UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS

INSTITUTO DE ECOLOGÍA

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EFECTO DE LA HIBRIDACIÓN DEL COMPLEJO *Q. glabrescens* × *Q. rugosa* y *Q. glabrescens* × *Q. obtusata* SOBRE LA COMUNIDAD DE INSECTOS FORMADORES DE AGALLAS Y SUS PARASITOIDES

TESIS

QUE PARA OPTAR POR EL GRADO DE:

DOCTOR EN CIENCIAS

PRESENTA:

M. en C. Elgar Castillo Mendoza

TUTOR PRINCIPAL DE TESIS: DR. EFRAÍN TOVAR SÁNCHEZ
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OFICIO CPCB/091/2019

Asunto: Oficio de Jurado para Examen de Grado.

M en C. Ivonne Ramírez Wence
Directora General de Administración Escolar, UNAM
P r e s e n t e

Me permito informar a usted, que el Subcomité de Biología Evolutiva Y Sistemática, en su sesión ordinaria del día 26 de noviembre de 2018, aprobó, el jurado para la presentación del examen para obtener el grado de **DOCTOR EN CIENCIAS**, del alumno **CASTILLO MENDOZA ELGAR** con número de cuenta: **512026680** , con la tesis titulada: **"EFECTO DE LA HIBRIDACIÓN DEL COMPLEJO *Quercus glabrescens x Q. rugosa* y *Q. glabrescens x Q. obtusata* SOBRE LA COMUNIDAD DE INSECTOS FORMADORES DE AGALLAS Y SUS PARASITOIDES"**, bajo la dirección del **DR. EFRAÍN TOVAR SÁNCHEZ**:

Presidente:	DR. ALEJANDRO ZALDÍVAR RIVERÓN
Vocal:	DRA. ROSA GABRIELA CASTAÑO MENESES
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Suplente:	DR. RICARDO REYES CHILPA

Sin otro particular, me es grato enviarle un cordial saludo.

A T E N T A M E N T E
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, Cd. Mx., a 24 de enero de 2019

DR. ADOLFO GERARDO NAVARRO SIGÜENZA
COORDINADOR DEL PROGRAMA

POSGRADO EN CIENCIAS BIOLÓGICAS
COORDINACIÓN

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ÍNDICE

Castillo-Mendoza, Elgar. 2018. Efecto de la hibridación del complejo *Q. glabrescens* × *Q. rugosa* y *Q. glabrescens* × *Q. obtusata* sobre la comunidad de insectos formadores de agallas y sus parasitoides. Tesis de Doctorado. Posgrado en Ciencias Biológicas e Instituto de Ecología, Universidad Nacional Autónoma de México.

RESUMEN

La hibridación en encinos es un proceso recurrente que afecta las características genéticas, fisiológicas, químicas y morfológicas de los taxones involucrados en dicho proceso y tiene implicaciones directas e indirectas sobre los grupos biológicos a las que se encuentran asociados. Particularmente los cinípidos, debido al grado de especificidad que las avispas tienen con los encinos, que han favorecido el desarrollo y radiación de Chalcidoidea parasitoides. *Q. rugosa*, *Q. glabrescens* y *Q. obtusata* presentan amplia distribución geográfica y gradiente altitudinal. Cuando estas tres especies de árboles se encuentran en alopatría pueden distinguirse morfológicamente. No obstante, en simpatría se detectan individuos con morfología atípica, sugiriendo eventos de hibridación. En este estudio se analizaron: a) los niveles y la dirección de la hibridación en áreas de simpatría entre *Q. glabrescens*, *Q. rugosa* y *Q. obtusata*, b) el perfil metabólico (ms) de genotipos puros de *Q. glabrescens*, *Q. rugosa* y *Q. obtusata*, c) la influencia de la hibridación sobre la expresión cualitativa y cuantitativa de diversos ms, y d) la influencia de la diversidad genética y los ms del taxón hospedero sobre la *S*, *H´*, abundancia y porcentaje de infestación de la comunidad de cinípidos y sus parasitoides asociados. Se muestrearon 180 individuos, 60 dentro de los sitios alopatridos (20/sitio/taxón [reconocidos morfológicamente como *Q. glabrescens*, *Q. rugosa* y *Q. obtusata*]) y 120 dentro de los sitios simpátridos (30/sitio/colecta aleatoria). Todos los individuos fueron analizados con ocho microsatélites (nSSR´s). Los resultados mostraron evidencia genética de hibridación en los cuatro sitios analizados formándose tres complejos híbridos: *Q. glabrescens* × *Q. rugosa*, *Q. glabrescens* × *Q. obtusata*, *Q. rugosa* × *Q. glabrescens* × *Q. obtusata*. Se encontró que el perfil químico que presentan estos tres taxones tiene alta similitud. Se documentó que al menos presentan un ms especie-específico (flavonoide). Se encontraron diferencias cuantitativas en todos los ms

analizados. En los híbridos, se encontró diferencias cuantitativas, pero no cualitativas en la expresión de ms. Se encontró que la diversidad genética del taxón hospedero tiene un efecto positivo y significativo sobre *H'*, *S*, abundancia y porcentaje de infestación de la comunidad de cinípidos y de la comunidad de parasitoides asociados al dosel de *Q. glabrescens*, *Q. rugosa* e híbridos. Asimismo, se encontró que las diferencias cualitativas y cuantitativas de los ms tienen influencias tanto positivas como negativas sobre la *S* y la abundancia de los cinípidos y los parasitoides asociados. Los resultados de este trabajo sugieren que la relación entre *Quercus*-cinípidos-parasitoides está fuertemente influenciada por la diversidad genética y el perfil metabólico que presentan los taxones hospederos. Por lo que, el mantenimiento de la mayor diversidad genética y específica puede tener efectos en cascada que ayuden a mantener las interacciones biológicas ciertamente estables, lo que puede ayudar a conservar la mayor biodiversidad posible.

ABSTRACT

Hybridization in oaks is a recurrent process that has the genetic, physiological, chemical and morphological characteristics of the taxa involved in the process and has direct and indirect implications in the biological groups. Particularly the Cynipidae inducers of galls, due to the degree of specificity that the wasps have with the oaks, which have favored the development and radiation of Chalcidoidea parasitoids. *Q. rugosa, Q. glabrescens* and *Q. Obtusata* are three white oaks that have a wide geographical distribution and altitudinal gradient. When these three tree species are found in allopathy, they can be distinguished morphologically. However, in sympatric, individuals with atypical morphology are detected, suggesting hybridization events. In this study we analyzed: a) The levels and direction of hybridization in the areas of sympatric between *Q. glabrescens*, *Q. rugosa* and *Q. obtusata*, b) The metabolic profile (sm) of pure genotypes of *Q. glabrescens*, *Q. rugosa* and *Q. obtusata*, c) the influence of hybridization on the qualitative and quantitative expression of various sm, and d) The influence of genetic diversity and the majority of the host taxon on the *S*, *H'*, abundance and the percentage of the infestation of the community of gall-forming wasps (Cynipidae) and their associated parasitoids (Chalcidoidea). 180 individuals were sampled, 60 within the allopatrid sites (20/site/taxon [morphologically recognized as *Q. glabrescens, Q. rugosa* and *Q. obtusata*]) and 120 within the sympatric sites (30/site/random collection). All individuals were analyzed with eight microsatellites (nSSR's). The results obtained show the genetics of the hybridization in the four analyzed sites forming three hybrid complexes: *Q. glabrescens* × *Q. rugosa*, *Q. glabrescens* × *Q. obtusata*, *Q. rugosa* × *Q. glabrescens* × *Q. obtusata*. It found that the chemical profile presented by these three taxa has high similarity. It was documented thar at least they present a sm analyzed. In the hybrids, quantitative differences were found, but not qualitative differences in the expression of sm. It was found that the genetic diversity of the host taxon has a positive and significant effect on *H'*, *S*, abundance and percentage of infestation of the community of wasps gall indicators and the community of parasitoids associated with *Q. glabrescens, Q. rugosa* and hybrids. We have also found that qualitative and quantitative of the sm differences have more positive and negative influences on the *S* and the

abundance of wasps and associated parasites. The results of this work have a relationship between *Quercus*-wasps galls-parasitoids are strongly influenced by the genetic diversity and the metabolic profile presented by the host taxa. Therefore, maintaining the greatest genetic and specific diversity can have cascading effects that help to maintain biological interactions that are certainly stable, which can help to conserve the greatest possible biodiversity.

INTRODUCCIÓN GENERAL

La hibridación se define como la cruza de individuos pertenecientes a grupos genéticamente distintos ya sea a nivel de poblaciones, subespecies, especies o géneros (Taylor et al. 2015, Quilodrán et al. 2018). La hibridación entre especies eucariotas es bastante frecuente. Según Mallet (2005) este proceso está relacionado con al menos el 25% de las especies de plantas y el 10% de las especies de animales. Aunque la hibridación es un evento recurrente, la proporción de individuos que se encuentran a nivel poblacional regularmente es baja. para algunas especies animales la tasa de hibridación no supera el 0.1% por generación en cualquier especie (Mallet 2005, Mallet 2007). Mientras que el porcentaje de individuos híbridos para algunas especies de plantas es significativamente más alto, pudiendo oscilar entre el 4.3 y 46% (Tovar-Sánchez y Oyama 2004, Valencia-Cuevas et al. 2015, Sullivan et al. 2016).

Por lo anterior se ha sugerido que entre el 50 y el 70% de las angiospermas pudieron haber pasado por este proceso (Whitham et al. 1991, Arnold 1994). Sin embargo, la frecuencia y la prevalencia de la hibridación se encuentra distribuida de manera irregular tanto a nivel de familias como de géneros. Un trabajo realizado por Whitney et al. (2010) documentó que de un total de 282 familias y 3212 géneros por lo menos el 40.4% de las familias y el 16.2% de los géneros analizados presentaban al menos un evento de hibridación. Dicho estudio documentó que 25 familias vegetales presentaban una alta propensión a la hibridación y que ésta estaba relacionada fuertemente con las propiedades intrínsecas de las especies, la cercanía filogenética así como factores ambientales, ya que los patrones de hibridación tienden a ser similares a través de las diferentes regiones en dichos taxones (Whitney et al. 2010).

Barreras reproductivas

La variación en los patrones de hibridación está fuertemente relacionada con la fortaleza de las barreras reproductivas debido a que estas pueden influenciar la “integridad” genética de las especies y la probabilidad de formar híbridos (Coyne y Orr 2004). Las barreras reproductivas pueden actuar antes, durante y después de

la formación del cigoto, dado que pueden ser pre o post cigóticas (Lowry et al. 2008a, Widmer et al. 2009, Baack et al. 2015). Aunque se ha documentado que de manera general las barreras precigóticas son significativamente más eficientes tanto para prevenir el flujo genético como para fortalecer las barreras reproductivas (Lowry et al. 2008) en algunas ocasiones las barreras poscigóticas (P. ej. Lowry et al. 2008a, b, Ishizaki et al. 2013) muestran una fortaleza mayor (Baack et al. 2015). En este sentido Rieseberg et al. (2006) menciona que cuando se analizan las barreras reproductivas poscigóticas aproximadamente el 70% de las especies biológicas presentan linajes reproductivamente aislados.

La formación del aislamiento reproductivo precigótico puede estar asociado a diferentes factores entre los que se encuentran: i) diferencias fenológicas, ii) interacción polen-estigma, iii) competencia polínica, iv) dirección de movimiento del polen, v) diferencias ecogeográficas e vi) inviabilidad del inmigrante (Baack et al. 2015, De la Torre 2015). En contraparte las barreras poscigóticas están fuertemente relacionadas con: i) la inviabilidad y esterilidad híbrida, ii) disponibilidad espacial, iii) la abundancia relativa de las especies parentales y iv) las condiciones ambientales particulares (Anderson 1948, Lepais et al. 2009, Ortego et al. 2014, De la Torre 2015). La contribución particular de cada barrera y su fortaleza específica es desconocida hasta el momento, aunque se puede sugerir que la mezcla particular y/o la suma de diferentes barreras contribuye al aislamiento reproductivo y al mantenimiento de la identidad e integridad específica a pesar de los eventos de hibridación. Diversos estudios con especies leñosas (p. ej. Wheeler et al. 2013, Vasilyeva y Semerikov 2014, Geraldes et al. 2014) han documentado que cuando las barreras reproductivas no son lo suficientemente fuertes el sobrelapamiento ecológico y geográfico habilita/posibilita el flujo genético interespecífico.

Implicaciones ecológicas y evolutivas de la hibridación

La importancia de la hibridación puede ser abordada desde diferentes perspectivas, y cada una de ellas da lugar a diferentes implicaciones ecológicas y evolutivas (frecuencia, tasas de especiación, riqueza específica, tasa de extinción y supervivencia entre otros). La hibridación tiene impacto sobre la estructura poblacional, la

constitución genética y la evolución fenotípica afectando directamente a las especies. En este sentido la hibridación puede ser considerada como una importante fuerza macroevolutiva (Folk et al. 2018) capaz de generar y/o incrementar la nueva diversidad biológica, al menos dentro de los sitios donde se lleva a cabo (Mallet 2007, Brumfield 2010). Por otra parte, la hibridación tiene importantes efectos en otros procesos biológicos como la modificación de la amplitud de distribución geográfica y la evolución del nicho ecológico (Folk et al. 2018). Dada la naturaleza de la "constitución híbrida" (heterocigotos para las especies parentales, Folk et al. 2018) que presentan los individuos híbridos, es posible esperar que en tiempos relativamente cortos dichos individuos híbridos desaparezcan, a menos que diferentes mecanismos ayuden a mantener o incrementar dicha heterocigosidad (p. ej. alopoliploidía, introgresión y/o la heterocigosis de traslocación permanente (Grant 1981, Holsinger y Feldman 1982, Harrison y Larson 2014).

La hibridación no necesariamente está relacionada con la introgresión, definida como la incorporación de una pequeña cantidad de genoma de una especie a otra por la constante cruza que se da entre ellas y sus híbridos (Harrison 1993). No obstante, si la introgresión se llega a producir ésta puede ser uni o bidireccional (Arnold 1997) y puede tener diversas implicaciones. Un término recientemente propuesto es el de "introgresión adaptativa" definido como el proceso mediante el cual se realiza un traspaso de pequeñas regiones genómicas de una especie que tienen consecuencias positivas para la adecuación de la especie receptora (Suárez-González et al. 2018). Lo anterior implica que la hibridación introgresiva es el resultado de un proceso selectivo (Harrison y Larson 2014). Los resultados potenciales de la introgresión adaptativa están determinados por una serie de factores ecológicos que modulan el grado de contacto interespecífico (Schmickl et al. 2017). Dichos factores pueden afectar/beneficiar el establecimiento de los genotipos híbridos provocando la formación de patrones complejos de mezcla genética (Hand et al. 2015). Por ejemplo; a) favoreciendo la adaptación local y/o especiación (Coyne y Orr 2004), b) formación de mezclas genéticas únicas que permitan el establecimiento de individuos híbridos con "arquitecturas genéticas" novedosas (Quilodran et al. 2018) y c) independientemente de que ésta no de como resultado la formación de un nuevo taxón la incorporación de genoma exoespecífico

puede modificar la estructura genética de los taxones involucrados. En contraparte, a) puede incorporar información que no presenta ninguna ventaja o que incluso puede ser perjudicial (Schmickl et al. 2017), b) algunos trasfondos genéticos pueden afectar las interacciones esenciales para el funcionamiento de los genes, inactivando la información de los genes extraños (Schmickl et al. 2017), c) el reforzamiento de las barreras reproductivas (Baack et al. 2015) y d) puede conducir a la extinción de especies raras debido al "hundimiento genético" (Gómez et al. 2015, Todesco et al. 2016).

Se espera que aquellos loci que presenten rasgos favorables sean seleccionados con mayor frecuencia (Whitney et al. 2006) mientras que aquellos que no representan ventajas y/o contribuyen al aislamiento reproductivo, se mezclen escasa o nulamente (Barton 2001). Sin embargo, la adaptación local en última instancia puede contribuir tanto al aislamiento reproductivo como al rompimiento de las barreras reproductivas, dependiendo si los alelos localmente adaptados contribuyen a las incompatibilidades genéticas (Nolte y Tautz 2017). En especies arbóreas se ha registrado que la hibridación contribuye a la adaptación local (Suárez-González et al. 2018) aunque las barreras a la introgresión en las especies arbóreas parecen estar fuertemente reguladas, existen diversos géneros como *Pinus* y *Quercus* que presentan cierta permeabilidad a nivel específico (Barcaccia et al. 2014, Christe et al. 2016, Leroy et al. 2017).

Implicaciones taxonómicas

Durante los eventos de hibridación que se dan entre diferentes especies y en diferentes grupos biológicos, uno de los elementos necesariamente involucrados es acerca de la definición y naturaleza de las especies. En la literatura frecuentemente se encuentran dos visiones claramente opuestas. Por un lado, existe la visión de que las especies son un grupo de individuos bien definidos que se encuentran unidos mediante el flujo genético específico y exclusivo que se encuentran reproductivamente aislados de otros grupos biológicos (Mayr 1942, Baum y Shaw 1995). En contraste, diversos investigadores sustentan que las especies son grupos biológicos que se encuentran aislados únicamente por pequeñas regiones genómicas que mantienen tanto la identidad como

la cohesión específica, evitando que las especies puedan "diluirse" cuando se mezclan con individuos que están filogenéticamente cercanos pero que a su vez mantienen su propia identidad específica (Wu 2001, Nosil et al. 2009, Harrison y Larson 2014). Lo anterior, intuye la necesidad de flexibilizar el concepto de especie para procesos como la hibridación en donde diversas especies no "cumplen con los requisitos" para pertenecer a una u otra categoría taxonómica.

Hibridación en *Quercus*

Uno de los grupos biológicos que mayor problema presenta en la clasificación taxonómica son los encinos, lo anterior debido a que en este grupo biológico se ha documentado frecuentes eventos de hibridación e introgresión interespecífica pero no intragenérica (González-Rodríguez et al. 2004, Albarrán-Lara et al. 2010, Lepais et al. 2013, Gailing y Curtu 2014, Fortini et al. 2015, Wei et al. 2015). Siendo una fuente de variación morfológica y genética (Tovar-Sánchez y Oyama 2004, Valencia-Cuevas et al. 2015). En los encinos tradicionalmente la utilización de caracteres morfológicos es empleada como método de clasificación específica. Por lo que, la presencia de individuos con morfología atípica frecuentemente ha sido interpretada como resultado de eventos de hibridación (Howard et al. 1997, Curtu et al. 2007, Burgarella et al. 2009). La tasa relativamente alta de hibridación que ocurre dentro del género ha provocado entre otras cosas que el empleo de marcadores morfológicos y moleculares no haya permitido una clasificación infragenérica sólida que permita la separación a escala fina de las especies debido principalmente a la amplia variabilidad genética y fenotípica (Aldrich y Cavender-Bares 2011, Denk et al. 2017, McVay et al. 2017). Lo que ha contribuido a incrementar la confusión taxonómica que caracteriza a las especies del género (Fortini et al. 2015). No obstante, la combinación del análisis de caracteres morfológicos y genéticos puede ayudar a delimitar de manera precisa a las especies de encinos (Rellstab et al. 2016).

Importancia de los metabolitos secundarios

Los metabolitos secundarios (ms) son compuestos químicos de bajo peso molecular derivados del metabolismo primario, que no están involucrados en el desarrollo y/o crecimiento normal de las plantas (Irchhaiya et al. 2014). Aunque no están involucrados directamente en las funciones básicas de las plantas los ms están relacionados con una serie de procesos que impactan en su supervivencia, defensa y reproducción. Se sugiere que pueden actuar como intermediarios en las interacciones que se dan entre herbívoros, polinizadores y depredadores, así como defensa contra el estrés abiótico (Iason et al. 2012). Los impactos que puede tener la presencia de un ms determinado rebasan al taxón que los produce llegando a modificar a diversos componentes en una escala ecosistémica. Por lo que se ha propuesto que pueden representar el enlace más importante entre los genes y los ecosistemas (Iason et al. 2012). Así, dependiendo de la función que realicen los ms dentro del ecosistema éstos pueden ser clasificados desde el punto de vista evolutivo como kairomonas (positivos para el taxón receptor), alelomonas (positivos para el taxón productor), sinomonas (positivos para productor y receptor) y feromonas (reproducción, socialización, alarma y localización de alimento) (Norlund y Lewis 1976, Blum 1996).

Funciones de los metabolitos secundarios

La presencia, concentración y los complementos de los ms presentes en un tejido particular pueden ser alterados por las diferentes señales de las rutas metabólicas (van Dam et al. 2008). Por ejemplo, las concentraciones de los ms pueden variar marcadamente a través de la planta dependiendo del "valor" del tejido donde se encuentren, de acuerdo con la teoría del valor óptimo (Hartley et al. 2012). Aunque las funciones que tienen los ms de manera individual tienen impacto sobre diferentes procesos y a diferentes escalas la diversidad que presenta cada planta puede tener diferentes implicaciones cuando actúan en sinergia y/o sus interacciones sirven como catalizadores/iniciadores de otros procesos biológicos (Barbehenn et al. 2006a, b). La variabilidad en la expresión de los ms puede actuar como el eslabón que relacione a las plantas

con los distintos organismos asociados mediante la diversidad genética y la "extensión del fenotipo" (Bailey et al. 2006). En este sentido la evolución de las plantas mediada por la hibridación puede ser un importante mecanismo de regulación que afecta a las comunidades asociadas así como a los ecosistemas (Bailey 2012).

La defensa química de las plantas provee de una importante fuente de protección contra una amplia gama de herbívoros. Sin embargo, la presencia de dichos ms ha favorecido la adaptación y especialización de diversos grupos de artrópodos como Acari, Lepidoptera e Hymenoptera (Stone et al. 2002, Skoracka et al. 2010, Dicke et al. 2012). Los artrópodos herbívoros especialistas como Heteroptera, Coleoptera y Hemíptera pueden utilizar a los ms como estimulantes alimenticios, señalizadores de sitios de oviposición y como mecanismo de defensa contra enemigos naturales (Aliabadi et al. 2002, Schoonhoven et al. 2005, Hopkins et al. 2009). Las comunidades de herbívoros tienen la capacidad de diferenciar tanto a nivel individual como específico la diversidad de ms (quimiotipos) (Nielsen 1997, van Leur et al. 2006). Los quimiotipos resultan de diferencias en un pequeño número de genes o alelos con la conversión de los diferentes ms durante la biosíntesis lo que deriva en el establecimiento de una comunidad de herbívoros particular (Nielsen 1997, van Leur et al. 2006, Dicke et al. 2012). Por otra parte, las plantas son capaces de diferenciar el ataque de diferentes herbívoros (especialistas vs. generalistas) reubicando y aumentando la concentración de los ms. Diversos estudios han documentado que el ataque temprano por parte de los herbívoros generalistas incrementa la preferencia de consumo por parte de los especialistas en comparación con aquellas hojas que no han sido atacadas por herbívoros generalistas previamente (van Zabdt y Agrawal 2004, Poelman et al. 2008, 2010). Las modificaciones en la expresión de ms en la planta hospedera tiene implicaciones sobre todos los miembros de la comunidad incluidos herbívoros, polinizadores, parasitoides y depredadores (Kessler y Halitschke 2007, Lucas-Barbosa et al. 2011). Debido a lo anterior, la variación en la regulación y expresión de los ms juega un papel fundamental en la composición y estructura de sus comunidades asociadas, llegando a afectar incluso la biodiversidad del ecosistema (Poelman et al. 2008, Schweitzer et al. 2008, Dicke et al. 2012).

Impacto de la hibridación sobre la expresión de metabolitos secundarios en *Quercus*

La hibridación es un proceso que puede modificar la expresión cualitativa y cuantitativa de los ms mediante la modificación de las rutas biogenéticas. No obstante, existen pocos estudios en encinos donde se ha evaluado como el intercambio de información genética entre especies cercanas puede impactar la expresión de ms. Por ejemplo, Yarnes et al. (2008a) utilizando un complejo híbrido formado por *Q. grisea*, *Q. gambelii* y el híbrido formado por estas especies, identificó mediante HPLC a 18 diferentes compuestos fenólicos los cuales difieren significativamente tanto en la concentración total como relativa entre los taxones parentales y el taxón híbrido (glucosidos flavonoides, proantocianidinas y elagitaninos). Asimismo, Yarnes et al. (2008b) documentaron el efecto que tienen los ms sobre cuatro especies de mariposas minadoras de hojas. En tal estudio se analizó el efecto de 10 ms pertenecientes a los elangitaninos y las proantocianidinas. Los autores encontraron que existen diferencias en la expresión cuantitatitativa entre los taxones parentales y el taxón híbrido. Los resultados muestran que la comunidad de mariposas responde significativamente a los cambios en la concentración de los ms, pero que dicha respuesta varía estacionalmente. Además, las concentraciones varían dependiendo del taxón analizado. Los resultados sugieren que la presencia de los ms puede tener una influencia negativa, positiva o neutra sobre el establecimiento de las mariposas.

La producción de los ms está fuertemente regulada por la información genética que contienen los taxones donde se producen y las variaciones intraespecíficas que pueden provocar que haya modificaciones en la expresión cualitativa y cuantitativa de los mismos (ver Glassmire et al. 2016). Considerando que la hibridación es un proceso que puede modificar la diversidad genética de los taxones y que dicha diversidad regula de manera importante la expresión de los ms, los estudios que se hacen para evaluar el efecto de la hibridación deben considerar que la interacción de los ms puede generar relaciones sinérgicas o antagónicas que impacten positiva o negativamente su expresión tanto cualitativa como cuantitativa.

En este sentido, Cheng et al. (2011) mostró que cualitativamente el 70.3% de los ms se expresan tanto en las especies parentales como en los individuos híbridos, 24.2% solo se expresaba en las especies parentales y 5.5%

fueron ms que solo se expresaron en los individuos híbridos. Por otra parte, la expresión cuantitativa mostró que en el 52% de los estudios, los ms no difieren entre híbridos y parentales, 28% tienen una expresión intermedia y el 20% presenta una expresión transgresiva. El amplio intervalo de respuestas que muestran los híbridos hace suponer que la hibridación puede favorecer la formación de ms y/o que la mezcla de los ms puede otorgar nuevas funciones a los ya existentes.

Finalmente, en un trabajo realizado por Yarnes et al. (2006) se menciona la presencia de 22 ms asociados a 10 especies de encinos blancos y dos especies de encinos rojos. Aunque en este trabajo no se evalúa el efecto que puede tener la hibridación sobre la expresión cualitativa y/o cuantitativa de los ms resulta de suma importancia porque es primer trabajo que se lleva a cabo en México. Además, este trabajo tiene una relevancia especial en el sentido de que evalúa de manera puntual la concentración absoluta y relativa de diversos ms en distintos taxones de encinos empleando técnicas cuantitativas que tienen gran precisión (HPLC), para lo que se requiere la obtención de compuestos puros que son obtenidos a partir del empleo de diferentes técnicas químicas. Finalmente, los resultados de este estudio permiten distinguir las diferencias en la expresión de ms (cualitativa, cuantitativa y específica) entre las dos secciones de encinos analizadas.

Variación-diversidad de metabolitos secundarios en especies del género *Quercus*

Son escasos los estudios que determinen a nivel fino la diversidad de compuestos químicos foliares presentes en encinos. Aunque se ha sugerido que están relacionados con la defensa ante la herbivoría en ninguno de los estudios llevados a cabo se ha documentado específicamente la relación entre algún ms y la defensa contra algún insecto ya sea generalista o especialista. Existen dos tipos de trabajos realizados en encinos por un lado estudios donde se ha caracterizado de manera muy fina la expresión de diferentes ms. Una revisión llevada a cabo por Glasby (1991) documentó el estudio de 23 especies de encinos en donde las familias de ms presentes son: compuestos alifáticos, esteroides, triterpenoides, hidrocarburos, taninos, glucósidos, compuestos fenólicos y benzofuranos. Yarnes et al. (2008a, b) reportan la diversidad de fenoles y flavonoides presentes en

tres taxones. Moctezuma et al. (2014) registran tanto taninos como flavonoides presentes en un taxón de forma detallada. Por último, Noori et al. (2015) mencionan una amplia diversidad de flavonoides para dos variedades de una especie de encino. En contraparte, Makkar et al. (1998) y Maldonado-López et al. (2015) han documentado la presencia de diversos ms, pero solamente a nivel de familia. Entre ellos se encuentran: fenoles totales, taninos condensados, Procianidólicos, flavonoides, flavan-4-ol, gallotaninos y proantocianidinas.

La variación en la caracterización de los ms puede estar relacionada particularmente con la capacidad técnica para separar a los ms, dado que por su naturaleza pueden identificarse desde el empleo de técnicas muy sencillas como la cromatografía en capa fina, hasta el uso de la cromatografía líquida de alta resolución (HPLC) y la resonancia magnética nuclear (RMN). En contraparte, la variación en el tipo de compuesto que se reporta para cada taxón está más relacionado con la información genética que dicho taxón presenta que con la capacidad para poderse separar, purificar e identificar. Lo anterior debido a que la diversidad de ms que producen puede dependiendo de su naturaleza formar complejos con una alta similitud o unirse de tal manera que no pueden ser separados para su identificación.

Relación entre diversidad genética y metabolitos secundarios

Estudios recientes muestran que la variación genética de diversas especies vegetales puede tener diferentes implicaciones a nivel de comunidades y a diversas escalas. Por ejemplo, diversas comunidades de artrópodos y microbianas, interacciones tritróficas y disponibilidad de nutrientes (Whitham et al. 2006, Johnson y Stinchcombe 2007, Hughes et al. 2008, Bailey et al. 2009b, Valencia-Cuevas et al. 2018). Dicha variación está directa e indirectamente relacionada con la capacidad que cada taxón vegetal tiene para producir diversos tipos de ms relacionados y a su vez está directamente relacionada con la variación en la diversidad genética y la relación que tenga con los insectos. Lo anterior podría suponer que una mayor diversidad genética puede otorgar la posibilidad de responder de diferentes maneras al estrés provocado por diferentes variables como variaciones en el ambiente, escases de nutrientes, procesos alelopáticos y las múltiples facetas que tienen en la

relación con los insectos pudiendo evolucionar en tiempos ecológicos relativamente cortos (Tovar-Sánchez et al. 2018).

La heredabilidad es un parámetro de la proporción de variación genética aditiva en el rasgo en el cual la selección puede actuar causando un cambio evolutivo poblacional (Falconer y Mackay 1996). Diversos estudios sugieren que la expresión de los ms puede variar dependiendo de los cambios ambientales (Donaldson y Lindroth 2007) y ontogénicos (Barton y Koricheva 2010). Por lo que la selección de ms con una alta heredabilidad (p. ej. compuestos fenólicos) para estudiar los efectos de la hibridación sobre los patrones de herbivoría puede establecer o ayudar a clarificar de manera muy puntual como el cambio en la estructura genética de las especies hospederas afecta los patrones de expresión de ms y, en consecuencia, el establecimiento de los insectos herbívoros y sus depredadores (parasitoides) naturales. Para que los ms tengan impacto sobre el establecimiento de los herbívoros no es necesario que haya una variabilidad en la expresión de los ms, pero si, que exista una relación causal directa entre su concentración y los impactos sobre los herbívoros (O´Reilly-Wapstra et al. 2013).

Implicaciones ecológicas de los metabolitos secundarios (herbivoría)

Dentro de la interacción planta-insecto los herbívoros raramente matan a las plantas. Sin embargo, pueden llegar a alterar sus características tales como la fisiología, la morfología y la química (Karban y Baldwin 1997; Ohgushi 2005). Los cambios en la fitoquímica pueden verse expresados en el metabolismo primario y secundario los cuales desempeñan un papel clave en las respuestas de alimentación y defensa contra la herbivoría (Iason et al. 2012, Betsiashvili et al. 2014, Harvey y Malcicka 2015). A menudo los aleloquímicos son inducibles, es decir se encuentran en niveles muy bajos dentro de las plantas, pero se incrementan significativamente después del daño tisular (Bourgaud et al. 2001, Schoonhoven et al. 2005). En consecuencia, los cambios evolutivos en las preferencias de alimentación de los herbívoros y las respuestas que las plantas

presentan ante la herbivoría pueden modificar la estructura de la comunidad de insectos asociados (Johnson et al. 2009, Utsumi 2015).

Las plantas responden de manera diferencial dependiendo del genotipo y la especie de herbívoro (Agrawal 2005, Kessler y Halitschke 2007). Kant et al. (2008) encontró que la variación genética del ácaro *Tetranychus urticae* puede inducir o reprimir la producción de ácido jasmónico (relacionado con la defensa de la planta hospedera). Asimismo, diversos estudios han encontrado que los insectos masticadores pueden incrementar la expresión de ácido jasmónico (Ali y Agrawal 2014, Weber y Agrawal 2014). Afectando directamente a los insectos que se alimentan de savia y que son sensibles a la defensa relacionada con el ácido salicílico (Felton y Korth 2000, Thaler et al. 2002). Lo anterior sugiere que las interacciones indirectas de las plantas mediadas por la especificidad en las respuestas inducidas son una de las principales fuerzas que estructuran a las comunidades de artrópodos herbívoros y niveles tróficos superiores (van Zandt y Agrawal 2004, Utsumi y Ohgushi 2009, Poelman et al. 2010). En este sentido, un estudio realizado por McCall y Fordyce (2010) mostró que las hojas jóvenes son más valiosas que las hojas viejas, y están mejor defendidas debido a que presentan una concentración más alta de ms. Dichos resultados son respaldados por la teoría de la defensa óptima (McKey 1974, Rhoades y Cates 1976), la cual menciona que la defensa de las plantas se debe asignar a aquellos tejidos con un valor más alto para el rendimiento de la planta. Por último, el impacto que los ms tienen sobre los herbívoros especialistas y generalistas difieren (Harvey y Malcicka 2015). Los insectos generalistas no poseen mecanismos refinados de defensa contra aleloquímicos por lo que han desarrollado mecanismos como la P_{450}-monooxigenasa, que son muy eficaces contra una amplia gama de ms (Berenbaum et al. 1996) pero no tanto como los mecanismos empleados por los insectos especialistas, los cuales han coevolucionado con las fitotoxinas en un proceso descrito como “guerra armamentista” (Ehrlich y Raven 1964). Dentro de este proceso, se ha encontrado que los niveles altos de ms favorecen la alimentación y oviposición de herbívoros especialistas (Schoonhoven et al. 2005).

Biodiversidad e importancia ecológica de los artrópodos

Los artrópodos constituyen el 78% de todas las especies descritas (Zhang 2013). Se estima que existen 6.8 millones de especies de insectos (Stork 2018) de las cuales el 50% son fitófagas (Barah y Bones 2015). Diversos estudios documentan que esta diversidad está relacionada principalmente con la estrecha relación que tienen con las plantas vasculares, con las que han tenido una relación relativamente estable en por lo menos 300 millones de años (Labandeira 2013). Durante el tiempo que se ha desarrollado la relación planta-insecto éstos últimos han evolucionado en diferentes direcciones formando diversos grupos funcionales como son herbívoros, detritívoros, parásitos, parasitoides, presas y polinizadores. La alta diversidad en los papeles ecológicos que los artrópodos desempeñan sugiere que este grupo biológico puede modificar la estructura y funcionamiento de las comunidades lo que finalmente puede tener impacto a niveles ecosistémicos (Maguire et al. 2015, Noriega et al. 2018, Schowalter et al. 2018).

Diversidad de avispas cinípidas

Con cerca de 1,300 especies descritas, Cynipidae (Hymenoptera) es la segunda familia en orden de importancia en número de especies inductoras de agallas, solo detrás de Cecidomyiidae (Diptera) y el principal grupo de insectos que ha radiado a partir de un solo subgénero vegetal (Ronquist y Liljeblad 2001, Csóka et al. 2005). Los datos sugieren que la radiación de Cynipidae se dio en el Cretácico, hace aproximadamente 85 millones de años (Raman 2005). Los cinípidos posiblemente divergieron en América, seguido por múltiples colonizaciones hacia la región Paleártica y algunas reinvasiones en la Neártica (Stone et al. 2002, Liljeblad 2002). Posiblemente, la mayor riqueza de especies de cinípidos se encuentre en la región neártica (principalmente en México, Raman 2005), donde se sugiere que pueden existir hasta 700 especies (Nieves-Aldrey 2001).

Los cinípidos han encontrado condiciones óptimas de vida en los encinos (*Quercus*), ya que prácticamente la totalidad de las especies conocidas de esta familia viven en ella (Liljeblad et al. 2008). Además, la diversificación y especialización en Cynipidae parece estar fuertemente relacionada con el área de distribución de las especies

hospederas (Avise 2007, Hardy y Cook 2010). En especies de encinos con una amplia distribución geográfica las avispas cinípidas son más diversas en comparación con aquellas especies no cinípidas (especies hermanas como los parasitoides [Hardy y Cook 2010]). En contraparte, los encinos con una distribución geográfica restringida pueden presentar una baja diversidad de especies de cinípidos, sin embargo, presentan una alta proporción de especies endémicas (Avise et al. 2007). Dado lo anterior, los patrones de similitud entre los cinípidos y los hospederos en diferentes sitios sugiere que ha habido flujo genético recientemente (Avise et al. 2007). Además, las diferencias en las comunidades de cinípidos puede deberse a que las diferencias genéticas intra e interespecíficas son tan fuertes que forman una barrera que impide el cambio de hospedero, incluso dentro de la misma especie (López-Vaamont et al. 2002, Avise et al. 2007).

