybrid plants (F_1) are intermediate between the herbivore resistances of the parental species. Examples of studies that showed the same patterns are: beetles on elms (Hall and Towsend 1987), *Pontonia* on European willows (Soetens et al. 1991), beetles on willows (Soetens et al. 1991), sawflies on birches (Hanhimäki et al. 1994), several insect species on oaks (Aguilar and Boecklen 1992; Boecklen and Larson 1994), several species of insects on American willows (Fritz et al. 1994, 1996).

In general, leaf mining (Schoonhove et al. 1998) and gall forming (Stone et al. 2002) insects are known to have narrow host preference. Oak gall wasps (Cynipidae) are obligate parasites and are considered as organ-species-specific; cynipid richness varied within oak taxa among sections: Quercus > Protobalanus > Lobatae. In this last section, are present *Q. crassifolia* and *Q. crassipes*, in spite of the low number of species registered in red oaks, we suggest that *Q. crassipes* presents a higher richness of gall forming insects, because it has been considered the oldest species of this complex, besides being the first that distributes trough the Eje Neovolcánico in relation to *Q. crassifolia* (Tovar-Sánchez and Oyama in press), therefore, it has been in contact with different gall forming insect species for more time (Lawton and Schöder 1977; Strong et al. 1984), contributing then to speciation and coevolution processes. On the other hand, *Q. crassifolia* registered the highest number of leaf mining in relation to *Q. crassipes*, probably, because trees are larger and canopy structure diverse, which represent more varied habitats and resources than do small trees host (Lawton 1978; Strong et al. 1984). For example, Simberloff and Stiling (1987) reported a positive relationship between leaf size and oviposition rate in leaf-mining moth (*Stilbosis quadricustatella*) on *Quercus geminate*, *Q. gambelii* compared to *Q. grisea*, and host plant height may increases the leaf mining oviposition of some species (Connor et al. 1983).

Variation in infestation levels and species diversity (gall forming and leaf mining insects) of hybrid plants in relation with its parental species among hybrid zones showed that genetic taxa and locality variation factors play a role in the infestation patterns of this system (Strauss 1994). The same resistance traits can be expressed differently among environments (hybrid zones). Therefore, environmental changes (e.g., temperature, precipitation, soil, humidity, solar radiation, etc.) could affect the expression of resistance (to parasites) among genotypes of many plant species (Fritz et al. 1996).

As we mentioned before, *Q. crassifolia* ranges from the Sierra Madre Occidental to the center of Mexico along the Eje Neovolcánico, while *Q. crassipes* ranges from the Sierra Madre Oriental to the Eje Neovolcánico, where both species overlap producing hybrid zones. The Eje Neovolcánico distributes in the central part of the country in an east–west direction, and is considered geologically the youngest mountain range in Mexico. It is hypothesized that its evolutionary process began during the Mid-Terciary with the occidental portion formation followed by the development of the central and oriental portion during the Quaternary-Pliocene (Ferrusquía-Villafranca 1993). Tovar-Sánchez and Oyama (2004) showed that in *Q. crassifolia* × *Q. crassipes* complex, the geographic proximity of hybrid plants to the allopatric site of a parental species increase its morphological and genetic similarity with its parental species. In this study, the similarity of endophagous insects in hybrid plants, increases with the nearest parental species of an allopatric site. Thus, the Eje Neovolcánico may act as a corridor where the proximity to an allopatric site favors the shift of parasite species (gall forming and leaf mining) associated to this parental to closely hybrid hosts.

Seasonal variation during a one year period did not affect infestation rate and species diversity of gall forming insects. We suggest that these results are due to the fact that wasps (Cynipidae) associated with the complex that develop galls on ephemeral structures (e.g. leaves, acorns, stems etc.) of the plant may prolong the life cycle of these structures in the host, extending the available period for insect development (Ananthakrishnan 1984; Stone et al. 2002).

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Figure legends

Fig. 1. Map of sampled populations of *Quercus crassifolia* × *Q. crassipes* complex. The mixed stands are represented by numbers, 1 = Cantera, 2 = Canalejas, 3 = Tlaxco, 4 = Acajete, 5 = Esperanza (located at the Eje Neovolcánico), 6 = Agua Blanca, 7 = Palo Bendito (located at the Sierra Madre Oriental).

Fig. 2. Percentage of infestation (mean±S.E.) of mining-insects and gall-forming wasps associated to *Quercus crassifolia* × *Q. crassipes* complex in Mexico. Different letters show significant differences with $P < 0.05$.

Fig. 3. Relationships between log infestation galls and log infestation mines in *Quercus crassifolia*, *Q. crassipes* and putative hybrids in Mexico.

Fig. 4. Percentage of infestation (mean±S.E.) of mining-insects and gall-forming wasps associated to *Quercus crassifolia* × *Q. crassipes* complex in seven hybrid zones in Mexico. Different letters show significant differences with $P < 0.05$.

Fig. 5. Cluster analysis of endophagous insects associated to *Quercus crassifolia* × *Q. crassipes* complex in seven hybrid zones in Mexico.

Table 1. Infestation of canopy endophagous insects (individuals/m^2±S.E.) for each taxa and seven hybrid zones in central Mexico.

Locality	Taxa	Leaf-mining	Gall-forming
Cantera	*Q. crassifolia*	3.42 ± 0.48	1.30 ± 0.16
	Hybrids	2.72 ± 0.38	2.30 ± 0.28
	Q. crassipes	1.76 ± 0.25	2.08 ± 0.23
	Total	2.63 ± 0.22	1.89 ± 0.14
Canalejas	*Q. crassifolia*	3.36 ± 0.43	1.27 ± 0.15
	Hybrids	2.89 ± 0.37	1.56 ± 0.20
	Q. crassipes	1.57 ± 0.19	2.81 ± 0.25
	Total	2.61 ± 0.21	1.91 ± 0.17
Tlaxco	*Q. crassifolia*	5.45 ± 0.58	1.08 ± 0.16
	Hybrids	3.52 ± 0.47	2.18 ± 0.27
	Q. crassipes	2.47 ± 0.36	2.31 ± 0.29
	Total	3.81 ± 0.29	1.86 ± 0.15
Acajete	*Q. crassifolia*	3.53 ± 0.49	1.78 ± 0.24
	Hybrids	2.25 ± 0.36	4.23 ± 0.50
	Q. crassipes	2.20 ± 0.27	4.76 ± 0.66
	Total	2.66 ± 0.23	3.59 ± 0.30
Esperanza	*Q. crassifolia*	4.91± 0.56	1.60 ± 0.19
	Hybrids	2.42 ± 0.38	2.71 ± 0.30
	Q. crassipes	2.25 ± 0.34	2.80 ± 0.32
	Total	3.19 ± 0.27	2.37 ± 0.16
Agua Blanca	*Q. crassifolia*	4.25± 0.55	1.88 ± 0.20
	Hybrids	3.73 ± 0.44	2.42 ± 0.32
	Q. crassipes	2.10 ± 0.29	3.35 ± 0.32
	Total	3.36 ± 0.26	2.55 ± 0.17
Palo Bendito	*Q. crassifolia*	3.88 ± 0.49	1.77± 0.18
	Hybrids	2.71 ± 0.41	2.28 ± 0.27
	Q. crassipes	2.28 ± 0.39	2.64 ± 0.29
	Total	2.96 ± 0.23	2.24 ± 0.15
Mean	*Q. crassifolia*	4.16 ± 0.19	1.53 ± 0.07
	Hybrids	2.95 ± 0.15	2.53 ± 0.12
	Q. crassipes	2.10 ± 0.117	2.96 ± 0.14
	Grand total	3.03 ± 0.09	2.34 ± 0.07

Table 2. Contingency table to compare canopy endophagous species between seasons for each taxa in seven hybrid zones in central Mexico.

Species	X^2	*P*
Cantera		
Q. crassifolia	15.91	> 0.05
Hybrids	6.24	> 0.05
Q. crassipes	18.45	> 0.05
Canalejas		
Q. crassifolia	4.04	> 0.05
Hybrids	3.20	> 0.05
Q. crassipes	10.42	> 0.05
Tlaxco		
Q. crassifolia	5.42	> 0.05
Hybrids	6.60	> 0.05
Q. crassipes	14.34	> 0.05
Acajete		
Q. crassifolia	15.23	> 0.05
Hybrids	18.91	> 0.05
Q. crassipes	35.89	> 0.05
Esperanza		
Q. crassifolia	7.65	> 0.05
Hybrids	8.23	> 0.05
Q. crassipes	13.74	> 0.05
Palo Bendito		
Q. crassifolia	15.36	> 0.05
Hybrids	7.94	> 0.05
Q. crassipes	10.4	> 0.05
Agua Blanca		
Q. crassifolia	4.98	> 0.05
Hybrids	111.42	< 0.001
Q. crassipes	8.72	> 0.05

Table 3. Contingency table to compare canopy endophagous species among taxa for season in each hybrid zone in central Mexico.

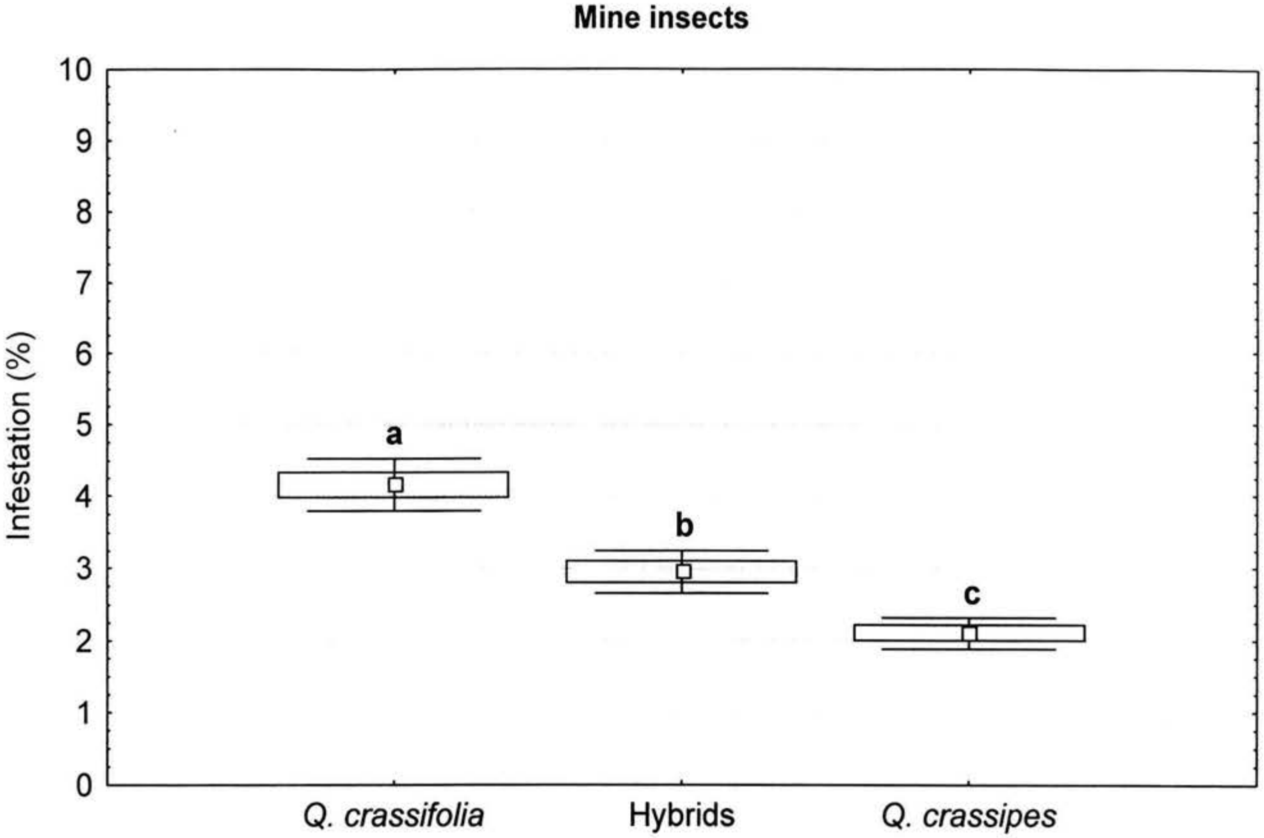
Month	χ^2	*P*
Cantera		
March	53.81	< 0.05
June	99.85	< 0.001
September	109.12	< 0.001
December	67.92	< 0.001
Canalejas		
March	46.86	< 0.05
June	71.55	< 0.001
September	73.44	< 0.001
December	48.85	< 0.01
Tlaxco		
March	52.95	< 0.001
June	106.85	< 0.001
September	108.01	< 0.001
December	68.85	< 0.001
Acajete		
March	81.26	< 0.001
June	106.45	< 0.001
September	117.05	< 0.001
December	95.87	< 0.001
Esperanza		
March	63.32	< 0.001
June	79.01	< 0.001
September	75.33	< 0.001
December	74.50	< 0.001
Palo Bendito		
March	66.42	< 0.001
June	138.14	< 0.001
September	135.15	< 0.001
December	114.14	< 0.001
Agua Blanca		
March	59.73	< 0.001
June	101.39	< 0.001
September	94.26	< 0.001
December	78.55	< 0.001

Table 4. Shannnon diversity index (H') and species richness (S) of gall forming insects for each taxa, seven hybrid zones, and four seasons in central Mexico.