La preferencia que tienen los cinípidos por los encinos puede atribuirse al hecho de que los encinos presentan crecimiento lento y tienen brotes que permanecen frescos por mucho tiempo, pudiendo dar refugio a una y hasta dos generaciones sucesivas de insectos en una temporada (Malyshev 1968). En este sentido, las avispas cinípidas atacan únicamente especies relacionadas dentro de una misma sección con ms, fisiología y fenología similar (Cornell 1986, Stone et al. 2002, Abrahamson et al. 2003), y existen muy pocas especies de algunos géneros (*Andricus* y *Callirhytis*) que alternan hospederos (Stone et al. 2008). Finalmente, aunque algunos estudios han sugerido que el perfil químico presente en los hospederos puede ser sumamente importante en el desarrollo de esta interacción (Abrahamson et al. 2003, Price 2004) y que dicho perfil es fundamental tanto en la elección del hospedero como en el rendimiento y supervivencia de la progenie (Abrahamson et al. 2003) aún no se explica la forma en que actúa cada uno de los ms que conforman el perfil químico de cada hospedero, en su relación con las diferentes especies de cinípidos.

Importancia de los parasitoides de Cynipidae

Las agallas forman una comunidad asociada muy estrecha de inquilinos (que incluye a los cinípidos, moscas, palomillas y escarabajos) y parasitoides, particularmente calcidoideos (Eulophidae, Torymidae, Eupelmidae,

Ormyridae, Eurotomydae y Pteromalidae [Pujade-Villar 2013]). Los parasitoides que atacan a los cinípidos son avispas que están clasificadas en tres superfamilias: Ichneumonidae, Braconidae y Chalcidoidea. Esta última es la más importante en términos de riqueza de especies y mortalidad infringida a Cynipide (Raman et al. 2005) Aunque se ha sugerido que las comunidades tienen bajas tasas de mortalidad por el ataque de parasitoides (Price 1988), algunos estudios documentan que los parasitoides pueden provocar una mortalidad que oscila entre el 31 y el 100% (Washburn y Cornell 1981, Wiebes-Rijks y Shorthouse 1992, Stone et al. 1995, Gibson 2006).

La estructura de la comunidad de parasitoides está determinada principalmente por la estructura de la agalla, la localización de la planta hospedera y la estación de crecimiento (Askew 1984). Por lo que, las especies de cinípidos que se desarrollan en la misma especie vegetal al mismo tiempo (cada ciclo) y que generan estructuras y tamaños similares constantemente tienden a presentar una comunidad de parasitoides similar (Askew 1984). Recientemente, se ha sugerido que el color, olor, forma y tamaño de la agalla también son factores importantes (Raman 2005). Asimismo, los parasitoides son capaces de localizar a su presa entre una gama de compuestos químicos liberados por plantas relacionadas con el hospedero pero que no contienen al herbívoro (Erb et al. 2010, Wäschke et al. 2014).

Finalmente, el éxito de los parasitoides de gallícolas radica en el hecho de que durante los primeros estadios de desarrollo las agallas son más blandas y pequeñas lo que las vuelve más vulnerables al ataque de los parasitoides (Nieves-Aldrey 1998). Dicha vulnerabilidad ha propiciado una presión “top-down” que ha permitido la radiación de diferentes especies de cinípidos y diversas formas de agallas (Price 1988, Stone y Schönrogge 2003, Bailey et al. 2009a).

Agallas como microcosmos evolutivo-ecológico

Se ha sugerido que las agallas de los cinípidos representan “hotspots” de diversidad que incluye a artrópodos, hongos, aves y mamíferos que se ven favorecidos directa o indirectamente en su desarrollo por la presencia de

las agallas (Askew 1984, Ronquist y Liljeblad 2001, Hayward y Stone 2005). En este sentido, las comunidades generadas a partir de un solo recurso como es el caso de los cinípidos son un sistema modelo para estudiar la estructura y función de las redes tróficas basadas en un solo recurso (Raman 2005). Una forma de estudiar la estructura de la comunidad es mediante el entendimiento de las causas que la estructuran a través de las interacciones tróficas (Berlow et al. 2004, Borer et al. 2005). Como resultado de la herbivoría las plantas pueden modificar mediante cambios en la aleloquímica la estructura celular y fisiología entre otros (Ohgushi 2008).

Muchos compuestos de defensa química vegetal son inducidos y no constitutivos (Karban y Baldwin 1997). Lo que sugiere que en los encinos la producción y/o incremento en el número de compuestos de defensa puede estar siendo determinado por el establecimiento de los cinípidos. Estas modificaciones pueden provocar grandes cambios "bottom-up" del genotipo de la planta o con respecto a la abundancia y/o preferencia de los herbívoros y los depredadores en los diferentes sitios en donde se encuentran las plantas hospederas (Ohgushi 2008). Por otra parte, existe evidencia de que los cambios "bottom-up" inician con las modificaciones que provocan los herbívoros y que se reflejan en las plantas hospederas y en los depredadores y/o parasitoides (Nakamura et al. 2006, Kaplan et al. 2007). Asimismo, dichas modificaciones incrementan la disponibilidad de los recursos para nuevas especies provocando un incremento en la riqueza específica (Martinsen et al. 2000, Lill y Marquis 2003, Nakamura et al. 2006).

Las interacciones tritróficas (planta-herbívoro-parasitoide) mediadas por compuestos químicos tienen importantes implicaciones ecológicas y evolutivas (Becerra et al. 2009, Wilson et al. 2012). La relación entre la diversidad genética vegetal y los niveles tróficos superiores está parcialmente regulada por los cambios en la diversidad química de las plantas (Richards et al. 2015). Estos cambios son heredables (Johnson et al. 2009, Barbour et al. 2015), y son capaces de responder a los cambios en el ambiente con consecuencias en las comunidades bióticas que se encuentran asociadas a ellas (Bailey et al. 2006, Tovar-Sánchez et al. 2018, Valencia-Cuevas et al. 2018). Los herbívoros son capaces de adaptarse a un perfil químico particular dentro de

una población. Dicha adaptación puede impedir que los artrópodos migren a otras poblaciones de hospederos, aunque sean de la misma especie (Wilson et al. 2012). En este sentido, las altas concentraciones de ms pueden afectar a los insectos herbívoros especialistas, beneficiando indirectamente a los parasitoides (Poelman et al. 2009, Richards et al. 2012, Glassmire et al. 2016). Lo que, en última instancia puede modificar la estructura y funcionamiento de las comunidades por las modificaciones en las interacciones que pueden llevarse a cabo con diversos grupos biológicos.

JUSTIFICACIÓN

Considerando que, los encinos presentan una alta propensión a la hibridación cuando están en simpatría, y que este proceso puede incrementar la diversidad genética de los taxones involucrados, se desconoce si la cercanía geográfica entre *Q. glabrescens*, *Q. rugosa* y *Q. obtusata* propicia eventos de hibridación. Asimismo, en caso de haber eventos de hibridación se desconoce el impacto que tiene este proceso sobre los siguientes aspectos: 1) si los niveles de hibridación entre *Q. glabrescens*, *Q. rugosa* y *Q. obtusata* son los mismos en los sitios de estudio, 2) si el impacto de la hibridación sobre los mecanismos de defensa química foliar (cualitativa y cuantitativa) es el mismo en los sitios de simpatría de los complejos *Q. glabrescens* × *Q. rugosa* y *Q. glabrescens* × *Q. obtusata*, 3) la influencia de la diversidad genética y el perfil metabólico del encino hospedero sobre la comunidad de cinípidos y parasitoides asociados y 4) el efecto del taxón hospedero (*Quercus rugosa*, *Q. glabrescens* e híbridos) sobre la estructura de la comunidad de cinípidos y parasitoides asociados.

OBJETIVO GENERAL

Evaluar el efecto de la hibridación, la diversidad genética y la defensa química del complejo *Quercus glabrescens* × *Q. rugosa* y *Q. glabrescens* × *Q. obtusata*, sobre la estructura de la comunidad de cinípidos y sus parasitoides asociados al dosel en la región centro de la Faja Volcánica Transmexicana.

Objetivos particulares:

1. Caracterizar los niveles de hibridación entre *Q. glabrescens*, *Q. rugosa* y *Q. obtusata* en cuatro zonas de sobrelapamiento.

2. Caracterizar la expresión química de las especies parentales (*Q. glabrescens*, *Q. rugosa* y *Q. obtusata*) y los individuos híbridos.

3. Caracterizar la estructura de la comunidad de cinípidos y sus parasitoides (en términos de abundancia, riqueza, diversidad y porcentaje de infestación) asociados al dosel de *Q. glabrescens*, *Q. rugosa* e híbridos.

4. Evaluar el efecto del genotipo de la planta hospedera (parentales e híbridos) sobre la estructura de la comunidad de cinípidos y sus parasitoides asociados al dosel.

5. Conocer la influencia de la diversidad genética y expresión (cualitativa y cuantitativa) de los metabolitos secundarios sobre la estructura de las comunidades de cinípidos y parasitoides asociados al dosel de *Q. glabrescens*, *Q. rugosa* e híbridos.

6. Evaluar si existen modificaciones (cualitativas y cuantitativas) en la configuración química de *Q. glabrescens*, *Q. rugosa* y *Q. obtusata* como resultado de sus diferencias genéticas.

7. Determinar si *Q. glabrescens*, *Q. rugosa* y *Q. obtusata* presentan más de un marcador químico específico dependiendo de la familia de metabolito secundario que se analice (flavonoides, terpenoides y/o cumarinas).

HIPÓTESIS

Si los hospederos genéticamente más diversos ofrecen una gama más amplia de recursos y condiciones para ser explotada por sus comunidades dependientes, se espera encontrar una relación positiva y significativa entre la diversidad genética de las poblaciones de *Quercus* y la diversidad de cinípidos asociados dado que estos últimos (Familia Cynipidae) son considerados especialistas de la especie hospedera, órgano y tejido que atacan.

Si la defensa química de las plantas ante la herbivoría está regulada por sus características genéticas, entonces se espera que las poblaciones más diversas genéticamente registren una mayor variedad de compuestos químicos.

Se espera que diferentes taxones de encinos (*Quercus rugosa*, *Q. glabrescens* e híbrido) presenten diferente estructura en la comunidad de cinípidos asociados dadas las diferencias genéticas que provocan cambios químicos y estructurales entre los taxones (variación interespecífica).

Sistema de estudio

Quercus glabrescens, *Q. obtusata* y *Q. rugosa* son tres especies dominantes de los bosques templados de México que, cuando se encuentran en alopatría pueden distinguirse fácilmente por sus caracteres morfológicos. Sin embargo, cuando estas especies se encuentran en simpatría muestran una morfología foliar atípica sugiriendo que parte de esa variación puede ser explicada por eventos de hibridación. A continuación, se describen algunos rasgos característicos de las especies parentales con base en las descripciones de Romero-Rangel et al. (2015)

Quercus glabrescens Benth. Es un árbol de 6 a 20 m de altura. Sus hojas tienen forma oblanceolada o elíptico-oblanceolada y un margen fuertemente revoluto, generalmente de dos a cuatro dientes de cada lado hacia la mitad apical de la hoja. Su haz es brillante y glabro, así como el envés, pero éste no es brillante y glabro en la nervadura. Sus amentos masculinos tienen 2 cm de largo en tanto que los femeninos contienen hasta tres flores de 1-1.8 cm de largo. Su fruto es solitario de 15 mm de largo.

Quercus obtusata Humb. & Bonpl. Es un árbol de 3 a 20 m de altura. Sus hojas tienen forma obovada, largamente obovada o elíptica y un margen engrosado y ligeramente revoluto regularmente presenta de tres a ocho dientes u ondulaciones de cada lado. Su haz es lustroso y tomentoso cerca de la base. Por su parte el envés es pubescente y con pelos glandulares. Presenta amentos masculinos de 3 cm de largo con muchas flores distribuidas a lo largo del raquis, los amentos femeninos de tres a seis o más flores distribuidas en la mitad distal de un pedúnculo de 2-3.5 cm de largo. Tiene frutos de uno a tres de 17 a 20 mm de largo c/u.

Quercus rugosa Née. Es un árbol de 3 a 25 m de altura. Tiene hojas con forma cóncava por el envés, obovadas o elíptico-obovadas y un margen engrosado generalmente con tres y hasta 17 dientes u ondulaciones hacia la mitad distal de la hoja. Su haz es lustroso y glabro el envés es tomentoso con pelos ramificados y pelos glandulares abundantes. Sus amentos masculinos tienen de 17 a 31 flores de 15-20 mm de largo pubescentes mientras que las flores femeninas se presentan de 2 a 20 en pedúnculos pubescentes. El fruto puede ser solitario o en grupos de 2 a 3, de 10 a 25 mm de largo c/u.

Tomando como base lo anterior, este trabajo de tesis se divide en tres capítulos, cada uno de los cuales aborda una línea de investigación como se describe a continuación:

Capítulo 1. HIBRIDACIÓN NATURAL ENTRE *Quercus glabrescens*, *Q. rugosa* y *Q. obtusata* (FAGACEAE): MARCADORES MICROSATÉLITES Y METABOLITOS SECUNDARIOS. Los objetivos de este estudio fueron; 1) caracterizar los niveles de hibridación entre *Q. glabrescens*, *Q. rugosa* y *Q. obtusata* empleando marcadores genéticos y 2) identificar la expresion química de las especies parentales (*Q. glabrescens*, *Q. rugosa* y *Q. obtusata*) y los individuos híbridos. Para alcanzar dichos objetivos, se utilizaron ocho primers de microsatélites (nSSR´s). En cada sitio alopátrido se colectaron 20 individuos por taxón reconocido morfológicamente. En cada sitio simpátrido, se colectaron 30 individuos que presentaban características morfológicas típicas de alguna de las tres especies parentales de manera aleatoria. Los resultados muestran que el porcentaje de hibridación varía entre sitios y entre combinaciones genéticas. Además, dentro de la caracterización química los taxones parentales mostraron la presencia de al menos un ms diagnóstico "exclusivo" mientras que los taxones híbridos no mostraron un ms, pero si mezclas de ms provenientes de los taxones parentales.

Capítulo 2. LA DIVERSIDAD GENÉTICA Y QUÍMICA DE LOS ROBLES BLANCOS AFECTA LA BIODIVERSIDAD DE LOS INSECTOS HERBÍVOROS DEL DOSEL Y LOS PARASITOIDES ASOCIADOS. Los objetivos de este capítulo fueron: 1) caracterizar la estructura de la comunidad de cinípidos y parasitoides asociados al dosel de tres taxones de encinos blancos, 2) evaluar el efecto del genotipo del taxón hospedero (parental e híbrido) sobre la estructura de la comunidad de cinípidos y parasitoides asociados al dosel y 3) conocer la influencia de la diversidad genética y expresión de los ms (cualitativa y cuantitativa) sobre la estructura de la comunidad de cinípidos y parasitoides asociados al dosel de tres taxones de encinos blancos. Para lograr los objetivos previamente mencionados se analizaron los mismos individuos del capítulo 1 con excepción de los individuos pertenecientes a *Q. obtusata* y aquellos que formaban parte del taxón híbrido formado por *Q. glabrescens* × *Q. obtusata* (debido a que el número de individuos híbrídos formados por dicho complejo, no fue suficiente para realizar las pruebas de ms y en consecuencia no se podría cumplir con los objetivos planeados en este capítulo). Los resultados muestran que la diversidad genética del taxón híbrido es mayor que la de los taxones parentales. Además, existen diferencias cualitativas y cuantitativas en la expresión de los ms entre taxones.

Finalmente, los resultados muestran que existe un efecto del taxón sobre la abundancia, riqueza y porcentaje de infestación sobre la comunidad de cinípidos y sus parasitoides asociados al dosel de *Q. rugosa*, *Q. glabrescens* y Qg × Qr.

Capítulo 3. Caracterización química de *Q. glabrescens*, *Q. obtusata* y *Q. rugosa* (Fagaceae: *Quercus*) dentro del Cinturón Volcánico Transmexicano. El objetivo de este capítulo fue: 1) caracterizar el perfil metabólico (flavonoides, terpenoides y cumarinas) de *Q. glabrescens*, *Q. rugosa* y *Q. obtusata*. Los resultados muestran que, algunos flavonoides son altamente específicos mientras que los terpenoides y las cumarinas se expresan de manera indiscriminada en los tres taxones de estudio.

CAPÍTULO I.

Hibridación natural, diversidad genética y expresión química en dos complejos de encinos blancos (*Q. glabrescens* × *Q. rugosa* y *Q. glabrescens* × *Q. obtusata*) en la región centro de la Faja Volcánica Transmexicana.

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RESEARCH PAPER

Natural hybridisation among *Quercus glabrescens*, *Q. rugosa* and *Q. obtusata* (*Fagaceae*): Microsatellites and secondary metabolites markers

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ABSTRACT

- Natural hybridisation has significant ecological, genetic and evolutionary consequences altering morphological and chemical characters of individuals. *Quercus glabrescens*, *Q. rugosa* and *Q. obtusata* are white oak species well separated by their morphological characters when they occur in allopatry in Mexican temperate forests. However, in sympatry, individuals with atypical morphology have been observed, suggesting hybridisation events.
- In this study, we determined, with microsatellites and secondary metabolites, if interspecific gene flow occurs when these three oak species coexist in sympatry. In total, 180 individuals belonging to seven populations [three allopatric (one for each parental species) and four sympatric sites] were analysed.
- Allopatric populations represent well-defined genetic groups and the sympatric populations showed genetic evidence of hybridisation between *Q. glabrescens* × *Q. rugosa* and *Q. glabrescens* × *Q. obtusata*. The hybridisation percentage varied between sites and combination of involved species. We registered the presence of unique flavonoid compounds for *Q. glabrescens* (caffeic acid and flavonol 2), *Q. rugosa* (flavonol 5) and *Q. obtusata* (flavonol 1). Three compounds (quercetin rhamnoside, flavonol 3 and alkyl coumarate) were expressed in all taxa. Finally, the hybrid genotypes identified in this study (*Q. glabrescens* × *Q. rugosa* and *Q. glabrescens* × *Q. obtusata*) showed specific chemical profiles, resulting from a combination of those of their parental species.
- These results show that hybridisation events between these oak species alter chemical expression of secondary metabolites, creating a mosaic of resources and conditions that provide the substrate for different combinations of foliar-associated species such as herbivores, endophytic fungi or epiphyte plants.

INTRODUCTION

Natural hybridisation is a frequent phenomenon in plants and has important genetic, ecological and evolutionary consequences (Soltis & Soltis 2009). Because this process involves the interspecific exchange of genetic material (Harrison 1990), hybridisation may promote the emergence of new chromosomal arrangements, yielding allelic and genotypic variants that favour genetic diversity of species (Rieseberg & Wendel 1993). Under this scenario, when fertile hybrid individuals cross with one or both parental species, they can generate a range of genetic combinations (*e.g.* backcrosses of various generations; Baack & Rieseberg 2007). Therefore, interspecific genetic flow can facilitate the transfer of genetic variants between species, contributing to the generation of new traits and to the segregation of transgressive traits (extreme), and can even facilitate adaptation and speciation events (Arnold 1992, 2004). The consequences of these genotypic and phenotypic changes promoted by interspecific genetic flow events in plant populations can create morphotypes that are able to invade new habitats or expand their distribution range (Rieseberg & Wendel 1993), with wider physiological and ecological tolerances and greater plasticity of the mating system (Rieseberg & Carney 1998) or with modified resistance patterns (Cheng *et al.* 2011). Moreover, the consequences of hybridisation in plants can escalate to other levels of ecological organisation, for example, may impact the diversity of different herbivore communities associated with hybridising populations or alter some process at the ecosystem level (Whitham *et al.* 2012).

Within sympatric zones, morphological characters may not be sufficient for the correct identification of all hybrid categories (*e.g.* F_1, backcrosses), because the individuals that are

generated between different species do not necessarily show intermediate morphology (Naisbit *et al.* 2003). Rieseberg & Ellstrand (1993) documented that the expression of morphological characters in F_1 hybrid individuals include intermediate, similar or transgressive values with respect to their parental species. These data illustrate the wide range of morphological characteristics that can be expressed by hybrid individuals and the inherent limitations of morphological identification of hybridisation (Mallet 2005).

Genetic markers have been recognised as the most robust tool available for describing and characterising the genetic structure of hybrid populations. Desired characters for this purpose are: (i) they are selectively neutral, (ii) their inheritance is strictly under Mendelian segregation ratios, (iii) usually, they show independence, and (iv) they are present in a large number within the genome (Rieseberg & Wendel 1993). Simple sequence repeats (SSRs), also known as microsatellites, have been the most commonly used genetic markers to diagnose hybridisation and introgression due to its codominant inheritance and its high sensitivity in detecting variation (Senan *et al.* 2014). Another advantage of SSRs is their rapid mutation rate, which promotes differentiation between closely related taxa and, consequently, differences in allelic frequencies and the appearance of species-specific variants, both of which are very useful traits for the identification of different genetic classes (pure and hybrid) and for the characterisation of the genetic structure of hybrid zones (Harrison & Larson 2014).

In addition, several studies have suggested the differential expression of secondary metabolites as a tool for identification of hybrid individuals and putative parental species (Nahrung *et al.* 2009; Savarese *et al.* 2009). Plant secondary metabolites are chemical compounds that play an important role in defence against herbivores (Wimp *et al.* 2007). In general, it has been shown that hybridisation creates qualitative and quantitative variation in secondary chemicals (Rieseberg & Ellstrand 1993; Staudt *et al.* 2004; Welter *et al.* 2012), promoting the occurrence of the following expression patterns in hybrid plants: (i) a combination of parental species metabolites, (ii) lack of parental metabolites and (iii) new metabolites not present in the parental species (Cheng *et al.* 2011). Although, the secondary chemistry should be used only as complementary tool of other markers [*e.g.* ADN markers (Kirk *et al.* 2004)]. Some secondary metabolites studied in plant species involved in hybridisation events are: alkaloid, flavonoid, phenolic and terpenoid compounds (López-Caamal & Tovar-Sánchez 2014). In particular, the flavonoids have been the most studied compounds due their high heritability and specificity (Caseys *et al.* 2015).

Temperate tree species are especially susceptible to hybridisation events because of the life history characteristics they present (*e.g.* long life cycle, wind pollination, cross-breeding and perennial habit; Petit & Hampe 2006). For example, hybridisation events among *Quercus* species (oaks: *Fagaceae*) have been widely documented (Peñaloza-Ramírez *et al.* 2010; Wei *et al.* 2015) and have been shown to have strong consequences in morphological and genetic variation of recombinant individuals (Tovar-Sánchez & Oyama 2004; Valencia-Cuevas *et al.* 2015). In fact, the presence of individuals with atypical morphologies has frequently increased the taxonomic confusion that characterises *Quercus* species (Fortini *et al.* 2015).

Despite the high frequency of hybridisation reported among *Quercus* species, the species involved in these events maintain their morphological, ecological and genetic differences, suggesting that they continue to preserve their integrity as individual species (Gailing & Curtu 2014). However, environmental variation based on the geographic distribution of species can promote differences in the strength of their reproductive barriers (Curtu *et al.* 2007; Jensen *et al.* 2009) and, consequently, differences in the occurrence and frequency of hybridisation events (Buerkle 2009). In this context, the following local factors have been considered important: (i) the spatial distribution of the species within sympatric zones (Curtu *et al.* 2015), (ii) the habitat conditions (Whitney *et al.* 2010); (iii) the proportion of conspecific pollen and the density of available males (Lagache *et al.* 2013); (iv) the differences in pollen dispersal capacity among species (Harrison & Larson 2014); and (v) the climate (Ortego *et al.* 2014).

Although little is known about the dynamics of genetic flow in multi-species oak complexes, evidence of hybridisation between almost all species of the same section that occur in sympatry has been reported (*e.g.* Dodd & Afzal-Rafii 2004; Peñaloza-Ramírez *et al.* 2010; Eaton *et al.* 2015). However, the percentage of hybridisation varies among the pairs of species and sites analysed (see Curtu *et al.* 2007; Lepais & Gerber 2011; Valencia-Cuevas *et al.* 2015).

Quercus glabrescens Benth., *Q. rugosa* Née and *Q. obtusata* Humb. & Bonpl. are three species of white oak (section *Quercus*) with close phylogenetic relationships (Hipp *et al.* 2017) which possess wide geographic distributions in the temperate forests of Mexico. These species can be easily differentiated when they are allopatric based on their foliar morphological characters (Romero-Rangel *et al.* 2015). However, when species convergence is sympatric, individuals with atypical morphology have been observed, suggesting that variation may be promoted by hybridisation events. The present study aims to: (i) determine the hybridisation levels in areas of sympatry among *Q. glabrescens*, *Q. rugosa* and *Q. obtusata* to (ii) characterise the secondary metabolites chemical profile of pure genotypes of *Q. glabrescens*, *Q. rugosa* and *Q. obtusata* and determine the influence of interspecific hybridisation events on the chemical profile.

MATERIAL AND METHODS

Study species

Quercus glabrescens is a species that can be recognised easily in the field by its oblanceolate or elliptic-oblanceolate leaves with strongly curled margins, usually with two to four teeth on each side towards the apical half of the leaf. The leaf's adaxial surface is bright and glabrous; the abaxial surface is glabrous or sparingly pubescent in the rib but not bright, and glandular hairs are absent. For its part, *Q. obtusata* can be recognised by its obovate, long obovate or elliptical leaves with a thickened margin and slightly curled, regularly presenting three to eight teeth or undulations on each side. The leaf's adaxial surface is lustrous and tomentose near the base; the abaxial surface is pubescent with glandular hairs. Finally, *Q. rugosa* is a species that can be recognised in the field because the abaxial surfaces of its leaves are concave, obovate or elliptic-obovate with thickened margins, generally with three to 17 teeth or undulations towards the distal half of the leaf. The leaf's adaxial surface is lustrous and glabrous, whereas the abaxial surface is tomentose

with branched hairs and abundant glandular hairs. An overlap in flowering time and altitudinal distribution between these white oak species have been reported in central Mexico (Romero-Rangel *et al.* 2015).

Population sampling

Seven locations in central Mexico were studied (Fig. 1). To minimise the environmental factors that can modify the genetic flow patterns and expression of the metabolic profile of the study taxa, seven localities with common characteristics were chosen: geological history [all localities belong to the Transmexican Volcanic Belt (TVB), whose formation process began during the Quaternary–Pliocene (Gómez-Tuena *et al.* 2007)], climate (temperate sub-humid), type of vegetation (mature oak), type of soil (volcanic origin or derived from igneous and sedimentary rocks), and these areas have no local disturbance. Oak populations in these locations are dominated by the species *Q. glabrescens*, *Q. rugosa* and *Q. obtusata* (Table 1). In each allopatric site, 20 adult individuals of each species were randomly sampled along a transect of approximately 1,000 m (n = 60). At 50-m intervals, the nearest individual of each species was sampled. In each sympatric site of *Q. glabrescens*, *Q rugosa* and *Q. obtusata*, 30 individuals whose leaf morphologies presented characteristics of one of the species or that possessed leaf characteristics atypical for the species were randomly selected (n = 120). This selection was performed by sampling the nearest individual along a transect of approximately 1,500 m at intervals of 50 m (Fig. 1, Table 1). The dominance of each parental species in the forest canopy (allopatric and sympatric zones) was estimated using a modification of Braun-Blanquet cover-abundance scale (1979; see Table 1).

Genetic analysis

Young leaves with no apparent damage were collected from 180 individuals. The leaf tissue was transported to the laboratory in a container with liquid nitrogen. Total DNA was extracted by means of a DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). The DNA concentration of each sample was obtained by spectrophotometric analysis (Biophotometer; Eppendorf, Hamburg, Germany), and the quality of the DNA was determined by electrophoresis on 0.8% agarose gels. Finally, all DNA samples were diluted to a concentration of 15 ng μl^{-1}.

The genetic analyses were performed with eight nSSR primers: ssrQpZAG110 (Steinkellner *et al.* 1997), ssrQpZAG11, ssrQrZag56 (Kampfer *et al.* 1998), Quru-GA-0A01, Quru-GA-0E09, Quru-GA-0C11, Quru-GA-1C08 and Quru-GA-1F07 (Aldrich *et al.* 2002). The use of these primers revealed polymorphisms among the individuals of *Q. glabrescens*, *Q. rugosa* and *Q. obtusata*. For each sample, the amplification mixture contained 20 mM Tris-HCl (pH 8.4), 50 mM KCl, 2 mM $MgCl_2$, 0.13 mM of each dNTP, 25 μM primer, 15 ng of genomic DNA and 0.8 U Taq polymerase in a final volume of 15 μl. The polymerase chain reaction (PCR) was performed on a Mastercycler (Eppendorf) thermocycler as follows: initial denaturation at 95 °C for 5 min; 30 cycles of 94 °C for 1 min, 1 min at the appropriate hybridisation temperature, and 30 s at 72 °C; and a final extension for 8 min at 72 °C.

The annealing temperatures for the nSSR primers were 58 °C for Quru-GA-1F07, 53 °C for ssrQpZAG110, ssrQpZAG11 and Quru-GA-0C11, 50 °C for Quru-GA-0A01 and Quru-GA-0E09, 48 °C for Quru-GA-1C08 and 46 °C for ssrQrZag46. The PCR products were visualised after electrophoresis on 4% agarose gels at 60 W for 1.5 h. Depending on the intensity of the bands obtained after agarose gel electrophoresis, the samples were diluted 1:10-1:90 in deionised water. The polymorphic fragments of the nSSRs obtained were measured on an ABI PRISM 3100 automated sequencer (Applied Biosystems, Foster City, CA, USA) using 9.5 μl formamide at 35 W for 80–90 min with 0.5 μl ROX-2500 as the standard size marker. The size of the fragments was recorded using Gene Mapper version 3.7 (Applied Biosystems, Foster City, CA, USA).

Assignment of hybrid and parental categories

Assignment of the genetic identity of morphologically recognised allopatric populations as distinct species, as well as determination of the proportion of ancestry of the individuals identified as *Q. glabrescens*, *Q. rugosa* and *Q. obtusata* within sympatric populations, was performed using the program STRUCTURE 2.3 (Pritchard *et al.* 2000) and the data obtained with eight nSSRs. This program uses Bayesian grouping to infer the structure of populations from genotypic data, and individuals are probabilistically assigned to *K* populations (genetic

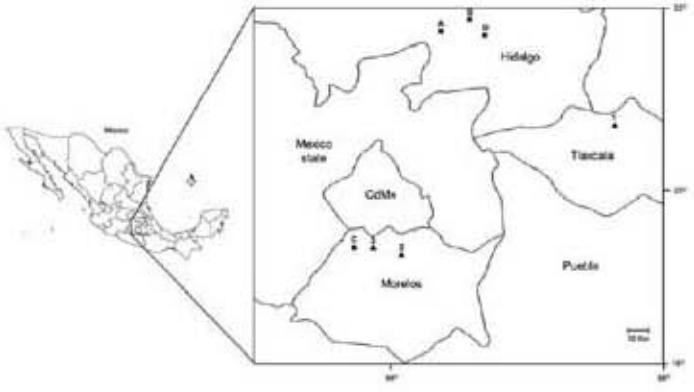


Fig. 1. Sampling populations, allopatric populations of white oak species (triangle): *Quercus glabrescens* (1), *Q. obtusata* (2), *Q. rugosa* (3). Sympatric populations of *Quercus glabrescens*, *Q. obtusata* and *Q. rugosa* (square): A = Mineral El Chico; B = Cardonal; C = Huitzilac; D = Omitlán de Juárez. See Table 1.

Table 1. Locality name, sample size (N), altitude (m) and white oak species by allopatric and sympatric sites in populations from the Transmexican Volcanic Belt. The numbers in parentheses represent the value of canopy cover of each oak species in allopatric and sympatric zones.

location	N	state	altitude (m)	species
Allopatric stand				
Tlaxco	20	Tlaxcala	2,588	*Q. glabrescens* (5)
Chamilpa	20	Morelos	1,655	*Q. obtusata* (4)
Coajomulco	20	Morelos	2,667	*Q. rugosa* (5)
Sympatric stand				
Mineral El Chico	30	Hidalgo	2,580	*Q. glabrescens* (5), *Q. obtusata* (3), *Q. rugosa* (3)
Cardonal	30	Hidalgo	2,898	*Q. glabrescens* (4), *Q. obtusata* (3), *Q. rugosa* (2)
Huitzilac	30	Morelos	2,318	*Q. glabrescens* (4), *Q. obtusata* (2), *Q. rugosa* (2)
Omitlán de Juárez	30	Hidalgo	2,522	*Q. glabrescens* (3), *Q. obtusata* (3), *Q. rugosa* (3)

Note. Canopy cover scale: 5 = Dominant (> 75%); 4 = Abundant (50–75%); 3 = Frequent (25–50%); 2 = Occasional (5–25%) and, 1 = Rare (< 5%), modified from Braun-Blanquet (1979).

clusters) based on their multilocus genotypes (Pritchard *et al.* 2000; Hubisz *et al.* 2009). For each individual, an admixture coefficient (Q) is calculated, and represents the proportion of an individual's genotype that originates from a given population. To determine the optimal number of genetic groups (K), the STRUCTURE program was run at K values ranging from one to ten (with ten runs for each value) to determine the K value with the highest posterior probability. The ΔK statistic was also used to evaluate the change in the probability value according to Evanno *et al.* (2005). For all runs, a burn-in period of 50,000 repetitions was used, followed by 100,000 iterations of the Markov Chain Monte Carlo (MCMC), which was confirmed to be satisfactory for the parameters to reach convergence. We employ the admixture model with correlated allele frequencies and the LOCPRIOR option off.

Since $K = 3$ was estimated in the aforementioned analysis, all individuals of *Q. glabrescens*, *Q. rugosa* and *Q. obtusata* were assigned to either one or another cluster. Subsequently, we evaluated the capacity of the microsatellite dataset to differentiate between multiple hybridisation events. The program Hybridlab (Nielsen *et al.* 2006) simulates crosses among populations by calculating the allele frequencies and by randomly drawing one allele at each locus of each parental population defined. We used all individuals from pure stands of *Q. glabrescens*, *Q. rugosa* and *Q. obtusata* to create 50 simulated hybrid genotypes from each of the next categories: (i) first generation hybrids (F_1), (ii) backcrosses (BC) of F_1 with *Q. glabrescens*, (iii) BC of F_1 with *Q. rugosa*, and (iv) BC of F_1 with *Q. obtusata*. Ten replicates were made with $K = 3$ with a burn-in period of 50,000 and 100,000 MCMC. The results of all five runs were merged in CLUMPP and the mean Q values were obtained for each hybrid category. F_1 hybrids are expected to have a Q value of 0.5, while backcrosses are expected to have Q values of 0.75 or 0.25. Finally, each individual was classified based on the model proposed by Vähä & Primmer (2006) and Lepais *et al.* (2009), in which all individuals with an assignment coefficient $Q = 0.9$ are considered pure individuals of *Q. glabrescens*, *Q. rugosa* or *Q. obtusata*. The results obtained with the STRUCTURE program were edited for processing in the DISTRUCT program (Rosenberg 2004).

Based on this analysis, we intended to assign individuals in the real dataset as hybrids or pure parental individuals. Then, we used these labels to study the chemical profile of all individuals in pure and mixed stands.

Preparation of extracts from *Quercus* species

Grouping individuals by site for the secondary metabolite analysis was done to control for potential differences in quantitative and qualitative metabolite expression associated with local environment differences among genotypes (Henriksson *et al.* 2003). To identify the most abundant compounds, leaves obtained from individuals of each genotype (pure and hybrid) per site were dried at room temperature and crushed to obtain 300 g of fine powder. The dried and ground material was extracted with acetone (1.5 l sample^{-1}) by maceration for 3 days and three times. The solvent was eliminated under reduced pressured distillation with a BUCHI R-114 rotary evaporator. The dried extracts were put together according to their chemical similarity per site and compared by thin-layer chromatography (TLC). Silica gel 60 and chromatographic plates from Merck KGaA (Darmstadt, Germany) were used.

In total, three pure genotypes (*Q. glabrescens*, *Q. rugosa*, *Q. obtusata*) and two hybrid genotypes (*Q. glabrescens* × *Q. obtusata* from the sympatric zone of Mineral El Chico and Huitzilac, and *Q. glabrescens* × *Q. rugosa* from the sympatric zone of Cardonal and Omitlán de Juarez) were chemically analysed. In addition, in the chemical analysis, the tri-hybrid genotype (*Q. glabrescens* × *Q. rugosa* × *Q. obtusata*) from the locality of Omitlán de Juarez was exduded because the individuals had few leaves, making it impossible to obtain 300 g of mature leaves. Finally, in this study we did not detect *Q. rugosa* × *Q. obtusata* hybrid genotypes.

Chromatographic analysis

The organic extracts for each genotype were analysed using traditional chromatographic methods such as TLC with NP-PEG reagents (2-aminoethyl diphenylborinate, for the detection of flavonoids) and the Komarovsky reaction (4-hydroxy-benzaldehyde for the detection of terpenes) reported as specific chemicals revealers from these compound families (Wagner *et al.* 1996). Flavonoid and terpenes compounds were clearly distinguished by TLC (Silica gel, 70–230 mesh). Extracts of these compounds (1 g) were subjected to column chromatography (Silica Gel 60, mesh 70–230, Merck) eluting with hexane, and mixtures of increasing polarity of hexane + acetone. All fractions were analysed through TLC. The presence of flavonoids and terpenes was confirmed using commercial standards (*e.g.* rutin, naringenin, quercetin; Sigma-Aldrich, Bellefonte, PA, USA).

Analysis with HPLC

High-pressure liquid chromatography (HPLC) analysis was performed on an HPLC system consisting of an Alliance 2695

(Waters) separation module equipped with a Waters 2695 photodiode array detector and Empower Pro software (Waters, Milford, MA, USA). Chemical separation was achieved using a Supelcosil LC-F column (4.6 mm, 250 mm diameter, 5 µm particle size; Sigma-Aldrich). The mobile phase consisted of a mixture of 0.5% trifluoroacetic acid (Solvent A) and acetonitrile (solvent B). The gradient system employed was as follows: 0–1 min, 0% B; 2–4 min, 10% B, 5–7 min, 20% B; 8–14 min, 30% B; 15–18 min, 40% B; 19–22 min, 80% B; 23–26 min, 100% B; 27–28 min, 0% B. The flow was maintained at 0.9 ml min^{-1}, and the sample injection volume was 10 µl. HPLC analysis was used for elucidate the identity of the pure compounds obtained.

RESULTS

Assignment of hybrid and parental categories

The Bayesian clusters obtained using the STRUCTURE program for allopatric and sympatric sites show three well-delimited genetic groups, in agreement with the three pure phenotypic species previously recognised (*Q. glabrescens*, *Q. rugosa* and *Q. obtusata*). These results were confirmed by the ΔK values, which indicate that $K = 3$ is the most likely number of genetic groups (Fig. 2). Likewise, analyses using the STRUCTURE program revealed a high proportion of ancestry ($Q > 0.9$) for individuals from the allopatric reference populations (*Q. glabrescens* $Q = 0.984 \pm 0.02$ (mean ± SD), *Q. rugosa* $Q = 0.969 \pm 0.03$ and *Q. obtusata* $Q = 0.975 \pm 0.02$). Several authors have documented that the appropriate number of nSSR for the adequate detection of the number of genetic groups depends on their exclusion power, efficiency and accuracy (*e.g.* Evanno *et al.* 2005; Burgarella *et al.* 2009; Gailing & Curtu 2014; Soto *et al.* 2018). Following Burgarella *et al.* (2009), we checked the suitability of our set of markers to assign virtual individuals to specific classes using Structure, and obtained the following values: $Q = 0.48 \pm 0.02$ between *Q. glabrescens* × *Q. obtusata* and $Q = 0.49 \pm 0.02$ between *Q. glabrescens* × *Q. rugosa*. On the other hand, hybrid genotypes between *Q. glabrescens* × *Q. obtusata* showed the next backcrosses: BC towards *Q. glabrescens* showed $Q = 0.28 \pm 0.02$, BC towards *Q. obtusata* $Q = 0.74 \pm 0.02$. Hybrid genotypes between *Q. glabrescens* × *Q. rugosa* showed the next backcrosses: BC towards *Q. glabrescens* showed $Q = 0.26 \pm 0.01$, BC towards *Q. rugosa* $Q = 0.73 \pm 0.02$. Due to the overlap between the Q values of the simulated hybrid categories, the microsatellite dataset was not able to fully discriminate between hybrid categories (F_1 hybrids from later introgressive forms; Fig. 3). Thus, assignment of individuals with Q values between 0.26 and 0.73 is equivocal, either repre-

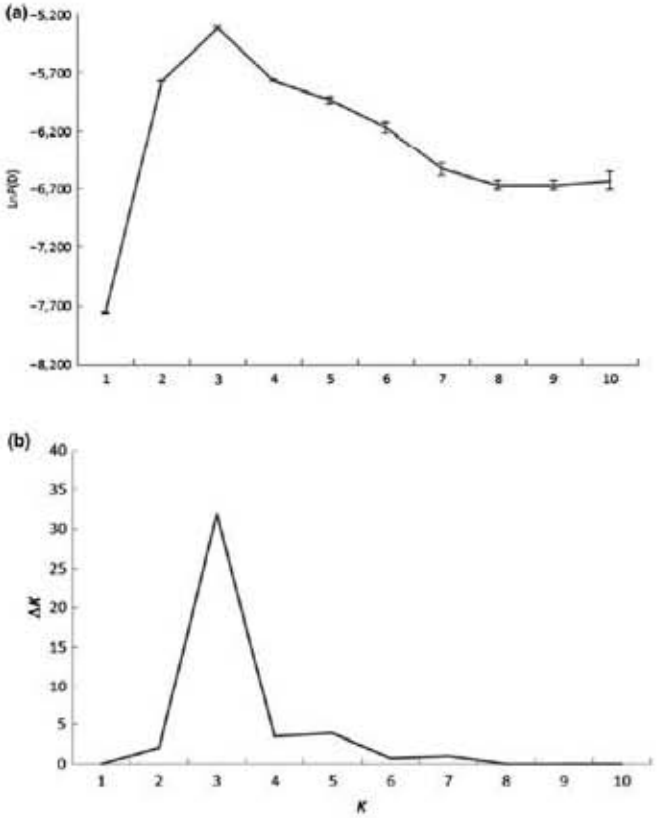
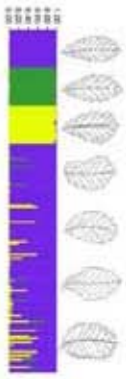


Fig. 2. Estimated genetic groups (K) by cluster analysis in the STRUCTURE program: (a) mean and SD of lnP (D) of ten independent runs of STRUCTURE, and (b) graph of the statistic ΔK with respect to genetic grouping K (from 1 to 10). In both cases, the peak indicates the most likely number of genetic groups.



Location	Morphological group	Genetic assignment		
		"*Q. glabrescens*"	"*Q. obtusata*"	"*Q. rugosa*"
Tlaxco	*Q. glabrescens*	0.984	0.004	0.005
Chamilpa	*Q. obtusata*	0.006	0.975	0.007
Coajomulco	*Q. rugosa*	0.009	0.006	0.969
Mineral El Chico	Sympatric	0.940	0.048	0.005
Cardonal	Sympatric	0.888	0.007	0.098
Huitzilac	Sympatric	0.910	0.034	0.040
Omitlán de Juárez	Sympatric	0.800	0.034	0.170

Fig. 3. Genetic assignment of three allopatric populations representing the pure species of *Quercus glabrescens*, *Q. obtusata* and *Q. rugosa*, as well as four sympatric populations, based on analysis of eight nuclear microsatellites (nSSRs) with the Bayesian method implemented in the STRUCTURE program. Each horizontal coloured line represents the individual probability of belonging to the group of that colour and each population is separated by a black vertical line. The ancestry ratio values for each population are given in the table.

senting F_1 or BC individuals. Therefore, we group F_1 hybrids and backcrosses together. Nevertheless, the set of markers is still useful to discriminate between 'pure' and hybrid individuals.

On the other hand, genetic analyses of 120 individuals randomly sampled in four sympatric zones revealed the presence of 25 individuals (20.8% of the total of individuals analysed) with indications of mixed ancestry (Fig. 3).

Frequency of hybridisation between sites and combinations between species

The results of Bayesian analysis show that the number of pure and hybrid genotypes varies among the sympatric zones, with a hybridisation percentage of 10% in Mineral El Chico, 20% in Cardonal, 14% in Huitzilac and 40% in Omitlán de Juárez. In general, the four sympatric zones show a dominance of pure *Q. glabrescens* genotypes and an absence of pure *Q. rugosa* and *Q. obtusata* genotypes (Fig. 3).

The Bayesian analysis of the samples in the sympatric zone Mineral El Chico identified 10% of the individuals (n = 3) as hybrid genotypes between *Q. glabrescens* × *Q. obtusata*. In Cardonal, 20% of individuals (n = 6) were identified as hybrid genotypes between *Q. glabrescens* × *Q. rugosa*. In Huitzilac, 7% of the individuals (n = 2) were identified as hybrid genotypes between *Q. glabrescens* × *Q. obtusata*, and 7% (n = 2) were identified as hybrid genotypes between *Q. glabrescens* × *Q. rugosa*. Also, in Omitlán de Juárez, 20% of individuals (n = 6) were identified as hybrid genotypes between *Q. glabrescens* × *Q. rugosa*, 10% (n = 3) were identified as hybrid genotypes between *Q. glabrescens* × *Q. obtusata*, and 10% (n = 3) were of genotypes identified as 'tri-hybrids' between *Q. glabrescens* × *Q. rugosa* × *Q. obtusata* (Figs 3 and 4). Finally, we did not find *Q. obtusata* × *Q. rugosa* hybrid genotypes in sympatric zones.

Secondary metabolite analysis

According to STRUCTURE software (Fig. 3), leaves of five individuals per genotype (*Q. rugosa*, *Q. obtusata*, *Q. glabrescens*) were randomly selected in each allopatric site (Table 1). In contrast, the power of the analysis to distinguish F_1 hybrids from later introgressive forms was limited. Therefore, we grouped F_1 hybrids and backcrosses together (hybrid genotypes). Hence, leaves of the hybrid genotypes in the sympatric sites were analysed. The number of hybrid individuals analysed by genetic combination varied as follows: *Q. glabrescens* × *Q. rugosa* [Cardonal (n = 6), Omitlán (n = 6)], *Q. glabrescens* × *Q. obtusata* [(Mineral El Chico (n = 3), Huitzilac (n = 2)] (Fig. 3).

Thin-layer chromatography analysis showed that there were differences in the expression of secondary metabolites among the different populations (Table 2). In addition, TLC showed that most of the compounds expressed in oak leaves were flavonoids (Fig. 5); thus, it was possible to confirm these differences by HPLC. Moreover, the differences in the number and type of compounds present at each study site could be determined.

Fingerprinting with HPLC of *Quercus* spp.

The chromatograms obtained from the acetone extracts of *Quercus* spp. were compared [Fig. 5: 1. Coajomulco (*Q. rugosa*); 2. Chamilpa (*Q. obtusata*); 3. Tlaxco (*Q. glabrescens*); 4. Mineral El Chico (*Q. glabrescens* × *Q. obtusata*), 5. Cardonal (*Q. glabrescens* × *Q. rugosa*), 6. Huitzilac (*Q. glabrescens* × *Q. obtusata*) and 7. Omitlán de Juárez (*Q. glabrescens* × *Q. rugosa*)]. The chromatograms showed the major chemical compounds present. Each compound had a unique retention time and UV spectrum. The distribution of phenolic compounds, the types of compounds present in these extracts and their retention times are shown in Table 2.

High-pressure liquid chromatography analysis of the acetone extracts of pure and hybrid *Quercus* genotypes at 312 nm demonstrated the presence of ten polyphenolic metabolites, including rutin, caffeic acid, quercetin glucoside and quercetin rhamnoside. These were identified by direct comparison of their retention times and UV spectra with those of commercial standards (Sigma-Aldrich).

Chemical characterisation within the study sites

In particular, flavonol 5 was considered a species-specific compound of *Q. rugosa* because it was found only in the allopatric

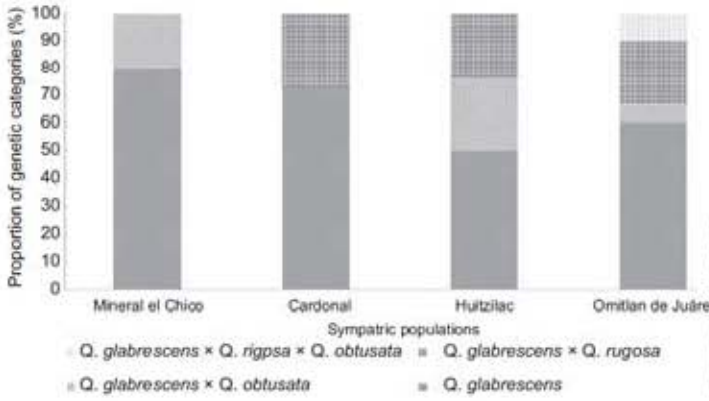


Fig. 4. Percentage of the different genetic category observed in each sympatric *Quercus glabrescens*, *Q. rugosa* and *Q. obtusata* population. Individuals were assigned to each category (*Q. glabrescens* pure genotype, *Q. glabrescens* × *Q. rugosa* hybrid genotype, *Q. glabrescens* × *Q. obtusata* hybrid genotype), depending on their individual coefficient of admixture derived from STRUCTURE.

Table 2. Phenolic compounds present in three allopatric populations (*Quercus glabrescens*, *Q. rugosa* and *Q. obtusata*) and four sympatric populations among *Quercus glabrescens*, *Q. rugosa* and *Q. obtusata* by HPLC at 312 nm. Rt = retention time. Qg × Qo = *Q. glabrescens* × *Q. obtusata*, Qg × Qr= *Q. glabrescens* × *Q. rugosa*.

locality	Coajomulco		Chamilpa		Tlaxco		Mineral El Chico		Cardonal		Huitzilac		Omitlán de Juárez	
genetic assignment	*Q. rugosa*		*Q. obtusata*		*Q. glabrescens*		Qg × Qo		Qg × Qr		Qg × Qo		Qg × Qr	
Phenolic compound (Pc)	Rt	Pc	Rt	Pc	Rt	Pc	Rt	Pc	Rt	Pc	Rt	Pc	Rt	Pc
① flavonol 1			8.774	①										
② rutin	9.152	②	9.105	②			9.137	②	9.136	②				
③ caffeic acid					9.320	③			9.296	③			9.307	③
④ quercetin glucoside	9.631	④			9.662	④	9.502	④	9.509	④	9.624	④	9.632	④
⑤ quercetin rhamnoside	10.035	⑤	9.997	⑤	9.957	⑤	10.017	⑤	10.007	⑤	10.045	⑤	10.034	⑤
⑥ flavonol 2					10.310	⑥								
⑦ flavonol 3	10.583	⑦	10.529	⑦	10.586	⑦	10.524	⑦	10.554	⑦	10.569	⑦	10.526	⑦
⑧ flavonol 4			12.090	⑧	12.070	⑧							12.017	⑧
⑨ flavonol 5	15.035	⑨												
⑩ alkyl coumarate	28.015	⑩	27.990	⑩	28.027	⑩	27.999	⑩	27.982	⑩	28.833	⑩	28.061	⑩

Specie-specific markers for *Q. rugosa* –⑨, *Q. obtusata* –①, and *Q. glabrescens* –③, ⑥.

population. Similarly, flavonol 1 was considered a species-specific compound of *Q. obtusata*, and caffeic acid and flavonol 2 were considered species-specific compounds of *Q. glabrescens*. In contrast, quercetin rhamnoside, flavonol 3 and alkyl coumarate were expressed in both pure genotypes and hybrids in all study populations; for this reason, these compounds were considered as genus-specific.

Quercetin glucoside was expressed in both pure genotypes and hybrids in all study populations except for *Q. obtusata* (Chamilpa). Rutin compound was detected in *Q. rugosa*, *Q. obtusata*, *Q. glabrescens* × *Q. obtusata* (Mineral El Chico) and *Q. glabrescens* × *Q. rugosa* (Cardonal) genotype. In *Q. glabrescens* × *Q. obtusata* (Mineral El Chico), the presence of quercetin glucoside, which had already been found within the allopatric zones of *Q. rugosa* and *Q. glabrescens*, and rutin, which had already been found within the allopatric zones of *Q. rugosa* and *Q. obtusata*, was documented. Caffeic acid was expressed in *Q. glabrescens* and *Q. glabrescens* × *Q. rugosa* (Omitlán de Juárez and Cardonal). Finally, flavonol 4 was expressed in *Q. obtusata*, *Q. glabrescens* and *Q. glabrescens* × *Q. rugosa* (Omitlán de Juárez) genotype. Comparison of the retention times and UV spectra associated with the chromatograms obtained through the analysis of phenolic compounds by HPLC did not indicate the presence of any compound that was expressed in a novel and/or exclusive manner in any of the four sympatric populations.

DISCUSSION

Genetic assignment of white oak species at allopatric sites

The field determination from the foliar-morphological characters of *Q. glabrescens*, *Q. rugosa* and *Q. obtusata* is supported by microsatellite analyses, which identify three well-defined genetic groups in allopatric conditions. This result indicates that these populations are useful as reference populations because they maintain their own genetic identities (Templeton 1989). Several authors have documented that the appropriate number of nSSR for the adequate detection of the number of genetic groups and for hybrid identification depends on their exclusion power, efficiency and accuracy (*e.g.* Burgarella *et al.* 2009; Soto *et al.* 2018). For this purpose, we evaluate the

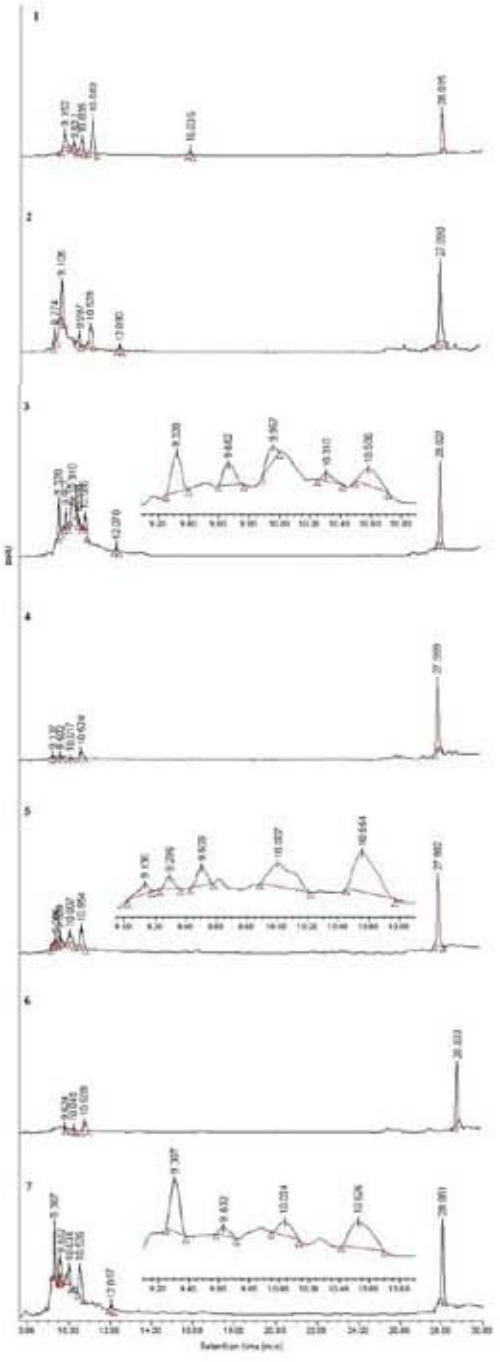


Fig. 5. Phenolic compounds in three allopatric (Coajomulco, Chamilpa and Tlaxco) and four sympatric (Mineral El Chico, Cardonal, O. Juárez and Huitzilac) oak populations by HPLC.

capacity of the microsatellite set to differentiate between pure and hybrid virtual genotypes created with HybridLab. According to these results, we followed the Vähä & Primmer (2006) criterion, using a threshold of $Q \geq 0.9$ to classify individuals as purebred, consistent with other studies (*e.g.* Valencia-Cuevas *et al.* 2015; Ortego *et al.* 2017a). The other individuals were classified as hybrids, since the marker set does not allow further discrimination.

We found evidence of a non-significative proportion ($Q < 0.1$; Vähä & Primmer 2006) of foreign genome in some individuals inside allopatric populations. One possible explanation is that with wind-pollinated species, such as oak trees, genetic flow can occur through tens of kilometres (Dodd & Afzal-Rafii 2004). These results agree with paternity-based studies on oaks showing that although pollen dispersal quickly decays with distance from paternal trees (*e.g.* Pluess *et al.* 2009), sporadic long-distance pollination events can still have some impact on the genetic structure and diversity of distant populations (*e.g.* Ortego *et al.* 2017a).

Genetic evidence of hybridisation between *Quercus glabrescens*, *Q. rugosa* and *Q. obtusata*

The hybridisation detected with the genetic markers used in this study supports the hypothesis that the atypical foliar morphology observed in the three species analysed can be attributed, in part, to interspecific genetic flow. As in other studies, atypical foliar morphology at sites of contact between oak species represents the first indication of hybridisation events; for this reason, it is considered a useful tool in the field (Peñaloza-Ramírez *et al.* 2010). In this sense, Hauser *et al.* (2017) documented introgressive hybridisation between *Q. parvula* var. shrevei and *Q. wislizeni* in California, which had a strong impact on phenotype (Goulet *et al.* 2017). In general, the morphometric analyses showed that genetically mixed plants exhibit atypical morphology in some characters (*e.g.* transgressive segregation in leaf size), giving phenotypic evidence of hybridisation events between these species. Nevertheless, in some cases morphological characters alone do not confirm unequivocally the existence of hybridisation (Mayol & Rossello 2001). For example, Craft *et al.* (2002) found that of the four trees with the highest probability of hybrid ancestry between *Q. douglasii* and *Q. lobata*, only one was identified as intermediate in appearance. The authors concluded that apparently intermediate phenotypes between these two species are not necessarily hybrids and that true hybrids are not necessarily intermediate in phenotype. Thus, the use of genetic markers which are selectively neutral, combined with other tools to identify hybridisation events is essential to increase the reliability of determining interspecific genetic flow among oak species (Gailing & Curtu 2014).

Genetic analyses of 120 individuals established at sites of sympatry between *Quercus glabrescens*, *Q. rugosa* and *Q. obtusata* revealed a 20.8% hybridisation rate. In a previous study, Sánchez-Ortiz (2012) reported the existence of a natural gradient of genetic diversity of *Q. glabrescens* through a species richness gradient of associated white oak species, suggesting that hybridisation was the responsible factor. Our results support the previous hypothesis because they show the presence of hybrid individuals resulting from the crossing of *Q. glabrescens*, *Q. rugosa* and *Q. obtusata* in sympatric sites. A similar pattern

was recently reported for a complex of red oaks in central Mexico in the TVB (Valencia-Cuevas *et al.* 2015), using *Q. castanea* as a focal species.

Frequency of hybridisation between sympatric sites

The variation in the hybridisation percentages of *Q. glabrescens*, *Q. rugosa* and *Q. obtusata* between sympatry sites ranged from 7% to 23%. These results coincide with values reported for oaks in other hybrid systems. For example, in a complex of four white oak species in Europe (*Q. robur*, *Q. petraea*, *Q. pyrenaica*, *Q. pubescens*) Lepais *et al.* (2009) and Lepais & Gerber (2011) reported percentages of hybridisation between 10.7% and 30.5%, and 2.7% to 34.5%, respectively, at different sites of sympatry. Finally, Valencia-Cuevas *et al.* (2015) found that hybridisation rate ranges from 15% to 30% among sites in a complex composed of *Q. castanea* and three red oaks (*Q. crassipes*, *Q. crassifolia* and *Q. laurina*) in Mexico.

The above-mentioned differences in the frequency and occurrence of cross-site hybridisation have been attributed to variation in ecological conditions at the local level ('ecological context of hybridization'; Buerkle 2009) and to differences in the strength of reproductive barriers. Consistent with this, it has been reported that hybridisation and introgression levels in oaks vary depending on the geographic location of the hybrid zone (Tovar-Sánchez & Oyama 2004), the establishment and survival of the hybrids (Harrison & Larson 2014) and the relative abundance (Lepais *et al.* 2009) and identity of the hybridising species (Harrison & Larson 2014). The spatial distribution of species within sympatry zones (Curtu *et al.* 2015), the habitat conditions (Ortego *et al.* 2017a), the proportion of conspecific pollen and the density of individuals available for crossing (Lagache *et al.* 2013) and differences in pollen dispersal capacity between species (Harrison & Larson 2014) have also been considered as important local factors. Finally, there are some studies that suggest genetic incompatibilities between oak species cannot be discarded (Steinhoff 1993; Ortego *et al.* 2017b; López De Heredia *et al.* 2018). Additionally, hybridisation events in some cases are preferentially directional and depend on the identity of the species that acted as maternal or paternal donor (Olrik & Kjær 2007; Lepais *et al.* 2013).

Also, it is suggested that differences in the ecological and geographic conditions at the analysed sites may promote plasticity in the efficiency of reproductive barriers between species and, consequently, differences in the frequencies of hybridisation among *Q. glabrescens*, *Q. rugosa* and *Q. obtusata*. In fact, plasticity of reproductive barriers in oak species as a result of variation in local ecological conditions has been reported (Gailing & Curtu 2014). For example, the rate of hybridisation between *Q. robur* and *Q. petraea*, two of the most important white oak species in Europe, vary across different sympatry sites (Curtu *et al.* 2007; Jensen *et al.* 2009; Lepais *et al.* 2009). In this sense, it is important to mention that all the sympatric sites analysed in this study are present in the TVB. This system is characterised by rugged topography and altitudinal and climatic diversity, which, combined with its geographic position, offers a range of environments, habitats and microhabitats (Challenger 1998). Therefore, it is suggested that the variation in the frequency of hybridisation among *Q. glabrescens*, *Q. rugosa* and *Q. obtusata* may be the result of variation in the strength of the mechanisms of reproductive isolation associated with the environmental variability that characterises the TVB. Similarly, differences in the hybridisation frequency between *Q. castanea* and tree oak species (*Q. crassipes*, *Q. crassifolia*, *Q. laurina*) at different sympatric sites were attributed to environmental variability within the scale of the TVB (Valencia-Cuevas *et al.* 2015).

Variation in the expression of phenolic compounds

Of the ten compounds identified in this study, four are expressed exclusively in the parental species (*Q. rugosa*, *Q. obtusata* and *Q. glabrescens*), three are expressed in two parental species and in at least one hybrid taxon, and three are present at all study taxa and sites (see Table 2). In general, studies have documented that variation in the expression of secondary metabolites after hybridisation events can be explained by at least three mechanisms: (i) polymorphism in the loci that control the expression of the metabolite in the parents; (ii) alterations in the biosynthetic pathways; and (iii) the prolongation of some steps of the metabolic pathway (Cheng *et al.* 2011).

In general, hybrids of the *Q. glabrescens* × *Q. rugosa* and *Q. glabrescens* × *Q. obtusata* complex have chemical characteristics that are additive and similar to those of their putative parental species, and very few compounds are exclusive to the parental species. Also, this study system did not document the presence of novel compounds in the hybrid individuals (Table 2). These results are in agreement with other reported studies, since the most frequent inheritance pattern described in hybrid plants, for approximately 70% of chemical traits, is the additive pattern (Cheng *et al.* 2011). Similarly, it has been reported that the synthesis of novel compounds in hybrids is a less frequent phenomenon, a fact that can explain the absence of new metabolites in hybrid genotypes of this oak complex. Despite that at the phenotypic level, the influence of hybridisation on secondary metabolite expression is known, the genetic causes of the high frequency of chemical additivity and of the rarity in the expression of new chemicals in hybrids continue to be largely unknown (Caseys *et al.* 2015). We also detected variation in the expression patterns of hybrid genotypes across geographically separated sympatric zones (Table 2). For example, *Q. glabrescens* × *Q. obtusata* hybrid genotype in the sympatric zone 'Mineral El Chico' expressed rutin and quercitin glucoside, while in Huitzilac we only detected the second chemical. A similar pattern was also observed in the *Q. glabrescens* × *Q. obtusata* hybrid genotype. These results can be explained considering that environment can play an import role in the expression of phytochemical traits (Xu *et al.* 2015; Glassmire *et al.* 2016). Illustrating the above, Caseys *et al.* (2015) documented geographic variation in the expression of flavonoids in hybridising European *Populus* species.

In this study, three compounds (flavonol 1, flavonol 2 and flavonol 5) were found to be exclusively expressed in parental species; these compounds were not expressed in any of the hybrid individuals, regardless of their genetic mix. This suggests that there is high specificity between these compounds and the oak species in which they are found; preventing these compounds from becoming part of the genetic mix that the hybrid individuals inherit. Thus, for example, Wink (2003), in a review of three plant families (*Fabaceae*, *Solanaceae* and *Lamiaceae*), found that there was a high reciprocal correspondence between the secondary metabolites and the analysed

plants. The author suggests that the results are the product of particular life strategies embedded in a particular phylogenetic framework and that a specific group of metabolites dominates in a single taxon, although other groups of metabolites may be present in smaller amounts. On the other hand, flavonol 4 is a compound that appears in the parental species *Q. glabrescens* and *Q. obtusata*; however, it was not detected in *Q. glabrescens* × *Q. obtusata* hybrids. Phenolic compounds arise biogenetically from the shikimate/phenylpropanoid pathway and/or the acetate/malonate pathway (Quideau *et al.* 2011). Several studies suggest that their expression and changes in expression are regulated by various mechanisms, among which are: oxidation (Appel 1993), epistatic genes (Hallgren *et al.* 2003), transcriptional processes (Hichri *et al.* 2011), and the integration of signalling molecules such as salicylic acid, jasmonic acid and its derivatives (Gould & Lister 2006; do Nascimento & Fett-Neto 2010). In addition, the results reported here suggest that the stimulation of certain metabolic pathways occurs when some genes are in homozygosis (at least, as species alleles), while others are suppressed in the heterozygous condition. This scenario could explain the presence of flavonols 1, 2 and 5 as species-specific compounds and the absence of flavonol 4 in *Q. glabrescens* × *Q. obtusata* hybrids.

In contrast, in this study, we documented the presence of three compounds (quercetin rhamnoside, flavonol 3 and alkyl coumarate) that are expressed in all taxa (parental and hybrids) and at all study sites. This may be due to the documented fact that phenolic compounds have high heritability in the plants with which they are associated. For example, Caseys *et al.* (2015) documented that there is high heritability of flavonoids and chlorogenic acid in *Populus alba*, *P. tremula* and hybrid individuals in Italy, Austria and Hungary. These authors showed that the variation in expression of phenylpropanoids is lower at the intraspecific level than at the interspecific level. It is possible that intraspecific variation is lower because the individuals derived by hybridisation between nearby species have metabolic profiles that are similar to both parental species (Bangert *et al.* 2006, 2008). In this sense, Hipp *et al.* (2017) showed that the three species in question are phylogenetically close. Therefore, it is to be expected that the changes resulting from hybridisation will be less striking when the parental species share similar genomes than when the genomes of two phylogenetically more distant species are mixed. In addition, if a compound is associated with various functions within a given species, the regulation of the compound may be very strict; therefore, processes such as hybridisation might not impact such compounds. This last reasoning can also explain the high degree of conservation in the expression of the three above-mentioned compounds.

Ecological and phytochemical implications of hybridisation

Natural hybridisation is a common phenomenon in plant species (Whitney *et al.* 2010), which has been recognised as a substantial evolutionary force that favours the transfer of genetic variants between species, contributing to the generation of new traits and to the segregation of transgressive traits (extreme), a condition that can facilitate adaptation and speciation events (Arnold 1992, 2004). These white oak species have a forest habitat, which unfortunately is affected by frequent disturbance events (agriculture, road construction, logging), a fact that may create opportunities for sympatry between the three species. To our knowledge, this is the first study where genetic and chemical evidence of genetic flow events between *Q. rugosa*, *Q. glabrescens* and *Q. obtusata* is shown. Thus, further studies are necessary to elucidate the possible adaptive function or impacts of hybridisation between these white oak species through time and space on their associated organisms.

The variation in plant genetic diversity involved in hybridisation events can result in differences in phenotype expression, including secondary metabolites. Although the expression of secondary metabolites is genetically determined, within the genetic regions encoding the expression of different metabolites there are certain genes that regulate differences in the expression of secondary metabolites (Nosil 2012; Lindtke *et al.* 2013). This group of genes may have dominant, over-dominant and/or epistatic effects within a locus that may eventually lead to evolution and diversification (Cheng *et al.* 2011). Thus, transfer of these genes by hybridisation may have diverse ecological and evolutionary implications for the species involved in these events (Arnold 2006), in various groups of associated organisms (*e.g.* specialist herbivores, fungi and plants), communities or ecosystems (Whitham *et al.* 2012). Therefore, the establishment of hybrid individuals that have a selective advantage of resistance or tolerance to environmental conditions or altered biotic interactions, as a result of hybridisation, may change the environment in which they occur and, as a result, change the resources and conditions affecting the biodiversity of different groups with which they interact.

Our study focused specifically on phenolic compounds, which show high heritability, are stable, have high variability and have frequently been associated with protection against environmental changes and attacks by pathogenic fungi (Varshney *et al.* 2012; Bernhardsson *et al.* 2013). Likewise, it has been suggested that phenolic compounds constitute one of the largest and most diversified groups of compounds used by plants as herbivore defence mechanisms (Usha-Rani & Jyothsna 2010; War *et al.* 2011). For example, flavonoids are commonly involved in plant–environment interactions and anti-herbivore activities involving insects (Treutter 2006) by altering their development and growth (Simmonds 2003). Thus, any change in the expression, concentration and/or alteration of the defence-associated phenolic compounds could be expected to have implications on the herbivory patterns of both generalist and specialist insects (*e.g.* leaf miners and gall wasps), inducing a change in the distribution, abundance and diversity of these arthropods (Pérez-López *et al.* 2016).

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CAPÍTULO II.

Efecto del genotipo, diversidad genética y metabolitos secundarios del complejo *Quercus glabrescens* × *Q. rugosa* sobre la estructura de la comunidad de cinípidos y sus parasitoides

Artículo original

Para ser enviado a Oikos en su versión en inglés

White oaks genetic and chemical diversity affect the community structure of canopy insects belonging to two trophic levels

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Running title: Genetic and chemical variation in *Quercus* drive arthropod communities

Resumen

La hibridación es un fenómeno recurrente en encinos que modifica la diversidad genética, la morfología y los perfiles químicos de los taxones involucrados. Dichas modificaciones pueden tener impacto en la estructura de las comunidades asociadas al dosel particularmente en organismos especializados como los insectos inductores de agallas y sus parasitoides. *Quercus rugosa* y *Q. glabrescens* son encinos bien representados en los bosques templados de México y presentan eventos de hibridación cuando se distribuyen en simpatría. En este estudio se evaluó el efecto de los niveles diversidad genética (*He*) y la variación cuantitativa de seis metabolitos secundarios (compuestos fenólicos) de *Q. rugosa*, *Q. glabrescens* e híbridos putativos (F1) sobre la estructura de la comunidad de avispas inductoras de agallas y sus parasitoides asociados. Este estudio se llevó a cabo en 100 árboles con un estatus genético conocido, identificados con marcadores genéticos (microsatélites).

La comunidad de insectos inductores de agallas está compuesta por 24 especies contenidas en seis géneros de Cynipidae los géneros con mayor riqueza fueron: *Andricus* > *Atrusca* = *Cynips*. La comunidad de insectos parasitoides de agallas está compuesta por seis géneros de Chalcidoidea, contenidos en cuatro familias: Eulophidae, Eurytomidae, Ormyridae y Torymidae. Los géneros más representativos fueron: *Galeopsomyia* > *Torymus* > *Ormyrus* > *Sycophila* = *Eurytoma* > *Baryscapus*. En general, la diversidad de insectos inductores de agallas registró el siguiente patrón: *Q. rugosa* = híbrido > *Q. glabrescens*. En contraste la diversidad de parasitoides mostró que *Q. glabrescens* > híbrido, mientras que *Q. rugosa* no difiere de ambos taxones. Con respecto a la abundancia, porcentaje de infestación y riqueza de especies inductores de agallas y sus parasitoides se encontró el siguiente patrón: *Q. rugosa* > híbrido = *Q. glabrescens*. La *He* de la planta hospedera es la variable que registró la mayor influencia sobre la expresión cuantitativa de ms (66.7%) y sobre la riqueza y abundancia de insectos inductores de agallas y sus parasitoides (100%). La influencia de ms sobre la comunidad de inductores de agallas y sus parasitoides mostró el siguiente patrón: escopoletina > quercitrina > rutina = ácido cafeico = quercitina. Se documentó una positiva y significativa relación entre la riqueza de insectos inductores de agallas y la riqueza de parasitoides, así como la abundancia de ambos gremios. La variación que como resultado de

hibridación se da en los patrones de expresión genética y química puede tener efectos sobre la estructuración de las comunidades de herbívoros especialistas, así como de sus parasitoides.

Palabras clave: flavonoides, interacciónes tritróficas, hibridación, *Quercus*

Introducción

Los encinos (Fagaceae: *Quercus*) son considerados el grupo leñoso más importante del hemisferio norte (Cavender-Bares et al. 2016) con un estimado de 500 especies a nivel mundial (Govaerts y Frodin 1998). Particularmente México destaca por contener el 32.2% de esta diversidad (161 especies) y es considerado el centro de diversificación más importante para el género (Valencia 2004). Además, se estima que los bosques de encino ocupan el 7.5% del territorio nacional (Jardel-Peláez 2012) siendo particularmente dominantes a través de la Faja Volcánica Transmexicana [FVT].

En términos ecológicos los encinos son relevantes dado que cumplen diversas funciones en los lugares en donde se distribuyen tales como la fertilización de suelo, el ciclaje de nutrientes y el balance de agua (Shrestha 2003, Bargali et al. 2014). Debido a su dominancia en los ecosistemas forestales y a su complejidad estructural también funcionan como hábitats para diversos grupos biológicos, entre los que se encuentran: plantas epífitas, mamíferos, aves, hongos y artrópodos (Bargali et al. 2015, Skarpaas et al. 2017). Entre los que podemos encontrar a los Cynipidae (Hymenoptera: Cynipidae: Tribu Cynipini) un grupo de avispas inductoras de agallas en género *Quercus,* atacando tejidos, órganos y especies de encino, siendo organismos específicos (Stone et al. 2002). Desde una perspectiva antropogénica los encinos son una fuente importante de recursos, por la obtención de madera, combustible, alimento, por mencionar algunos (Kremer et al. 2012 Petit et al. 2013, Bargali et al. 2014).