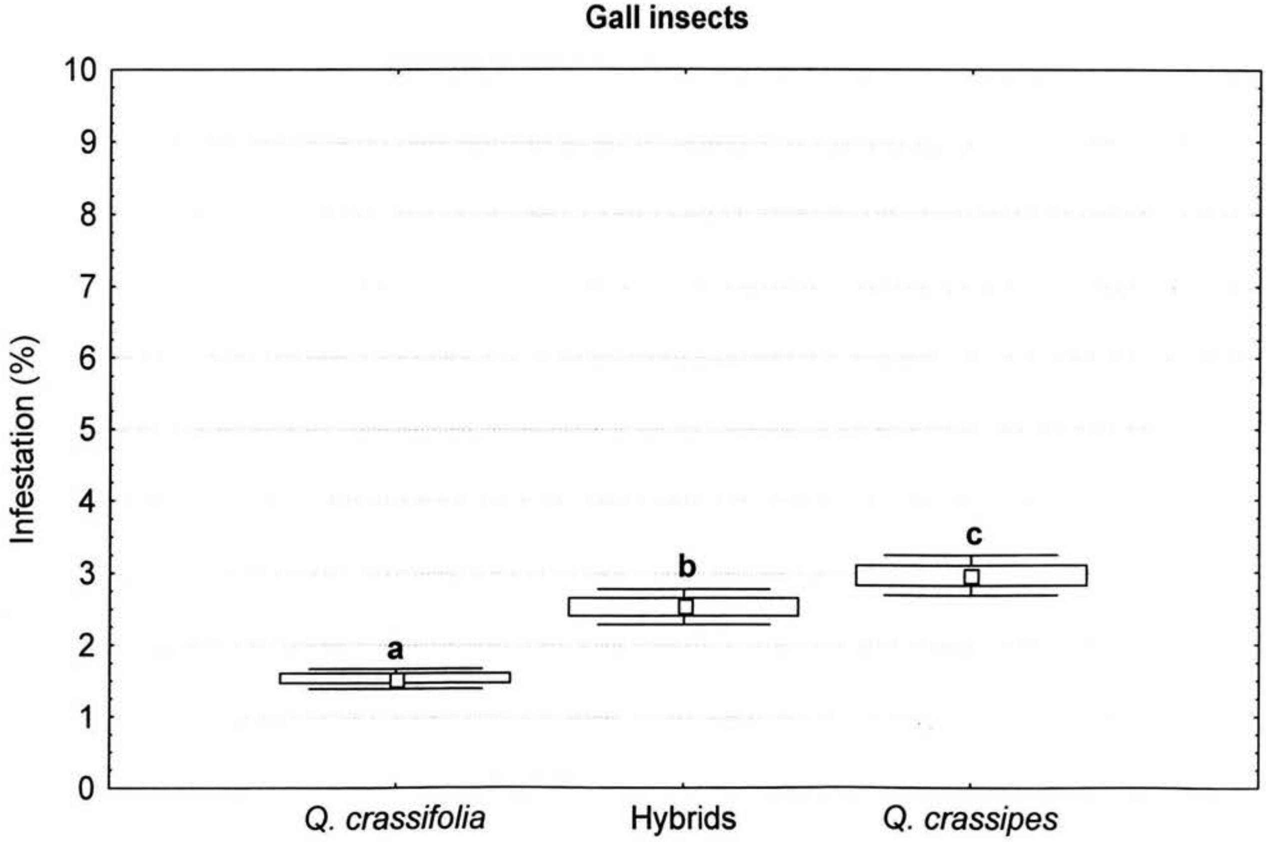
Taxa	March		June		September		December		Total	
	H'	*S*	*H'*	*S*	*H'*	*S*	*H'*	*S*	*H'*	*S*
Cantera										
Q. crassifolia	0.87	9	0.79	11	0.72	11	0.71	11	0.77	11
Hybrids	1.05	9	0.88	9	0.92	9	0.86	9	0.92	9
Q. crassipes	0.95	14	0.84	15	0.79	15	0.79	13	0.87	15
Canalejas										
Q. crassifolia	0.41	3	0.47	4	0.50	4	0.32	3	0.48	4
Hybrids	0.55	5	0.51	6	0.55	6	0.55	6	0.56	6
Q. crassipes	0.89	12	0.86	12	0.93	12	0.88	11	0.91	12
Tlaxco										
Q. crassifolia	0.66	5	0.69	6	0.69	6	0.64	5	0.70	6
Hybrids	0.81	8	0.77	8	0.75	8	0.80	8	0.77	8
Q. crassipes	0.74	8	0.76	9	0.72	9	0.72	8	0.76	9
Acajete										
Q. crassifolia	0.71	9	0.65	10	0.63	10	0.65	9	0.67	10
Hybrids	0.82	11	0.67	12	0.66	12	0.67	12	0.70	12
Q. crassipes	0.88	12	0.82	15	0.84	14	0.83	13	0.87	15
Esperanza										
Q. crassifolia	0.45	5	0.56	6	0.56	6	0.55	6	0.54	6
Hybrids	0.82	9	0.74	10	0.77	10	0.75	9	0.78	10
Q. crassipes	0.67	7	0.71	8	0.74	8	0.74	8	0.72	8
Agua Blanca										
Q. crassifolia	0.59	6	0.35	6	0.44	6	0.29	6	0.44	6
Hybrids	0.63	8	0.50	9	0.53	9	0.51	7	0.55	8
Q. crassipes	0.78	7	0.80	8	0.82	8	0.78	7	0.81	9
Palo Bendito										
Q. crassifolia	0.16	3	0.21	3	0.21	3	0.20	3	0.20	3
Hybrids	0.61	5	0.56	6	0.49	6	0.41	4	0.54	6
Q. crassipes	0.72	6	0.73	7	0.69	7	0.73	7	0.73	7


Sierra Madre Occidental
(SMOc)
N
Sierra Madre Oriental
(SMOr)
7
6
Eje
Neovolcánico
1
2
3
4
5
18º
108º
100 km
● Mixed stand