Una de las características distintivas de *Quercus* es la alta frecuencia de eventos de hibridación e introgresión entre especies de la misma sección (e.g., Tovar-Sánchez et al. 2004, Núñez-Castillo et al. 2010, Petit et al. 2013, Valencia-Cuevas et al. 2015; Ortego et al. 2017). Se atribuye a débiles mecanismos de aislamiento reproductivo entre especies (Manos y Standford 2001) resultado de la alta similitud filogenética y baja divergencia específica intrasección (Hubert et al. 2014, Pollock et al. 2015). Debido a que la hibridación es una forma de intercambio genético, varios estudios en encinos han documentado un incremento en la diversidad genética de las especies involucradas en estos eventos (González-Rodríguez et al. 2004; Tovar-Sánchez et al. 2008, Peñaloza-Ramírez et

al. 2010; Valencia-Cuevas et al. 2015). Esta condición puede promover variación en la expresión de características morfológicas, químicas (calidad nutricional, metabolitos secundarios [ms]), fenológicas, entre otras (Curtu et al. 2007, Yarnes et al. 2008a, b, Cheng et al. 2011; Song et al. 2015), en las especies de encinos interactuantes. Teniendo consecuencias importantes a nivel de la estructura de las comunidades asociadas a éstos y en los procesos ecosistémicos en los que participan dichas especies (Whitham et al. 2003, 2006, 2012; Crutsinger, 2016). Se ha reportado que los artrópodos responden a características como: biomasa, calidad nutricional foliar y ms de su planta hospedera atributos que tienen una base genética (Dyer et al. 2014, Tovar-Sánchez et al. 2015, Kostenko et al. 2017). Lo anterior puede tener implicaciones en las preferencias de oviposición y alimentación de los artrópodos asociados a encinos modificando su distribución y abundancia (Becerra 2015, Moreira et al. 2016).

En el caso particular de los ms los estudios han reportado que la hibridación natural modifica las rutas metabólicas de las especies interactuantes generando diferencias en su expresión (tipo, concentración, mezcla y calidad) (Arnold y Martin 2010). Se ha documentado que la expresión de ms en híbridos puede variar a nivel cualitativo y cuantitativo (Rieseberg y Ellstrand 1993). A nivel cualitativo los híbridos pueden expresar todos, algunos o nuevos ms con respecto a los taxones parentales siendo el primer patrón de expresión el más frecuentemente reportado (Orians 2000, Cheng et al. 2011). En términos cuantitativos la variación en la concentración en los ms puede ser mayor, intermedio, menor o similar a uno o ambos parentales (Orians et al. 2000; Cheng et al. 2011). En este caso los patrones de expresión más comunes son concentraciones similares a uno de los taxones parentales o intermedias con respecto a ambos (Cheng et al. 2011). Los ms son compuestos químicos de bajo peso molecular derivados del metabolismo primario que no están involucrados en el desarrollo y/o crecimiento normal de las plantas (Irchhaiya et al. 2014). No obstante, dichos compuestos están fuertemente relacionados con su supervivencia y aptitud (Sepúlveda-Jiménez et al. 2003, Irchhaiya et al. 2014) y cumplen con diversas funciones (protección contra la luz UV, pigmentación, perfil aromático y hormonas vegetales) (Crawley y Harborne 2005). En la mayoría de los casos los ms están relacionados con la defensa contra la herbivoría

(Betsiashvili et al. 2014, Caseys et al. 2015, Glassmire et al. 2016). La variación en los ms generada por hibridación puede repercutir en la resistencia a la herbivoría. Se ha propuesto que la resistencia en híbridos puede seguir los mismos patrones de expresión ya mencionados en términos cuantitativos de los ms siendo una menor resistencia a los herbívoros el patrón más común (Fritz et al. 1999; Orians et al. 2000; Cheng et al. 2011). El perfil metabólico puede ser considerado un vínculo importante entre la genética de la planta hospedera (modificada en un escenario de hibridación) y la organización/dinámica de las comunidades de herbívoros asociados (Cheng et al. 2011).

Estudios en *Quercus* han registrado que la hibridación promueve variación en la expresión cualitativa y cuantitativa de ms (Klaper et al. 2001, Madritch y Hunter 2002, Li et al. 2016, Usié et al. 2016). Los ms más frecuentemente encontrados en las hojas de encino son flavonoides, terpenoides, taninos y compuestos alifáticos (Makkar et al. 1998, Chauhan et al. 2004, Yarnes et al. 2008a, b, Moctezuma et al. 2014, Noori et al. 2015, Castillo-Mendoza et al. 2018). Los cuales pueden actuar como atrayentes de insectos, inhibidores de la alimentación, reguladores de la cadena respiratoria (terpenoides-taninos), citotóxicos, reguladores del desarrollo larval (flavonoides) y toxinas (compuestos alifáticos), que pueden afectar a una gran cantidad de herbívoros generalistas y algunos especialistas (Jansen et al. 2009, War et al. 2012, Irchhaiya et al. 2014).

Se ha propuesto que el nivel de sensibilidad de los cinípidos les permite detectar los pequeños cambios a nivel de desarrollo, fisiológico, químico o fenológico que presentan sus encinos hospederos lo que les permite "elegir" el sitio de oviposición y/o consumo (Stone et al. 2002, Abrahamson et al. 2003, Raman 2007, Evans et al. 2012). Por lo que se ha sugerido que cada especie de cinípido establece una relación única con su especie de encino hospedero y los recursos y condiciones que de ésta obtiene (Abrahamson et al. 1998). Por ejemplo, la comunidad de insectos inductores de agallas asociados al dosel del complejo *Q. crassipes* × *Q. crassifolia* Tovar-Sánchez y Oyama (2006a) evidenciaron que la identidad taxonómica del encino hospedero (parental vs hibrido) tuvo un efecto significativo sobre la composición, densidad y diversidad de las comunidades de estos insectos. En particular, se detectó que el taxón híbrido actúa como un centro de biodiversidad albergando un mayor número

de especies incluyendo especies de ambas especies parentales, así como un número considerable de especies raras.

Las agallas producidas por cinípidos forman un microcosmos de gran actividad ecológica (Hayward y Stone 2005) en donde se alberga una comunidad muy estrecha de inquilinos (Hymenoptera: Cynipidae: Synergini), cinípidos que no inducen agallas, así como parasitoides de las familias Eulophidae, Torymidae, Eupelmidae, Ormyridae, Eurotomydae y Pteromalidae (Pujade-Villar 2013). Los parasitoides asociados a cinípidos son específicos para el grupo (Hayward y Stone 2005), y se ha documentado que sus comunidades pueden verse afectadas por los atributos genéticos o los mecanismos de defensa de las plantas (Ode 2006, Stireman 2016), a través de efectos genéticos indirectos (Shuster et al. 2006). Por ejemplo, Valencia-Cuevas et al. (2018) documentaron que el incremento en la diversidad genética individual del encino hospedero *Q. castanea* (resultado de eventos de hibridación) tuvo un efecto positivo y significativo sobre la riqueza y la densidad de su comunidad de insectos endófagos asociados al dosel y que este efecto se extendió sobre la comunidad de parasitoides asociados. Los autores sugieren que la diversidad genética de la planta hospedera tuvo un efecto indirecto sobre la riqueza y densidad de la comunidad de insectos parasitoides mediado por el efecto positivo de la diversidad genética de la planta sobre la riqueza de insectos agalleros. Sin embargo, aún son escasos los trabajos que han abordado el estudio de este tipo de interacciones en encinos p. ej., Wimp et al. 2007, Tovar-Sánchez y Oyama 2006a, Tovar-Sánchez et al. 2015). El entendimiento de los procesos que estructuran a las comunidades de parasitoides cobra relevancia debido a su contribución en términos de su biodiversidad y porque participan de manera importante en la regulación de las comunidades de sus insectos huésped (Wash-Burn y Cornell 1981, Wiebes-Rijks y Shorthouse 1992, Stone et al. 1995). Bajo este escenario los encinos, cinipidae y parasitoides representan un excelente sistema para estudiar las consecuencias de la variación genética y química que son el resultado de eventos de hibridación sobre las comunidades de cinípidos y sus parasitoides asociados.

Como parte del proyecto doctoral, en el primer capítulo Castillo-Mendoza et al. (2018) reportaron evidencia genética (microsatélites) y química (flavonoides) de eventos de hibridación en un complejo de encinos blancos

formados por *Q. glabrescens* y *Q. rugosa* en el centro de México. Los autores detectaron marcadores químicos específicos para cada uno de los taxones parentales (ácido cafeico y flavonol 2 en *Q. glabrescens* y flavonol 5 en *Q. rugosa*) y un patrón complementario en la expresión cualitativa en los híbridos es decir la presencia de flavonoides presentes en las especies parentales.

En este segundo capítulo, el objetivo fue determinar la importancia relativa de la diversidad genética y ms (variación cualitativa y cuantitativa) del complejo *Q. glabrescens* × *Q. rugosa* sobre la estructura de la comunidad de insectos inductores de agallas y parasitoides asociados al dosel en términos de riqueza (*S*) y diversidad (*H'*) de especies, así como en el porcentaje de infestación. En particular se plantearon las siguientes preguntas: 1) ¿la hibridación entre *Q. glabrescens* y *Q. rugosa* promueve diferencias cuantitativas en el patrón de expresión de compuestos fenólicos?, 2) ¿existe relación entre la variación genética y química (cualitativa y cuantitativa) del complejo *Q. glabrescens* × *Q. rugosa* y la comunidad de insectos inductores de agallas (Cinipidae)?, 3) ¿Cuál es la magnitud y dirección de la relación entre la genética, química y la comunidad de cinípidos? y 4) ¿El efecto de los atributos genéticos y químicos del encino hospedero escala a la comunidad de parasitoides asociados a cinípidos? Esta investigación es relevante porque relaciona de manera cuantitativa y cualitativa la relación existen entre la diversidad genética y la expresión de ms y como este binomio tiene impacto sobre la riqueza y diversidad de cinípidos y sus parasitoides asociados.

Materiales y métodos

Especies de estudio

Quercus glabrescens Benth. es un árbol de 6 a 20 m de altura que se reconoce por la escasa pubescencia de las ramillas y envés de las hojas, presenta dientes mucronados en el borde de la hoja hacia la parte apical. En los sitios de estudio florece de febrero a junio y fructifica en octubre. Se distribuye en nueve estados de la República Mexicana (Valencia 2004). ***Quercus rugosa*** Née. es un árbol de 3 a 25 m de altura que se reconoce por presentar hojas coriáceas y cóncavas mucrones largos y envés con tricomas glandulares y depósitos de mucilago. En los

sitios de estudio florece en agosto y fructifica de septiembre a noviembre. Se distribuye en 21 estados de la República Mexicana (Valencia 2004). Ambas especies se distribuyen principalmente en las cadenas montañosas de la FVT (Valencia 2004). Son elementos dominantes del dosel de los bosques donde habitan (Castillo-Mendoza et al. 2018). En el primer capítulo de esta tesis y, mediante el uso de marcadores moleculares (microsatélites) y químicos (flavonoides) Castillo-Mendoza et al. (2018) documentaron eventos de hibridación entre ambas especies en sitios de simpatría en la FVT.

Sitios de estudio y muestreo

Las poblaciones muestreadas en este estudio son las mismas en donde previamente Castillo-Mendoza et al. (2018) detectaron eventos de hibridación natural entre *Q. glabrescens* y *Q. rugosa* en la FVT (Huitzilac, Morelos y Omitlán de Juárez, Hidalgo) mediante el uso de marcadores genéticos y químicos. Se muestrearon 100 individuos, 40 pertenecientes a dos poblaciones alopátridas [una por especie parental (20/sitio)] y 60 individuos pertenecientes a las dos zonas de simpatría (30/sitio), en donde fue variable el número de individuos parentales e híbridos por sitio (Tabla 1). Los individuos muestreados fueron individuos maduros que no presentaban ningún daño aparente. Para minimizar la influencia del ambiente y factores espaciales sobre ambas comunidades de insectos todos los sitios presentaron las siguientes características en común: bosques maduros con clima templado subhúmedo, suelo de origen volcánico o derivado de rocas ígneas o sedimentarias. En los sitios no hay evidencia de disturbios a nivel local, todos los sitios comparten la misma historia geológica (están incluidos en la FVT).

Datos moleculares

Ocho primers nucleares de microsatélites (nSSRs) fueron utilizados para la caracterización genética previa de los 100 individuos analizados en el primer capítulo de esta tesis (Castillo-Mendoza et al. 2018). Dichos marcadores permitieron evidenciar que los individuos colectados en las poblaciones alopátridas de cada especie son

individuos puros. Los autores reportaron tres poblaciones simpátridas con individuos con ancestría mezclada entre *Q. glabrescens* y *Q. rugosa*.

Datos químicos

Además de la caracterización genética de los individuos del complejo *Q. glabrescens* × *Q. rugosa* Castillo-Mendoza et al. (2018) realizaron la caracterización química cualitativa de los ms mediante HPLC, evidenciando la presencia de nueve flavonoides y una cumarina. En general, el patrón de expresión detectado mostró marcadores químicos específicos para cada uno de los taxones parentales (ácido cafeico y flavonol 2 en *Q. glabrescens* y flavonol 5 en *Q. rugosa*) y un patrón complementario de expresión en los híbridos es decir la presencia de flavonoides presentes en las especies parentales. Siguiendo la metodología empleada en el primer capítulo de esta tesis, en este estudio se identificaron cualitativamente dos compuestos más (kaemferol y escopoletina). Considerando que los eventos de hibridación promueven variación en la expresión cuantitativa de los ms (Orians 2000, Cheng et al. 2011) y que los insectos pueden responder a estos cambios, en este trabajo se midió la concentración de seis ms, cuatro identificados previamente para el complejo *Q. glabrescens* × *Q. rugosa* (ver tabla 2, Castillo-Mendoza et al. 2018) y los dos ms caracterizados en el presente capítulo. Finalmente, el resto de los compuestos documentados en el primer capítulo no fueron analizados cuantitativamente debido a que no fue posible identificar a nivel especifico a estos compuestos (flavonoles 1-5, alkil cumarato, ver Tabla 2 en Castillo-Mendoza et al. 2018). Para este capítulo, 48 individuos [*Q. glabrescens* (n= 18), *Q. rugosa* (n= 18), híbridos (n= 12)] del complejo *Q. glabrescens* × *Q. rugosa* fueron analizados cuantitativamente usando los mismos extractos a partir de los cuales se hizo la caracterización cualitativa en el primer capítulo. Tales extractos fueron purificados mediante cromatografía de columna obteniendo 10 mg de compuesto puro/población.

Para determinar las concentraciones de los flavonoides se elaboraron curvas de calibración utilizando estándares comerciales conocidos de cada uno de los compuestos mediante HPLC [ácido cafeico, quercitina, rutina, quercitrina, kaemferol y escopoletina (Sigma-Aldrich chemical Co., St. Louis, MO, EUA)]. El método estándar

interno que se utilizó para la construcción de las curvas de calibración presentó las siguientes concentraciones: 12.5, 25, 50, 100 y 200.0 µg/mL-1 realizándose por triplicado. La separación química se llevó a cabo en una columna supelcosil fase reversa (rp-18, 25 cm, 4 um) con un gradiente TFA/acetonitrilo. flujo= 0-9 mil/min; vol inyección 10 ul, longitud de onda 350 nm. Posteriormente se realizó la medición del área de cada uno de los estándares conocidos y se trazó una gráfica relacionando el área del pico con su masa. Finalmente, la concentración de cada compuesto puro aislado por taxón estudiado fue analizada mediante HPLC. Se efectuó una regresión lineal de las áreas que fueron medidas frente a las concentraciones obtenidas. Las concentraciones de cada uno de los compuestos en las dos poblaciones se determinaron por extrapolación de la curva de calibración que se obtuvo a partir de los compuestos estándares puros. La concentración de los ms fue calculada como promedio ± error estándar en $\mu g/mL^{-1}$ sobre la base de peso seco. Para la realización de los análisis estadísticos, las muestras donde no se detectó la concentración del ms, se utilizó la mitad de los valores de límite de detección para cada ms.

Comunidades de cinípidos y parasitoides asociados

Después de la caracterización genética y química de los taxones del complejo *Q. glabrescens* × *Q. rugosa* la comunidad de insectos inductores de agallas (Cynipidae) y los parasitoides asociados fue muestreada por taxón en 100 individuos [*Q. glabrescens* (n= 40), *Q. rugosa* (n= 40) e híbridos (20)]. Con la finalidad de tener bien representada a la comunidad de insectos agalleros y parasitoides se realizaron dos colectas la primera en el mes de abril y la segunda en diciembre del 2016. Los árboles seleccionados para el muestreo fueron aquellos cuyo dosel no se traslapará con el de otros individuos con una talla de entre 10-12 m y con una cobertura del dosel de 17-19.5 m. La comunidad de insectos fue muestreada en cuatro ramas elegidas al azar en la parte media del dosel (tomando como referencia los cuatro puntos cardinales). Para cada árbol hospedero el porcentaje de infestación fue estimado (número de insectos cinípidos/200 hojas × 100) sobre las cuatro ramas. Las agallas colectadas en cada encino hospedero fueron separadas a nivel de morfoespecie, colocadas en recipientes

etiquetados de plástico y transportadas al laboratorio en donde se esperó que los insectos adultos (agalleros y parasitoides) emergieran. Ambos grupos de insectos fueron identificados al nivel taxonómico más fino posible.

Análisis estadísticos

Diversidad genética del encino hospedero

Para estimar la diversidad genética del complejo de encinos *Q. glabrescens* × *Q. rugosa* se utilizó el parámetro heterocigosis esperada (*He*). Esta medida permitió comparar los resultados de otros trabajos en encinos debido a que es frecuentemente empleada para evaluar la magnitud de la diversidad genética (e.g., Tovar-Sánchez et al. 2013; Valencia-Cuevas et al. 2014). Los estimados de *He* fueron obtenidos con el programa Popgene v. 1.31 (Yeh et al. 1999). Un análisis de varianza de Kruskal-Wallis fue usado para determinar el efecto del taxón sobre los índices de diversidad genética.

Estructura de la comunidad y niveles de infestación de los insectos inductores de agallas y parasitoides asociados al dosel

Un análisis de varianza (ANOVA) fue realizado para evaluar diferencias en la *S* y abundancia de las comunidades de insectos entre taxones de encino (Zar 2010). La diversidad de la comunidad de insectos fue estimada usando el índice de diversidad de Shannon-Wiener (*H'*). Posteriormente este índice fue comparado entre pares de taxones de encino con un proceso de aleatorización descrito por Solow (1993). Esta prueba re-muestrea 10 000 veces a partir de la distribución de las abundancias producida por la suma de las dos muestras.

Por otro lado, cada valor de infestación del encino hospedero fue estimado como: [(número de agallas /200 hojas) × 100)] sobre las cuatro ramas. Análisis de varianza (ANOVA, Modelo III; Zar 2010) fue usado para determinar diferencias en los niveles de infestación entre taxones (*Q. glabrescens*, *Q. rugosa* e híbridos). Los datos de infestación fueron corregidos como X= arcsin (%)1/2 y datos discontinuos fueron transformados como X= (x) 1/2 + 0.5 (Zar 2010). Finalmente, una prueba de Tukey fue usada para identificar entre que datos existían

las diferencias significativas entre los valores promedio de las poblaciones (Zar 2010). El paquete usado para los análisis estadísticos fue STATISTICA 8.0 (Statsoft 2007).

Influencia de la diversidad genética y metabolitos secundarios del complejo *Q. glabrescens* × *Q. rugosa* sobre la estructura de la comunidad de insectos asociados al dosel.

Se construyó un modelo de ecuación estructural (análisis de redes) para estimar las relaciones causales entre la diversidad genética del complejo de encinos (*He*), los ms y dos variables de la estructura de la comunidad: *S* y abundancia de insectos cinípidos y parasitoides. Previamente se propusieron las rutas causales que pueden ser importantes tomando como referencia un trabajo previo (Castillo-Mendoza et al. 2018) y revisión de literatura. Para el modelo, la diversidad genética del encino hospedero fue considerada como variable independiente. Las variables dependientes que fueron examinadas fueron: ms, *S* y abundancia de los insectos inductores de agallas y sus parasitoides.

Una relación causal (X → Y) se cuantifica utilizando el método de regresión lineal donde una variable independiente A produce efectos en la variable dependiente B. El factor de correlación se usa comúnmente en el análisis de regresión simple para indicar si la variable independiente responde a los cambios de la variable dependiente. Sin embargo, nuestro objetivo es cuantificar la tendencia de las relaciones causales. Por lo tanto, la tendencia estará representada por la pendiente de la línea recta interpolada derivada del método de regresión lineal. La línea recta interpolada se representa mediante la expresión (1) a continuación:

$Y = Bo + B1X$ ---------- (1)

dónde, Bo representa el valor de Y cuando X = 0, y B1 representa la pendiente de la línea recta interpolada.

El coeficiente B1 es una constante que representa el valor tangente de la pendiente de la línea recta interpolada que se muestra en la ecuación 1. Por lo tanto, no tiene unidades asociadas (kilogramos, kilómetros, etc.). Sin embargo, el valor de la pendiente puede cuantificar la tendencia de la relación en una dirección ascendente o descendente. Aprovechamos esta característica para situar las tendencias de las relaciones dentro de un

contexto de zonas con cierto nivel de influencia donde los valores de las tendencias podrían caer facilitando así su interpretación. Este concepto se deriva del siguiente método: la pendiente B1 se da en valores tangentes, que se representan entre 0 y ∞ (para valores positivos). Sin embargo, los valores de tangente se pueden transformar en valores angulares utilizando la siguiente función: angtan (α) = θ o tg-1 (α) = θ, que se puede leer de la siguiente manera: θ es el ángulo cuya tangente es α. Por lo tanto, en lugar de usar valores entre 0 y ∞, usaremos valores entre 0° y 90°, que es más fácil de interpretar. Del mismo modo los valores del rango [0°, 90°] se pueden convertir en valores normalizados entre 0 y 1 de la siguiente manera: tendencia-valor-normalizado = θ/90, donde θ representa el valor angular actual de la pendiente a ser normalizado

Una función sigmoidal para analizar el comportamiento de las relaciones.

Está claro que los cambios en las relaciones entre las variables dependientes e independientes no coinciden con un comportamiento lineal. Hemos seleccionado la función sigmoide representada en la Figura 1 para modelar el comportamiento de las relaciones que definen cinco zonas que representan la influencia de la variable independiente X en la variable dependiente Y. Se describe a continuación: 1) en el rango [0°, 20°] la influencia de A en B es muy baja; 2) en el rango [20°, 40°] la influencia es baja; en el rango [40°, 60°] hay una influencia media; 4) en el rango [60°, 80°) la influencia es alta; en el rango [80°, 90°] la influencia es muy alta. El comportamiento de la influencia de X en Y es exponencial en el rango [0°, 40°), que se compone de la muy baja influencia y las zonas de baja influencia. Un comportamiento exponencial similar se observa en el rango [60°, 90°], que está compuesto por las zonas de influencia alta y muy alta. En particular, en la zona muy baja, los cambios de la variable independiente X no ejercen efectos importantes sobre la variable dependiente. Sin embargo, un comportamiento exponencial tiene lugar en el rango [20°, 40°]. Se produce un comportamiento lineal en la zona de influencia media. Finalmente, un comportamiento exponencial tiene lugar en el rango [60°, 90°].

RESULTADOS

Diversidad genética de los tres taxones de encinos hospederos

Los análisis genéticos del complejo *Q. rugosa* × *Q. glabrescens* usando ocho microsatélites nucleares mostraron que los niveles de diversidad genética medida como *He* presentaron el siguiente patrón: taxón híbrido (0.669) > *Q. rugosa* (0.637) > *Q. glabrescens* (0.447). El análisis de varianza de Kruskal-Wallis reveló que existen diferencias significativas en estos valores, mientras que la prueba de Tukey evidenció que los valores más altos encontrados en el taxón híbrido difieren estadísticamente de ambos parentales (Tabla 2).

Variación cualitativa y cuantitativa de los metabolitos secundarios

En este estudio se identificaron cualitativamente un compuesto fenólico (kaemferol) y una cumarina (escopoletina) que se suman a otros cuatro compuestos fenólicos (flavonoides) reportados para el complejo *Q. rugosa* × *Q. glabrescens* en un estudio previo (Castillo-Mendoza et al. 2018). En total se tienen identificados seis compuestos presentes en los tres taxones analizados (Tabla 2). Asimismo, se realizó la caracterización cuantitativa de estos seis compuestos a nivel de taxón (mg/g extracto). En particular se encontró que *Q. rugosa* y *Q. glabrescens* presentan diferencias significativas en la concentración de todos los compuestos analizados, además de que *Q. rugosa* presentó los mayores valores en la concentración de todos los ms. Cabe mencionar que los compuestos más importantes en términos de concentración para *Q. rugosa* son: quercitrina, kaemferol y escopoletina, mientras que para *Q. glabrescens* son: quercitrina y kaemferol (Tabla 2). Con respecto al taxón híbrido se encontró que la concentración de ácido cafeico difiere de *Q. rugosa* pero no de lo encontrado para *Q. glabrescens*, mientras que para el caso del kaemferol el patrón fue inverso. Con respecto a la quercitina su concentración fue intermedia con respecto a ambas especies parentales. Por su parte, quercitrina y escopoletina presentaron concentraciones por debajo de lo encontrado en ambas especies parentales. Es importante mencionar que los ms más importantes en términos de concentración en el hibrido son: kaemferol y la quercitina, respectivamente. Los resultados sugieren: a) un efecto del taxón en la expresión cuantitativa de los ms analizados y b) tres patrones de herencia en esta expresión cuantitativa en el taxón híbrido: un patrón de herencia dominante en dos compuestos (ácido cafeico y kaemferol) pues sus concentraciones son similares a lo

encontrado para alguna de las especies parentales, un patrón de herencia intermedia en la expresión de la quercitina y un patrón de subexpresión para el caso de quercitrina y escopoletina.

Composición de la comunidad de insectos inductores de agallas y sus parasitoides

La comunidad de artrópodos asociados al dosel de complejo *Q. rugosa* × *Q. glabrescens* se caracterizó a partir de 1082 agallas pertenecientes a 24 especies de avispas agalleras (Cynipidae). En *Q. rugosa* se documentó la presencia de 29 especies, en *Q. rugosa* × *Q. glabrescens* 18 especies y en *Q. glabrescens* 10 especies que están agrupadas en seis géneros (Tabla 3, Fig. 2). Los géneros más representativos en términos de las especies que albergan son: *Andricus* con 11 especies y *Atrusca* y *Cynips* con cuatro especies cada una. En términos de abundancia, las especies que más individuos presentaron fueron: *Disholcaspis* sp. 3 (386), *Neuroterus* sp. 1 (339) y *Andricus georgei* (177). Asimismo, en el 2.5% del total de agallas colectadas emergieron avispas inquilinas *Synergus* sp, las cuales no fueron incluidas en los análisis. Del total de agallas colectadas el 24.3% presentó parasitoides. Las familias, Eulophidae y Eurytomidae fueron las más representativas al presentar dos géneros cada una. Se encontraron seis géneros, todos representados por una sola especie (Tabla 3).

Efecto del taxón y la expresión de metabolitos secundarios sobre las comunidades de insectos inductores de agallas y parasitoides

Los resultados muestran que *Q. rugosa* es el taxón que presenta los mayores valores en todos los parámetros analizados y que éstos difieren estadísticamente de lo reportado para *Q. glabrescens* y el taxón híbrido (Tabla 4). En contraste, este último no muestra diferencias significativas en ninguno de los parámetros de ambas comunidades con respecto a *Q. glabrescens*. Los resultados sugieren: a) un efecto del taxón hospedero sobre los parámetros de la comunidad de inductores de agallas y parasitoides y b) un patrón de herencia dominante en la susceptibilidad del taxón híbrido a sus artrópodos asociados al dosel pues no difiere de lo encontrado para su especie parental *Q. glabrescens*.

Los resultados del análisis de redes muestran que la diversidad genética del complejo *Q. glabrescens* × *Q. rugosa* tuvo un efecto significativo sobre la expresión cuantitativa de cuatro de los seis ms analizados. En particular, quercitina, kaemferol y escopoletina se vieron influenciadas positivamente mientras que para el ácido cafeico la influencia fue negativa (Fig. 2). Asimismo, se encontró que *He* tuvo un efecto positivo y significativo sobre la abundancia y *S* de especies de insectos inductores de agallas y parasitoides. Sin embargo, de acuerdo con los valores de Bn la diversidad genética del encino hospedero presenta el siguiente patrón en la magnitud de su influencia: abundancia y *S* de insectos agalleros > abundancia y *S* de insectos parasitoides > ms (Fig. 2). Por otra parte, se evidenció que el 83.33 % (cinco de los seis ms analizados) tuvieron influencia sobre alguno de los parámetros de la comunidad de agalleros. Específicamente se encontró que escopoletina, quercitina y ácido cafeico tuvieron una influencia positiva y significativa sobre la abundancia de estos insectos. De manera similar rutina y quercitina afectaron positivamente la riqueza de especies de inductores de agallas. En contraste, quercitrina y rutina tuvieron una influencia negativa sobre la abundancia de insectos agalleros (Fig. 2). Cabe mencionar que en términos de magnitud (Bn) la influencia de los ms presenta el siguiente patrón: abundancia > *S* de insectos agalleros independientemente de su dirección. Finalmente se encontró que sólo el 33.33% (dos de seis) de los ms analizados tuvieron influencia sobre los parámetros de la comunidad de insectos parasitoides. Específicamente se encontró que quercitrina tiene una influencia positiva sobre la abundancia y *S* de parasitoides mientras que la escopoletina afecta de forma negativa a ambos parámetros (Fig. 2). Cabe mencionar que en los casos en donde se listan dos o más ms éstos fueron presentados considerando el valor de Bn (magnitud de la influencia de mayor a menor) de *He* y ms sobre los parámetros de la comunidad de insectos.

DISCUSIÓN

Diversidad genética y expresión de metabolitos secundarios

En este trabajo se analizó de manera simultánea el impacto que tiene la diversidad genética (modificada por la hibridación) y la expresión (cualitativa y cuantitativa) de seis ms en tres taxones de encinos sobre la estructura

de la comunidad de avispas inductoras de agallas y sus parasitoides asociados. Los resultados muestran que tanto la diversidad genética como la expresión (cualitativa y cuantitativa) de compuestos fenólicos en las especies hospederas tienen influencia sobre el establecimiento de las avispas agalleras y sus parasitoides. Recientemente diversos trabajos han analizado la diversidad genética (*He*) de algunos taxones de encinos mexicanos en donde se ha registrado que dicha diversidad oscila entre 0.25 y 0.88 (Valencia-Cuevas et al. 2014, Wehenkel et al. 2017, Oyama et al. 2018). En este trabajo se documentó que *Q. rugosa*, *Q. glabrescens* e híbridos presentan una *He* de 0.43, 0.47 y 0.69 respectivamente. Los resultados muestran que el taxón híbrido tiene una diversidad genética (*He*) más alta que cualquiera de los taxones parentales. Los resultados son consistentes con los presentados en otros estudios donde se ha analizado el efecto de la hibridación sobre la diversidad genética (p. ej., Rieseberg 1997, Mallet 2007, Valencia-Cuevas et al. 2014).

Estos tres taxones se encuentran distribuido dentro de la FVT la cual ha sido propuesta como centro de diversificación para el género (Nixon 1993, Tovar-Sánchez et al. 2008, Peñaloza-Ramirez et al. 2010, Hipp et al. 2018) dadas las condiciones orográficas, climáticas y de simpatría de diversas especies filogenéticamente cercanas. En este sentido diversos estudios muestran que la diversidad genética de los encinos mexicanos es en promedio más alta que la presentada por algunas especies de encinos europeos (p. ej. Müller-Starck et al. 1993, Gomory et al. 2001, Zanetto et al. 1994, pero ver: Curtu et al. 2014, Antonecchia et al. 2015). Cabe señalar que la hibridación no homogeniza los niveles de divergencia en todo el genoma (Wolf y Ellegren 2017) y que la información adquirida mediante hibridación no necesariamente tiene impactos inmediatos ya que dicha información puede almacenarse de forma críptica y expresarse cuando las condiciones ambientales cambien (Paaby y Rockman 2014). No obstante, aunque la hibridación no dé como resultado la formación de un nuevo taxón, la incorporación de genoma exoespecífico tiene la capacidad de modificar la estructura genética de los taxones involucrados.

En este estudio se documentó que la hibridación afecta la expresión cualitativa y cuantitativa de diversos compuestos fenólicos, aunque no se detectó la presencia de nuevos ms. Lo anterior podría deberse a que los

compuestos que de manera novedosa se expresan en los híbridos generalmente lo hacen en concentraciones demasiado bajas por lo que muchas veces no pueden ser detectados (Kirk et al. 2005). Además, tomando como base la arquitectura genética de las especies parentales se pueden formar genotipos híbridos que presentan características muy particulares, aunque también se puede evitar el traspaso de ciertos genes que se encuentran ligados (Bouck et al. 2005, 2007), lo que explicaría la expresión novedosa (el incremento o reducción de la concentración también puede ser considerado expresión novedosa en los taxones híbridos) y/o la supresión de diferentes rasgos en los individuos híbridos.

Aunque diversos trabajos se ha reportado gran variedad de ms en encinos como resultado de la diversidad genética, en dichos trabajos no se realizó el análisis correspondiente (p. ej., Madritch y Hunter 2002, Yarnes et al. 2008a, b). Debido a lo anterior, en este trabajo se analizaron compuestos fenólicos (flavonoides y cumarinas) debido a que se ha documentado que su expresión y su heredabilidad (medida de la reproducibilidad del fenotipo dentro de un conjunto de genotipos (Lynch y Walsh 1998]) están determinadas fuertemente por factores genéticos (Scioneaux et al. 2011, Barbour et al. 2015, Caseys et al. 2015, Tsai y Schmidt 2017, Barker et al. 2018). En este sentido, y desde nuestro conocimiento existen pocos trabajos en donde de manera directa se ha intentado evaluar de manera objetiva y cuantitativa la relación existente entre la genética y el perfil metabólico de los encinos. Klaper et al. (2001) encontró que al menos siete compuestos fenólicos tienen una alta probabilidad de ser heredables. Es decir, su expresión obedece a la constitución genética aditiva. Por su parte, Zanetto et al. (2013) documentó que existe mayor heterocigosidad en las enzimas que codifican para el metabolismo secundario que para el metabolismo primario. Lo anterior sugiere dos cosas: 1) que la expresión de los ms en encinos está regulada por factores genéticos y 2) que las rutas metabólicas que regulan la expresión pueden ser extremadamente complejas en el sentido que no necesariamente sigue una linealidad con respecto a la diversidad genética.

Se encontró que el taxón híbrido muestra tres patrones de herencia (dominante, intermedia y subexpresión) dependiendo del ms analizado. Los resultados encontrados en este estudio pueden ser explicados mediante

diferentes mecanismos. Por ejemplo, Rehill et al. (2006) documentan que la heredabilidad en la expresión de los compuestos fenólicos en *Populus* está controlada por genes dominantes. Por otra parte, algunos estudios sugieren que en los híbridos la expresión de compuestos fenólicos puede estar regulado por controles de expresión aditiva (Crawford 1974).-La no detección de rutina, así como la subexpresión de la quercitrina y la escopoletina puede explicarse bajo tres escenarios posibles: a) la baja concentración que dicho compuesto presenta en el tejido foliar que la hace indetectable (límite de detección), b) la hibridación modificó la ruta metabólica que conduce a su expresión y c) mutaciones puntuales en genes biosintéticos como resultado del flujo genético interespecífico. Lo anterior debido a que se ha sugerido que la hibridación es el principal factor que determina la calidad y la cantidad de los compuestos fenólicos (Rehill et al. 2006, Scioneaux et al. 2011).

Caracterización de las comunidades de agalleros y sus parasitoides

En este estudio se documentó la presencia de 24 especies de avispas gallícolas y seis especies de parasitoides. De acuerdo con una revisión realizada por Pujade-Villar y Ferrer-Suay (2015) existen 183 especies de avispas agalleras asociadas especies de encinos mexicanos. Los géneros más importantes en términos de riqueza de especies son *Andricus*, *Atrusca* y *Cynips*. En este estudio, se documentó el 13.11% de especies de avispas agalleras siendo los géneros más importantes los mismos mencionados en el trabajo de Pujade-Villar y Ferrer-Suay (2015). Aunque falta de la avispa inductora impidió la descripción específica de cada una de las morfoagallas se pudo caracterizar a morfoespecie a los integrantes de los diferentes géneros esto bajo la premisa de que cada avispa agallera desarrolla una forma particular de agalla. Tomando como base que México ha sido considerado como el principal centro de diversificación del género *Quercus* puede sugerirse que el número de especies de cinípidos asociados a encinos mexicanos puede incrementarse considerablemente, si se realizan más estudios con un enfoque taxonómico cuidadosamente dirigido.

De manera general existen pocos ecológicos en donde se describa de manera fina la riqueza específica en la mayoría de los trabajos se describe únicamente a nivel de morfoagalla (p. ej. Maldonado-López et al. 2016,

Pascual-Alvarado et al. 2017, Rodríguez-Rivera et al. 2017). Lo anterior dificulta la comparación y la obtención de patrones de riqueza y diversidad entre los distintos taxones de encinos mexicanos. Sin embargo, en dichos trabajos se ha documentado que existe una alta especificidad a nivel genérico entre las avispas agalleras y sus taxones hospederos (Abrahamson 1998). Que el mayor número de especies de agalleros está asociado a la sección *Quercus* (Pujade-Villar et al. 2009) pudiendo ser aproximadamente el doble (en comparación con la sección Lobatae) (Rodríguez-Rivera et al. 2017). Que el establecimiento de avispas agalleras depende de diferentes factores entre los que se encuentran el rango de distribución del taxón hospedero (Stone y Schonrogge 2003), variables ambientales, altitudinales y geográficas (Clark-Tapia 2013, Rodríguez-Rivera et al. 2017), el grado de perturbación (Maldonado-López et al. 2015) y si entre las especies hospederas existen eventos de hibridación (Pérez-López et al. 2016).

Dentro de la sección *Quercus*, *Q. rugosa* ha sido la especie donde se registrado el mayor número de avispas agalleras (33 especies [Pujade-Villar et al. 2009]) mientras que para *Q. glabrescens* solo existen dos trabajos en donde se ha reportado la presencia de cuatro especies pertenecientes al género *Atrusca* y una especie de inquilino (Pujade-Villar et al. 2016, Lobato-Vila y Pujade-Villar 2017). Los resultados obtenidos en este trabajo confirman la alta prevalencia de avispas agalleras establecidas en *Q. rugosa*. En contraparte, *Q. glabrescens* con una menor prevalencia de establecimiento, aunque las especies colectadas pertenecían al mismo número de géneros que *Q. rugosa*. Cabe señalar que en un trabajo realizado por Pascual-Alvarado et al. (2017) se menciona que no se encontraron agallas asociadas a *Q. glabrescens* por lo que es muy posible que las morfoespecies encontradas en este estudio sean nuevos registros y/o nuevas especies. Para el taxón híbrido se documentó una prevalencia intermedia entre ambos taxones parentales. En este taxón las especies de agalleros fueron agrupadas en seis géneros y dado que nos hay estudios previos para este taxón puede mencionarse que las 16 especies de agalleros son al menos registros nuevos.

La variación en la riqueza de avispas asociadas podría deberse al intervalo de distribución que presentan las especies de encinos. Por ejemplo *Q. rugosa* se distribuye en 21 estados en México y a través de diferentes

ambientes lo que puede facilitar el establecimiento de diferentes especies de agalleros a través del continuo flujo genético intraespecífico que mantiene una gama de recursos y condiciones similares y que pueden otorgar microambientes para un amplio número de avispas agalleras. Mientras que *Q. glabrescens* se distribuye en nueve estados en México y en altitudes superiores a los 2500 m, lo anterior podría limitar el establecimiento de las avispas agalleras debido a la potencial reducción de las áreas de colonización. Asimismo, dadas las condiciones ambientales y geográficas en las que se distribuye *Q. glabrescens* las especies de agalleros que se establecen deberían presentar características muy particulares que les permitan establecerse. Para el taxón híbrido, aunque solo fueron analizados dos sitios de estudio, la riqueza de avispas agalleras encontrada hace suponer que la hibridación ha jugado un papel fundamental en el traspaso de información genética a los individuos híbridos que provoco la formación y/o modificación de los recursos y/o condiciones que pueden ser aprovechados por diferentes especies de avispas agalleras. Posiblemente las condiciones ambientales de los sitios de estudio presenten una serie de condiciones que favorecen su establecimiento.

Un trabajo realizado por Serrano-Muñoz (2016) documentó la presencia de nueve géneros (agrupados en cuatro familias) de parasitoides asociados a seis géneros de avispas agalleras (en 17 especies de encinos). Asimismo, en un trabajo realizado por Valencia-Cuevas et al. (2018) se documentó la presencia de 10 géneros (agrupados en siete familias) asociados a 18 especies de agalleros (en *Q. castanea*). En este trabajo se encontraron seis géneros de parasitoides (agrupados en cuatro familias) asociados a 24 especies de agalleros. La variación en la riqueza de especies de parasitoides puede estar directamente relacionada con el recurso (agalleros) que obtienen. Se ha documentado que responden a los cambios en las especies vegetales (Schädler et al. 2010) por lo que si el recurso alimenticio no está disponible difícilmente podrán establecerse. Existen tres géneros (*Torymus*, *Ormyrus* y *Eurytoma*) que se encuentran en los tres estudios previamente mencionados además de *Sycophila* que también ha sido reportado en encinos rojos por lo que podría suponerse que se trata de géneros generalistas en el sentido de que atacan a los agalleros independientemente de si se establecen en encinos rojos o blancos. Para el caso

de *Baryscapus* y *Galeopsomyia* se encontraron asociados a agalleros establecidos únicamente en encinos blancos por lo que puede suponerse cierto grado de especificidad genérica.

Efecto de la diversidad genética y química sobre la comunidad de insectos inductores de agallas y sus parasitoides

En este trabajo se documentó que las comunidades de agalleros y parasitoides responden significativamente tanto a la diversidad genética como a la expresión cualitativa y cuantitativa de los ms en cada uno de los taxones hospederos. Diversos estudios han documentado que la diversidad genética vegetal repercute en diversos aspectos y tiene implicaciones ecológicas y evolutivas que incluyen desde el individuo hasta el ecosistema y que además dicha diversidad no es afectada por factores ambientales (Vellend y Geber 2005, Hughes et al. 2008, Bailey et al. 2009b, Lamy et al. 2017, Des Roches et al. 2018). Aunque dicho impacto difiere dependiendo del nivel biológico de organización, así como los taxones estudiados (Raffard et al. 2018). Se ha documentado que el impacto ecológico de la diversidad genética a está relacionado con el ensamblaje de la comunidad y a nivel ecosistémico se ve reflejado principalmente en los artrópodos (Raffard et al. 2018). Por otra parte, los compuestos fenólicos regulan la interacción que las plantas tienen con el ambiente, los procesos ecosistémicos y con diversos organismos (Moore et al. 2014). Por lo anterior, el análisis tanto cualitativo como cuantitativo de diferentes ms puede ser importante para determinar la actividad específica y la dirección del flujo metabólico, así como el uso de los recursos (Arnold y Schultz 2002, Yarnes et al. 2008b). La diferencia en la expresión específica de diversos ms puede resultar adaptativa al permitir que las plantas superen las disyuntivas de la transferencia de información química y la relación que esta tiene con depredadores y mutualistas (parasitoides) que están mediadas por la misma expresión (Kessler y Halitschke 2009, Heil y Karban 2010).

La variación en la expresión cualitativa y cuantitativa, que en principio está regulada por la diversidad genética de la planta hospedera puede tener impacto no solo sobre los herbívoros que dependen directamente de ella, sino que también pueden afectar el establecimiento y desarrollo de los parasitoides ya sea por la detección de

presas potenciales, por la calidad nutricional que los herbívoros pueden presentar y por la producción de ms que pueden tener efectos negativos sobre su desarrollo.

Legenda de figuras

Figura 1. La función sigmoidal que modela el comportamiento de las relaciones representadas en la red en relación con las influencias positivas.

Figura 2. Especies de avispas y agallas inducida en el complejo *Q. glabrescens* × *Q. rugosa*.

Figura 3a. Análisis de red para relacionar la influencia positiva entre variables de la planta de encino hospedero [diversidad genética (*He*), metabolitos secundarios (rutina, ácido cafeico, quercitina, quercitrina, kaemferol, escopoletina)] y la riqueza y abundancia de insectos inductores de agallas y sus parasitoides.

Figura 3b. Análisis de red para relacionar la influencia negativa entre variables de la planta de encino hospedero [diversidad genética (*He*), metabolitos secundarios (rutina, ácido cafeico, quercitina, quercitrina, kaemferol, escopoletina)] y la riqueza y abundancia de insectos inductores de agallas y sus parasitoides.

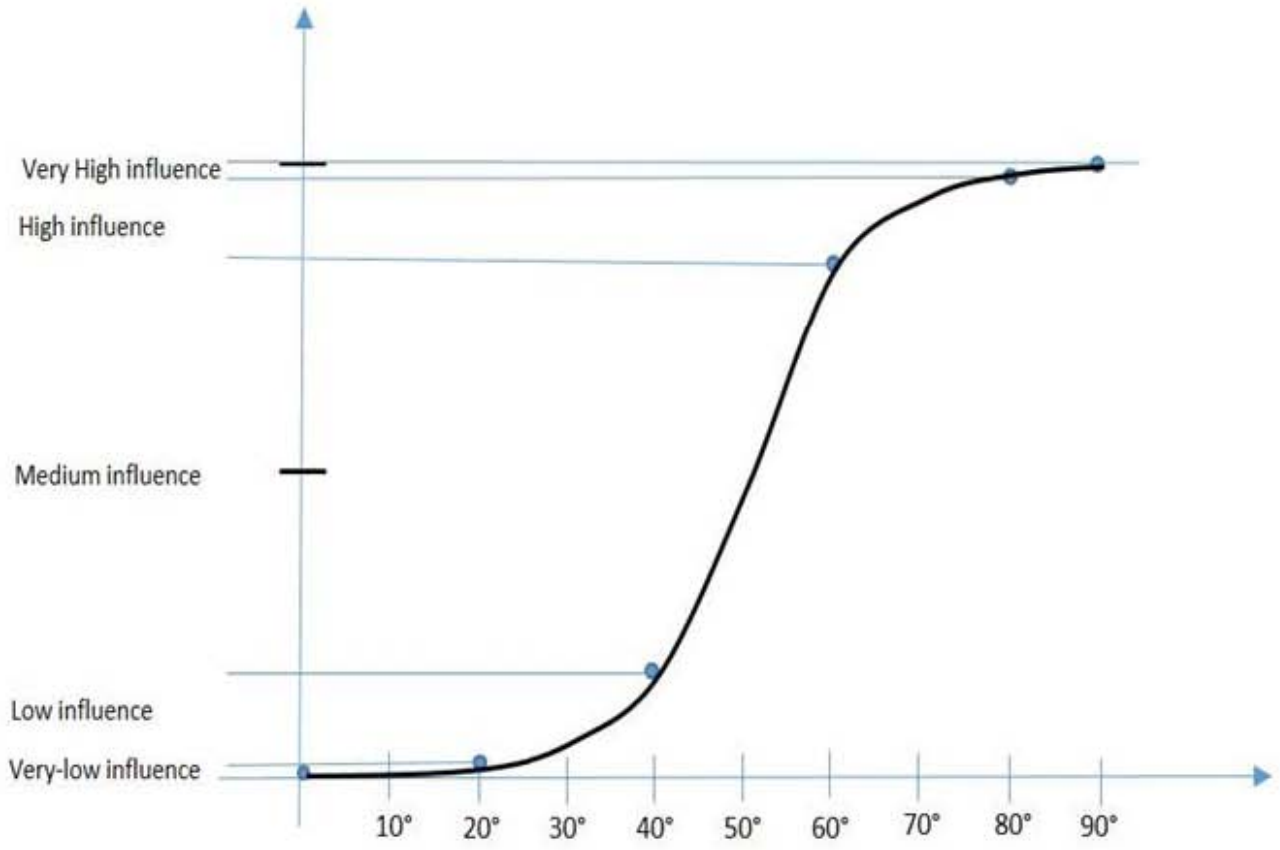
Very High influence
High influence
Medium influence
Low influence
Very-low influence
10°
20°
30°
40°
50°
60°
70°
80°
90°

Figura 1.

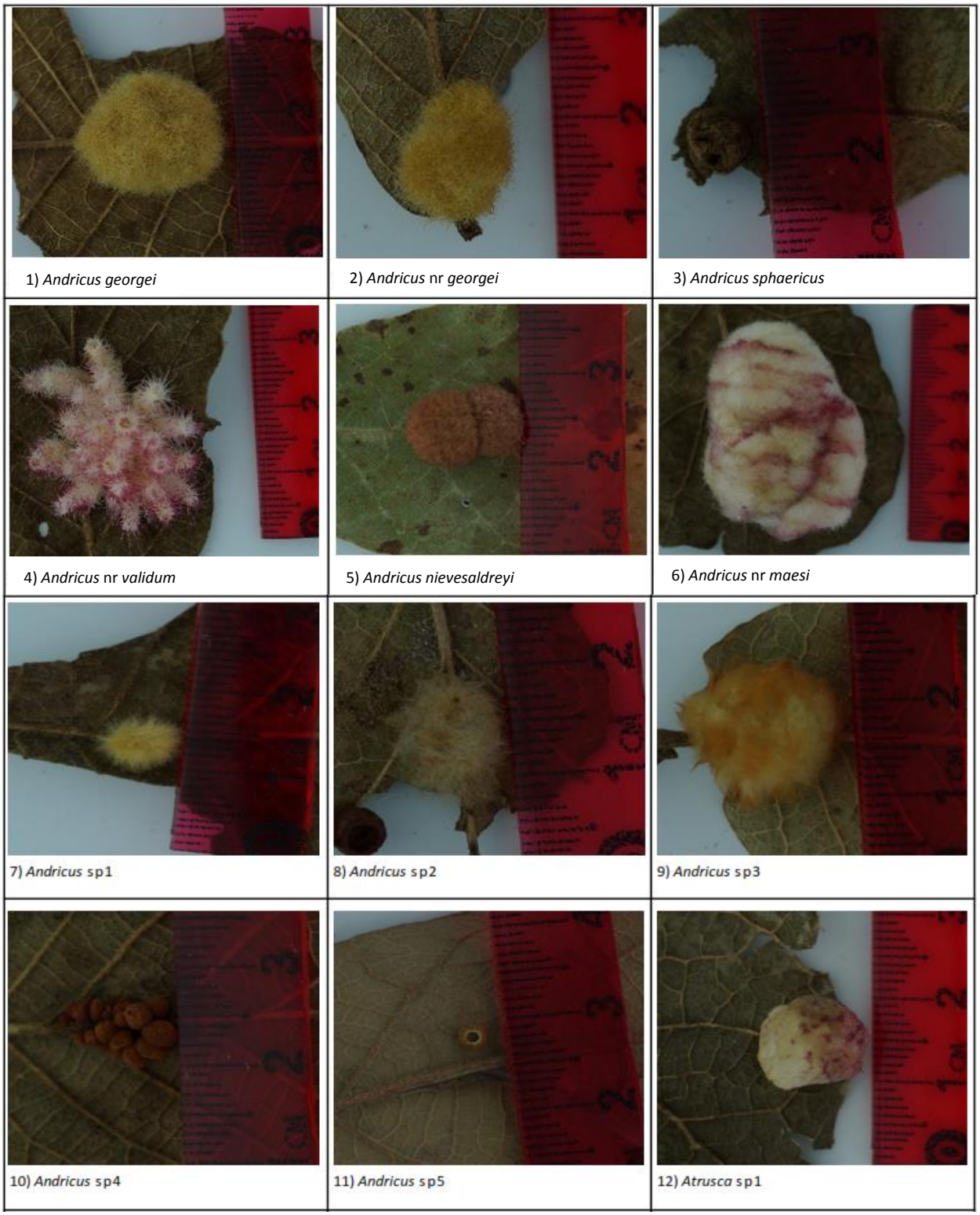
1) Andricus georgei
2) Andricus nr georgei
3) Andricus sphaericus
4) Andricus nr validum
5) Andricus nievesaldreyi
6) Andricus nr maesi
7) Andricus sp1
8) Andricus sp2
9) Andricus sp3
10) Andricus sp4
11) Andricus sp5
12) Atrusca sp1

Figura 2

Continuación figura 2

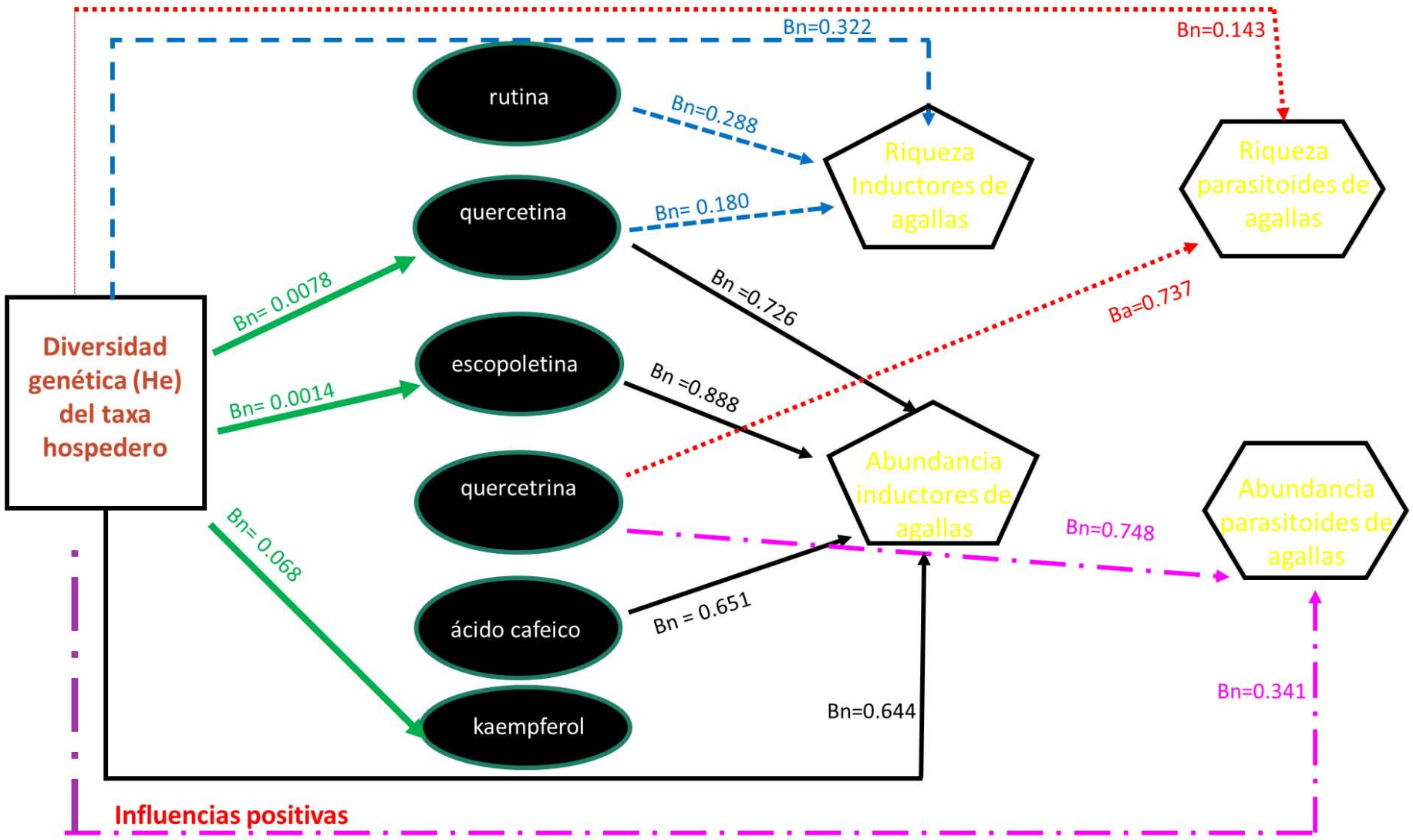
Bn=0.322
Bn=0.143
rutina
Bn=0.288
Riqueza Inductores de agallas
Riqueza parasitoides de agallas
quercetina
Bn= 0.180
Bn =0.726
Ba=0.737
Bn= 0.0078
Diversidad genética (He) del taxa hospedero
escopoletina
Bn =0.888
Bn= 0.0014
Abundancia inductores de agallas
quercetrina
Abundancia parasitoides de agallas
Bn=0.748
Bn= 0.068
ácido cafeico
Bn = 0.651
Bn=0.341
Bn=0.644
kaempferol
Influencias positivas

Figura 3a.

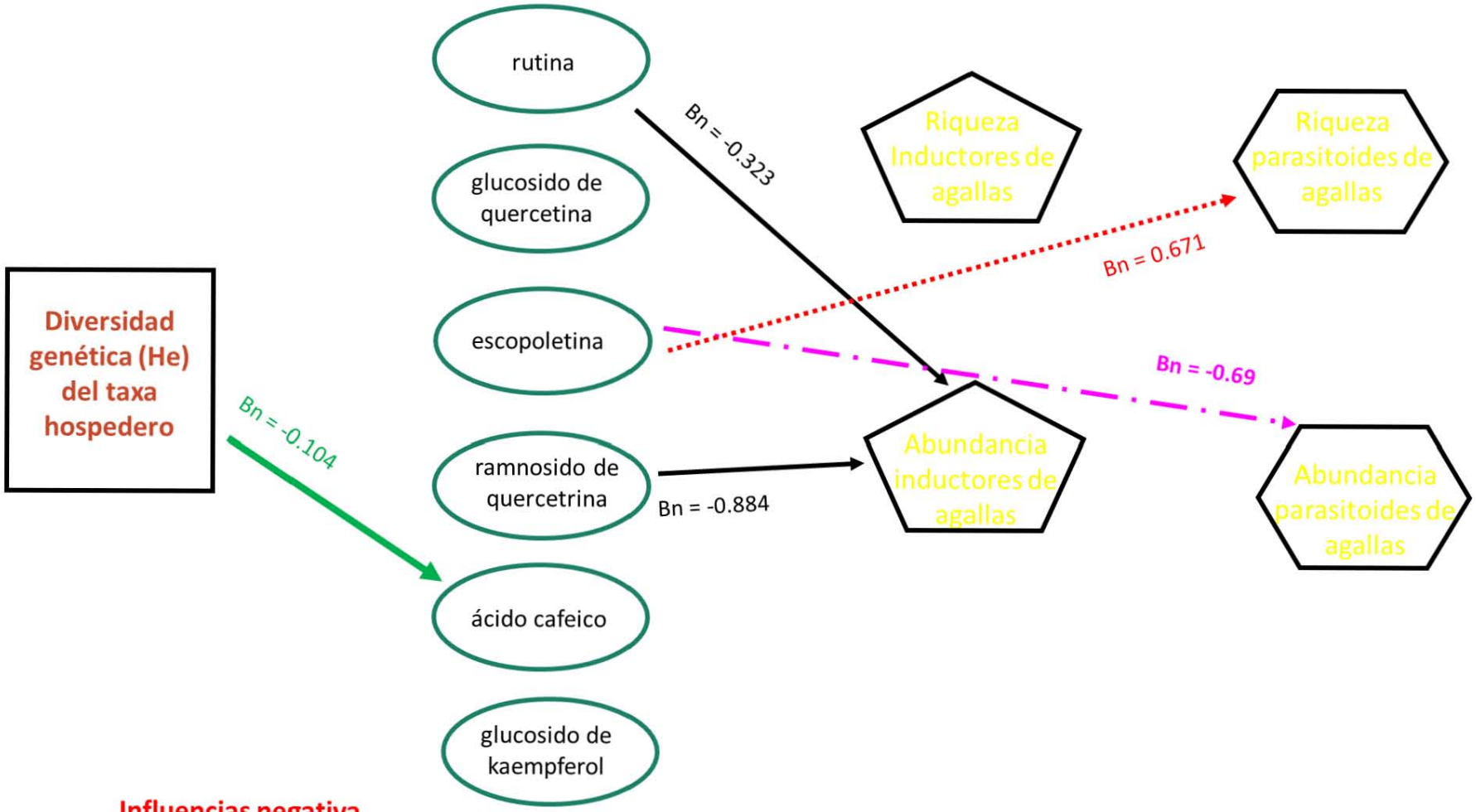
rutina
glucosido de quercetina
escopoletina
ramnosido de quercetrina
ácido cafeico
glucosido de kaempferol
Diversidad genética (He) del taxa hospedero
Bn = -0.104
Bn = -0.323
Bn = -0.884
Riqueza Inductores de agallas
Riqueza parasitoides de agallas
Bn = 0.671
Bn = -0.69
Abundancia inductores de agallas
Abundancia parasitoides de agallas
Influencias negativa

Figura 3b.

Tabla 1. Nombre de localidad, tamaño de muestra (N), altitud (m), coordenadas geográficas y taxón de encino [*Quercus glabrescens*, *Q. rugosa* y *Q. glabrescens* × *Q. rugosa* (Qg×Qr)] por localidad en la Faja Volcánica Transmexicana.

Tabla 2. Análisis no paramétrico de varianza (Krusskal-Wallis) para evaluar el efecto del taxón sobre la diversidad genética (*He*) y concentración [promedio ± d.e.] de compuestos fenólicos (flavonoides y cumarina) en el complejo *Quercus glabrescens* × *Q. rugosa*.

Letras diferentes denotan diferencias significativas P<0.05. *= P<0.05, **P=<0.01, ***P=<0.001. ND = no detectado.

Tabla 3. Listado de avispas inductoras de agallas y sus parasitoides asociados al dosel del complejo *Q. glabrescens* × *Q. rugosa*.

P es igual a presente, sin letra es igual a ausente.

Tabla 4. Promedios (±d.e.) y análisis de varianza para detectar el efecto del taxón (*Q. glabrescens*, *Q. rugosa* e híbrido) sobre la abundancia, riqueza y porcentaje de infestación de cinípidos y parasitoides asociados al dosel. Análisis de delta para detectar diferencias significativas entre pares de índices de diversidad (Shannon-Wiener, *H'*) de insectos inductores de agallas y sus parasitoides.

NOTA: letras minúsculas diferentes denotan diferencias significativas con una P < 0.05 (prueba de Tukey). Letras mayúsculas denotan diferencias significativas con una P < 0.05 (Solow, 1993; δ test).

Tabla 1

Localidad	N	Estado	Altitud (m)	Coordenadas (N-W)	Taxón
Sitio alopátrido					
Tlaxco	20	Tlaxcala	2,588	19°41'44.7'' - 98°4'49.1''	*Q. glabrescens*
Coajomulco	20	Morelos	2,667	19°2'3.5'' - 99°11'54.1''	*Q. rugosa*
Sitio simpátrido					
Huitzilac	30	Morelos	2,318	19°1' 57'' - 99°16' 34''	*Q, glabrescens*, *Q. rugosa*, híbrido
Omitlán de Juárez	30	Hidalgo	2,522	20°9'57'' - 98°39'16''	*Q, glabrescens*, *Q. rugosa*, híbrido

Tabla 2

	Taxón	Diversidad genética (*He*)	rutina ($H_{2, 48}$)	ácido cafeico ($H_{2, 48}$)	quercitina ($H_{2, 48}$)	quercitrina ($H_{2, 48}$)	kaemferol ($H_{2, 48}$)	escopoletina ($H_{2, 48}$)
Límite de detección (mg/g)			3.701	1.232	3.234	1.348	2.374	1.455
	Q. rugosa	0.637 a	4.90±0.20 a	3.59±0.35 a	5.78±0.23 a	16.46±1.53 a	10.65±2.00 ab	8.25±0.96 a
	Q. glabrescens	0.447 b	3.37±0.40 b	2.75±0.35 ab	3.55±0.03 b	6.64±0.39 b	5.95±0.51 b	2.62±0.16 b
	híbrido	0.699 c	0.0 c	1.87±0.44 b	4.17±0.12 c	2.44±0.09 c	8.47±0.29 a	1.49±0.24 c
	Kruskal-Wallis	73.794***	28.852***	6.411*	37.880***	38.470***	7.639*	34.455***

Tabla 3

Superfamilia	Familia	Género	Especies	*Q. rugosa*	*Q. glabrescens*	*Qg* × *Qr*
		Avispas agalleras				
Cynipoidea	Cynipidae	*Andricus*	*A. georgei*	P		P
			A. nievesaldreyi	P	P	
			A. nr georgei	P		P
			A. nr maessi	P		
			A. nr validum	P		
			A. sphaericus	P		P
			A. sp1	P		P
			A. sp2	P		
			A. sp3	P		P
			A. sp4	P		P
			A. sp5	P		
		Atrusca	*A*. grupo bulboides	P	P	P
			A. sp1	P		P
			A. sp2	P		P
			A. sp3	P		
		Cynips	*C*. sp1	P		P
			C. sp2	P	P	P
			C. sp3	P		P
			C. sp4	P		
		Disholcaspis	*D*. sp1	P	P	P
			D. sp2	P		P
			D. sp3	P	P	
		Dros	*D. perlentum*			P
		Neuroterus	*N*. sp1	P	P	P
		Parasitoides				
Chalcidoidea	Eulophidae	*Galeopsomyia*	*Galeopsomyia* sp	P	P	P
		Baryscapus	*Baryscapus* sp	P		
	Eurytomidae	*Sycophila*	*Sycophila* sp	P		
		Eurytoma	*Eurytoma* sp	P	P	
	Ormyridae	*Ormyrus*	*Ormyrus* sp	P	P	
	Torymidae	*Torymus*	*Torymus* sp	P	P	P

Tabla 4

Taxón		Abundancia	Porcentaje de infestación	Riqueza	Diversidad (*H´*)
	Avispas agalleras				
Q. rugosa		31.85±4.44 a	15.95±2.22 a	6.30±0.51 a	2.492 A
Q. glabrescens		12.35±2.20 b	6.17±1.10 b	1.90±0.39 b	0.821 B
Híbrido		5.35±1.35 b	2.67±0.67 b	2.25±0.39 b	2.429 A
Anova ($F_{2,97}$)		14.979***	15.717***	29.644***	
	Parasitoides				
Q. rugosa		6.50±1.15 a	23.16±3.49 a	1.42±0.14 a	0.620 AB
Q. glabrescens		0.70±0.27 b	9.46±3.88 b	0.25±0.07 b	0.905 A
Híbrido		0.75±0.42 b	11.38±5.80 b	0.25±0.09 b	0.362 B
Anova ($F_{2,97}$)		41.359***	6.282*	89.138***	

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CAPÍTULO III.

Caracterización química de *Q. glabrescens*, *Q. obtusata* y *Q. rugosa* (Fagaceae: *Quercus*) dentro de la Faja Volcánica Transmexicana

Artículo original

Para ser enviado a Journal Agricultural and Food Chemistry en su versión en inglés

Marcadores químicos de *Quercus rugosa*, *Q. glabrescens* y *Q. obtusata* en la Faja Volcánica Transmexicana

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Resumen

El estudio fitoquimico de las tres especies de encinos blancos (Fagaceae: *Quercus rugosa*, *Q. glabrescens* y *Q. obtusata*) permitió el aislamiento e identificación de nueve compuestos conocidos: β-sitosterol (**1**), β-amirina (**2**), ácido usólico (**3**), rutina (**4**), quercitina (**5**), ácido cafeico (**6**), escopoletina (**7**), glucósido de kaemferol (**8**) quercitrina (**9**). Las técnicas de identificación fueron CCF, HPLC y RMN de 1 y 2D.

Palabras clave: encinos, metabolitos secundarios, cumarinas, flavonoides, terpenoides

Introducción

De manera natural las plantas producen una gran cantidad de metabolitos secundarios que, aunque no están directamente relacionado con funciones esenciales si se encuentran asociados con otras funciones que impactan en su sobrevivencia y capacidad reproductiva. Por ejemplo, los metabolitos secundarios pueden cumplir con las siguientes funciones en las plantas que los producen: protección contra la luz UV, pigmentación, perfil aromático, hormonas vegetales, cofactores en funciones enzimáticas (catalíticos), compuestos alelopáticos, defensa contra la herbivoría o tener influencia sobre otro tipo de interacciones que establecen las plantas con diversos grupos biológicos [1-3]. Considerando la estructura química su solubilidad, así como la ruta biosintética a partir de la cual se desarrollan, los metabolitos secundarios pueden agruparse en familias tales como: terpenoides, flavonoides, compuestos fenólicos y polifenólicos aliados, alcaloides que contienen nitrógeno y compuestos que contienen azufre [4].

A nivel mundial el género *Quercus* (Fagaceae) está representado por aproximadamente 500 especies [5]. Se distribuye principalmente en las zonas templadas, subtropicales y semiáridas [6]. México contiene el 30.3% de esta diversidad (161 especies) y es considerado uno de los principales centros de diversificación para el género [7]. Además, se estima que los bosques de encino ocupan el 7.5% del territorio nacional [8] siendo particularmente dominantes dentro de la Faja Volcánica Transmexicana [FVT]. Recientemente se ha sugerido que la diversificación de este grupo vegetal en México está relacionada con la heretogeneidad geológica, gradientes climáticos y disponibilidad de hábitats que pueden ser ocupados por poblaciones de encinos provocando una alta variabilidad del género [7, 9, 10]. Finalmente, los encinos interactúan con diversos grupos biológicos (p. ej. plantas epifitas, artrópodos, aves, mamíferos pequeños), cumplen diversas funciones ecológicas (p. ej. reguladores climáticos, aportan biomasa para el ciclaje de nutrientes y balance hídrico) y son importantes en términos económicos para las poblaciones humanas (p. ej. alimentación, combustible, construcción) [5, 11-15].

A pesar del uso extensivo de algunas especies de encino en la industria vinícola, maderera y de alimentación animal (aves de corral y cerdos) [p.ej., 16-18] existen pocos estudios en donde se haya caracterizado su perfil

químico. En general, en dichos trabajos se ha documentado la presencia (cualitativa) de diversos metabolitos secundarios mayoritarios particularmente: taninos, fenoles, flavonoides, compuestos alifáticos, esteroides, glucósidos, hidrocarbonos y triterpenos [p. ej., 19-22].

Considerando que México ha sido propuesto como uno de los principales centros de diversificación del género *Quercus* y que existe un conocimiento limitado sobre la diversidad de metabolitos secundarios que este género puede presentar. El análisis de los perfiles metabólicos que presentan los encinos es una tarea de gran importancia dada las implicaciones ecológicas y económicas que dichos perfiles pueden tener, así como por las potenciales aplicaciones a la salud humana.

Quercus rugosa, *Q. glabrescens* y *Q. obtusata* son tres especies de encinos blancos (sección: *Quercus*) que se distribuyen ampliamente en México principalmente en la FVT a través de un amplio gradiente altitudinal (entre 620 y 3300 msnm), filogenéticamente cercanas [10] con una alta diversidad genética y con reportes de hibridación interespecífica [23-24]. Nuestro grupo de investigación ha trabajado en el aislamiento, purificación e identificación de diversos metabolitos secundarios que tienen implicaciones ecológicas, principalmente en la interacción planta-insecto. Con base en lo anterior, el objetivo de este trabajo fue caracterizar el perfil metabólico (compuestos mayoritarios) de tres especies de encinos blancos mediante el empleo de técnicas de identificación química (TLC, HPLC y RMN). Particularmente el estudio se centró en la expresión química de tres grupos de metabolitos secundarios: flavonoides, terpenoides y cumarinas.

Materiales y métodos

Material vegetal

Las poblaciones muestreadas en este estudio son las mismas previamente analizadas por Castillo-Mendoza et al. (23). Se utilizaron como poblaciones parentales (alopátridas) para evidenciar un proceso de hibridación natural entre *Q. glabrescens*, *Q. obtusata* y *Q. rugosa* en la FVT (Tlaxco, Chamilpa y Coajomulco) mediante el uso de marcadores genéticos y químicos. En total, se muestrearon 60 individuos (20/sitio/taxón). Todos los individuos muestreados fueron individuos maduros que no presentaban ningún daño aparente. Para la caracterización de los perfiles metabólicos se colectaron únicamente hojas maduras ya que se ha documentado

que este tipo de hojas presentan una alta concentración de metabolitos secundarios. Finalmente, para minimizar la influencia del ambiente así como de factores espaciales en la expresión de los metabolitos secundarios todos los sitios de colecta en donde se muestreo el material vegetal presentaron las siguientes características en común: bosques maduros con clima templado subhúmedo, suelo de origen volcánico o derivado de rocas ígneas o sedimentarias, en estos sitios no hay evidencia de disturbios a nivel local y todos los sitios comparten la misma historia geológica (están incluidos en la FVT).

Obtención de los extractos de acetona

Para identificar los compuestos mayoritarios, las hojas obtenidas de individuos de cada especie/sitio *Q. rugosa*, *Q. glabrescens* y *Q. obtusata* fueron secadas a temperatura ambiente y se trituraron para obtener 300 g de polvo fino de cada especie. El material seco y molido se extrajo con acetona (1.5 L, MERCK) mediante maceración dejándolo reposar 24 horas. Posteriormente el disolvente se eliminó con destilación a presión reducida con un evaporador rotatorio BUCHI R-114, este procedimiento se realizó por triplicado y de la misma manera para las otras dos especies. El extracto de acetona fue disuelto en una mezcla de acetona/metanol (1:1) y fue analizado por cromatografía en capa fina (CCF). Se utilizaron gel de sílice 60 y placas cromatográficas de Merck KGaA (Darmstadt, Alemania). El rendimiento obtenido para cada una de las especies fue de 8% para *Q. rugosa* (eaQr), 10% para *Q. glabrescens* (eaQg) y 11 % para *Q. obtusata* (eaQg]).