Fig. 1



Mine insects



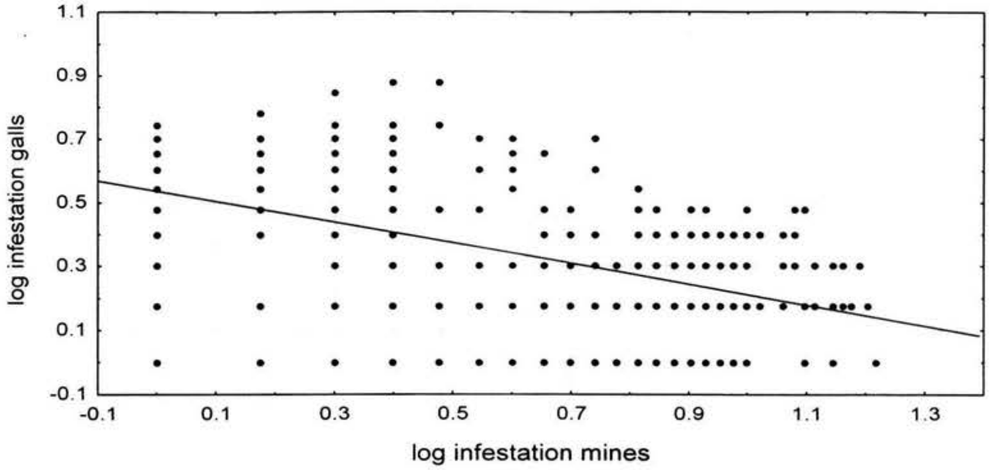
Gall insects

Fig. 2

Q. crassifolia

log infestation galls = 0.54 - 0.33 * log infestation mines

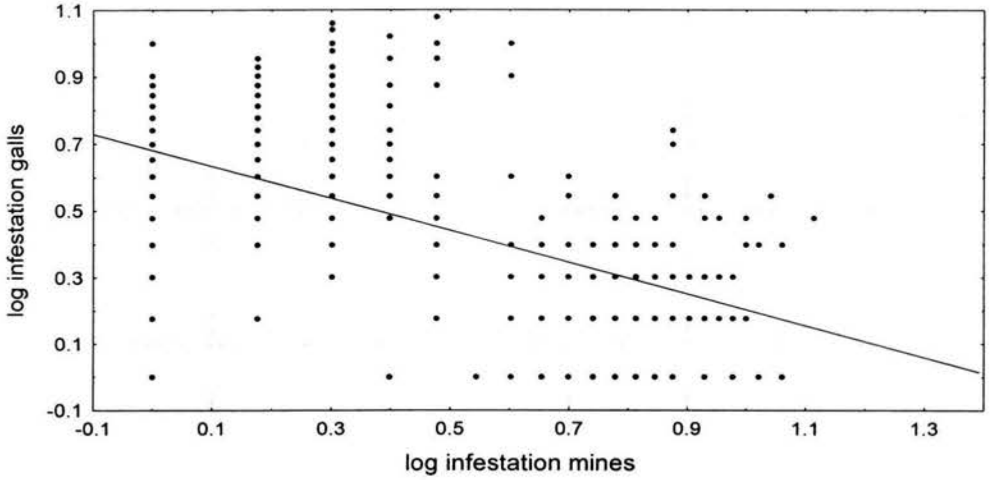
$r = -0.49, r^2 = 0.24$



Hybrids

log infestation galls = 0.68 - 0.48 * log infestation mines

$r = -0.55, r^2 = 0.31$



Q. crassipes

log infestation galls = 0.71 - 0.50 * log infestation mines

$r = -0.51, r^2 = 0.26$

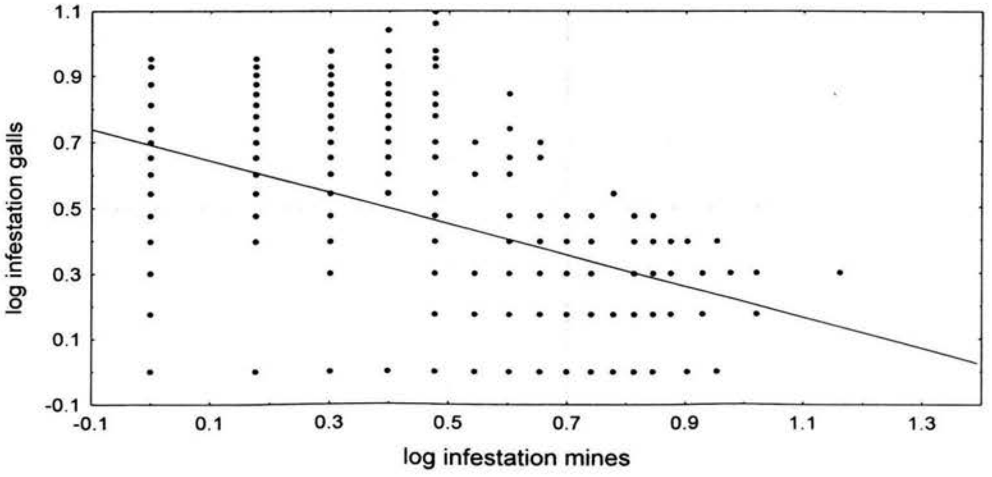


Fig. 3

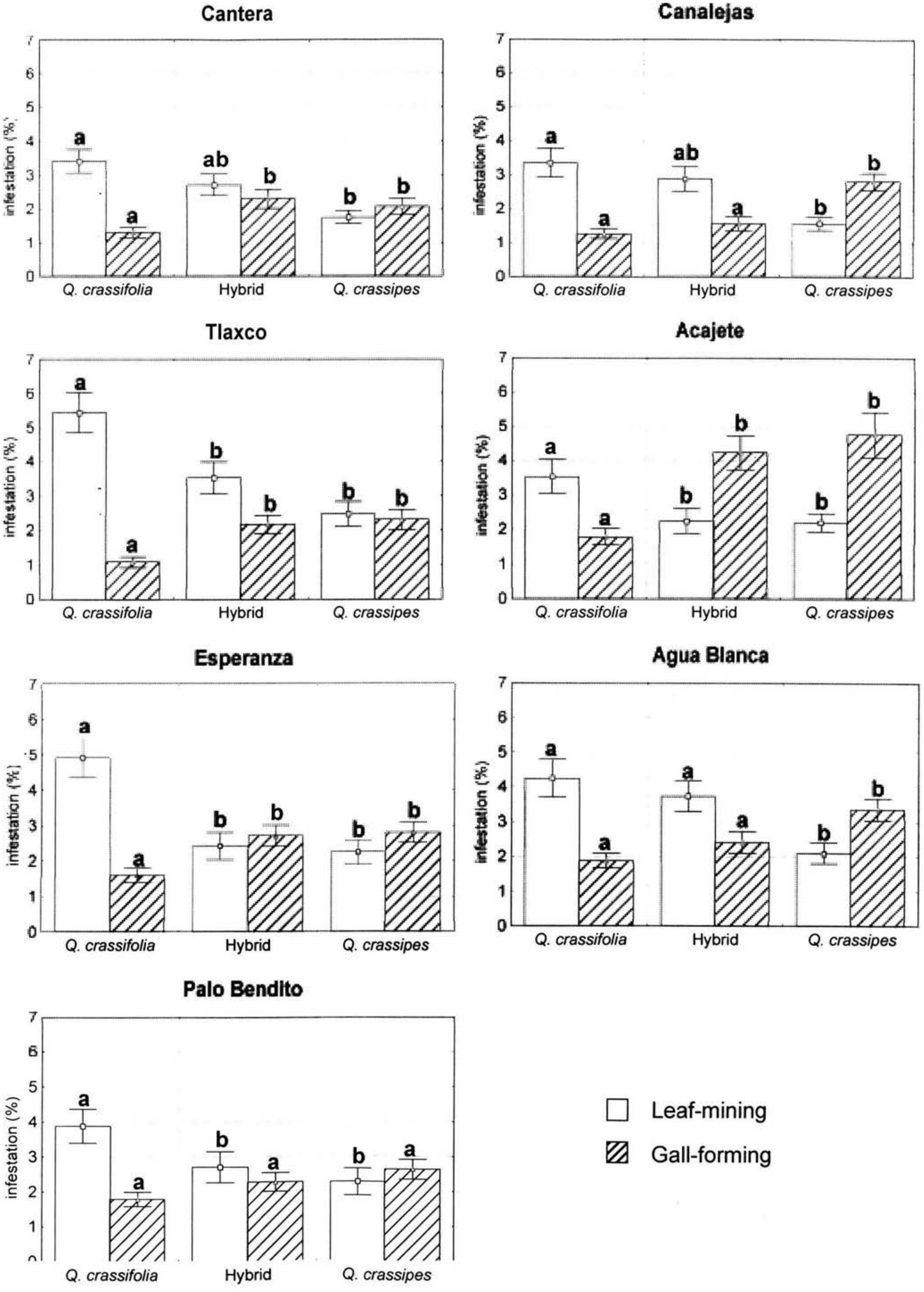
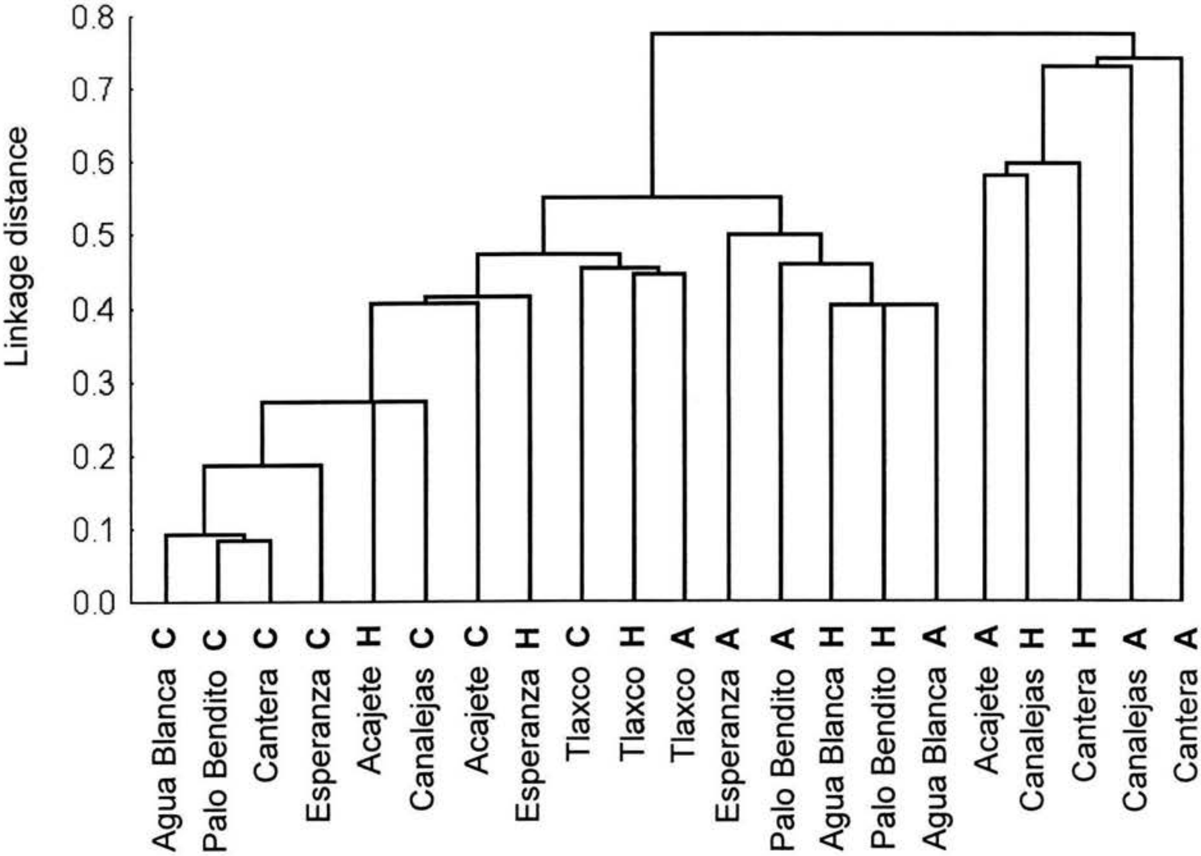

Cantera
infestation (%)
0
1
2
3
4
5
6
7
a
a
ab
b
b
b
Q. crassifolia
Hybrid
Q. crassipes
Canalejas
infestation (%)
0
1
2
3
4
5
6
7
a
a
ab
a
b
b
Q. crassifolia
Hybrid
Q. crassipes
Tlaxco
infestation (%)
0
1
2
3
4
5
6
7
a
a
b
b
b
b
Q. crassifolia
Hybrid
Q. crassipes
Acajete
infestation (%)
0
1
2
3
4
5
6
7
a
a
b
b
b
b
Q. crassifolia
Hybrid
Q. crassipes
Esperanza
infestation (%)
0
1
2
3
4
5
6
7
a
a
b
b
b
b
Q. crassifolia
Hybrid
Q. crassipes
Agua Blanca
infestation (%)
0
1
2
3
4
5
6
7
a
a
a
a
b
b
Q. crassifolia
Hybrid
Q. crassipes
Palo Bendito
infestation (%)
0
1
2
3
4
5
6
7
a
a
b
a
b
a
Q. crassifolia
Hybrid
Q. crassipes
☐ Leaf-mining
▨ Gall-forming

Fig. 4



A = *Quercus crassifolia*

H = Hybrids

C = *Quercus crassipes*

Fig. 5

Capítulo IV.

ESTRUCTURA DE LA COMUNIDAD DE ARTRÓPODOS ASOCIADOS AL DOSEL DEL COMPLEJO

Community structure of canopy arthropods associated to *Quercus crassifolia* × *Quercus crassipes* complex

Efraín Tovar-Sánchez and Ken Oyama

Centro de Investigaciones en Ecosistemas, UNAM. Campus Morelia. Antigua Carretera a Pátzcuaro No. 8701. Col. San José de la Huerta. Morelia, 58190, Michoacán, México.

Author for correspondence: E. Tovar-Sánchez. Fax: 52 (55) 56232719; E-mail: efratosa@yahoo.com

Manuscript received_______________; revision accepted_________________.

The authors thank Susana Valencia, Patricia Mussali, Mauricio Mora, and Maribel Paniagua for technical assistance. This research was supported by a PAEP-UNAM grant and a CONACYT scholarship to Efraín Tovar Sánchez.

Abstract *Quercus crassifolia* and *Q. crassipes* are dominant species in temperate forests of central Mexico and hybridize between each other when they occur in sympatry. Oak canopies contain a considerable portion of arthropod diversity and the hybrid zones can provide new habitats to epiphyte fauna. We tested if the establishment of hybrids in contact zones with their parental hosts increases the species diversity of canopy arthropods assuming that hybrid trees constitute new genotypes of potential new habitats to small organisms. We examined the effect of hybridization on some community structure parameters (diversity, composition, similarity, and density of arthropod fauna) of canopy arthropods compared to their parental species in a hybrid zone located in central Mexico. We employed 17 leaf morphological traits and six diagnostic RAPD primers to identify parental and hybrid plants. The RAPDs marker showed unidirectional introgression towards *Q. crassifolia*, and were detected hybrid (F_1), backcrosses and introgression individual trees. In total, 30 oak canopies were fogged during rainy and dry season. In total, we recognized 532 taxa of arthropods belonging to 22 orders associated with tree canopies. The taxonomic status of host-trees may be an important factor in the arthropod community structure and that seasonality (dry and rainy) is not a factor that could modify their organization. Trees of *Q. crassipes* registered the highest densities of arthropod fauna followed by hybrid hosts (F_1); trees originated by backcrosses towards *Q. crassifolia* registered a significant less arthropod density than F_1 hybrids; and trees of *Q. crassifolia* had the lowest density. Hybrid plants and *Q. crassipes* individuals had higher diversity (*H'*) of arthropods than *Q. crassifolia* plants. Hybrid plants had also more rare species in both seasons in comparison with parental species. This study suggests that hybrid oaks act as a center of biodiversity by accumulating arthropods of both parental and different species including a considerable number of rare species.

Key words. Hybridization, canopy arthropods, *Quercus*, species diversity.

Introduction

Hybridization between plants is a very common phenomenon (Barton and Hewitt 1985, Rieseberg and Brunsfeld 1992, Arnold 1997), and is an important process in plant evolution, an estimated between 50% and 70% of all angiosperm arose by hybridization (Stance 1987, Wendel et al. 1991). Hybrid zones have been studied to understand the effects of plant hybridization on plant-insect interactions (Whitham 1989, Boecklen and Spellenberg 1990, Aguilar and Boecklen 1992, Floate et al. 1993, Moorehead et al. 1993, Preszler and Boecklen 1994, Boecklen and Larson 1994, Hjältén 1997, Fritz et al. 1994, 1996, 1998). Hybrid plants provide an ideal system to study the responses on variation in morphological (Simberloff and Stiling (1987), chemical (Orians and Fritz 1995), phenological (Floate et al. 1993), and genetic (Dungey et al. 2000) traits between parental species and derived hybrids on the diversity and community structure of canopy arthropods. Ecologists have investigated community level responses to the varied host genotypes inherent to hybrid zones (Fritz et al. 1987, Maddox and Root 1987, 1990, Fritz and Price 1988, Linhart 1989, Fritz 1992, Whitham et al. 1994, 1999, Dickson and Whitham 1996, Dungey et al. 2000), but almost all studies to date have concentrated on endophagous (e.g. gall forming and leaf mining insects), partially because they leave permanent records of their presence and fate.

Community level studies of different organisms between parental and hybrid taxa, have showed three different results. First, and more frequently species on hybrid plants showed to be greater (Fritz et al. 1994, Whitham et al. 1994) abundant to parental plants. The authors suggest an increased genetic susceptibility of hybrids due to hybrid breakdown; increased stress in the hybrid zone resulting in greater plant susceptibility; and a greater diversity of resources in the hybrid zone which could support more species. Second, equal species abundance on hybrid plants and parental plants (Fritz et al. 1994, Messina et al. 1996, Fritz et al. 1998), generally the difference in species number is when comparing different habitats over an elevation gradient, and that common garden studies at different elevations revealed no differences in hybrid susceptibility. Third, less species abundance to parental plants (Boecklen and Spellenberg 1990, Aguilar and Boecklen 1992, Fritz 1999). They propose as possible causes affecting patterns of herbivore the host architecture, leaf morphology, and geographic range of the plant. The lack of clear patterns is due to the complex interactions between several factors such as: geographical range of hybrid zones, environmental gradients, hybrid zone age, and genetic status of hybrids (Boecklen and Spellenberg 1990, Hanhimäki et al. 1994, Strauss 1994).

The genus *Quercus* (Fagaceae) is one of the most diversified groups of temperate trees with more that 500 species worldwide distributed. Mexico is considered one of the centers of diversification of *Quercus* (oaks) (Muller and McVaugh 1972, Rzedowski 1978, Nixon 1993) with 150 species and 86 of these endemic (Nixon 1993). The *Quercus* genus which is known for its easiness to hybridize (Palmer 1948, Muller 1952, Tucker 1961, McVaugh 1974, Cottam et al. 1982, Boecklen and Spellenberg 1990, Whitemore and Schaal 1991, Rushton 1993, Preszler and Boecklen 1994, Spellenberg 1995, Spellenberg and Bacon 1996, Howard et al. 1997, González-Rodríguez et al. 2004, Tovar-Sánchez and Oyama 2004), has complex patterns of inter- and intra-specific genetic and morphological variation (Anderson 1949, Van Valen 1976, Rieseberg and Wendel 1993). The existence of hybridization in oaks has been detected based on morphological characters (Stebbins et al. 1947, Barlett 1951, Tucker 1961, Benson et al. 1967, Hardin 1975, Cottam et al. 1982, Rushton 1993, Bacon and Spellenberg 1996), but direct methods of DNA analysis had given the most satisfactory results to detect interspecific hybridization and introgression (Howard et al. 1997, Samuel 1999, Bruschi et al. 2000, Craft et al. 2002, Ishida et al. 2003, González-Rodríguez et al. 2004, Tovar-Sánchez and Oyama 2004).

Quercus crassifolia and *Q. crassipes* are two red oak species (*Erythrobalanus*) that can be recognized easily in the field by its leaf characteristics such as shape, size, coloration, and pubescence. Both species overlap their geographic distribution at the Eje Neovolcánico (central Mexico) where trees with intermediate morphological characters occur; they have been characterized morphologically and genetically as hybrids (Tovar-Sánchez and Oyama 2004).

In this study, we compared the community structure of canopy arthropods between trees of *Q. crassipes*, *Q. crassifolia* and their derived hybrids occurring in a hybrid zone in Mexico. We tested if hybrid trees modify the patterns of species richness, diversity, rareness, and composition of canopy arthropods. Also, we determined if the genetic nearness of hybrid plants to a parental species will increase its similarity of arthropod species, and to know if the hybrid plants are acting as new habitats for of arthropod biodiversity.

Materials and methods

Study sites

We chose Jilotepec hybrid zone at 2600 m altitude because it contained a great number of intermediate individuals of this complex. Three species of oaks are common at Jilotepec, *Q. crassifolia*, *Q. crassipes* and *Q. rugosa*. The firsts belong to the "red oaks" section *Lobatae*, whereas *Q. rugosa* belongs to the distantly related "white oaks" section *Quercus* (Nixon 1993). Only the "red oaks" species are candidates for hybridization.

Morphological data

Seventeen leaf morphological characters (Table 1) were selected, scored, and analyzed. In the hybrid zone, 76 trees were chosen (*Q. crassipes* = 35, *Q. crassifolia* = 31, hybrids = 10) within an area approximately 1.5 km^2. Thirty mature leaves without any apparent damage of each tree were randomly sampled; in total, 2280 leaves were measured.