Separacion química de los tres extractos de acetona de (*Quercus rugosa*, *Q. glabrescens* y *Q. obtusata*)

Los extractos eaQg, eaQr y eaQo fueron sometidos a un proceso de fraccionamiento químico aplicando técnicas cromatográficas convencionales en forma consecutiva, se utilizó el modelo de cromatografía en columna abierta (CCA) para la obtención de compuestos mayoritarios presentes en *Quercus rugosa*, *Q. glabrescens* y *Q. obtusata* utilizando silica gel fase normal (70-230 mesh, Merk) y de fase reversa POLYGOPREP® 50 C_{18} (fase reversa, MACHEREY-NAGEL). Para el método de cromatografía en capa fina (CCF) se utilizaron placas de aluminio recubiertas de sílica gel 60 F_{254} (fase normal, Merck) y sílica gel RP-18 F_{254} (fase reversa,

Merck). Para la fase móvil se utilizaron diferentes solventes en mezclas como metanol (CH_3OH, BAKER), acetona (CH_3COCH_3, Merck), diclorometano (CH_2Cl_2, Baker), acetato de etilo (AcOEt, J. T. BAKER), *n*-hexano (Hex, Merck), metanol-HPLC (CH_3OH-HPLC, TECSIQUIM), agua-HPLC (H_2O-HPLC, Merck), acetonitrilo-HPLC (CH_3CN-HPLC, TECSIQUIM) y ácido trifluoroacético-HPLC (TFA, Merck). Las placas fueron reveladas con una lámpara de luz ultravioleta (UVGL-58, UVP, 254-365 nm UV, Cambrige, UK) y para la detección física de terpenos y flavonoides se utilizaron los reactivos de Komarovsky (KOM), sulfato sérico y NP/PEG (Natural Products-polyethylenglycol). El reactivo KOM contiene 1 ml de ácido sulfúrico-etanólico al 50% y 10 ml de 4-hidroxibenzaldehído metanólico al 2% los cuales son mezclados antes de ser usados. Una vez rociada la placa de aluminio con el reactivo KOM o sulfato sérico se calienta a 65°C durante 1-2 min, estos reactivos dan pruebas positivas para terpenos, saponinas etc. El reactivo NP-PEG contiene difenilboroloxietilamina (NP) y polietilenglicol-400 (PEG) disueltos en 40 ml de etanol. Después del revelado las CCF son puestas en la lámpara de UV-365 nm en donde si dan positivos para compuestos fenólicos y flavonoides hay una fluorescencia (Wagner y Bladt 1996).

También se utilizó el método HPLC que consta de un sistema cromatográfico de módulo de separación (Waters 2696) y un detector de serie de fotodiodos (Waters 2996) y una columna Licrosphere® (100 rp-18, 250 x 4 mm, 5 µm). se utilizaron como estándares de referencia al ácido cafeico, ácido ursólico, rutina y escopoletina. Se utilizó un método (flavonoides) en HPLC para analizar los extractos acetónicos de las tres especies de encinos, las fracciones y los compuestos. Una muestra (3 mg) de cada especie fue disuelta en metanol y fueron analizadas por separadas eaQg, eaQr y eaQo. Tanto las fracciones y los compuestos fueron disueltos a una concentración de 0.5 mg/ml. El tiempo de duración del método fue de 30 min con un flujo de 0.9 ml por minuto y la inyección de la muestra fue de 10 µl (ver cuadro 1). Se realizó un barrido de longitud de onda (λ) de 200-600 nm.

Cuadro 1. Método en el HPLC para flavonoides.

	Tiempo (min)	Flujo	%A	%D
1		0.90	100.0	0.0
2	1.00	0.90	100.0	0.0
3	2.00	0.90	95.0	5.0
4	3.00	0.90	95.0	5.0
5	4.00	0.90	70.0	30.0
6	20.00	0.90	70.0	30.0
7	21.00	0.90	50.0	50.0
8	23.00	0.90	50.0	50.0
9	24.00	0.90	20.0	80.0
10	25.00	0.90	20.0	80.0
11	26.00	0.90	0.0	100.0
12	27.00	0.90	0.0	100.0
13	28.00	0.90	100.0	0.0
14	30.00	0.90	100.0	0.0

Aislamiento e identificación de los compuestos (1-9).

El fraccionamiento cromatográfico de eaQr, eaQo y eaQg, se realizó de la siguiente manera (Columna 1, fase normal); se disolvieron 5 g de eaQr en acetona y se adsorbieron en 7 g de sílica gel fase normal. Se realizó dicho fraccionamiento en una columna de vidrio (30 x 1 cm) empacada con 60 g de silica gel (malla 70-230, Merck) con un sistema de gradientes, iniciando la elución con 100% diclorometano y un aumento de polaridad de 5% con metanol. Se realizaron colectas de 180 ml y se concentraron. Se obtuvieron 43 fracciones las cuales fueron analizadas por CCF y se reunieron de acuerdo con la similitud de sus compuestos (cuadro 2) obteniendo 9 reuniones ($eaQrC_1R_1$ A $eaQrC_1R_9$).

Cuadro 2. Resumen cromatográfico de eaQr

Sistema de elución CH_2Cl_2-MeOH	Fracciones reunidas	clave
100:0	17-21	$eaQrC_1R_1$
95:5	22	$eaQrC_1R_2$
90:10	23-24	$eaQrC_1R_3$
85:15	25-26	$eaQrC_1R_4$
80:20	27-29	$eaQrC_1R_5$
75:25	30-31	$eaQrC_1R_6$
70:30	32-35	$eaQrC_1R_7$
50:50	36-42	$eaQrC_1R_8$
0:100	43	$eaQrC_1R_9$

La presencia de los terpenos conocidos como β-sitosterol (**1**), β amirina (**2**) y ácido usólico (**3**) fue establecida por CCF en las fracciones QrC_1R_1 y QrC_1R_2 por comparación directa de estándares. Estos compuestos también

se encuentran presentes en las otras dos especies (*Q. glabrescens* y *Q. obtusata*). En la fracción eaQrC$_1$R$_5$ de la columna 1, se identificaron por HPLC, a la llamada rutina (**4**), a la quercitina (**5**) y al ácido cafeico (**6**) y por comparación directa en CCF con una muestra estándar, la presencia de una cumarina denominada escopoletina (**7**), en la fracción eaQrC$_1$R$_8$ se identificó por HPLC a la quercetrina (**9**) con un estándar comercial y por RMN de ^{1}H y ^{13}C.

La fracción eaQrC$_1$R$_4$ se le realizó una separación cromatográfica en una columna, con un sistema de gradientes en fase reversa agua-acetonitrilo, con colectas de 10 ml. Se obtuvieron 37 fracciones. El análisis cromatográfico se muestra en la siguiente tabla:

Cuadro 2. Resumen cromatográfico de eaQrC$_1$R$_4$

Sistema de elución H_2O-C_2H_3N	Fracciones reunidas	clave
100:0	17-21	eaQrC$_2$R$_1$
95:5	22	eaQrC$_2$R$_2$
90:10	23-24	eaQrC$_2$R$_3$
85:15	25-26	eaQrC$_2$R$_4$
80:20	27-29	eaQrC$_2$R$_5$
75:25	30-31	eaQrC$_2$R$_6$
0:100	32-35	eaQrC$_2$R$_7$
100	37	

Debido al contenido mayoritario de los compuestos se decidió separar a la fracción eaQrC$_2$R$_6$ en una columna en fase reversa (C$_2$FR$_2$) quedando el análisis cromatográfico de la siguiente manera:

Cuadro 3. Resumen cromatográfico de eaQr.

Sistema de elución H_2O-C_2H_3N	Fracciones colectadas	clave
100:0	1-5	eaQrC$_3$FR$_1$
90:10	6-10	eaQrC$_3$FR$_2$
80:20	11-15	eaQrC$_3$FR$_3$
70:30	16-20	eaQrC$_3$FR$_4$
60:40	21-25	eaQrC$_3$FR$_5$
50:50	26-30	eaQrC$_2$FR$_6$
40:60	31-35	eaQrC$_2$FR$_7$
30-70	36-40	eaQrC$_2$FR$_8$
20:80	41-45	eaQrC$_2$FR$_9$
10:90	46-55	eaQrC$_2$FR$_{10}$
5:95	56-67	eaQrC$_2$FR$_{11}$
0:100	68-70	eaQrC$_2$FR$_{12}$

En la fracción eaQrC$_2$FR$_4$ se observó un polvo amarillo soluble en metanol y de acuerdo a RMN de una y dos dimensiones se identificaron a la mezcla de glucósido de kaemferol (**8**) y quercitrina (**9**) en menor proporción. Para las otras dos especies en estudio (*Q. glabrescens* y *Q. obtusata*) se les realizó el mismo procedimiento.

Análisis con HPLC

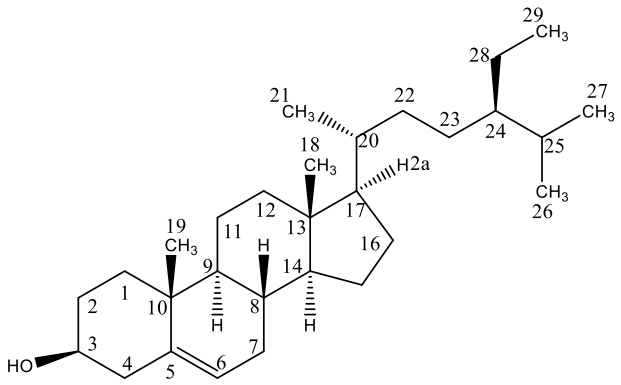
El análisis de los compuestos puros obtenidos se realizó en un módulo de separación Alliance 2695 (Waters) equipado con un detector de matriz de fotodiodos Waters 2695 y el software Empower Pro (Waters, Milford, MA, EE. UU.). La separación química se logró utilizando una columna Supelcosil LC-F (4,6 mm, 250 mm de diámetro, 5 µm de tamaño de partícula; Sigma-Aldrich). La fase móvil consistió en una mezcla de ácido trifluoroacético al 0.5% (disolvente A) y acetonitrilo (disolvente B). El sistema de gradiente empleado fue el siguiente: 0–1 min, 0% B; 2–4 min, 10% B, 5–7 min, 20% B; 8–14 min, 30% B; 15-18 minutos, 40% B; 19–22 min, 80% B; 23-26 min, 100% B; 27–28 min, 0% B. El flujo se mantuvo a 0,9 ml min^{-1}, y el volumen de inyección de la muestra fue 10 µl.

Los cromatogramas obtenidos de los extractos de acetona de *Quercus* spp. fueron comparados (*Q. rugosa*, *Q. obtusata* y *Q. glabrescens*). Cada compuesto tenía un tiempo de retención único y un espectro UV. La distribución de los compuestos fenólicos, los tipos de compuestos presentes en estos extractos y sus tiempos de retención se muestran en la Tabla 2. El análisis por HPLC de los extractos acetónicos a 312 nm mostró la presencia de seis metabolitos polifenólicos. Estos se identificaron por comparación directa de sus tiempos de retención y espectros UV con los de los estándares comerciales (Sigma-Aldrich).

Resultados

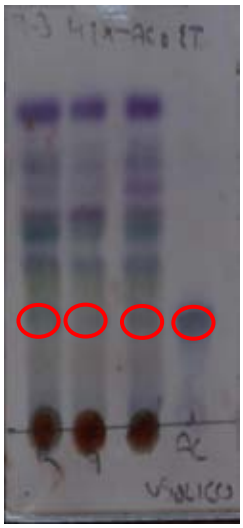
El β-sitosterol (**1**), fue obtenido como un polvo incoloro. Su fórmula molecular es $C_{29}H_{50}0$. El análisis de los espectro de RMN ^{1}H en $CDCl_3$ (600MHz, TMS, ppm) mostro señales en: δ 3.53 (1H, m, H-3), 5.35 (1H, d, J= 5.35Hz, H-6), 0.68 (3H, s, H-18), 1.01 (3H, s, H-19), 0.92 (3H, d, J=6.8 Hz, H-21), 0.81 (3H,d, J=6.8 Hz, H-26), 0.82 (3H,d, J=6.32 Hz, H-27), 0.84 (3H, t, H-29), y en el espectro de RMN ^{13}C mostró los siguientes desplazamientos (150 MHz, CDCl3; ppm): δ 37.25 (C-1), 31.66 (C-2), 71.81 (C-3), 42.30 (C-4), 140.75 (C-5), 121.72 (C-6), 31.91 (C-7), 31.91 (C-8), 50.13 (C-9), 36.51 (C-10), 21.09 (C-11), 39.77 (C-12), 42.32 (C-13), 56.77 (C-14), 24.30 (C- 15), 28.24 (C-16), 56.06 (C-17), 11.86 (C-18), 19.40 (C-19), 36.14 (C-20), 18.77 (C-21), 33.95 (C-22), 26.08 (C-23),

45.84 (C-24), 29.15 (C-25), 19.03 (C-26), 19.81 (C-27), 23.07 (C-28), 11.98 (C-29). (ver espectros en anexos Fig1A-5A). Los datos espectroscópicos fueron comparados con los descritos en la literatura (25-26).



1

La identificación de β-amirina (**2**) y ácido ursólico (**3**) fue a través de CCF comparada con muestras comerciales.

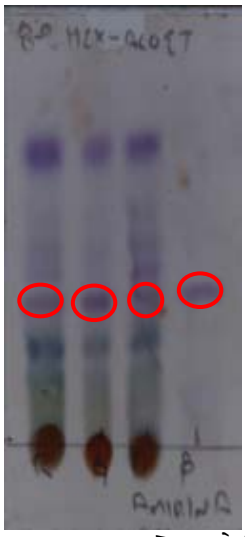


Fase normal

Sistema 8-2:

n-hexano-acetato de etilo

Q. rugosa
Q. glabrescens
Q. obtusata
Ácido usólico, referencia

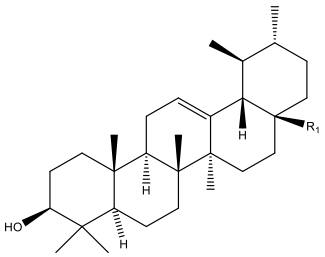


Fase normal

Sistema 7-3:

n-hexano-acetato de etilo

Q. rugosa
Q. glabrescens
Q. obtusata
B-amirina, referencia



2 R_1=CH_3

3 R_1 = COOH

Finalmente, la identificación de los compuestos rutina (**4**), quercitina (**5**), ácido cafeico (**6**), escopoletina (**7**), glucósido de kaemferol (**8**) y quercitrina (**9**). Se realizó a través de HPLC.

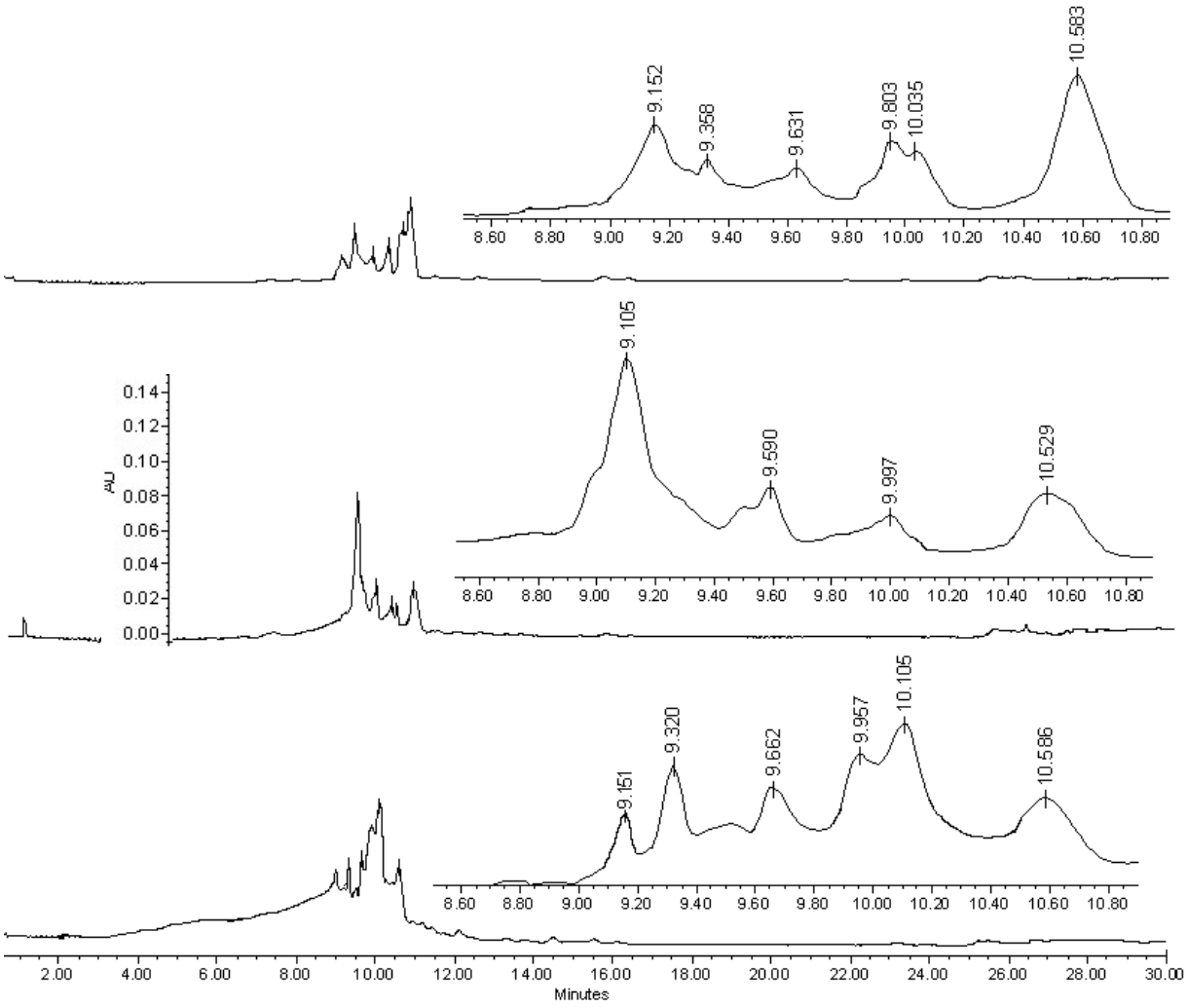
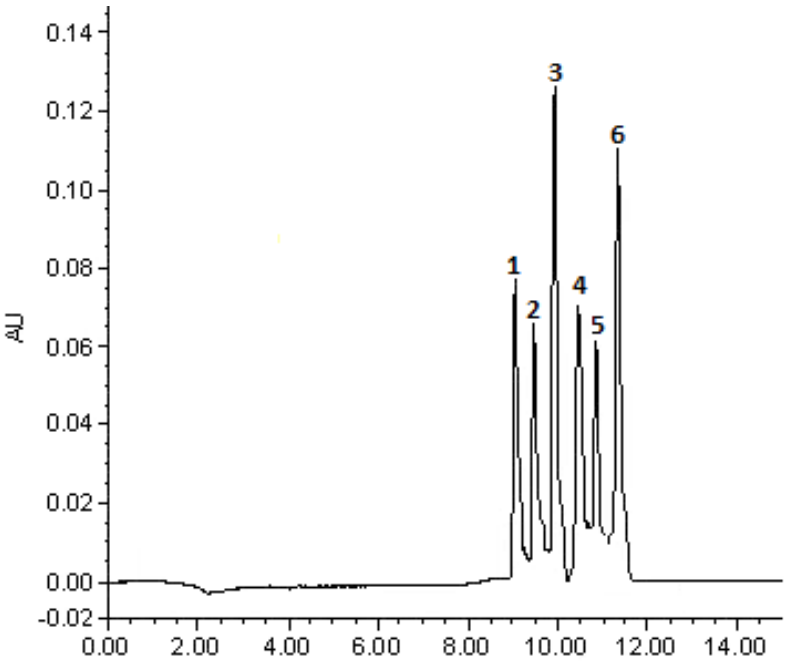
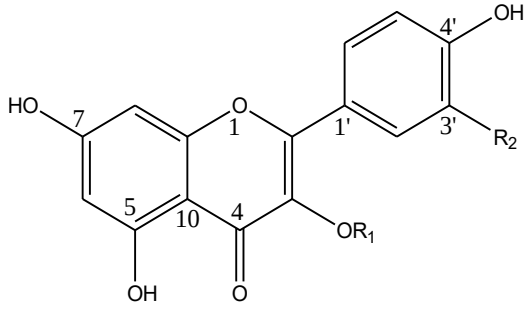
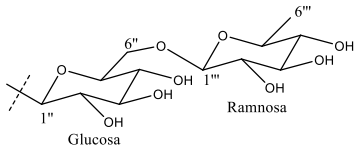


Tabla 1. compuestos fenólicos mayoritarios presentes en tres especies de encinos blancos y referencia comercial.

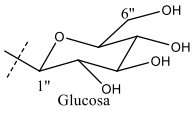
Especie	quercetin-3-rutinósido (1)	ácido cafeico (2)	quercitina (3)	3-*O*-glucosido de kaemferol (4)	3-O-ramnósido de quercitina (5)	escopoletina (6)
Q. rugosa	9.152	9.358	9.631	9.803	10.035	10.583
Q. obtusata	9.105	ND	ND	9.590	9.997	10.529
Q. glabrescens	9.151	9.320	9.662	9.957	10.105	10.586
Referencia comercial	9.071	9.226	9.530	9.960	10.067	10.524

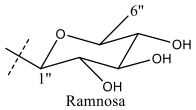


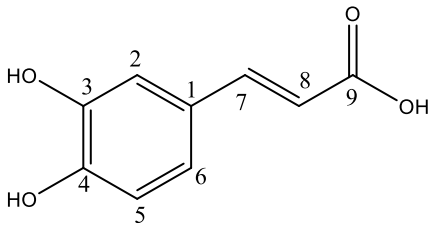


4 R_1 =  R_2 = OH

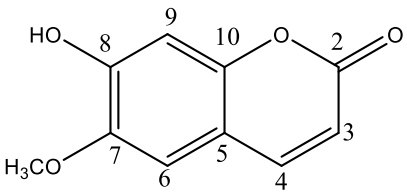
5 R_1 = H R_2 = OH

8 R_1 =  R_2 = H

9 R_1 =  R_2 = OH



6



7

Cromatograma 2. Picos generados por las referencias comerciales utilizadas para la confirmación de la presencia de metabolitos secundarios mayoritarios presentes en tres especies de encinos blancos. En la

imagen: (1) rutina, (2) ácido cafeico, (3) quercitina, (4) glucósido de kaemferol, (5) quercitrina y (6) escopoletina.

El compuesto **8** y **9**, fueron obtenidos como un polvo amorfo amarillo identificados como la mezcla de glucósido de kaemferol (**8**) y quercetrina (**9**) en menor concentración.

El análisis de RMN de ^{1}H en CD_3OD (600MHz, TMS, ppm), mostraron señales características de un flavonol; δ 8.06 (2H, d, J=8.39 Hz, H-2' y H-6'), 6.89 (2H, d, J=8.39 Hz, H-3' y H-5'), 6.38 (1H, br, s, H-8) y 6.19 (1H, br, s, H-6): adicionalmente un azúcar en δ 5.24 (1H, d, J=7.6 Hz, H-1''), 3.46 (1H, dd, J=9.1, 9.1 Hz, H-2''), 3.42 (1H, dd, J=9.1, 9.1 Hz, H-3''), 3.31 (1H, m, H-4''), 3.23 (1H, m, H-5''), 3.69 (1H, dd, J=2.2, 12.2 Hz, H-6a'') y δ 3.56 (1H, dd, J=6.1, 12.2 Hz, H-6b'') correspondiendo a la glucosa. Los datos del espectro de RMN de ^{13}C mostraron los siguientes desplazamientos δ 157.57 (C-2), 134 (C-3), 178 (C-4), 161.6 (C-5), 98.6 (C-6), 165.1 (C-7), 93.4 (C-8), 157.1 (C-9), 104.1 (C-10), 121.3 (C-1'), 138.8 (C-2' y C-6'), 114.6 (C-3' y C-5'), 160.14 (C-4'), 102.7 (C-1''), 74.3 (C-2''), 76.0 (C-3''), 69.9 (C-4''), 76.97 (C-5''), 61.20 (C-6''). ver espectros en anexos (Fig. 6A-11A).

Discusión

Los cambios en la expresión metabólica de las tres especies se encuentran en la tabla (1). A nivel mundial existen diversos trabajos donde se ha documentado la expresión de diferentes metabolitos secundarios presentes en las hojas de los encinos. Aunque la caracterización de los perfiles metabólicos se ha realizado empleando diferentes técnicas y con diferentes propósitos de manera general, dichos estudios se han realizado desde dos perspectivas: por un lado, se ha caracterizado de manera muy fina la expresión de diferentes metabolitos secundarios. Por ejemplo, una revisión llevada a cabo por Glasby (19) documentó el estudio de 23 especies de encinos en donde las familias de metabolitos secundarios presentes son: compuestos alifáticos, esteroides, triterpenoides, hidrocarburos, taninos, compuestos fenólicos y benzofuranos.

Asimismo, Yarnes et al. (20-21) reportan la diversidad de metabolitos secundarios presentes en individuos híbridos del complejo *Q. gambelii* × *Q. grisea*: fenóles (ácido cumarilquinico, ácido vescavaloninico, ácido castavaloninico etc.) y flavonoides (quercetina, glucósido de kaemferol, etc.). además, Noori et al. (27), con

Quercus brantii, reportó una amplia diversidad metabolitos secundarios flavonoides: apigenina, glucósido de kaemferol, rutina, etc.). En contraparte, Makkar et al. (28) documentaron la presencia de diversos metabolitos secundarios, pero solamente a nivel de familia. Entre ellos se encuentran: fenoles totales, taninos condensados, Procianidólicos, flavonoides, flavan-4-ol, gallotaninos y proantocianidinas. Finalmente, una revisión hecha por Vaca-Sánchez et al. (29) documentó la presencia de diferentes taninos expresados en 17 especies: ácido castavaloninico, ácido. vescavaloninico, acutisimina A/B, castalagina, catequinas, cocciferina, elagitanina, gallotaninos, mongolinina A, oenotenina B, pedunculagina, proantocianidinas, procianidinas, prodelfinidinas y vescalagina.

Para el caso de encinos mexicanos, pocos estudios han documentado la presencia de metabolitos secundarios tanto a nivel fino como a nivel de familia. A continuación, se presentan en orden cronológico dichos trabajos. Yarnes et al. (30) en 12 especies de encinos documentó la presencia de 23 tipos de metabolitos secundarios pertenecientes a 3 distintos grupos: ácidos fenólicos, elagitaninos y glucósidos flavonoides. Por su parte, Moctezuma et al. (31), mencionan la presencia de taninos y flavonoides presentes en *Q oleoides* de forma detallada: Hexahudroxydifenoilglucosa, Di-hexahidroxydifenoilglucosa, Vescalagina, Flavan-3-oles, catequinas, glucósido de kaemferol y quercetrina.

Asimismo, Maldonado-López et al. (32) describieron la presencia de diversos metabolitos secundarios a nivel de familia. Entre las que se encuentran: fenoles totales, taninos condensados, Procianidólicos, flavonoides, flavan-4-ol, gallotaninos y proantocianidinas. Por último, Castillo Mendoza et al. (23) documentaron la presencia de 10 diferentes tipos de compuestos fenólicos (p. ej. rutina, ácido cafeico, quercetina, quercetrina) en tres especies de encinos blancos. Lo anterior dificulta la comparación con los resultados obtenidos en este estudio, aunque de manera general se puede concluir que los encinos presentan una amplia diversidad de flavonoides, taninos y terpenoides.

El análisis de los metabolitos secundarios mediante el empleo de diferentes técnicas permitió observar de manera fina la constitución de los compuestos mayoritarios que presentan las especies de estudio. Aunque es posible que diversos grupos o familias de metabolitos secundarios falten por ser caracterizados en las especies

analizadas en este estudio, el acercamiento alcanzado en este trabajo resulta importante pues abre la posibilidad de realizar más análisis para estas u otras especies de encinos. Lo que a corto o mediano plazo contribuirá a conocer las variaciones cualitativas y en los perfiles metabólicos de los encinos, así como sus posibles implicaciones ecológicas y/o económicas.

En pocos trabajos se ha realizado el análisis específico del perfil metabólico que presentan las especies de encinos, posiblemente por las dificultades técnicas, económicas, así como la falta de conocimiento (en estudios ecológicos) pueden limitar fuertemente el análisis de los metabolitos secundarios (mayoritarios) que conforman a las diferentes especies de encinos. La identificación específica de cualquier metabolito secundario requiere de: a) el aislamiento y purificación de dicho compuesto (mediante cromatografía de columna) y b) una concentración mínima de cada compuesto puro (para realizar cromatografía líquida de alta resolución y resonancia magnética nuclear [HPLC, RMN]). Por lo que, en los análisis donde se requiere conocer la identidad específica de cualquier metabolito secundario, se realiza únicamente con la identificación de compuestos mayoritarios.

Dependiendo de la especie de estudio, así como la parte analizada dentro de la misma (rama, tronco, hojas o frutos), las especies de encinos muestran variaciones cualitativas en el perfil metabólico que presentan (33-38). De manera general, se ha reportado que las especies de encinos presentan metabolito secundario con diversas actividades biológicas que tienen impacto en su relación con otros grupos biológicos: atrayentes de insectos, inhibidores de la alimentación, reguladores de la cadena respiratoria, citotóxicos, reguladores del desarrollo larval (39-40).

En conclusión, en las hojas de los encinos puede encontrarse una amplia diversidad de metabolitos secundarios que pertenecen principalmente a tres grupos: taninos, flavonoides y terpenoides. La similitud en la expresión de los compuestos en las diferentes especies de encinos puede estar relacionada con: a) la cercanía filogenética que presentan (10). Por lo que podría suponerse que comparten las mismas rutas metabólicas. B) dados los frecuentes eventos de hibridación que se presentan dentro del género (23) podría haber un constante flujo de información que conduce a una expresión similar en diversos metabolitos secundarios y c)

condiciones ambientales similares podrían favorecer la expresión de perfiles metabólicos similares. Dado lo anterior, el análisis del perfil químico en los encinos puede ayudar a entender diversos procesos biológicos o ecológicos en los que están involucradas tanto las especies vegetales como los organismos asociados a ellas. Por ejemplo, Castillo-Mendoza et al. (datos no publicados) encontraron que la expresión cualitativa y cuantitativa de diferentes metabolitos secundarios puede afectar de manera positiva y/o negativa tanto a las comunidades de insectos herbívoros especialistas como a los parasitoides asociados.

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Anexos

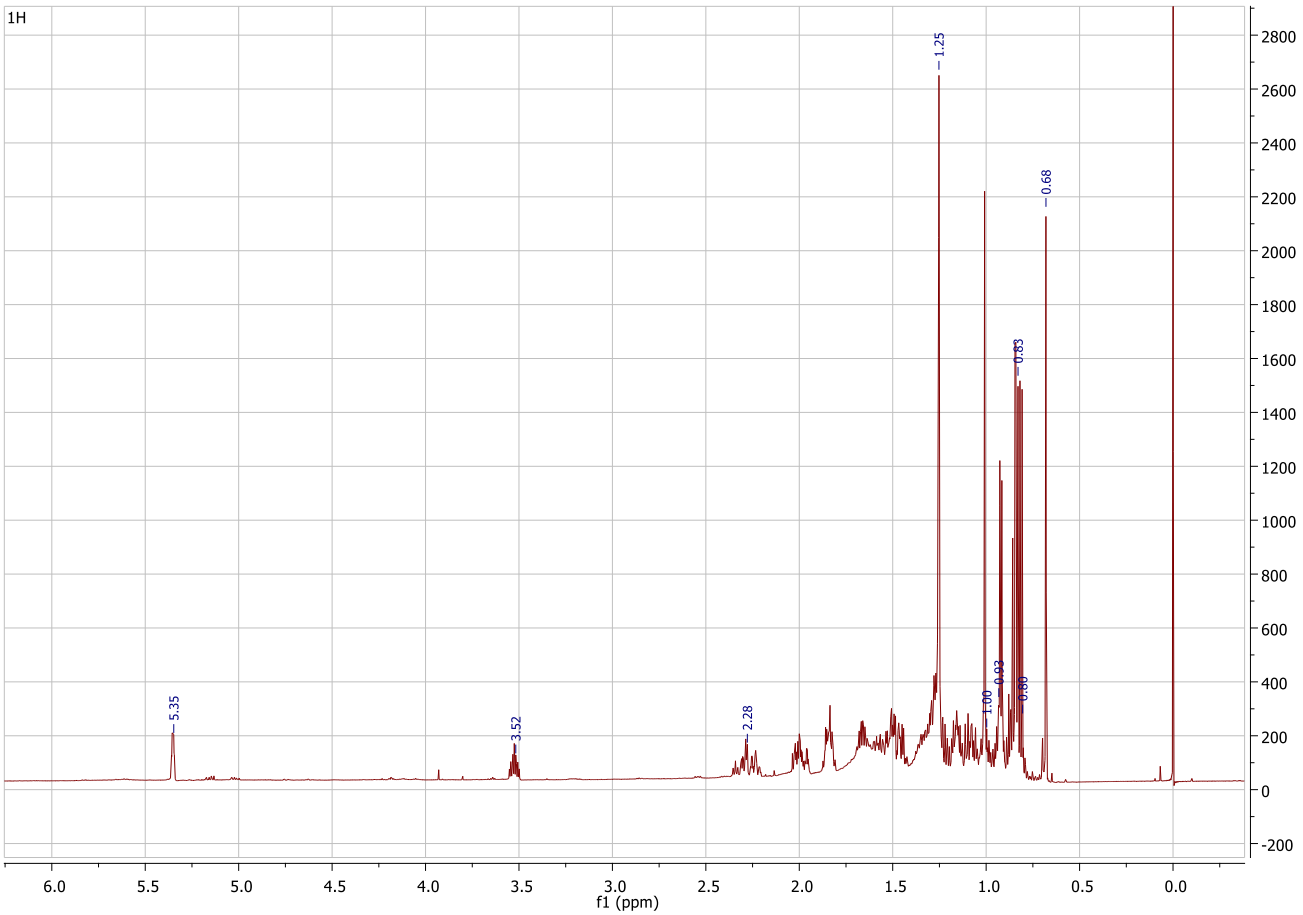

Figura 1A. Espectro de RMN ^{1}H (600 MHz, $CDCl_3$) del β-sitosterol (**1**)

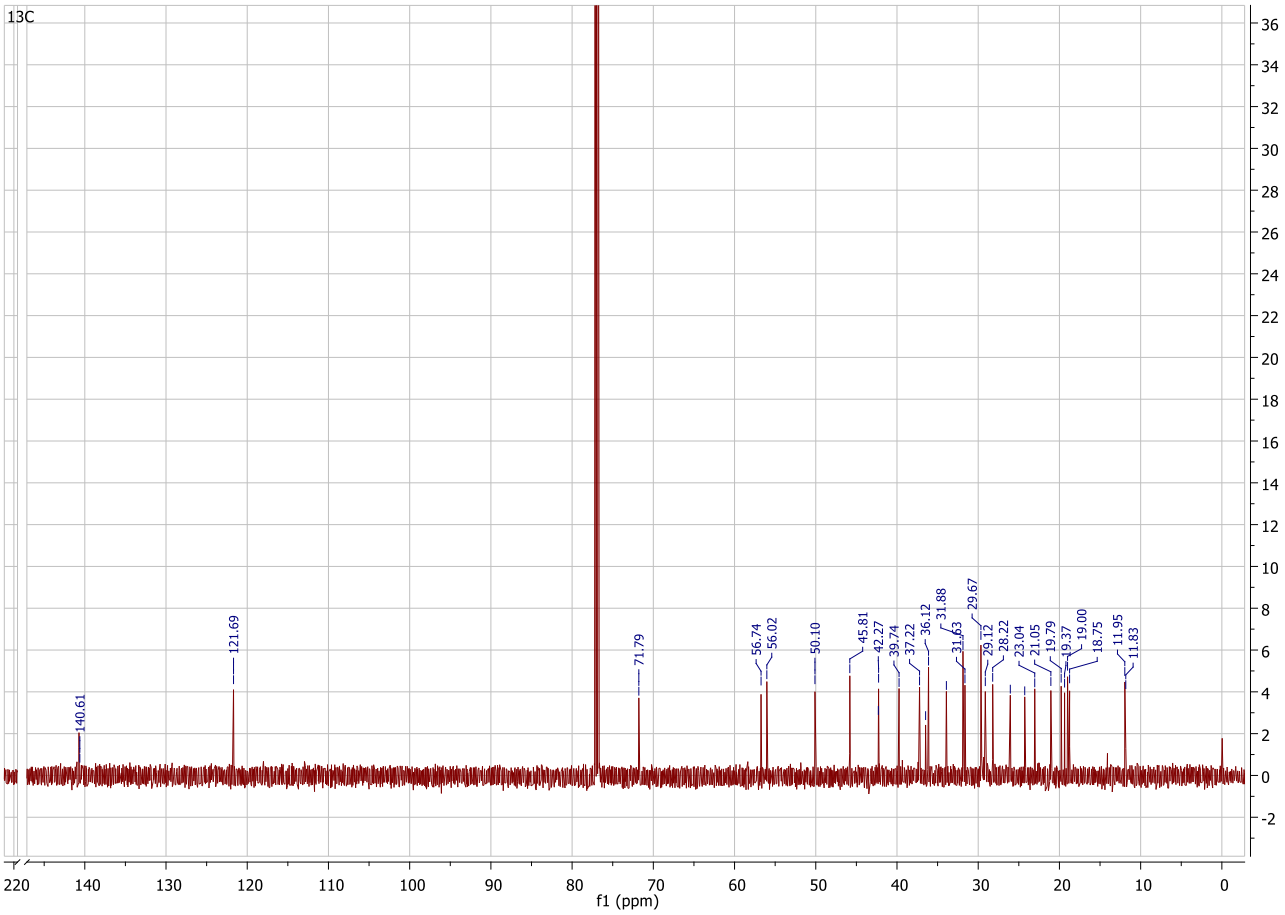

Figura 2A. Espectro de RMN ^{13}C (150 MHz, $CDCl_3$) del β-sitosterol (**1**)