Molecular data

Leaf tissue was collected in the field from permanently tagged specimens and returned to the laboratory for DNA extraction. Total DNA was extracted and purified using DNAeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) to remove proteins and other secondary compounds. The DNA concentration of each sample was obtained by fluorometric analysis, and the DNA quality was estimated by comparing the intensity of bands with known standards of lambda DNA on an agarose gel at 0.8%. We performed a genetic analysis using Randomly Amplified Polymorphic DNA (RAPD) (Williams et al. 1990, Welsh and McClelland 1990), and the polymerase chain reaction (PCR) technique. RAPD markers were selected, amplified, and scored. Six diagnostic markers fixed for one or the other parental species were selected to characterize the genetic status of the 76 individuals collected. The DNA fragments were separated by means of electrophoresis on agarose gel at 1.5%, stained with ethidium bromide and developed on an UV-table. The molecular weight of the DNA fragments was estimated by utilizing a 1 kb DNA ladder.

Canopy arthropod community

In the hybrid zone we chose 10 trees for each taxon (*Q. crassifolia*, *Q. crassipes* and putative hybrids), ranged between 8 and 10 m (mean 8.7 m) in height and between 28.5 and 37.2 m^2 (mean 31.3 m^2) of crown cover. We used a ground-based technique to know the canopy arthropods associated to *Quercus crassifolia* × *Q. crassipes* complex. The arthropods were collected by fogging with 750 ml of non-persistent insecticide (AquaPy, AgrEvo, Mexico), the whole canopy of a single tree. The insecticide is composed by 30 g pyrethrine / 1 and 150 g piperonyl-but-oxide / 1 in a concentration of 30% vol. / vol. Five trees of each taxa were sampled once during the dry season (March, 2001) and once during the rainy season (August, 2001) giving a total of thirty trees. Arthropods fallen from each fogged tree were collected in 10 plastic trays (each 0.10 m^2 area) located randomly under tree crowns. The arthropods were separated into morphospecies and sent to specialists for taxonomic identification.

Statistical analysis

Variance analyses were conducted (ANOVA) to determine the effects of oak taxa, locality, and individual (trees) on the morphological leaf variability of each of the 17 studied characters (Table 1). Percentage data were corrected as $X = \arcsin (\%)½$, and discontinuous data were transformed as $X = (x)½ + 0.5$ (Zar 1999).

All variables were used in a discriminant analysis to assess the most useful character for taxonomic discrimination between *Q. crassifolia* and *Q. crassipes* and to determine how well morphological characters separate individuals into the groups.

A hybrid index to identify intermediate individuals, backcrosses and structure of a hybrid swarm was estimated with the maximum likelihood (ML) hybrid index using RAPD markers and the Hardig-Hybrid computer program (Hardig et al. 2000). The results obtained were represented in a frequency histogram.

Analysis of variance (ANOVA) was used to test differences in arthropod fauna densities among taxa, and seasons (Zar 1999). Arthropod densities values were log-transformated ($X' = \log X + 1$).

The abundance of arthropod orders were organized in contingency tables and differences among taxa and seasons were estimated with a chi-square test. The diversity of canopy arthropod community was estimated using the Shannon-Wiener index (H'), and after the index was compared between pairs of trees with a *t*-test with a Bonferroni adjustment ($\alpha = 0.01$) (Magurran 1988, Zar 1999).

Principal component analysis (PCA) was used to group individual trees based on canopy arthropod composition (at the order level). Also, to know the importance degree of each arthropod order in the ordination model, the *Pearson* (*r*) correlation was conducted between each variable (arthropod order) and each principal component ($\alpha < 0.05$).

A cluster analysis was conducted to group host trees according to taxonomical similarity of canopy arthropods. The cluster was elaborated using the Jaccard's index (Magurran 1988).

A rare species was defined as a species represented by a single individual in the collections (Basset 1997).

Results

Morphological analysis of parental and hybrid plants

Discriminant function analysis for size and shape variation showed that in the hybrid zone analyzed, the hybrids had intermediate characters between their parents. For the *Q. crassifolia* × *Q. crassipes* comparison, one highly significant ($P<0.001$) discriminant axis accounted for 89.42% of the taxa variation. This axis chiefly represented overall leaf size (Fig. 1a). The shape variables, LL/MWL and LLW/MWL also produced a highly significant ($P < 0.001$) discriminant axis. The axis described a gradient of leaf shape from the elliptic-narrowed of *Q. crassipes* and the hybrids, to the obovate leaves of *Q. crassifolia* (Fig. 1b). The shape variable also produced two significant discriminant axes and the ratio of the sums of eigenvalues of the hybrid zone indicated that approximately 4.48/7.56 = 59.25% of the total variation in size and shape was attributable to shape alone.

Genetic analysis (RAPD) of parental and hybrid plants

The RAPD analysis showed to be a powerful tool for characterizing hybrid individuals between *Q. crassifolia* and *Q. crassipes*. Only one plant showed perfect marker additivity as may be expected in an F_1 (AB4), and five individuals were interpreted as F_1, two individuals deviated by only one character (0.50-0.562), and three plants deviated by only two characters (0.563-0.625). Four plants were interpreted as backcrosses towards *Q. crassifolia* (0.687-0.750) (Fig. 2).

The mean ML hybrid index score for *Q. crassifolia* individuals was 0.86 (SD 0.07), for *Q. crassipes* individuals was 0.10 (SD 0.06), and for the hybrid individuals was 0.45 (SD 0.12).

Canopy arthropods associated to oaks

Composition of arthropods

Canopy arthropod communities in *Q. crassifolia* × *Q. crassipes* complex are conformed by 532 morphospecies belonging to 22 orders: Araneae, Arthropleona, Astigmata, Coleoptera, Cryptostigmata, Dermaptera, Diptera, Hemiptera, Homoptera, Hymenoptera, Isoptera, Lepidoptera, Mecoptera, Mesostigmata, Neuroptera, Opiliones, Orthoptera, Pseudoscorpiones, Prostigmata, Symphypleona, Thysanoptera, and Trichoptera (nomenclature based on Borror et al. 1992; Evans 1992). In general, the number of morphospecies were lower in the dry season that in the rainy season. Hybrid hosts supported the greatest morphoespecies abundance in both seasons (dry = 49, rainy = 226, total = 275), while *Q. crassipes* supported the intermediate abundance, (dry = 47, rainy =162, total = 209), and *Q. crassifolia* the lowest abundance in both seasons (dry = 33, rainy = 76, total = 109).

The relative abundance of morphospecies grouped in orders of arthropods changed between seasons and among taxa (Fig. 3). During the dry season, Hymenoptera and Cryptostigmata were the most important orders in *Q. crassipes* and hybrid plants, but in *Q. crassifolia*, Prostigmata and

Lepidoptera were the orders with the most relative abundance. In the rainy season, Arthopleona and Cryptostigmata were the most common in *Q. crassipes* and hybrids while in *Q. crassifolia* the most common orders were Arthopleona, Lepidoptera, and Prostigmata. In general, the host taxa had significantly differences in canopy arthropod composition among taxa (in dry season: χ^2 = 12.54, $P < 0.01$; and in rainy season: χ^2 = 118.37, $P < 0.01$), and between seasons (*Q. crassifolia*: χ^2 = 23.79, $P < 0.01$; hybrids: χ^2 = 19.67, $P < 0.01$; and *Q. crassipes*: χ^2 = 13.52, $P < 0.01$).

Density of arthropods

The density of canopy arthropod species differ significantly among taxa (F = 4.73, d.f. = 2, $P < 0.01$), between seasons (F =231.6, d.f. = 1, $P < 0.01$). Also, interaction season × taxa (F = 53.1, d.f. = 2, $P < 0.01$) was significant. In general, the arthropod densities were lower in the dry season that in the rainy season. *Quercus crassifolia* supported the lowest arthropod densities (mean±S.E. individuals m^{-2}) in both seasons (dry = 27.5±3.4, rainy = 146.5±15.3), while *Q. crassipes* supported the greatest densities, (dry = 58.0±8.2, rainy = 494.5±44.6). The hybrids supported intermediate densities of arthropods in both seasons compared with the parental species (dry = 40.9±6.2, rainy = 329.5±38.7) (Table 2), and the backcrosses (dry = 28.7±5.7, rainy = 243.0±41.3) supported significantly lower arthropod densities than the F_1 (dry = 49.0±6.5, rainy = 387.1±44.2) (F = 5.8, d.f. = 1, $P < 0.01$).

In the hybrid zone, the average density of each arthropod order for both seasons showed differences among host trees using the PCA (Fig. 4). Both seasons the PCA analysis showed the separation among taxa for canopy arthropod communities. In the dry season, the PC1 explained approximately 50.1% of the variation among trees and the orders of arthropods that presented a better correlation with this component were: Isoptera (r = -0.90, F = 53.2, $P < 0.01$), Hymenoptera (r = 0.74, F = 15.3, $P < 0.01$), and Homoptera (r = -0.72, F = 14.0, $P < 0.01$). The PC2 explained the 25.9% of variation and the better correlated orders were: Lepidoptera ($r = 0.90$, $F = 56.2$, $P < 0.01$), Dermaptera ($r = 0.89$, $F = 51.8$, $P < 0.01$), and Cryptostigmata ($r = -0.88$, $F = 43.3$, $P < 0.01$). On the other side, for the rainy season the PC1 explained the 68.3% of the variation and the better correlated order with the PC1 was Arachneae ($r = -0.77$, $F = 12.4$, $P < 0.01$), and the PC2 explained 19.7% of variation and the better correlated orders were: Hymenoptera ($r = 0.65$, $F = 9.64$, $P < 0.01$), Mecoptera ($r = -0.62$, $F = 8.25$, $P < 0.01$), and Prostigmata ($r = -0.61$, $F = 7.71$, $P < 0.01$).

Diversity of arthropods

Species richness and diversity were higher in the rainy than in the dry season to *Q. crassipes* and *Q. crassifolia* complex (Table 3). During the dry season the diversity differed significantly among taxa. The hybrid plants registered the highest diversity index (3.8), followed by *Q. crassipes* (3.4), and *Q. crassifolia* (2.8). In the dry season and the total, the diversity did not differ significantly between *Q. crassipes* (rainy = 4.5, total = 4.9) and hybrids (rainy = 4.7, total = 5.0) which showed the higher index, while the lowest diversity was registered in *Q. crassifolia* (rainy = 3.6, total = 4.1) (Table 3). In general, the hybrid hosts registered the highest species richness in both seasons and for the total, followed by *Q. crassipes*, and *Q. crassifolia* (Table 3).

Abundance of individuals per species

Figure 5 shows the distribution of individuals per species. In general, the hybrids registered the greatest percentage of rare species (collected only once) (dry = 51%, rainy = 50%, total 50%), followed by *Q. crassifolia* (dry = 33%, rainy = 46%, total = 42%) and *Q. crassipes* (dry = 38%, rainy = 40%, total = 40%) for both seasons and for the total (dry + rainy). During the dry season, the orders with the highest number of rare species in *Q. crassipes* and hybrid host were

Hymenoptera and Araneae, and in *Q. crassifolia*, Lepidoptera and Araneae. In the rainy season, the most important orders in *Q. crassipes* were Diptera and Homoptera, in hybrid hosts Coleoptera, Homoptera, and Lepidoptera, and in *Q. crassifolia* Lepidoptera and Coleoptera.

Similarity among taxa

Cluster analysis using the similarity (Jaccard) values showed that the canopy arthropod communities associated to the *Q. crassipes* × *Q. crassifolia* complex are grouped in host trees which present the same taxonomic affinity in both seasons. In other words, canopy arthropod fauna is more similar among trees of the same taxa (Fig. 6).

Discussion

The molecular analysis (RAPD) of *Quercus crassipes*, *Q. crassifolia* and their hybrid individuals showed that this complex presents high variability, and registered an unidirectional introgression towards *Q. crassifolia.* In general, the morphological and molecular results are congruent to determine intermediate hybrids. Also, the RAPD markers were a reliable marker to determine hybrid individuals, and probably backcrosses. Unidirectional introgression towards *Q. crassifolia* might result from the nearness of the hybrid zone to the allopatric sites of *Q. crassifolia* (in Sierra Madre Occidental) as suggested by Tovar-Sánchez and Oyama (2004).

Composition

The canopy arthropod communities in *Q. crassipes* × *Q. crassifolia* complex in the hybrid zone are conformed by 532 morphospecies belonging to 22 orders. In this oak forest the hybrids are an important reservoir of arthropod species. Probably, due to differences in canopy structure, also the genetic, phenological and historical events might be responsible. A separation of taxa during both seasons, suggesting a different community structure on hybrid and parental plants was demonstrated by the principal component analyses (PCA). These results, suggested that the tree host and their canopy arthropod communities associated to parental oaks and hybrids are organized in stable cores that are strongly related, and that seasonality is not a factor that could modify their organization. Therefore, taxonomic status of host-trees may be an important factor when structuring arthropod communities.

The canopy arthropod composition changed between trees, taxa, and seasons. The high fluctuation in abundance of Arthropleona, Cryptostigmata, Prostigmata, Hymenoptera and Lepidoptera can not be fully interpreted. It is known that Collembola migrate vertically along tree trunks probably due to higher soil moisture (Bowden et al. 1976); therefore, the highest abundance of individuals was registered during the rainy season for all taxa (*Q. crassifolia*, *Q. crassipes* and putative hybrids). The higher occurrence of Lepidoptera (caterpillar) in some tree-crowns might be correlated by high nutritional quality of young leaves that they use as food. On the other side, the high variability among individuals of the same taxa shown by morphological and molecular data might be correlated with the fluctuation in abundance of Hymenoptera, where the wasps (Cynipidae) were the most representative guild and the females are highly specialized and selective in their host plant and oviposition site, because the subsequent gall development can be sensitive to host attributes including genotype, age, size, phenology, and nutrition status (Ejlersen 1978, Askew 1984, Stille 1985, Eliason and Potter 2000, Pires and Price 2000). The abundance of Cynipidae illustrated an unidirectional response, which was: *Q. crassipes* > F_1 hybrid > backcrosses > *Q. crassifolia*, this pattern was obtained in *Q. grisea* × *Q. gambelii* hybrid zone for leaf mining (Lepidoptera: Nepticuliade and Gracillariidae) and gall-forming herbivores (Hymenoptera: Cynipidae) (Aguilar and Boecklen 1992, Boecklen and Larson 1994), and in *Q. dumosa* × *Q. engelmanii* hybrid zone for Cynipidae wasp (Moorehead et al. 1993). Probably, intermediate inherence of leaf morphology and genetic traits in hybrid host should have very similar characteristics (e.g., leaf morphology, defense, phenology and secondary compounds) to both parental species, which facilitates the change of the endophagous insects from the parental species to the hybrid individual, also, combination of similar morphologies may invest hybrids with appropriate ovipositional cues for the parasites of both parental species (Dupont and Crivelli 1988).

Density

The canopy arthropod densities illustrate an increasing gradient from *Q. crassifolia,* passing trough the backcrosses towards *Q. crassifolia*, followed by intermediate hybrids (F_1s) and finally to *Q. crassipes.* This pattern supports Aguilar and Boecklen results (1994) who studied the herbivores density in the *Quercus grisea* × *Q. gambelii* hybrid complex.

Backcrosses towards *Q. crassifolia* registered lower arthropod densities than F_1 hybrids, suggesting a genetic resistance in host hybrids, this similar pattern were found in *Eucalyptus* in Tasmania, where F_1 hybrids were compared with backcrosses and parental plants, often backcrosses plants were intermediate between F_1 and the parentals (Whitham et al. 1994). This suggests that the introgression towards *Q. crassifolia* could give advantage to the hybrid individuals over the more susceptible parental species (*Q. crassipes*), therefore, the effect of arthropods, specially the herbivores guild on hybrid, could favor the introgression of certain resistance genes between plant species (Rieseberg and Ellstrand 1993).

Quercus crassifolia registered the lowest canopy arthropod densities, followed by the backcrosses towards *Q. crassifolia*, and the F_1 hybrids. The apparent additive inheritance of secondary chemical metabolites in hybrid plants and backcrosses might show this lowest density. These results support Whitham's proposal (1989), that backcrossing might lead to the breackdown of resistance traits of backcross plants compared to parentals or F_1s. However, if the resistance characters were additively inherited, backcrossing towards the parental with the higher concentration should lead to the recovery of resistance traits of the parental. This in turn should result in a parallel herbivore response rather than a further breakdown.

Arthropod densities to the complex may be affected by seasonal effect, *Q. crassipes* and *Q. crassifolia* are deciduous drought, and their highest density levels were shown in the rainy season. This data is supported by the studies of Tovar-Sánchez et al. (2003) who found a significantly increase in arthropod densities during rainy season on six oak species in central Mexico, including *Q. crassifolia* and *Q. crassipes*. Probably, leaf production (Basset 1996) and an increase in resource availability (Cytrynowicz 1991) during the rainy season are correlated with increase of herbivores in forest canopies.

Diversity and rare species

Hybrid trees acted as centers of species richness (with higher number of rare species) and supported significantly more species of arthropods than their parental plants. This has been called susceptibility response. On the other hand, *Q. crassifolia* consistently supported the least diverse arthropod community. This increased susceptibility of hybrid host to canopy arthropods may be caused by genetic recombination disrupting co-adapted gene complexes controlling resistance (Whitham 1989). Change in plant characters through hybridization may represent habitats with architectural diversity (Martinsen and Whitham 1994), increase food resources, and diluted resistance mechanisms (probably chemical) or phenological traits (Fritz et al. 1994, Strauss 1994, Fritz 1999) for canopy arthropod community that is distinct from that of adjacent parental plant species. Also, we thought that the environmental gradient in this hybrid zone, where *Q. crassipes* occur principally in low elevation and *Q. crassifolia* prefer the high zones could influence the resistance of the hybrid plant, because the hybrid trees are frequently growing where the boundaries of two species overlap. This could occur at the limits of their physiological tolerance where plant stress could lead to elevated susceptibility (Whitham et al. 1994). The same pattern has been reported where the hybrid zones sometimes have both higher herbivore abundance and diversity (Whitham 1989, Floate et al. 1993, Morrow et al. 1994, Whitham et al. 1994). Also, the highest number of rare species registered in host hybrid plants could be due to the disturbance and fragmentation of the hybrid zones habitat. Producing habitats with different environmental parameters in relationship with its parental species.

The seasonal effect showed a strong role in the diversity index, species richness levels, and rare species levels, illustrating their highest values in rainy season. Leaf production (Basset 1997) and an increase in resource availability (Gilbert and Smiley 1978, Cytrynowicz 1991) during the rainy season are strongly correlated with the increase of arthropods in forest canopies. The same pattern was shown in canopy arthropod communities of Mexican oaks (Tovar-Sánchez et al. 2003).

Conclusion

Our results indicate that hybrid zones act as important reservoirs of canopy arthropod fauna. The data showed that the hybrid plants increase species richness, diversity, and rare species of arthropod community, they act as centers of biodiversity as suggested in others studies of the *Eucalyptus* hybrid system (Morrow et al. 1994, Whitham et al. 1994, Dungey et al. 2000). Also, the genetic structure (degree of introgression, gene flow, unidirectional or bidirectional introgression) of the plant population may also play a major role in the structure of canopy arthropod community, in addition to other factors such as, competition, predation, disturbance, fragmentation, and abiotic factors (Diamond and Case 1986, Ricklefs and Schluter 1993, Tovar-

Sánchez et al. 2003). The host hybrid plants are formed by distinct canopy arthropod communities in relation with their parental species, so they should be conserved as a new habitat.

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Figure legends

Fig. 1. Discriminant function analysis for size **(a)** and shape **(b)** variation in leaf morphology for the *Quercus crassifolia* x *Q. crassipes* complex. Characters measured by size LL, NV, MWL, HMW, PD, MD, and character measured by shape LLW/MWL, LL/MWL, see abbreviation in Table 1.

Fig. 2. Histograms of Hardig hybrid index for *Quercus crassifolia* and *Q. crassipes* hybrid zone.

Fig. 3. Relative species richness of arthropod fauna in *Quercus crassifolia* × *Q. crassipes complex* in Jilotepec, Mexico state. COL = Coleoptera, ART = Arthropleona (Collembola), CRY = Cryptostigmata (Acari), DIP = Diptera, HOM = Homoptera, HYM = Hymenoptera, LEP = Lepidoptera, PRO = Prostigmata (Acari), OTHERS = Araneae, Astigmata, Dermaptera, Hemiptera, Isoptera, Mecoptera, Mesostigmata, Neuroptera, Opiliones, Orthoptera, Pseudoscorpiones, Symphypleona, Thysanoptera, Trichoptera.

Fig. 4. Principal component plot for PC1 and PC2 in relation to canopy arthropod density by orders for the *Quercus crassifolia* × *Q. crassipes* complex in Jilotepec, Mexico state.

Fig. 5. Number of species per abundance class in fogging collections.

Fig. 6. Cluster analysis of species of arthropods associated with *Quercus crassifolia* × *Q. crassipes* complex in Jilotepec, Mexico state.

Table 1. List of leaf morphological characters examined for *Q. crassifolia* × *Q. crassipes* complex in Jilotepec, Mexico.

Macromorphological characters	
LP	Length of petiole
LL	Length of lamina
TLL	Total leaf length (LL + LP)
MWL	Maximal width of lamina
HMW	Height of maximal width (length of lamina from base to widest part)
PD	Petiole diameter
MD	Midvein diameter
NV	Number of veins
LWB	Leaf width at basal 1/3 of leaf
LWA	Leaf width at apical 1/3 of leaf
NA	Number of aristae
LLW	Length of lamina from base to widest part (LL - HMW)

Combinations of characters	
P%	Length of petiole × 100/total leaf length
HW%	Height of maximal width × 100/total leaf length
DW%	Length of lamina from base to widest part × 100/ total leaf length
LL/MWL	Length of lamina/maximal width of lamina
LLW/MWL	Length of lamina from base to widest part/ maximal width of lamina

Table 2. Density of canopy arthropods (individuals/m² ± standar error) for each individual tree, associated to *Q. crassipes* x *Q. crassifolia* complex in two seasons.

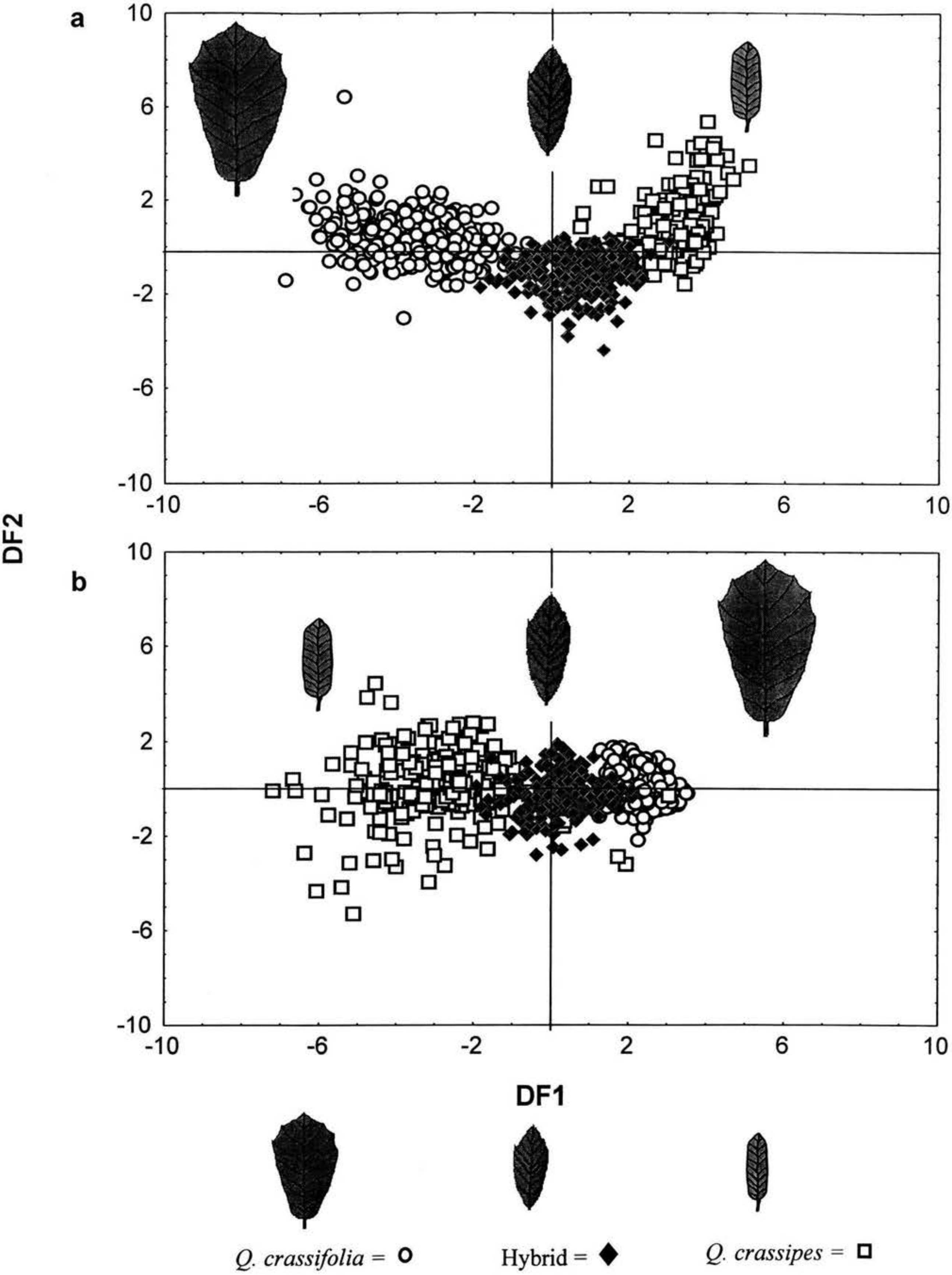
Tree number	Complex					
	Q. crassipes		*hybrid*		*Q. crassifolia*	
	Dry	Rainy	Dry	Rainy	Dry	Rainy
1	26.2±4.1	356.2±54.1	45.3±13.4	397.7±36.0	43.2±4.7	117.3±34.1
2	76.5±10.3	413.6±80.3	43.1±4.9	342.4±34.7	21.6±3.2	146.2±19.8
3	45.7±5.6	657.3±75.8	58.6±9.7	421.3±44.9	18.3±3.6	126.4±40.3
4	60.3±7.5	576.4±65.5	**27.4±5.6**	**209.8±28.3**	38.7±6.5	183.1±30.4
5	81.1±9.8	468.8±58.9	**29.9±6.2**	**276.1±54.2**	15.8±7.4	159.6±36.6
Total	58.0±8.2	494.5±44.6	40.9±6.2	329.5±38.7	27.5±3.4	146.5±15.3
	(a)	(A)	(b)	(B)	(c)	(C)

Total = overall average for each species within a season. Mean values with the same letter for each season did not differ at $P < 0.05$ after a multiple comparison test (Tukey's test) (dry season = small letters; rainy season = capital letters). Values in bold indicate the backcrosses trees to *Q. crassifolia.*

Table 3. Shannon-Wiener diversity index (*H'*), and species richness (*S*) for the communities of canopy arthropods associated to *Q. crassipes* x *Q. crassifolia* complex.