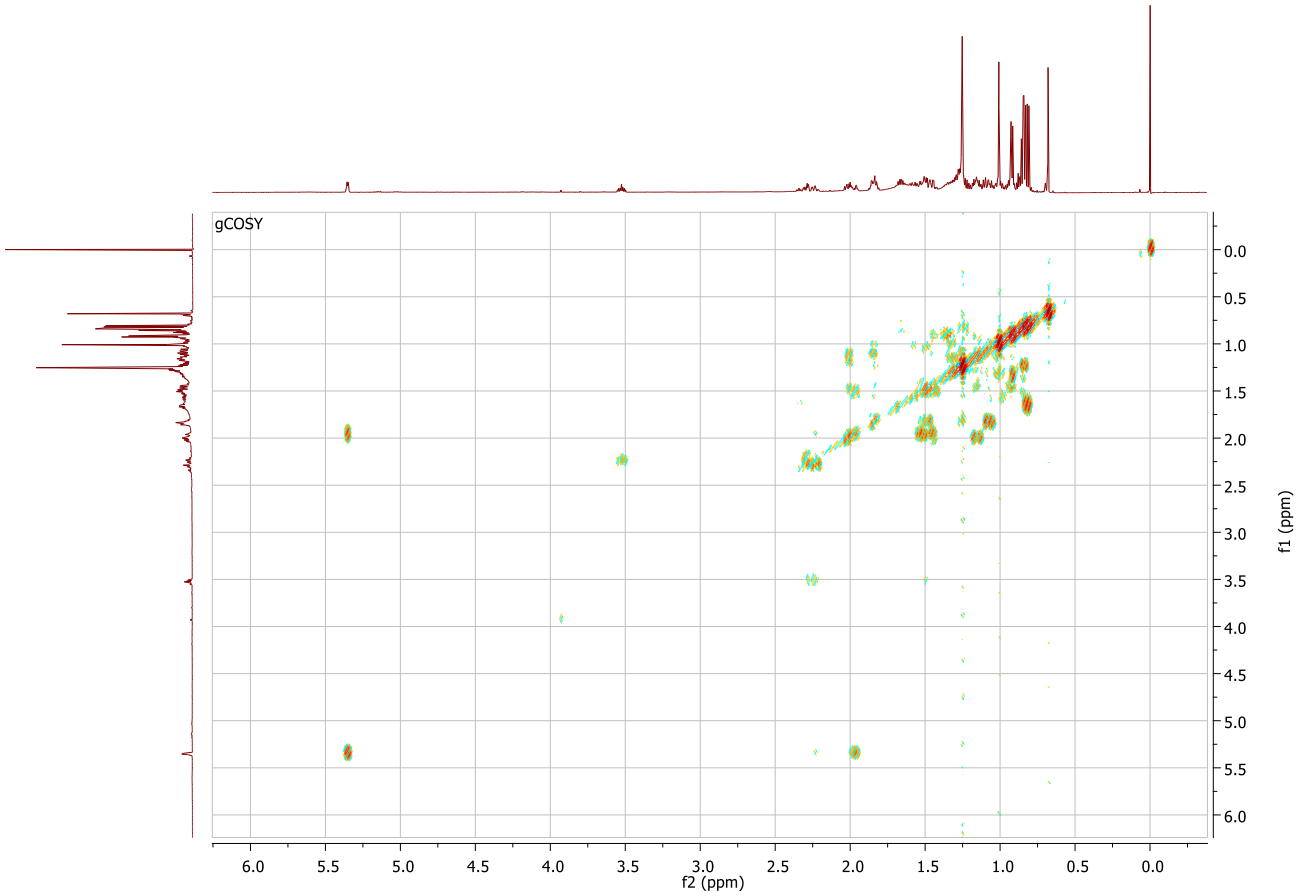

Figura 3A. Espectro de RMN ^{1}H-^{1}H (COSY, 600 MHz, $CDCl_3$) del β-sitosterol (**1**)

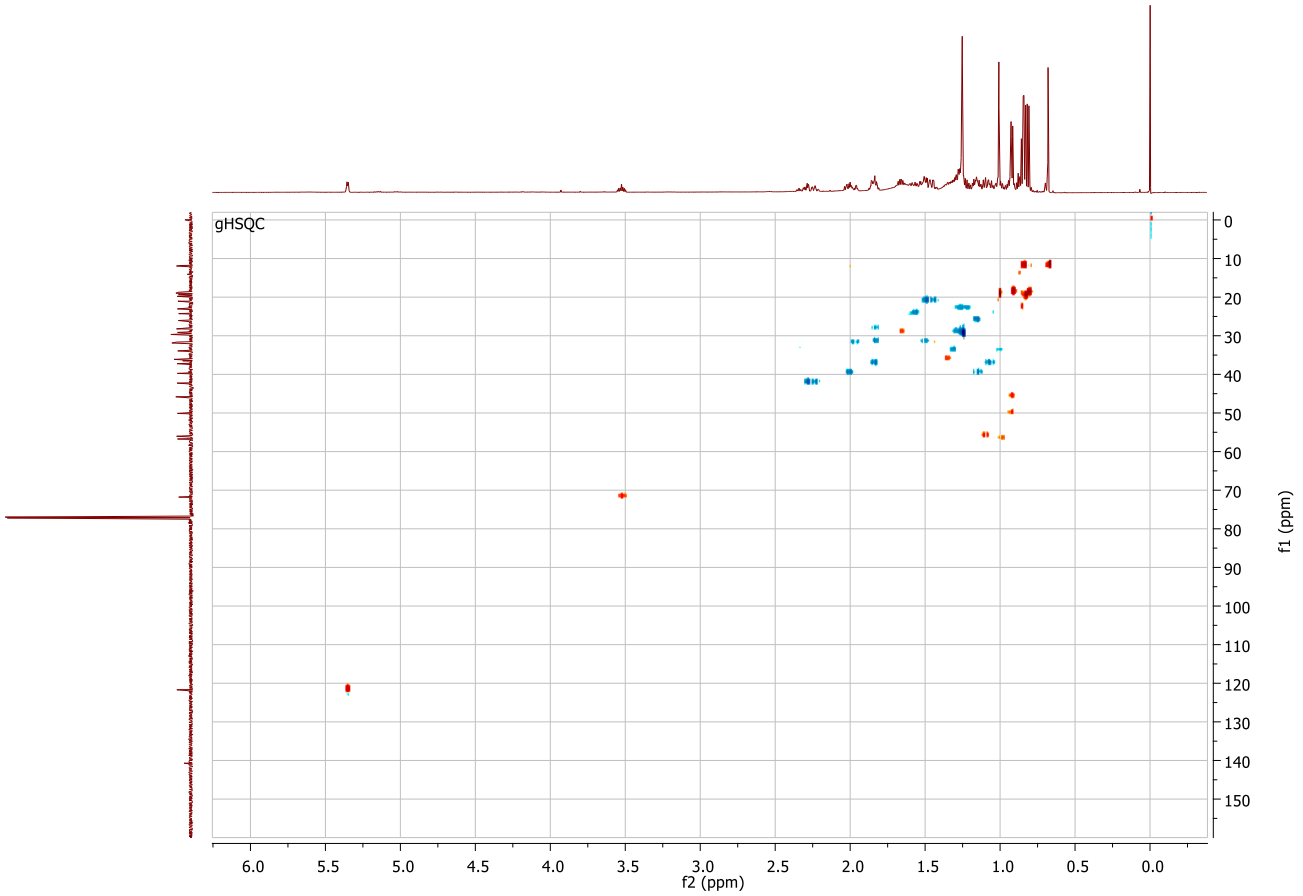

Figura 4A. Espectro de RMN de ^{1}H-^{13}C (HSQC, 600 MHz, $CDCl_3$) del β-sitosterol (**1**)

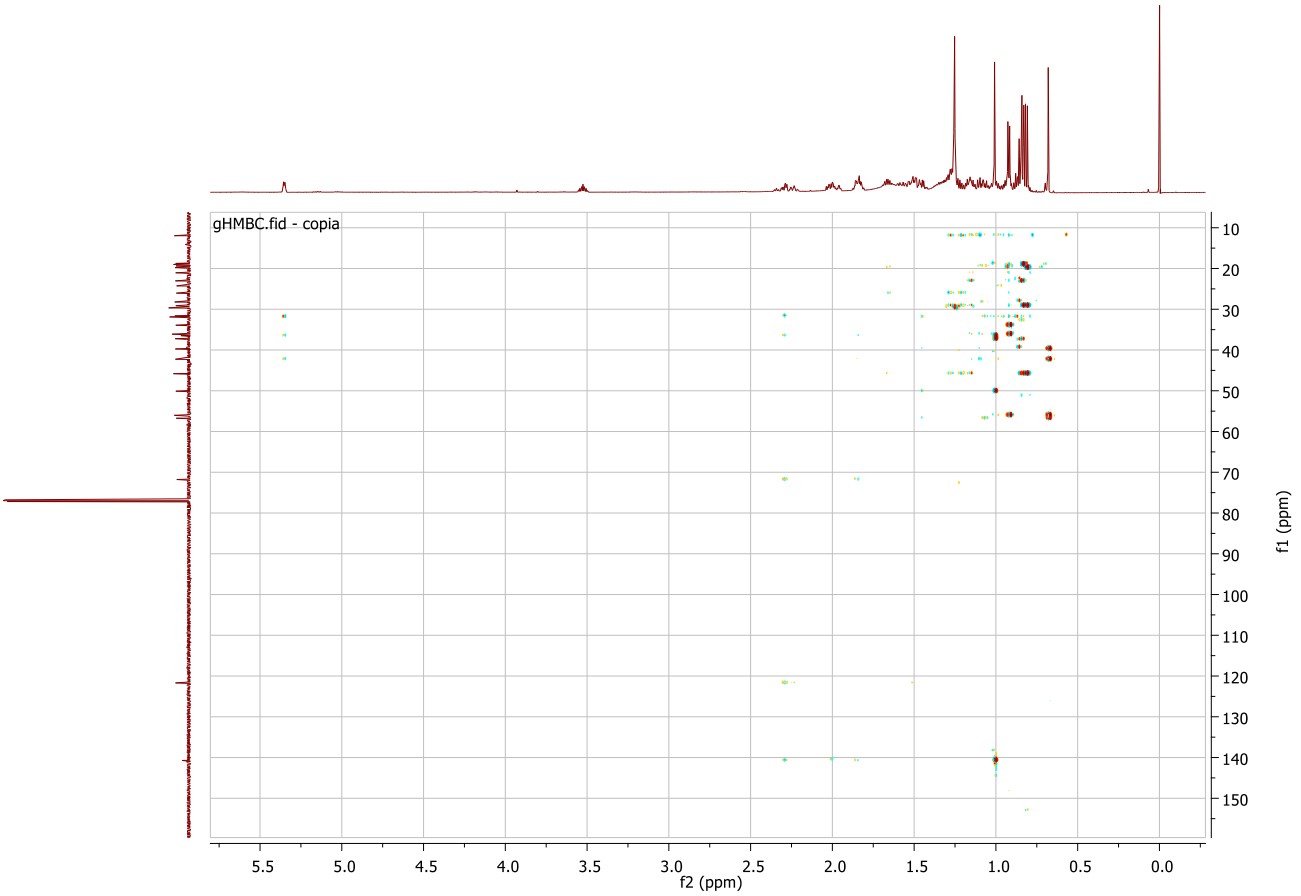

Figura 5A. Espectro de RMN de ^{1}H-^{13}C (HMBC, 600 MHz, $CDCl_3$) del β-sitosterol (**1**)

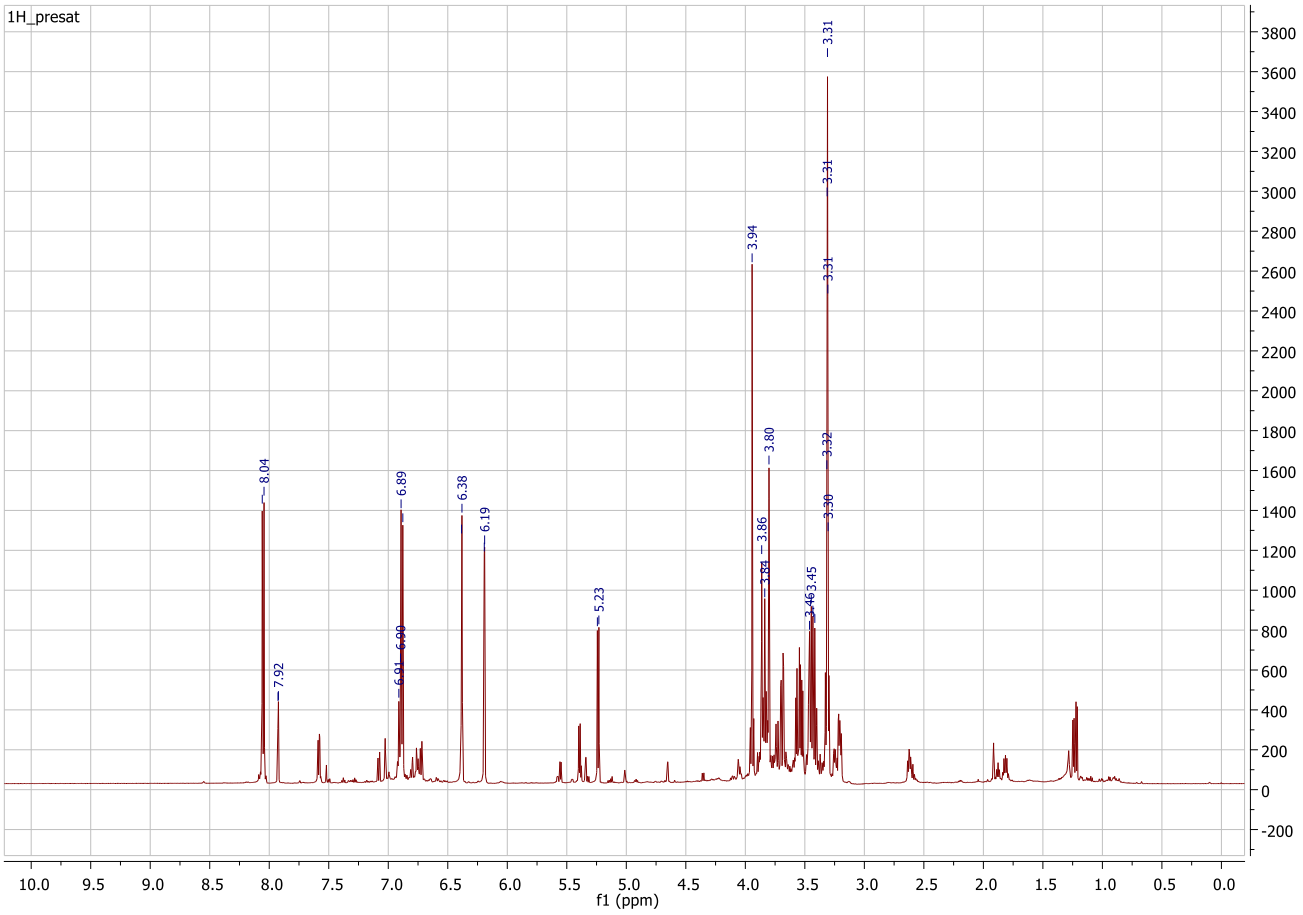

Figura 6A. Espectro de RMN ^{1}H (600 MHz, CD_3OD) del 3-*O*-glucósido de kaemferol (**8**)

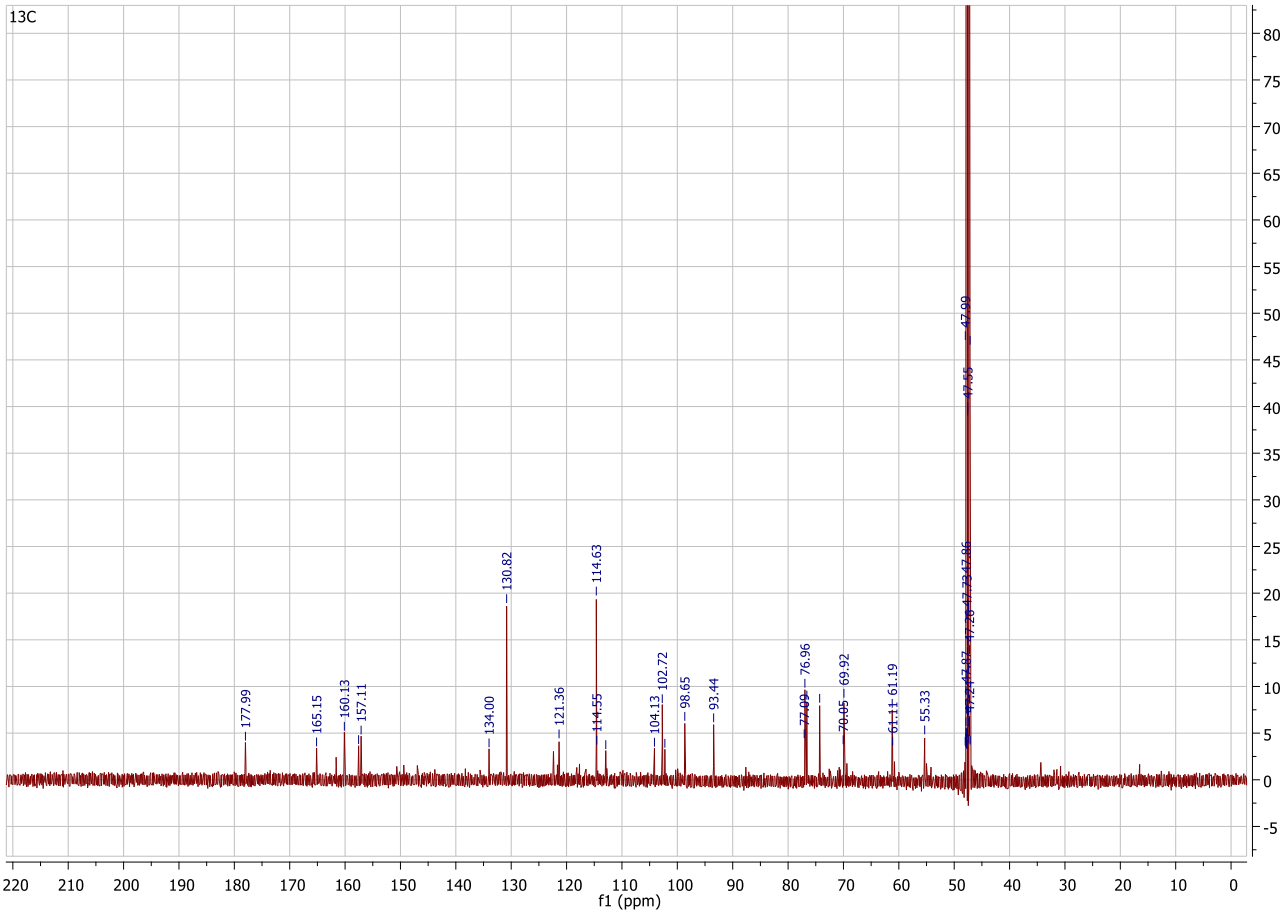

Figura 7A. Espectro de RMN ^{13}C (150 MHz, CD_3OD) del 3-*O*-glucósido de kaemferol (**8**)

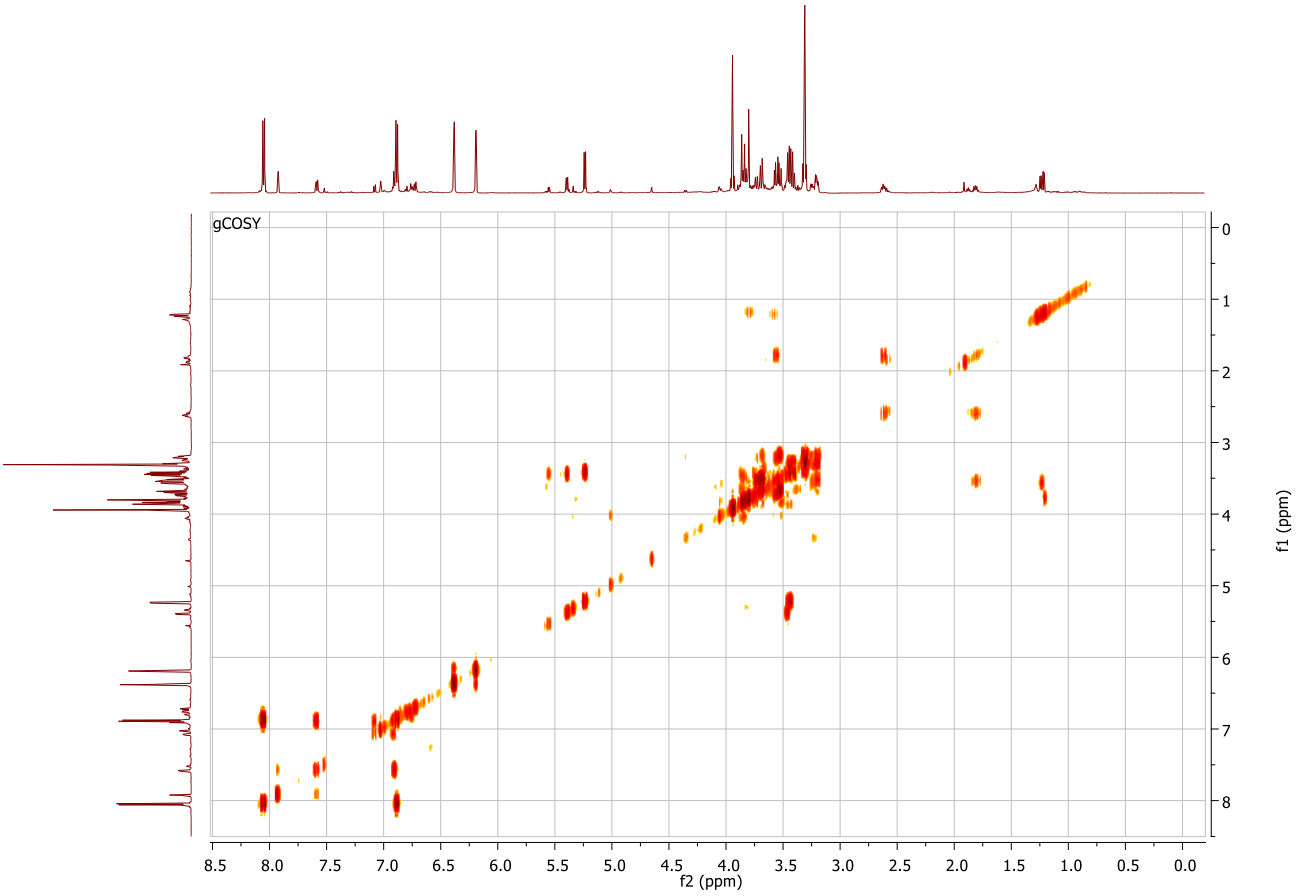

Figura 8A. Espectro de RMN ^{1}H-^{1}H (COSY, 600 MHz, $CDCl_3$) del 3-*O*-glucósido de kaemferol (**8**)

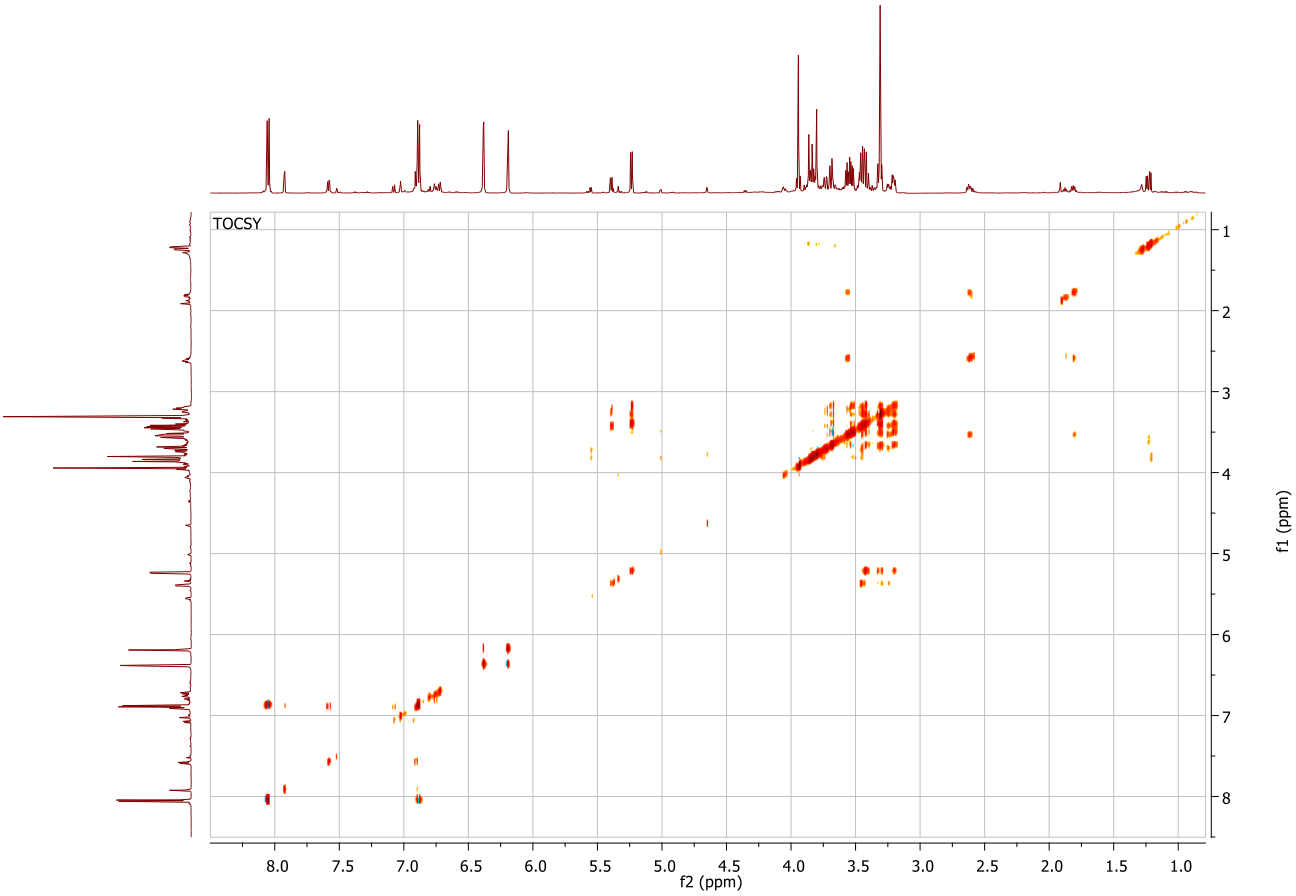

Figura 9A. Espectro de RMN ^{1}H-^{1}H (TOCSY, 600 MHz, $CDCl_3$) del 3-*O*-glucósido de kaemferol (**8**)

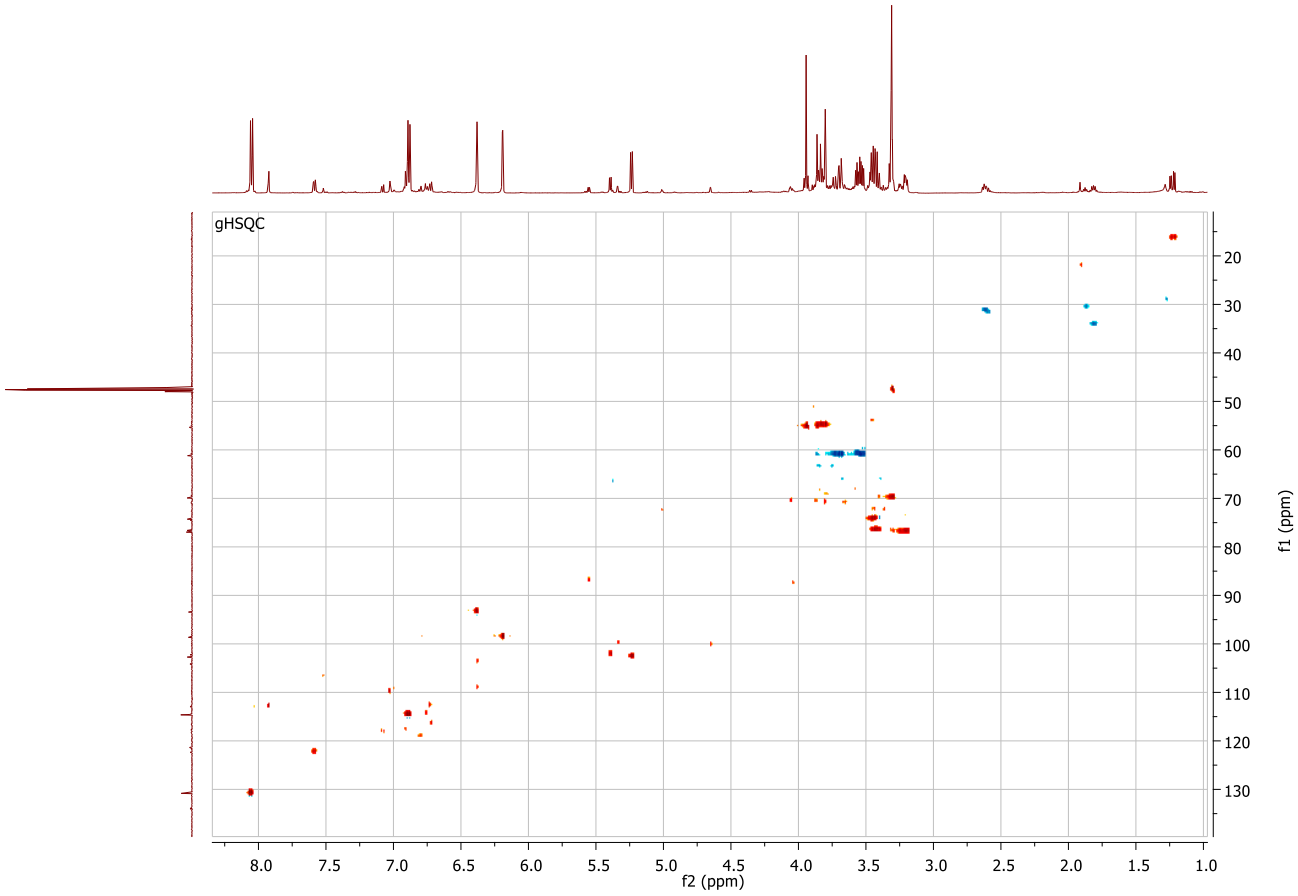

Figura 10A. Espectro de RMN ^{1}H-^{13}C (HSQC, 600 MHz, $CDCl_3$) del 3-*O*-glucósido de kaemferol (**8**)

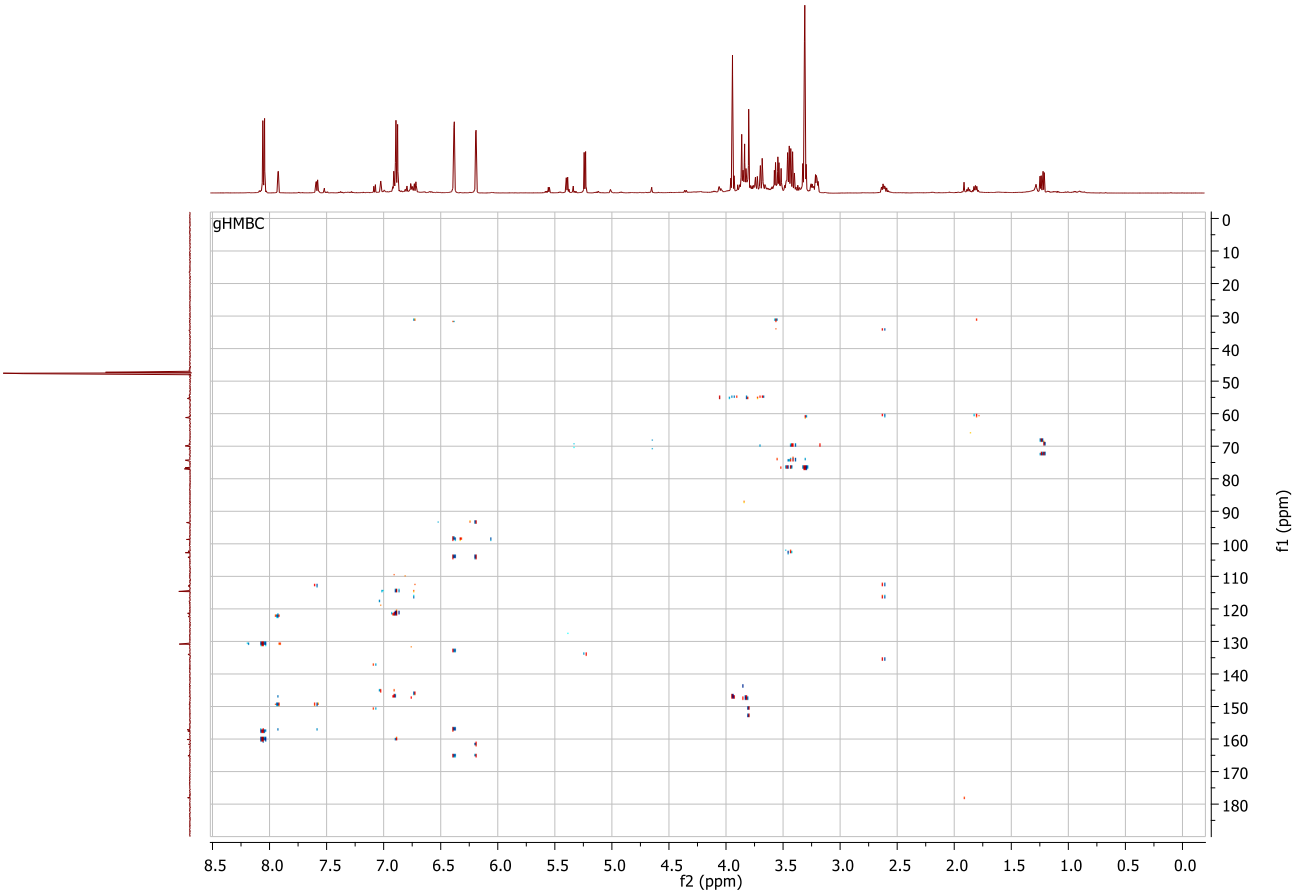

Figura 11A. Espectro de RMN ^{1}H-^{13}C (HMBC, 600 MHz, $CDCl_3$) del 3-*O*-glucósido de kaemferol (**8**)

DISCUSIÓN GENERAL

El género *Quercus* ha sido muy estudiado debido a las características que presenta que lo han definido como un género ampliamente propenso a eventos de hibridación. Debido a la dificultad que representa la detección de este proceso en condiciones naturales, su análisis se ha llevado a cabo utilizando diferentes características que presentan los taxones involucrados (genéticas, químicas, morfológicas etc.) a través del empleo de diferentes técnicas (RAPD´s, SSR´s, RAD, EST-SSR, SNP, caracterización química, secuenciación etc.). En este sentido, Ortego et al. (2017) sugieren que en los estudios sobre hibridación, la presencia de individuos dentro de una misma localidad con diferentes niveles de ancestría indica que la mezcla genética encontrada es resultado de hibridación.

En este estudio se analizaron eventos de hibridación en dos complejos formados por tres especies de encinos blancos (*Q. glabrescens*, *Q. rugosa* y *Q. obtusata*) mediante el empleo de nSSR´s y ms (compuestos fenólicos) que presentan una alta heredabilidad (definida como la medida de la reproducibilidad de un fenotipo al interior de un grupo de genotipos y/o la fracción de la variación fenotípica total explicada por un locus específico [Lynch y Walsh 1998, Soltis y Kliebestein 2015]), por lo que se pueden emplearse como marcadores específicos en eventos de hibridación. Por otra parte, se analizó el efecto que tiene la hibridación y la expresión de diversos ms sobre la comunidad de cinípidos y sus parasitoides asociados (en términos de riqueza, diversidad, porcentaje de infestación y abundancia). Este trabajo aporta información valiosa con respecto a las consecuencias que tiene la hibridación sobre la diversidad genética y la expresión de ms asimismo cómo el binomio formado por estos componentes regula de manera importante la configuración de la estructura de las comunidades de avispas inductoras de agallas y sus parasitoides asociados.