Taxa	Season								
	Dry			Rainy			Total		
	S	H'		S	H'		S	H'	
Q. crassipes	47	3.4	a	162	4.5	a	209	4.9	a
Hybrids	49	3.8	b	226	4.7	a	275	5.0	a
Q. crassifolia	33	2.8	c	76	3.6	b	109	4.1	b

Diversity index (*H'*) with the same letter for each season and total did not differ at $P < 0.01$ after a *t*-test with a Bonferroni adjustment.


a
10
6
2
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-10
-6
-2
2
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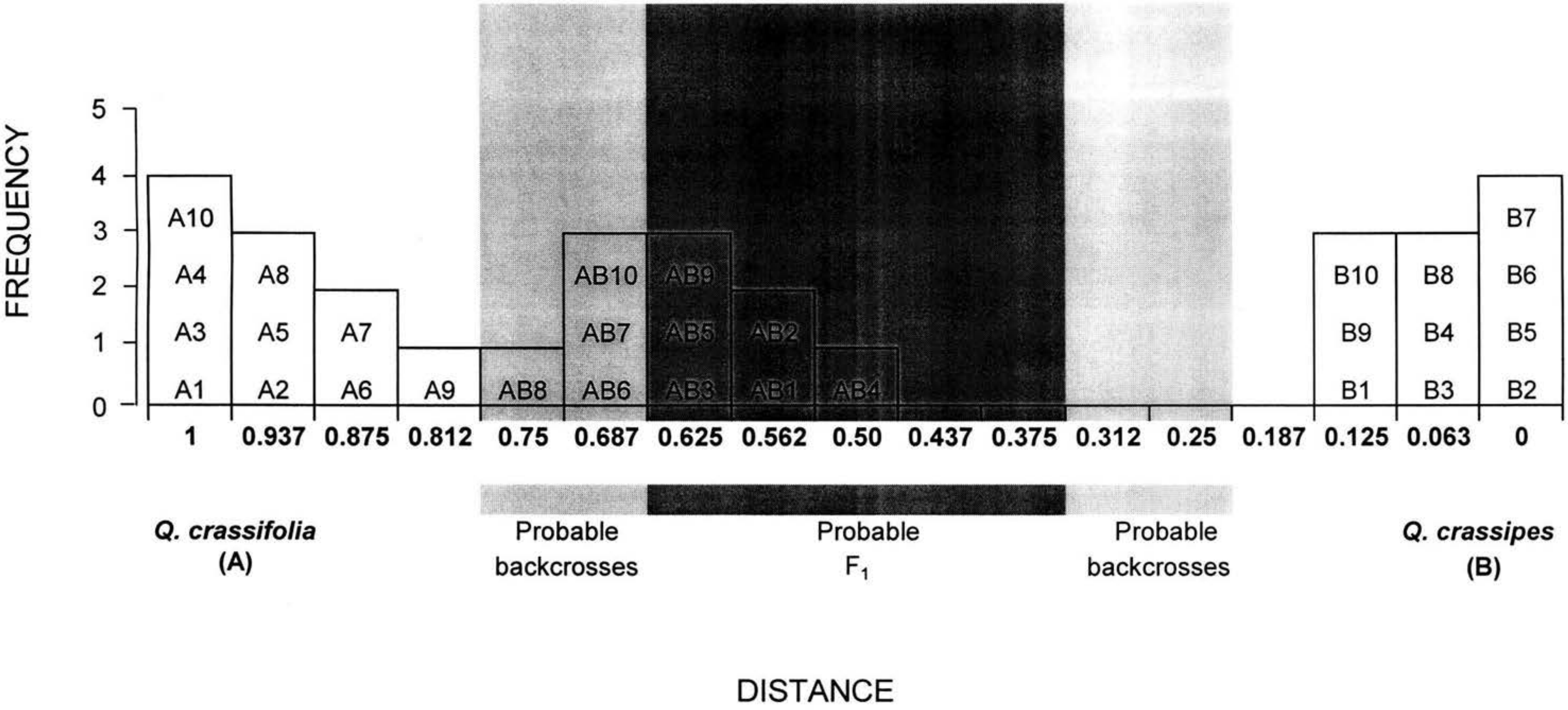
10
DF2
b
10
6
2
-2
-6
-10
-10
-6
-2
2
6
10
DF1
Q. crassifolia = ○
Hybrid = ◆
Q. crassipes = □

Fig. 1


FREQUENCY
0
1
2
3
4
5
A10
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A8
A5
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AB8
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AB9
AB5
AB3
AB2
AB1
AB4
B10
B9
B1
B8
B4
B3
B7
B6
B5
B2
1
0.937
0.875
0.812
0.75
0.687
0.625
0.562
0.50
0.437
0.375
0.312
0.25
0.187
0.125
0.063
0
Q. crassifolia (A)
Probable backcrosses
Probable F_1
Probable backcrosses
Q. crassipes (B)
DISTANCE

Fig. 2

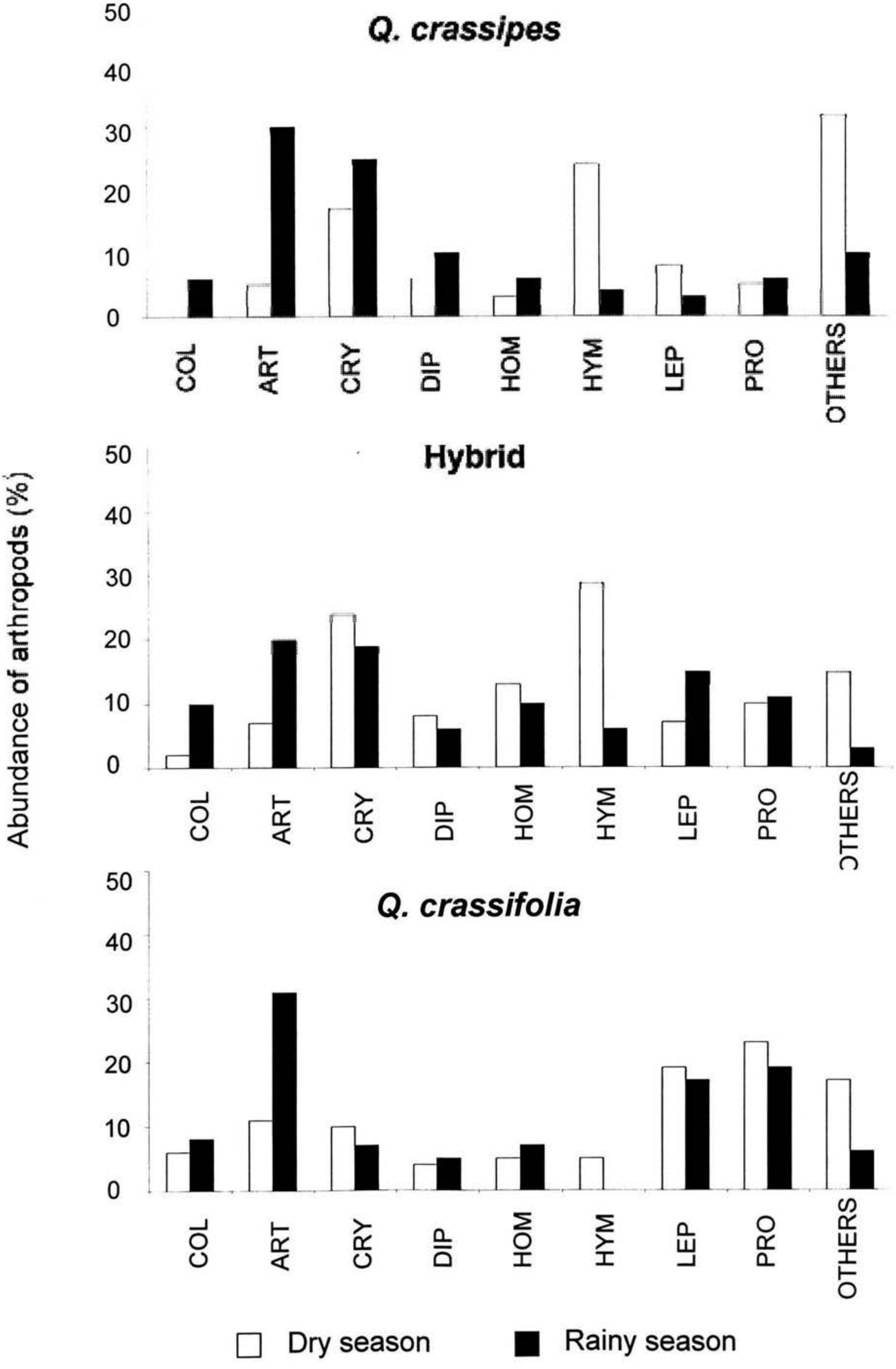

Q. crassipes
Hybrid
Q. crassifolia
Abundance of arthropods (%)
0
10
20
30
40
50
COL
ART
CRY
DIP
HOM
HYM
LEP
PRO
OTHERS
☐ Dry season
■ Rainy season

Fig. 3

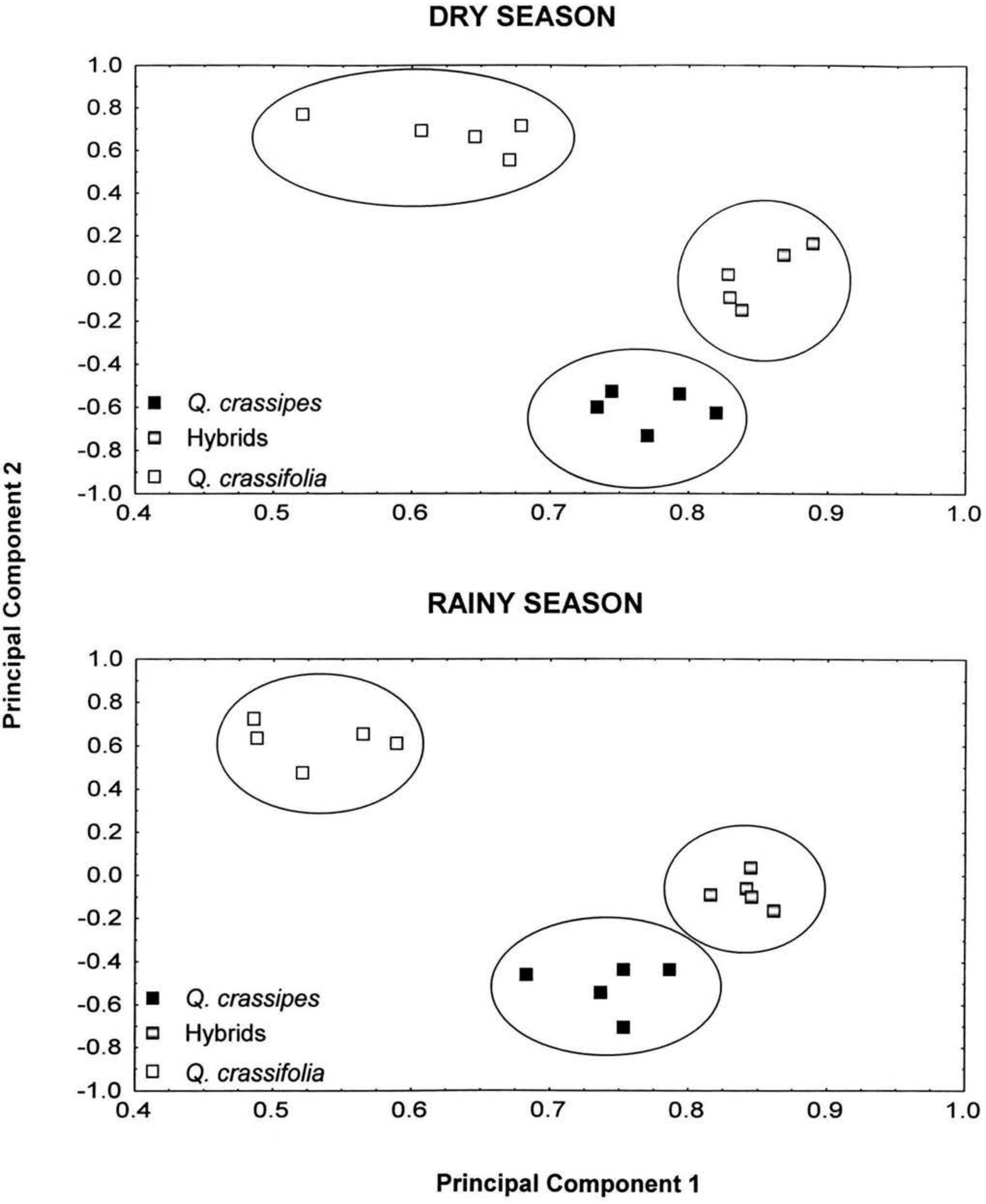

DRY SEASON
RAINY SEASON
Principal Component 2
Principal Component 1
■ Q. crassipes
▨ Hybrids
☐ Q. crassifolia
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
0.4
0.5
0.6
0.7
0.8
0.9
1.0

Fig. 4

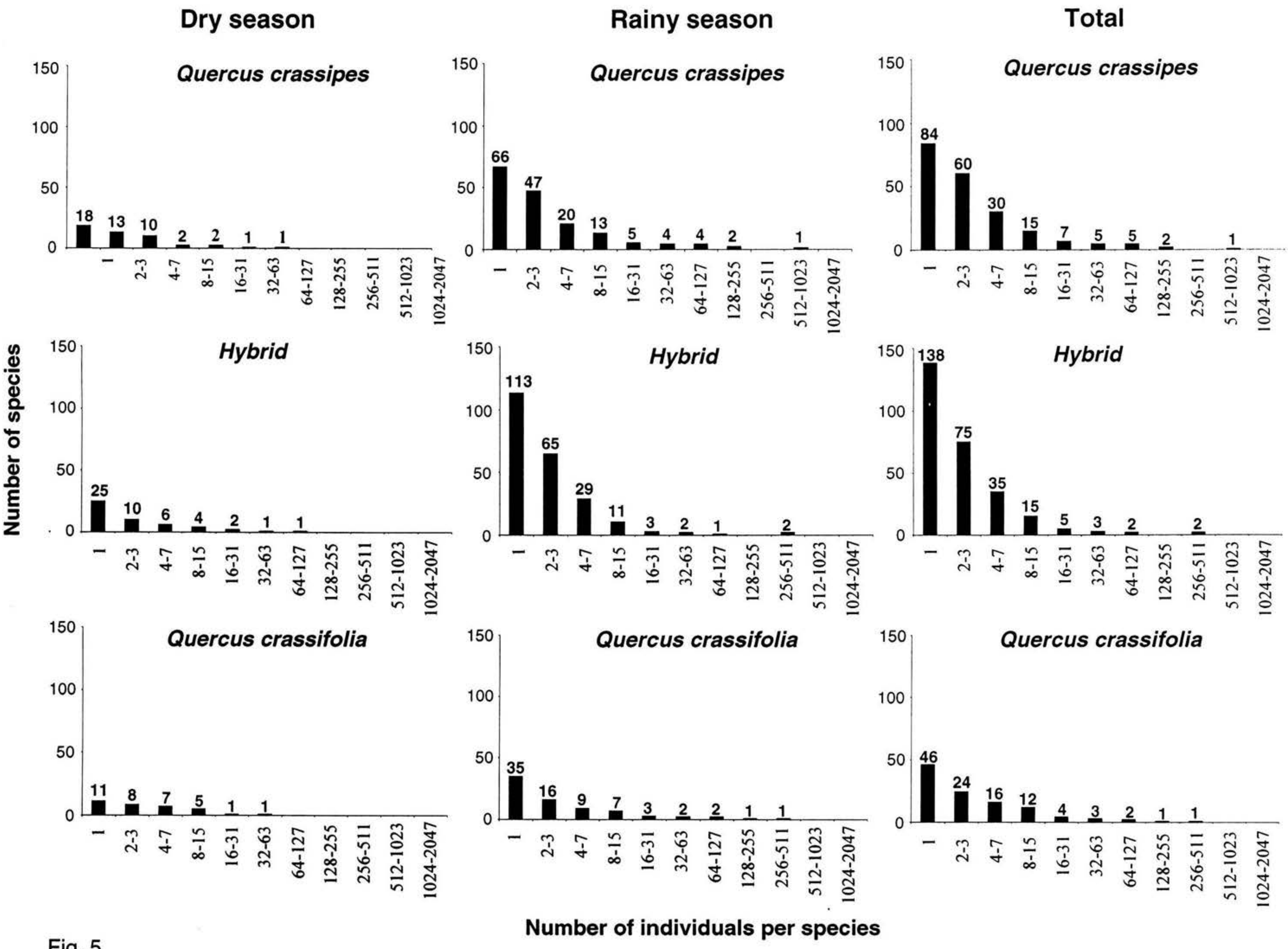
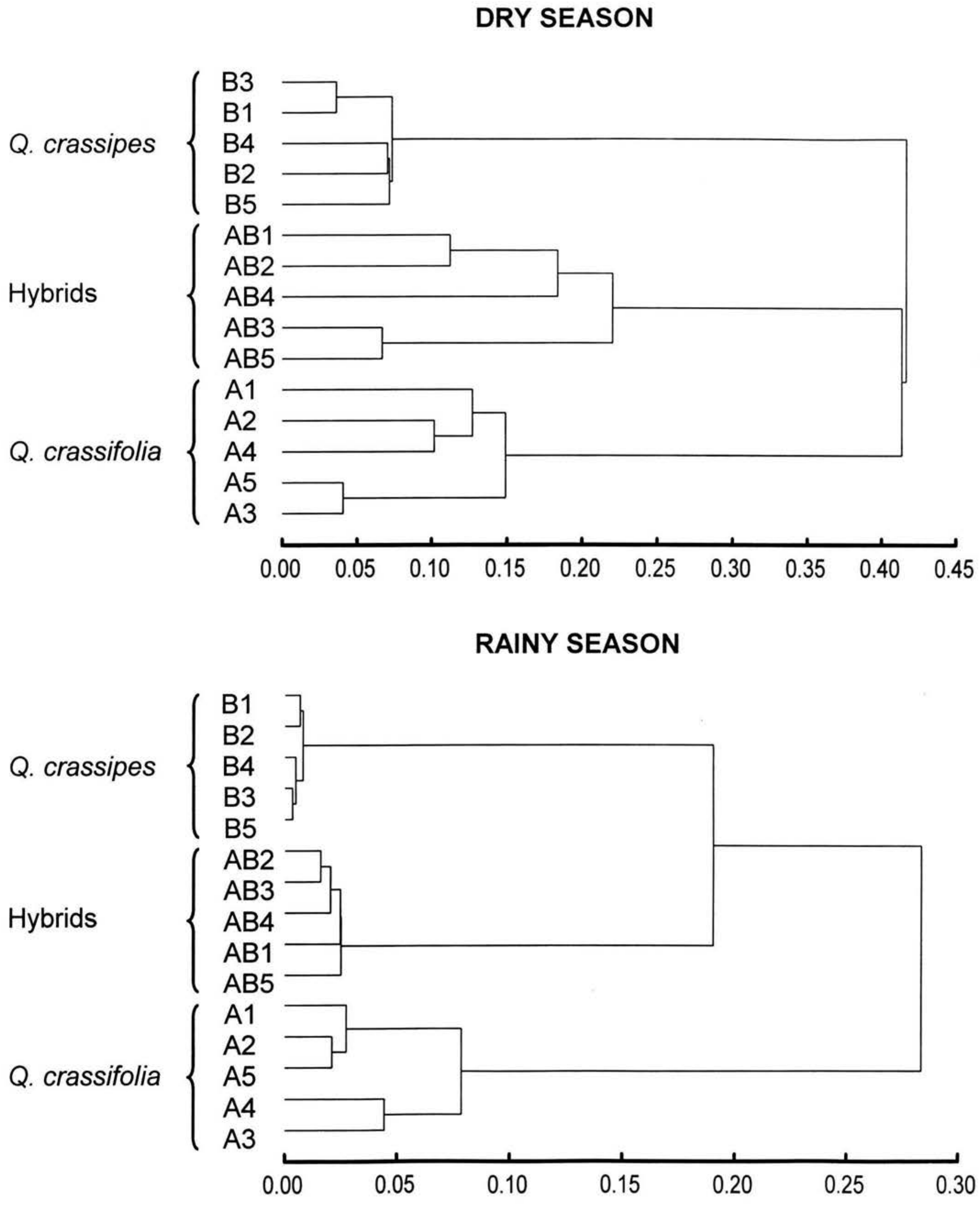

Dry season
Rainy season
Total
Quercus crassipes
Hybrid
Quercus crassifolia
Number of species
Number of individuals per species
0
50
100
150
1
2-3
4-7
8-15
16-31
32-63
64-127
128-255
256-511
512-1023
1024-2047
18 13 10 2 2 1 1
66 47 20 13 5 4 4 2 1
84 60 30 15 7 5 5 2 1
25 10 6 4 2 1 1
113 65 29 11 3 2 1 2
138 75 35 15 5 3 2 2
11 8 7 5 1 1
35 16 9 7 3 2 2 1 1
46 24 16 12 4 3 2 1 1

Fig. 5



DRY SEASON
Q. crassipes
B3
B1
B4
B2
B5
Hybrids
AB1
AB2
AB4
AB3
AB5
Q. crassifolia
A1
A2
A4
A5
A3
0.00
0.05
0.10
0.15
0.20
0.25
0.30
0.35
0.40
0.45
RAINY SEASON
Q. crassipes
B1
B2
B4
B3
B5
Hybrids
AB2
AB3
AB4
AB1
AB5
Q. crassifolia
A1
A2
A5
A4
A3
0.00
0.05
0.10
0.15
0.20
0.25
0.30

Fig. 6

DISCUSIÓN GENERAL

Procesos de hibridación entre *Q. crassifolia* y *Q. crassipes*

La hibridación es inferida frecuentemente por la morfología intermedia de los árboles, así los caracteres morfológicos de tipo foliar constituyen una fuerte herramienta para la detección de individuos híbridos dentro del género *Quercus* (Rieseberg y Ellstrand, 1993; Kleinschmit *et al.*, 1995; Bacon y Spellenberg, 1996; Hardig *et al.*, 2000; Bruschi *et al.*, 2000; Gozález *et al.*, 2004). En muy pocos casos la morfología intermedia de los árboles no ha reflejado hibridación, por ejemplo, Craft *et al.* (2002) analizaron individuos con características foliares intermedias entre *Q. lobata* y *Q. douglasii*, y los resultados fueron corroborados con marcadores moleculares (microsatélites). Los autores concluyeron que los aparentes fenotipos intermedios entre estas dos especies no son necesariamente híbridos y que los híbridos verdaderos no son necesariamente fenotípicamente intermedios. En nuestro estudio la hibridación fue fuertemente corroborada por los caracteres morfológico foliares, los cuales señalaron que *Q. dysophylla* es un híbrido derivado por el entrecruzamiento de *Q. crassifolia* y *Q. crassipes*. En general, los árboles híbridos fueron escasos y se localizaron en los sitios de simpatría de las especies parentales putativas. Además se observó que en las siete zonas híbridas estudiadas (cinco en el Eje Neovolcánico y dos en la región más sureña de la Sierra Madre Oriental) la perturbación ha sido un factor que promueve la hibridación (Arnold *et al.*, 1990; Klier *et al.*, 1991). Las principales formas de disturbio en los sitios de estudio fueron: deforestación, introducción de ganado, incendios, urbanización, apertura de claros para campos de cultivo y tala de árboles para carbón. Arnold (1997) sugiere que los individuos híbridos que se forman en ambientes perturbados podrían ser eliminados si el ambiente regresara a un estado de predisturbio. Lo anterior sería muy poco probable en las zonas híbridas estudiadas ya que en las visitas constantes que se realizaron durante tres años se observó un incremento sostenido en la extensión del área perturbada.

Por otro lado, los marcadores moleculares RAPDs (ADN total) y SSR (ADN de cloroplasto) corroboraron los datos morfológicos y ayudarnos a inferir los procesos de introgresión bidireccional. A pesar de que el fenómeno de hibridación e introgresión se registró en todas las zonas híbridas, las especies parentales permanecen distintas. Una posible explicación está dada por los estimadores de diferenciación genética (F_{ST} y R_{ST}) obtenidos con cpSSR, los cuales fueron significativos entre *Q. crassifolia* y *Q. crassipes*. Indicando que las dos especies difieren tanto en talla como en la distribución de frecuencia de alelos. Slatkin (1995) propone que estos cambios pueden presentarse después de un largo periodo de divergencia ocasionando cambios en sus frecuencias de alelos, lo que sugiere que *Q. crassifolia* y *Q. crassipes* han tenido bajos niveles de introgresión durante su desarrollo histórico (Craft *et al.*, 2002).

Nuestros resultados sugieren que los caracteres morfológicos de tipo foliar son una fuerte herramienta para determinar el fenómeno de hibridación, principalmente en el reconocimiento de individuos híbridos en el campo, así como en la localización de zonas híbridas y este hecho es especialmente importante porque coinciden de manera sustancial con los marcadores moleculares. Estos últimos (RAPD y SSR), ofrecieron evidencias sólidas sobre los procesos de introgresión y retrocruza. Las ventajas de los RAPDs sobre otros marcadores moleculares como RFLPs y microsatélites son (1) la relativa facilidad de la técnica que no requiere de un conocimiento previo de la secuencia de ADN, (2) no es necesaria la construcción o mantenimiento de una librería genómica, (3) el número de loci que pueden ser examinados es ilimitado, (4) no requiere de pruebas radiactivas (Reiter *et al.*, 1992; Whitkus *et al.*, 1994), y (5) pueden revelar altos niveles de polimorfismo intra- e inter – específico (Van Heusden y Bachmann, 1992). Por su parte, los microsatélites son secuencias de nucleótidos con grupos de uno a cinco pares bases (motivos en tandem), son marcadores codominantes (Anzidei *et al.*, 1999), presentan altos niveles de polimorfismo (Jarne y Lagoda, 1996), su estructura molecular se encuentra ampliamente caracterizada (Jarne y Lagoda, 1996), se considera que mutan una base en cada cambio y siguen el modelo de mutación de un paso (SMM), son selectivamente neutros y por tanto, compatibles con los supuestos de genética de poblaciones (Golstein y Pollock, 1994; Vendramín *et al.*, 1996). Para hacer estudios de microsatélites es necesario conocer la secuencia, la cual es una región repetitiva bien definida (el microsatélite), responsable de la variación observada y además es homóloga para diferentes especies o incluso géneros (Golstein *et al.*, 1995).

Las técnicas morfológicas y moleculares utilizadas sugieren que para obtener un mejor reconocimiento de la zona híbrida, así como de los procesos de hibridación e introgresión, es necesaria la combinación de éstas.

Por último, nosotros proponemos que los individuos previamente nombrados como *Q. dysophylla* no debe ser considerada con el estatus de especie, pero debe ser reconocida como una entidad de potencial importancia evolutiva y debe ser nombrada como *Quercus* × *dysophylla* Benth. pro sp. (Tovar-Sánchez and Oyama, 2004)

Filogeografía de los encinos del complejo *Quercus crassifolia* × *Q. crassipes* en el Eje Neovolcánico

Se estima que la colonización de las coníferas de ambientes templados y géneros de latifoliadas como *Quercus*, *Carpinus*, *Ostrya*, *Magnolia* y *Liquidambar* se realizó durante el Oligoceno, a mediados del periodo Terciario, hace unos 35 millones de años. Los registros de hojas fósiles de *Quercus* encontradas en Texas, que datan del Oligoceno y tienen mucha semejanza con los encinos mexicanos modernos (i.e., *Q. sartorii*) (Daghlian y Cerpet, 1982), sugieren que la ruta de ingreso a México fue por la Sierra Madre Oriental (SMOr), procedente de las montañas Apalaches, en el oriente de los Estados Unidos (Martin y Harrell, 1975).

Quercus crassifolia se distribuye sobre la Sierra Madre Oriental (SMOr), Sierra Madre Occidental (SMOc), Sierra Madre del Sur, Eje Neovolcánico y Sierra de Chiapas y es a lo largo del eje Neovolcánico y la región más sureña de la Sierra Madre Occidental donde se sobrelapa con *Q. crassipes* produciendo zonas híbridas. Nosotros sugerimos que *Q. crassifolia* ingresó a México por la SMOc y posteriormente se distribuyó por el Eje Neovolcánico, mientras que *Q. crassipes* lo hizo por la SMOr y después ingresó al Eje Neovolcánico. Nuestra propuesta es que los encinos siguieron la ruta de colonización de los pinos (Styles, 1993), en la que, se sugiere que los encinos son especies pioneras capaces de colonizar rápidamente los flujos de lava resultantes de la intensa actividad volcánica que caracterizó a gran parte de México a partir del Mioceno (Granham, 1993). Lo anterior debió permitir que los distintos taxa que se encontraban en la SMOr y SMOc hasta entonces aislados, hicieran contacto a través de las montañas del Eje Neovolcánico. Este último sistema orográfico atraviesa al país de costa a costa por su parte central en dirección este-oeste, y es considerado geológicamente como las montañas más jóvenes de México. Se cree que su evolución comenzó durante el Terciario Medio con la formación de la porción occidental, seguida del desarrollo de la porción central y oriental durante el Plioceno-Cuaternario (Ferrusquía-Villafranca, 1993).

Los resultados genéticos (cpSSR) apoyan nuestra hipótesis de que *Q. crassifolia* ingresó a México por la SMOc y *Q. crassipes* lo hizo por la SMOr y posteriormente se distribuyeron por el eje Neovolcánico. Los sitios alopátricos de ambas especies son más antiguos que los registrados en las siete zonas híbridas, además *Q. crassipes* es más antigua que *Q. crassifolia.*

Por otro lado, los resultados genéticos de RAPD (ADN total) indican que los procesos de introgresión están presentes en ambas especies (bidireccional), pero la dirección cambia dependiendo de la localización de la zona híbrida. Las zonas híbridas más cercanas a la SMOr (Cantera y

Canalejas) registraron una introgresión unidireccional hacia *Q. crassifolia*, mientras que los híbridos de la localidad intermedia entre las dos Sierras (Tlaxco) mostraron una introgresión bidireccional, y los híbridos localizados más cercanamente a la SMOr (Acajete y Esperanza) registraron una introgresión unidireccional hacia *Q. crassipes*. Lo anterior sugiere que la cercanía de los híbridos a un sitio alopátrico de una especie parental está directamente relacionada con su similitud morfológica y genética. Por tanto, el Eje Neovolcánico actúa como un corredor donde la proximidad a un sitio alopátrico favorece la introgresión de los híbridos hacia la especie parental, diluyendo así los límites con la especie parental del sitio alopátrico.

Naturaleza de la comunidad de los artrópodos asociados al dosel de encinos

La comunidad de artrópodos del dosel en el complejo *Q. crassipes* × *Q. crassifolia* está conformada por 532 morfoespecies contenidas en 22 ordenes. Los análisis de componentes principales durante ambas estaciones (lluvias y sequía) detectaron una separación de taxa (*Q. crassifolia*, *Q. crassipes* e híbridos), señalando una diferente estructura de la comunidad sobre híbridos y sobre cada una de las especies parentales putativas. Estos resultados sugieren que la comunidad de artrópodos del dosel asociados a sus árboles hospederos están organizados en unidades estables fuertemente relacionadas y la estacionalidad no es un factor que pueda modificar su organización. Por tanto, el estatus taxonómico de los árboles hospederos pueden ser un importante factor cuando se estructuran las comunidades de artrópodos y el dosel de las especies de encinos se comportan como comunidades naturales, en las que la fauna de artrópodos no se establece de manera azarosa, sino que existe una organización de las especies, es decir, que la comunidad de artrópodos del dosel de los árboles no es una abstracción hecha por el investigador.

Efecto de la hibridación de los insectos epifitos y endófagos asociados al dosel

La comunidad de artrópodos del dosel asociada al complejo *Q. crassipes* × *Q. crassifolia* en Canalejas, Estado de México, está conformada por 532 morfoespecies. En general, la hibridación de la planta hospedera favorece la riqueza específica, diversidad y densidad de insectos ectófagos. Probablemente, porque los herbívoros polífagos presentan una gran habilidad de ampliar su ámbito de hospederos, pudiendo alimentarse de diferentes familias de plantas, varios géneros o de especies que se encuentran distantes filogenéticamente dentro del mismo género (Arnold, 1992). Muchos autores sugieren la posibilidad de que consumir una gran variedad de alimentos permite evitar que se alcance la dosis letal de cada compuesto secundario (Freland, 1975; Simpson y Simpson, 1990) y optimiza el balance de nutrientes (Whittaker, 1979; Lee, 1990). Asimismo, se ha demostrado que especies generalistas del orden Orthoptera crecen más rápido cuando son capaces de seleccionar entre una gran variedad de alimento que, cuando están restringidos a sólo uno de ellos (MacFarlane y Thorsteinson, 1980; Lee, 1990). Además, como se mencionó anteriormente, los cambios en las características de las plantas a través de la hibridación pueden representar hábitats con mayores posibilidades de colonización.

Por otro lado, la comunidad de insectos endófagos de siete zonas híbridas del complejo *Q. crassifolia* × *Q. crassipes* está conformada por 35 especies de insectos agrupadas en dos gremios (insectos minadores y formadores de agallas). En general, los híbridos soportan niveles intermedios de infestación tanto de insectos agalleros como minadores en relación con sus parentales, apoyando la predicción realizada por Fritz *et al.* (1996), en la que sugieren que hay un efecto genético de la hibridación de los árboles hospederos soportando densidades intermedias de herbívoros. Nuestros resultados mostraron un creciente gradiente unidireccional hacia *Q. crassifolia* para los niveles de infestación de insectos minadores y hacia *Q. crassipes* para los niveles de infestación de insectos formadores de agallas.

Los insectos monófagos son favorecidos a cambiar de planta hospedera cuando ha ocurrido un fenómeno de hibridación (Drake, 1981; Whitham, 1989; Boecklen y Spellenberg, 1990; Floate y Whitham, 1993) y la nueva planta hospedera comparte características genéticas, morfológicas, químicas y de arquitectura del dosel con su planta hospedera actual (Denno y McClure, 1983). Es probable que los herbívoros monófagos salgan más beneficiados de un cambio de hospedero a partir de la hipótesis del "*puente híbrido*" (Floate y Whitham, 1993) que insectos polífagos, probablemente debido a que los insectos monófagos mantienen una estrecha relación con su planta hospedera, lo que los hacen menos probables de expandir su ámbito alimentario en la ausencia de híbridos intermediarios.

Conservación de zonas híbridas

A pesar de la importancia ecológica y evolutiva que tiene la hibridación, frecuentemente los especialistas en conservación la consideran como "destructiva" (O'Brien y Mayr, 1991), debido a que puede "desintegrar la organización genética de las especies en contacto". La hibridación en plantas es un fenómeno muy común (Barton y Hewitt, 1985; Rieseberg y Brunsfeld, 1992; Arnold, 1997), el cual ha originado entre el 30% y 80% de las especies de angiospermas (Stance, 1987; Wendel *et al.*, 1991). Sólo por lo antes mencionado las zonas híbridas deberían de ser conservadas como un centro de evolución de plantas, así como un recurso de variedad de plantas económicamente importantes y como agentes importantes de biocontrol (Whitham *et al.*, 1991).

En nuestro estudio, los árboles híbridos actúan como un centro de riqueza de especies y contienen significativamente más especies de artrópodos que sus especies parentales putativas. El incremento en la susceptibilidad de las plantas híbridas hospederas hacia los artrópodos, puede ser el resultado de una recombinación genética que interrumpe un complejo de genes co-adaptados que controlan la resistencia (Grant, 1971; Whitham, 1989). Cambios en las características de las plantas a través de la hibridación puede representar hábitats con una arquitectura más diversa (Martinsen y Whitham, 1994), incrementando los recursos alimentarios y diluyendo los mecanismos de resistencia (i.e., metabolitos secundarios) o caracteres fenológicos (Fritz *et al.*, 1994; Strauss, 1994; Fritz, 1999) para artrópodos del dosel, en relación con las especies parentales putativas.

Además, se ha observado que la alteración en la arquitectura de plantas híbridas ha tenido un efecto sobre taxa de vertebrados, los cuales representan un mayor interés para el público. Por ejemplo, Martinsen y Whitham (1994) registraron un significativo incremento en la densidad de anidación de aves en una zona híbrida producidas por la entrecruza de *Populus fremontii* y *P. angustifolia.*

Continuamente se incrementa el número de especialistas en conservación que reconocen su limitado conocimiento sobre insectos a pesar de que éstos son los principales componentes de cualquier ecosistema terrestre debido a su riqueza, densidad, biomasa y papel ecológico (Martín-Piera, 1998). Generalmente, las medidas conservacionistas suelen aplicarse para proteger algún ambiente en particular o grandes vertebrados, mientras que los insectos escapan a su efecto o se benefician indirectamente de ellas. Si se pretende preservar la diversidad biológica, no es posible ignorar a los insectos (Pyle *et al.*, 1981), aunque para ello haya que vencer los prejuicios humanos.

Debido a que los insectos hasta el momento son el grupo más diverso sobre la Tierra es evidente que cuando se habla de extinción de especies o pérdida de diversidad nos referimos casi por completo a los insectos y otros grupos como ácaros, anélidos y hongos. Sin embargo, es difícil conocer con exactitud el número de especies extintas cuando la mayoría de éstas ni siquiera se han descrito. May *et al.* (1995) proponen que la tasa de extinción anual se sitúa entre las 10,000 y 25,000 especies, es decir, de 24 a 72 especies diarias. La deforestación es una de las principales causas de este alarmante incremento en la extinción de especies. En México, los encinos son parte integral de los bosques y la asociación encino-pino cubre una tercera parte (15 millones ha) de las actuales áreas boscosas del país. Durante la década de 1980 y 1990 la tala de estos bosques, llegó a ser de 163 000 ha/año

(Masera *et al.*, 1990; 1992). Las regiones templadas de México son consideradas como centro de diversidad del género *Pinus* y *Quercus*, además, aunque la diversidad de especies vegetales asociadas al dosel de pinos y encinos es relativamente baja, la diversidad de los estratos herbáceos y arbustivos suelen ser muy altas, y lo mismo ocurre con la heterogeneidad global de ecotipos del bosque dentro de toda la zona, lo que hace de esta la más biodiversa de todas las zonas ecológicas de México (Challenger, 1998).

En los ecosistemas boscosos, los artrópodos incrementan su importancia dado el papel ecológico que desempeñan, por ejemplo, son componentes importantes en la regulación del ciclaje de nutrientes (Kimmins, 1972; Chew, 1974; Mattson y Addy, 1975; Wickman, 1980; Wickman y Starr, 1990). Swank *et al.* (1981) reportan que los eventos que producen los insectos defoliadores sobre el dosel de los árboles son (a) decremento en la producción de madera pero incremento en la producción de hojas, (b) altos incremento del mantillo, (c) incrementos de nitrógeno por la caída de hojarasca durante el periodo de defoliación y (d) incremento significativo en la cantidad de nutrientes disponibles (especialmente nitrógeno) en la capa superficial del suelo. Risley y Crossley (1988) y Risley (1986) encontraron que las hojas verdes que se caen como resultado de la herbivoría presentaron una mayor concentración de nitrógeno que las hojas senescentes que se encontraban en el suelo, por lo tanto, se sugiere que estas hojas son más apetecibles para los organismos descomponedores. Por lo anterior, se puede pensar que los insectos defoliadores del dosel aceleran el ciclaje de nutrientes (Risley y Crossley, 1992).

Lo anterior nos permite entender el papel que juegan las perturbaciones debidas sobre todo a la deforestación que genera el hombre provocando una disminución en la diversidad y riqueza de las especies de artrópodos asociados al dosel. Además, ahora contamos con datos de diversidad y riqueza específica de artrópodos asociados al dosel que nos ayudan a entender la importancia de conservar los bosques de encinos (en particular las zonas híbridas) y lo que implicaría la pérdida de éstos en México.

Es importante la conservación de estos bosques ya que cada uno de éstos posee todo un mosaico de microhábitats en el dosel para la fauna de artrópodos que no se pueden repetir en ningún otro tipo de bosque. Por ejemplo, Winchester y Ring (1996) encontraron datos que sugieren que los microhábitats explotados por los artrópodos de dosel no se repiten en ningún otro sitio del bosque que fue examinado.

Muy pocos insectos en el mundo se encuentran protegidos y cada día se incrementa la pérdida de diversidad en el mundo, por lo que se recomienda hacer un esfuerzo en las siguientes direcciones. En primer lugar, es necesario describir e inventariar la diversidad de insectos. En segundo lugar, es necesario investigar los factores que causan las fluctuaciones poblacionales de insectos así como conocer la respuesta que tienen las comunidades a tales perturbaciones. En tercer lugar, se deben difundir los avances que se tienen sobre la diversidad de artrópodos del dosel y el papel ecológico que tienen en los ecosistemas terrestres. Por último, como actualmente se tiene muy poca información sobre la diversidad de artrópodos del dosel y las causas de esta alta diversidad, es necesario conservar la mayor cantidad de paisajes que sean posibles que garanticen la conservación de un mayor número de especies.

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