Hibridación en tres especies de encinos blancos

Con la finalidad de documentar si la simpatría entre tres especies de encinos blancos (*Q. glabrescens*, *Q. rugosa* y *Q. obtusata*) generaba eventos de hibridación, en este estudio se emplearon ocho nSSR´s y 10 ms

mayoritarios. Los resultados muestran que en los sitios de simpatría existen eventos de hibridación formándose tres complejos (*Q. glabrescens* × *Q. rugosa*, *Q. glabrescens* × *Q. obtusata* y *Q. glabrescens* × *Q. rugosa* × *Q. obtusata*). Aunque la hibridación es frecuente entre diferentes especies del género existe evidencia que los patrones de hibridación cambian según las especies involucradas y dentro/entre poblaciones, existiendo muchas zonas híbridas formadas principalmente por individuos híbridos F1 (De la Torre 2015). Los resultados obtenidos en estudio (capítulo 1) muestran una alta variación (entre complejos y entre sitios) en los porcentajes de hibridación, sin embargo, la totalidad de los individuos híbridos fue catalogada como híbridos F1. Lo anterior podría deberse a que existen barreras pre y postcigóticas que pueden impedir tanto la formación de retrocruzas avanzadas o que la introgresión uni o bidireccional genera combinaciones genéticas que no son capaces de desarrollarse y/o establecerse en los diferentes sitios donde se lleva a cabo este proceso. Además, existe la posibilidad de que el tamaño de muestra analizado en este estudio no haya sido lo suficientemente grande para confirmar que no existen generaciones híbridas posteriores a F1 y en caso de existir, conocer su abundancia relativa. Sin embargo, la variación en el número de híbridos obtenidos en este estudio hace suponer que puede existir variación en las mezclas genéticas que se dan entre estos taxones.

En este sentido, la hibridación repercute en la formación modificación y/o alteración de diversas combinaciones alélicas que los híbridos presentan y que pueden modificar el área de distribución de distintos genets en diferentes áreas donde se encuentran diferentes especies favoreciendo un comportamiento invasivo a través de la "invasión del genoma" (Mallet 2005). Aunque el éxito de dicha invasión puede verse minimizado/incrementado por las condiciones ambientales donde los individuos híbridos pueden establecerse potencialmente (Lihová et al. 2007, Tucker y Behm 2011, Ortego et al. 2017). Por otra parte, este estudio encontró que el contacto entre taxones genéticamente compatibles no garantiza que habrá eventos de hibridación. Lo anterior podría deberse al hecho de que las condiciones ambientales particulares de cada sitio pueden tener una fuerte influencia sobre la formación y/o desarrollo del híbrido o como se mencionó anteriormente, ciertas combinaciones genéticas que se dan entre los mismos taxones parentales no generen

individuos híbridos capaces de establecerse. Lo anterior podría también explicar el relativamente bajo número de híbridos encontrados.

La estructuración de las zonas híbridas está determinada por las características particulares que presentan tanto las especies que hibridan como los sitios donde lo hacen (De la Torre 2015). En *Quercus*, se ha propuesto que la hibridación actúa como un mecanismo de dispersión (Petit et al. 2003). Si este proceso funciona como un mecanismo efectivo de dispersión entonces mediante el mismo, la información para adaptarse a diferentes sitios puede encontrarse fuertemente regulada. Dado lo anterior, las zonas híbridas están formadas por diferentes genotipos que son el resultado de la mezcla de dos o más genotipos en diferentes ocasiones y aunque sean las mismas especies parentales se pueden obtener diferentes combinaciones que pueden afectar la adecuación de los individuos híbridos provocada por la variación en la expresión de diversos caracteres.

Los híbridos que logran establecerse pueden presentar una mezcla de características particulares y/o novedosas debido a las combinaciones genéticas exitosas que contienen. En este trabajo se encontró que las zonas híbridas estaban formadas únicamente por individuos híbridos F1. Sin embargo, los cambios en la expresión de diferentes caracteres que se expresan en mayor o menor grado pueden verse reflejados desde la primera generación. P. ej., Adams y Wendel (2005) encontraron que los patrones de expresión de los genes que codifican para enzima alcohol deshidrogenasa cambian inmediatamente después del primer evento de hibridación y diferentes alelos fueron silenciados en diferentes órganos. Por tanto, la hibridación puede actuar como un mecanismo con implicaciones ecológicas y evolutivas de efecto inmediato tanto en los taxones involucrados como en las comunidades asociadas a ellos (organismos generalistas y/o especialistas).

Variación en la expresión de ms en encinos

En este estudio se encontró que los taxones parentales tenían al menos un marcador químico específico mientras que los individuos híbridos tenían una mezcla de ms que presentaban las especies parentales. Además, no se detectó la presencia de ms novedosos en los híbridos. Los resultados muestran que la

hibridación tiene un efecto sobre la expresión cuantitativa pero no cualitativa de los ms. el patrón documentado en este estudio coincide con lo registrado por Cheng et al. (2011), donde se encontró que la expresión cualitativa en los ms es en más del 70% similar a las especies parentales.

En general *Q. rugosa* presentó los valores más altos de concentración en los ms con respecto a *Q. glabrescens* y el taxón híbrido. Lo anterior podría estar relacionado con el intervalo de distribución que presentan las especies parentales en México (21 estados *Q. rugosa*, 9 estados *Q. glabrescens*, Valencia 2004) y a través de una amplia gama de condiciones ambientales, así como un amplio intervalo altitudinal. Posiblemente el flujo genético intraespecífico que de manera natural se da en *Q. rugosa* y en *Q. glabrescens* podría estar incorporando en diferentes poblaciones información genética que le permita expresar una mayor variedad (cualitativa y cuantitativa) de ms. En este estudio, se lograron aislar e identificar únicamente a seis compuestos mayoritarios, sin embargo, debido a las diversas funciones que tienen los ms es posible considerar que la gama contenida (principalmente de compuestos minoritarios y/o cuya puede modificarse cuando se extraen las hojas del árbol) en las especies parentales es mucho más amplia. Aunque en el taxón híbrido no hubo expresión cualitativamente novedosa, si se detectaron diferencias en la expresión cuantitativa con respecto a sus taxones parentales y dicha variación también puede ser considerada novedosa en el sentido de las posibles modificaciones a las rutas metabólicas que provocan patrones de expresión variados dependiendo del ms analizado. En este sentido, Los resultados podrían estar relacionados con el tipo de herencia que presentan los ms purificados en este estudio ya que se ha sugerido que la expresión de los compuestos fenólicos (flavonoides y cumarinas [Clarke 1995, Irchhaiya et al. 2014]) está regulada por genes dominantes (Rehill et al. 2006) y que en procesos como la hibridación presentan un tipo de herencia aditiva (Crawford 1974). Finalmente, se ha sugerido que la hibridación es el principal factor que determina la calidad y la cantidad de los fenólicos (Rehill et al. 2006, Scioneaux et al. 2011) (capítulo 1 y 2).

Aunque se ha sugerido que la expresión de los algunos ms en las plantas es afectada por variaciones en las condiciones ambientales, interacciones sinérgicas o antagónicas con otros ms, así como interacciones

alelopáticas (Hamilton et al. 2001, Baldwin et al. 2006, Mithofer y Boland 2012). La elección de ms con las características que presentan los compuestos fenólicos puede ayudar a entender como diversos procesos (como la hibridación) que incrementan la diversidad genética pueden tener un impacto directo sobre la expresión cualitativa y cuantitativa de los ms. Además, este estudio analizó sitios dentro de la FVT que presentaron condiciones homogéneas (dentro de las posibilidades, cuando se hace un estudio de campo) y que también pueden contribuir a la obtención de conocimiento específico sobre los efectos que tienen tanto de la diversidad genética como la expresión química (no afectada por el ambiente) sobre las interacciones que se dan entre planta-herbívoro-parasitoide (capítulo 2).

De manera general, existen pocos trabajos en donde se haya documentado de manera fina la variación en la expresión de ms en encinos. Aunque se ha sugerido que la expresión de los ms tiene una fuerte base genética la dificultad para separar, purificar e identificar a los diferentes ms parece ser la limitante principal en los estudios de identificación. Como se mencionó previamente, la elección de los ms (flavonoides y cumarinas) en este estudio fue considerando la heredabilidad que presentaban dichos compuestos, además de las implicaciones ecológicas que presentan en las plantas (defensa contra estrés ambiental, alta intensidad lumínica, bajas temperaturas, infección por patógenos [bacterias y hongos], herbivoría (insectos) y deficiencia de nutrientes) (Lattanzio 2013).

La constitución química que presentan los encinos tiene diversas implicaciones ecológicas y económicas (p. ej., Ríos-Villa 2006, Raffard et al. 2018). No obstante, existen pocos trabajos donde se haya realizado el análisis específico del perfil metabólico que presentan las especies de encinos, como se mencionó anteriormente las dificultades técnicas, económicas, así como la falta de conocimiento (para el caso de los estudios ecológicos) pueden limitar fuertemente el análisis de los ms (mayoritarios) que conforman a las diferentes especies de encinos. Aunque existen diversas técnicas que pueden ayudar a la identificación (casi siempre subjetiva, en el sentido de que se realiza por observación directa de la coloración y la comparación con estándares conocidos) de algunos ms presentes en concentraciones relativamente bajas (principalmente cromatografía en capa fina

[TLC]). La identificación específica de cualquier ms requiere de: a) el aislamiento y purificación de dicho compuesto (mediante cromatografía de columna) y b) una concentración mínima de entre 18 y 20 mg de cada compuesto puro (que será ocupado para realizar cromatografía líquida de alta resolución y resonancia magnética nuclear [HPLC, RMN]). Por lo que, en los análisis donde se requiere conocer tanto la identidad específica de cualquier ms, así como su concentración se realiza únicamente con la identificación de ms mayoritarios (capítulo 3).

Influencia de la diversidad genética y los metabolitos secundarios sobre la comunidad de cinípidos y sus parasitoides asociados

Diversos estudios han documentado que la variación genética de las especies vegetales tiene implicaciones directas e indirectas sobre la estructuración de las comunidades de insectos asociados (generalistas y/o especialistas) y que dicha variación puede llegar a afectar a las comunidades de parasitoides asociadas (interacciones tri-tróficas). En este estudio se encontró que el incremento en la diversidad genética (como resultado de hibridación) tiene una fuerte influencia (positiva o negativa) sobre la concentración del 66.66% de los ms mayoritarios identificados y sobre las comunidades de cinípidos y sus parasitoides. Finalmente se documentó que dependiendo del ms analizado este puede tener una influencia positiva/negativa sobre la riqueza y/o diversidad tanto de cinípidos como de parasitoides (capítulo 2). De manera general, los ms no actúan de manera aislada en la defensa contra la herbivoría ya que, dependiendo de las modificaciones a la ruta metabólica, diversos ms pueden actuar como precursores de otros ms (p. ej., ácido cafeico) potencializando las respuestas que las plantas tienen contra la herbivoría. Asimismo, existen reportes que indican que existe un sinergismo entre los ms y otros mecanismos como la calidad nutricional, complejidad estructural y una gran cantidad de compuestos con un alto peso molecular (p. ej., celulosa y lignina) (Bryant et al. 1987, Pastor et al. 1993).

La hibridación es una forma de recombinación genética que entre otras consecuencias favorece el incremento de la diversidad genética de las especies involucradas en dicho proceso. Diversos estudios con encinos muestran este patrón (p. ej., Tovar-Sánchez et al. 2008, Hata et al. 2011, Tovar-Sánchez et al. 2015, Valencia-Cuevas et al. 2015, capítulo 2 de esta tesis). En general, dichos estudios han cuantificado la diversidad genética a nivel poblacional (p. ej., *He*, número de genotipos), e individual (p. ej., IR). El incremento en la diversidad genética puede estar relacionada con el hecho de que la mezcla de diferentes pools génicos incrementa las posibilidades de formar nuevas combinaciones, incrementando con ello la posibilidad de desarrollo de novedades genéticas que bajo un escenario de selección natural pueden establecerse y formar nuevas especies (Rieseberg y Willis 2007, Wisseman 2007). Por otra parte, la relativa adecuación de los genotipos híbridos permite potencialmente el establecimiento de individuos que eventualmente diversificaran y se podrían adaptar a nuevas condiciones (Arnold et al. 2012). Se ha sugerido que, para el caso de los genotipos híbridos, la interacción genotipo x ambiente impacta directamente en el tipo de adecuación relativa que presentarán (Arnold et al. 2012). Además, dependiendo del tipo y la cantidad de información que se mezcla dentro de los individuos híbridos se esperaría la adaptación a un ambiente particular por las características particulares que presentan.

Los resultados obtenidos en este estudio (capítulo 2) muestran que la diversidad genética tiene un impacto directo sobre la expresión cualitativa de los ms y estos a su vez tienen una influencia directa sobre la riqueza y diversidad de cinípidos y parasitoides. Diversos estudios han documentado que los cambios en los ms son particularmente importantes debido a que interactúan directamente con el medio donde se desarrollan y por tanto están relacionados con los herbívoros, patógenos y competidores (p. ej., Moore et al. 2013, Hughes et al. 2008a, b, Bailey et al. 2009, Des Roches et al. 2018). En este sentido, las agallas producidas por avispas de la familia Cynipidae pueden ser utilizadas como un modelo de estudio para interpretar las respuestas que las plantas presentan ante el establecimiento de los cinípidos y/o sus parasitoides asociados. Esto, debido a que el nivel de sensibilidad de los agalleros es tan alto que les permite detectar los pequeños cambios que presentan

las plantas a nivel fisiológico, químico, desarrollo y fenología para poder ovipositar en las especies y/u órganos específicos (Abrahamson et al. 1998, Raman 2005). En este sentido, Mccalla (1962) mostró que la formación de la agalla depende principalmente de la correcta colocación del huevo, la cantidad y tipo de compuestos presentes en la saliva del cinípido. Lo anterior supone que el establecimiento depende de la interacción química entre la especie hospedera y el cinípido, en donde cualquier modificación hecha por cualquiera de las partes involucradas puede alterar la relación y/o suspenderla, al menos provisionalmente.

El perfil químico de *Quercus* está fuertemente relacionado con la distribución de Cynipidae (Abrahamson et al. 2003, capítulo 2). Los cinípidos pueden formar comunidades únicas en las especies de *Quercus* a las que se establecen (Abrahamson et al. 2003, capítulo 2) y dichas comunidades pueden estar estructuradas con base en la filogenia que presentan las especies de agalleros (Li y Hsiao 1973, Solomon 1983, Abrahamson et al. 2003). Abrahamson et al. (2003) sugieren que uno o dos compuestos fenólicos (o la mezcla de fenólicos específicos) pueden ser claves en la oviposición de los cinípidos. Diversos estudios han sugerido que los perfiles químicos vegetales frecuentemente alteran el patrón de consumo de los herbívoros por lo que los cambios que presentan las plantas pueden estar dirigidos hacia un insecto fitófago particular (Schuman y Baldwin 2016). Dada la especificidad de la relación *Quercus*-Cynipidae se ha sugerido que el cambio de hospedero en cinípidos está influenciado fuertemente por el perfil químico de la planta hospedera (Kessler y Baldwin 2001). Lo anterior, refuerza la teoría de que la única forma de cambiar de hospedero es a través del mecanismo denominado “puente híbrido” (cambio de especie hospedera mediante introgresión bidireccional [Floate y Whitham 1993]). Ya que, se ha sugerido que las hembras de cinípidos son mucho más específicas como para “equivocarse” de hospedero (Ronquist y Liljeblad 2001). Por otra parte, se ha propuesto que la alta especificidad de los cinípidos sobre *Quercus* está regulada fuertemente por la presión top-down que ejercen los parasitoides (Ronquist y Liljeblad 2001).

Todos los parasitoides asociados a cinípidos son específicos para el grupo (Hayward y Stone 2005) y solo atacan a los géneros que pertenecen a una sola tribu particular de cinípidos. Sin embargo, diferentes tribus de

cinípidos pueden atraer a una sola tribu de parasitoides (Jones 1983). Diversos estudios han documentado que la presión que ejercen los parasitoides ha provocado la modificación de los sitios de oviposición y/o consumo lo que ha provocado la diversificación de los cinípidos al reducir la competencia interespecífica e incrementando la coexistencia (Bruce 2014, Schuman et al. 2016). Por su parte, la estructura de la comunidad de parasitoides está determinada por la estructura de la agalla, la localización de la planta hospedera, la estación de crecimiento, el color, olor, forma y tamaño de la agalla (Askew 1984, Raman 2005). Las plantas pueden afectar a las comunidades de parasitoides asociados a los herbívoros mediante a alteración y/o modificación de la calidad nutricional o los mecanismos de defensa (Ode 2006, Stireman 2016). Finalmente, los estudios en la interacción *Quercus*-Cynipidae-parasitoides puede ayudar a dilucidar importantes procesos ecológico-evolutivos que pueden extrapolarse en la teoría de la interacción planta insecto.

CONCLUSIONES GENERALES

Con base en los resultados generales, se formulan las siguientes conclusiones:

1. Los taxones híbridos (*Q. glabrescens* × *Q. rugosa* y *Q. glabrescens* × *Q. obtusata* [datos no mostrados]) mostraron los valores más altos de diversidad genética en comparación a los taxones parentales.

2. Dentro de los compuestos fenólicos, existe al menos un marcador especie específico para cada taxón parental (*Q. rugosa*, *Q. glabrescens* y *Q. obtusata*).

3. La expresión de los compuestos fenólicos está fuertemente regulado por la diversidad genética del taxón que los produce.

4. Se encontró que, de manera general los metabolitos secundarios en los taxones híbridos varían cuantitativa pero no cualitativamente con respecto a los taxones parentales.

5. La presencia, frecuencia y proporciones de mezcla (híbridos) entre las diferentes combinaciones entre *Q. rugosa*, *Q. glabrescens* y *Q. obtusata* difiere entre localidades simpátridas.

6. La coexistencia de *Q. rugosa*, *Q. glabrescens* y *Q. obtusata* no es condición suficiente para que haya flujo genético interespecífico.

7. La diversidad genética de los taxones (*Q. rugosa*, *Q. glabrescens* e híbrido) tiene influencia (positiva o negativa) sobre la expresión cualitativa y cuantitativa de los compuestos fenólicos.

8. La diversidad genética de los taxones (*Q. rugosa*, *Q. glabrescens* e híbrido) tiene influencia (positiva o negativa) sobre la diversidad y abundancia de cinípidos y de los parasitoides asociados.

9. La variación en la expresión cuantitativa de los compuestos fenólicos tiene influencia (positiva o negativa) sobre la diversidad y abundancia de los cinípidos y de los parasitoides asociados.

10. Dada la especialización de los cinípidos dentro del género *Quercus* y la fortaleza de dicha relación, los cinípidos responden de manera puntual a los cambios genéticos y químicos que el taxón hospedero presenta.

11. Las modificaciones en la diversidad genética y química del taxón vegetal pueden tener impacto sobre diversos niveles biológicos asociados a ellas (parasitoides) mediante efectos genéticos indirectos.

PERSPECTIVAS

El presente estudio reveló que *Q. rugosa*, *Q. glabrescens* y *Q. obtusata* son tres especies de encinos blancos que cuando se encuetran en simpatría pueden tener eventos de hibridación. Asimismo, se documento que la hibridación entre estos tres taxones de encinos blancos puede modificar sus niveles de diversidad genética y modificar la expresión cuantitativa de los ms, lo que en última instancia puede tener impacto sobre la diversidad y abundancia de sus comunidades de insectos especialistas asociados. Estos resultados abren la posibilidad de abordar diferentes temas de investigación. Por ejemplo, sería interesante evaluar la influencia de la hibridación sobre las comunidades de insectos generalistas y/o pertenecientes a otros grupos funcionales, para conocer si dichos grupos funcionales tienen los mismos patrones de respuesta. Otro aspecto importante es determinar si las barreras reproductivas presentes en *Q. rugosa*, *Q. glabrescens* y *Q. obtusata* están impidiendo la formación de generaciones híbridas avanzadas (F2 y retrocruzas) o si las condiciones ambientales tienen impacto sobre la formación de dichos individuos. Por lo que, un monitoreo detallado de la fenología de las especies, experimentos de polinización y el análisis de su dirección mediante análisis de paternidad a nivel local, podrían ser de utilidad para identificar barreras al flujo genético interespecífico entre *Q. rugosa*, *Q. glabrescens* y *Q. obtusata*.

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Anexo 1

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ORIGINAL ARTICLE

Hybridization of *Quercus castanea* (Fagaceae) across a red oak species gradient in Mexico

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Abstract Interspecific gene flow between more than two species is a common phenomenon in oaks, which can occur simultaneously among different species, promoting the transfer of genetic material across species boundaries. However, the hybridization dynamics in multispecies hybrid zones remain unknown. In this study, we provide genetic evidence of hybridization and introgression of *Quercus castanea* across a natural gradient of red oak species richness. We analyzed five populations recognized morphologically as "pure" *Q. castanea*, one allopatric and four sympatric populations, where the number of red oak species associated with *Q. castanea* ranged from one to four. Also, one allopatric population of each red oak species that occurs in sympatry with *Q. castanea* was chosen as reference population (*Q. crassipes*, *Q. laurina*, *Q. mexicana* and *Q. crassifolia*). In total, six nSSRs were used in 10 and 20 individuals from each allopatric and sympatric populations, respectively. Our results showed that allopatric populations formed completely distinct genetic clusters. In sympatric populations, we found evidence of hybridization and introgression among *Q. castanea* and three of its associated red oak species. However, the occurrence and frequency of hybrids between *Q. castanea* and these species varied among stands. Our analyses provide evidence and new insights into hybridization and introgression dynamics within a Mexican red oak species complex, through a focal species, *Q. castanea*.

Keywords Interspecific gene flow · Multispecies hybrid zones · Nuclear microsatellites · Oaks

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Introduction

Natural hybridization and subsequent introgression are important processes in plant evolution and speciation (Barton 2001; Coyne and Orr 2004). For example, the movement of genes across species boundaries can promote genetic recombination and an increase in genetic diversity levels (Rieseberg 1997), the presence of new lineages (Seehausen 2004), adaptive solutions (Rieseberg et al. 2003), or colonization abilities (Potts and Reid 1988; Petit et al. 2004). In contrast, genetic pollution by alien alleles may disturb the endemism of rare species (Ellstrand and Elam 1993; Arnold 1997; Wolf et al. 2001; López-Caamal et al. 2013). It is thus important to clarify the direction in which species or populations are driven by natural hybridization, to predict their future status in the context of evolutionary and conservation genetics.

Oaks (*Quercus*) represent good models for hybridization studies, because reproductive barriers between some species appear to be weak (Williams et al. 2001; Abadie et al. 2012; Lagache et al. 2013) and hybridization is common. Interspecific gene exchange within this genus has caused

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much debate about species concepts, suggesting that the biological species concept is inappropriate for oaks (Burger 1975; Coyne 1994). Also, oaks have played a central role in questions concerning the importance of introgression in plant evolution (González-Rodríguez et al. 2004; Tovar-Sánchez and Oyama 2004; Lepais et al. 2009), stimulating discussions about the role of ecological factors to promote or limit hybridization events (Buerkle 2009) and have served as a model in the development of a species concept that relies on ecological criteria (Muller 1952; Van Valen 1976). In general, the existence of plants morphologically and ecologically intermediate between recognized oak species frequently has been explained as the result of interspecific hybridization (Howard et al. 1997; González-Rodríguez et al. 2004; Burgarella et al. 2009; Peñaloza-Ramírez et al. 2010). However, the wide intraspecific variability of leaf and acorn morphology within the genera *Quercus* commonly limits its utility for hybridization diagnosis (Curtu et al. 2007). In recent studies, valuable information to detect and evaluate the level of hybridization and introgression direction has been obtained using genetic markers such as microsatellites, a complementary tool to study hybridization and introgression in oaks (e.g., Muir et al., 2000; Valbuena-Carabaña et al. 2005; Curtu et al. 2007; Burgarella et al. 2009; Lepais et al. 2009; Peñaloza-Ramírez et al. 2010; Neophytou et al. 2011; Lagache et al. 2013). In general, hybridization has been intensively studied in white oaks (section *Quercus*); however, few studies have examined gene flow in red oaks (section *Lobatae*, Moran et al. 2012). Red oaks are an important part of the North American flora and there is evidence that suggests that species barriers may be weaker than in white oaks (Guttman and Weigt 1989; Kashani and Dodd 2002; Aldrich et al. 2003).

Despite of a high frequency of interspecific gene flow that has been inferred from many combinations in oaks, most of the studies have been carried out in mixed stands consisting of only two parental taxa and their hybrids (e.g., Tovar-Sánchez and Oyama 2004; Valbuena-Carabaña et al. 2005; Albarrán-Lara et al. 2010; Neophytou et al. 2011; Lagache et al. 2013). Nevertheless, other species complexes of simultaneous hybridization among more parental taxa have been studied in nature (Dodd and Afzal-Rafii 2004; Curtu et al. 2007; Lepais et al. 2009; Peñaloza-Ramírez et al. 2010; Moran et al. 2012). As a consequence, the dynamics of gene flow in multispecies hybrid zones are poorly known (Lepais et al. 2009). Also, most of these studies have analyzed the hybridization dynamics in a restricted area.

Recently, rates of hybridization in *Quercus* have been estimated by means of multilocus microsatellite genotypes combined with Bayesian statistical procedures. In these studies, the results have been contrasting. For example, a study in three species of Mexican oaks (*Q. hypoleucoides* Camus, *Q. scytophylla* Liebm., and *Q. sideroxyla* Bonpl.) found that hybrids, including backcrosses and probable triple hybrids, were dominant in the contact zone (Peñaloza-Ramírez et al. 2010). Also, a study in four species of European oaks (*Q. robur* L., *Q. petraea* (Matt) Liebl., *Q. pyrenaica* Willd., and *Q. pubescens* Willd.) found that the percentage of hybrid individuals ranged from 10.7 to 30.5 % in different stands (Lepais et al. 2009). A recent study in four species of American red oaks (*Q. rubra* L., *Q. velutina* Lam., *Q. falcata* Michx. and *Q. coccinea* Münchh.) found that the percentage of hybrid individuals was 20 %. In contrast, other studies have reported lower rates of hybridization. For example, analysis of interspecific gene flow between *Q. robur*, *Q. petraea*, *Q. pubescens*, and *Q. frainetto* Ten. in Romania showed that the level of hybridization varied from 1.7 to 16.2 % between species pairs (Curtu et al. 2007). Burgarella et al. (2009) found that the hybrids between two Mediterranean evergreen oaks, *Q. suber* L. and *Q. ilex* L., comprised less than 2 % of adults in areas where their ranges overlap. In a study of *Q. virginiana* Mill. and *Q. geminata* Small, two common species in the southeastern United States, 5.5 % of adults showed mixed ancestry (Cavender-Bares and Pahlich 2009).

These earlier studies have contributed important insights to the issue of hybridization in oaks, and at the same time show the complexity of this phenomenon. For instance, it has been reported that hybridization not only depends on the intrinsic characteristics of the species involved, but also on the environmental context. Particularly, it has been documented that habitat conditions (Williams and Ehleringer 2000; Williams et al. 2001; Himrane et al. 2004; Lagache et al. 2013), geographical localization of the hybrid zone (Tovar-Sánchez and Oyama 2004), establishment and survivorship of hybrid individuals (Valbuena-Carabaña et al. 2007), different rates of gene flow (Curtu et al. 2007), relative abundance and identity of species (Lepais et al. 2009), spatial structure of species (Salvini et al. 2009), or proportion of conspecific pollen and the density of individuals available to mating (Lagache et al. 2013) can influence the levels of hybridization and introgression in oaks. Also, recent studies have reported that the reproductive barriers that operate among oak species involved in hybridization events changes between species pair (Curtu et al. 2007; Jensen et al. 2009; Lepais et al. 2009, 2013), depending on which species act as maternal or paternal parental (Boavida et al. 2001; Olrik and Kjær 2007; Lepais et al. 2013) and in response to environmental variation (Lepais and Gerber 2011; Abadie et al. 2012; Lepais et al. 2013). In consequence, variation in the richness of the local oak community and in ecological and geographical factors among sites could promote

differences in the occurrence, types of hybrids and their frequency. A better understanding of the conditions that enable the hybridization process to occur as well as the prediction of when and where hybridization is likely to happen, still today, is therefore an important research goal (Lepais and Gerber 2011; Lagache et al. 2013).

Mexico is considered the center of diversification of the genus *Quercus* (Nixon 1993), including 161 species, out of which 68 % are endemic to the country (Valencia 2004). Of the total, 76 species belong to the section *Lobatae* (red oaks), considering 61 as endemic (Valencia 2004). Several studies conducted on Mexican red oaks have focused on hybridization between two (González-Rodríguez et al. 2004; Tovar-Sánchez and Oyama 2004) or three (Peñaloza-Ramírez et al. 2010) species. However, oak species complexes constituted of more species have been described in Mexico (Valencia 1994). The consideration of such multispecies interactions is fundamental to understand the dynamics and consequences of hybridization, because this is the way that the phenomenon occurs in nature. In this context, the high number of oak species that coexist naturally at different sites plus the complex topography, altitude and climatic diversity of the temperate forest of Mexico, altogether provide a great opportunity to investigate the dynamics of gene flow in multispecies hybrid zones and how the frequency and types of hybrids vary among forests and ecological settings. *Quercus castanea* Neé is a species that presents a wide geographical distribution and it is a dominant element of Mexican temperate forests. This species presents morphological diagnostic characteristics in allopatric conditions. However, individuals with atypical leaf shapes have been detected when other red oaks species occur in sympatry with this species. Inside sympatric areas, the overlap in flowering phenology among red oaks is common, a fact that suggests that the phenomenon of hybridization may explain the observed variation. In a previous study using 14 microsatellites (SSRs) primers (six nSSRs and eight cpSSRs) we showed that the genetic diversity of *Q. castanea* populations increases as the number of associated red oaks species also increases in sympatric sites (Valencia-Cuevas et al. 2014). We have suggested that this result is the consequence of interspecific genetic exchange.

In this work, we analyzed if interspecific gene flow between *Q. castanea* and its associated species across a natural red oak species gradient occurs. The specific aims of this study were: (1) to determine if *Q. castanea* individuals hybridize with other red oaks species that grow in sympatric stands, (2) to detect the level of hybridization and introgression between *Q. castanea* and their associated species across the gradient and (3) to determine the influence that local red oak community richness has on hybridization patterns.

Materials and methods

Species description

Quercus castanea Neé (*Lobatae*: red oaks) includes trees from 5 to 15 m in height with a trunk diameter of 30–60 cm. These trees can be recognized easily in the field by its leaf characteristics such as shape (obovate, oblanceolate), underside veins conspicuously elevated and reticulate, margins [with 2–5 (–6) short teeth], secondary veins (8–12 on each side of the midvein), coloration (gray-greenish), and trichomes (fasciculate sessile). The flowering season is from April to May and fruiting from August to December (Valencia 1995; Vazquez 2006). It is located between 1,180 and 2,600 m a.s.l., and it distributes along all the major Mexican mountain ranges (Sierra Madre Oriental, Sierra Madre Occidental, Sierra Madre del Sur

Table 1 Locality name, state, and number of red oak species associated with *Quercus castanea* in populations from the Transmexican Volcanic Belt

Population	Locality	State	Oak species
Allopatric stand			
A	Coajomulco	Morelos	*Q. castanea*
B	Piñón	San Luis Potosí	*Q. crassipes*
C	Xuchitepec	Oaxaca	*Q. laurina*
D	Cuesta Colorada	Hidalgo	*Q. mexicana*
E	Cuesta Blanca	Durango	*Q. crassifolia*
Sympatric stand			
PNT	Parque Nacional El Tepozteco	Morelos	*Q. castanea, Q. crassipes*
PECM	Parque Ecológico de la Ciudad de México	Mexico city	*Q. castanea, Q. crassipes, Q. laurina*
PBT	Parque Barranca de Tarango	Mexico city	*Q. castanea, Q. crassipes, Q. laurina, Q. mexicana*
PLP	Parque Ecológico Las Peñas	Mexico state	*Q. castanea, Q. crassipes, Q. laurina, Q. mexicana, Q. crassifolia*

and Transmexican Volcanic Belt; Valencia 2004). It is found frequently in perturbed areas with a xerophytic shrub type of vegetation, it is also localized in mountain cloud forests (Rzedowski and Rzedowski 2001). Also, when other red oak species occur in sympatry with *Q. castanea* the existence of individuals with atypical leaf shapes has been detected.

The red oak species that were identified coexisting with *Q. castanea* through the species gradient were: *Q. crassifolia* Bonpl., *Q. crassipes* Bonpl., *Q. laurina* Bonpl., and *Q. mexicana* Bonpl. (Table 1). All four oak species are broadly distributed in Mexico (Valencia 2004).

Quercus crassipes, includes trees up to 17-m tall and 0.40–1 m in trunk diameter. Leaves are deciduous, coriaceous, narrowly elliptic and lanceolate, their surface is barely lustrous and the lower surface is tomentose, white grayish and with revolute margins (Rangel et al. 2002). *Quercus laurina* includes trees between 10 and 30 m in height with a truck diameter of 50 cm or more. Leaves are coriaceous, lanceolate or elliptic oblanceolate, their surface is green and lustrous. The lower surface is lustrous, slightly yellow with glandular hairs persisting in the axils of the larger veins (Valencia 1994). *Q. mexicana* includes trees between 3 and 15 m in height. Leaves are deciduous, coriaceous, elliptic, lanceolate or oblong, their surface is dark with stellate hairs scattered like dusty dots (Rangel et al. 2002). *Q. crassifolia* includes large trees up to 23 m in height with a trunk diameter of 1 m. Leaves are deciduous, aristate, ovate, obovate or elliptic, with coriaceous upper surface, the lower surface is yellow tomentose, orange or brown (Rangel et al. 2002).

Study sites and sampling

The Transmexican Volcanic Belt (TVB) is an orographic system that traverses the central part of the country in an east–west direction, whose formation process began during the Quaternary-Pliocene (Gómez-Tuena et al. 2007). It is considered the youngest mountain range in Mexico and contains valleys higher than 2,000 m in altitude and the highest mountains in Mexico (Ferrusquía-Villafranca 1998). In particular, in the central region of the TVB, the predominant climate is temperate subhumid, with annual summer rainfall in excess of 1,000 mm and average temperatures ranging between 3 and 22 °C, the dominant altitudinal zone is between 1,500 and 2,500 m, the soil type origin is volcanic or derived from igneous and sedimentary rocks (Ferrusquía-Villafranca 1998). Trees recognized morphologically as "pure" *Q. castanea* were sampled from five populations (20 trees/site), one allopatric population of *Q. castanea* (population A) and four sympatric stands between *Q. castanea* and other red oaks species (population 1–4, Fig. 1) through the central part of the TVB. The number of associated species with *Q. castanea* in each

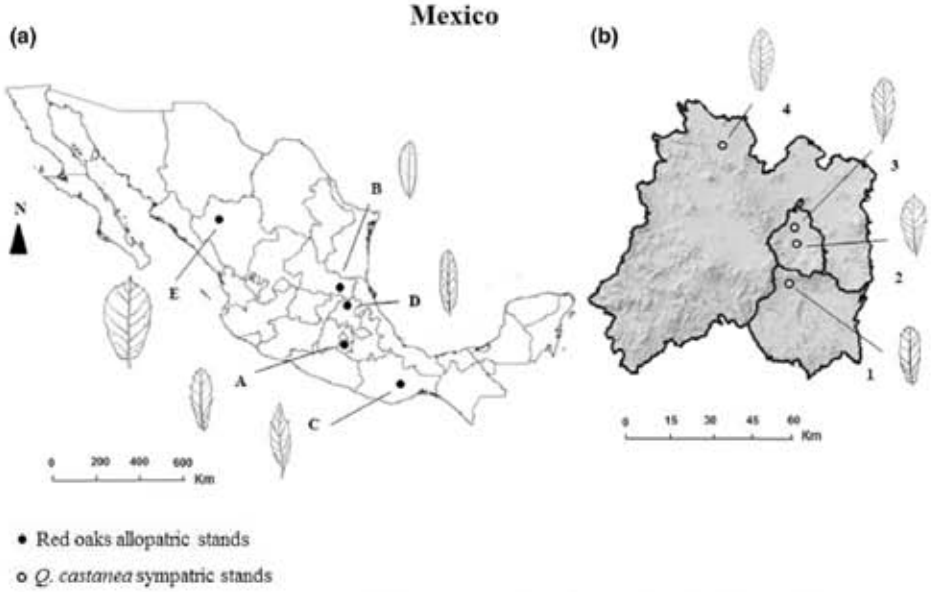


Fig. 1 Sampling populations. **a** Allopatric populations of red oak species: *Quercus castanea* (*A*), *Q. crassipes* (*B*), *Q. laurina* (*C*) *Q. mexicana* (*D*), *Q. crassifolia* (*E*). **b** Sympatric populations of *Q. castanea* with different red oak species richness. *1* PNT, *2* PECM, *3* PBT, *4* PLP. See Table 1

sympatric locality ranged from one to four (Table 1). These species were: *Q. crassipes* (B), *Q. laurina* (C), *Q. mexicana* (D), and *Q. crassifolia* (E) (Fig. 1). Ten individuals from one allopatric population of each red oak species co-occurring in sympatry with *Q. castanea* were sampled (Table 1). Allopatric and sympatric populations were selected based on typical diagnostic characters of each species. Valencia-Cuevas et al. (2014) documented that the oak species density (individuals/hectare) per sympatric site was Parque Nacional El Tepozteco [*Q. castanea* (56.7), *Q. crassipes* (44.1)], Parque Ecológico de la Ciudad de México [*Q. castanea* (149.0), *Q. crassipes* (135.2), *Q. laurina* (127.9)], Parque Barranca de Tarango [*Q. castanea* (161.6), *Q. crassipes* (120.1), *Q. laurina* (140.4), *Q. mexicana* (84.0)], Parque Las Peñas [*Q. castanea* (186.7), *Q. crassipes* (150.0), *Q. laurina* (202.2), *Q. mexicana* (133.1), *Q. crassifolia* (189.7)]. Three transects of 1,000 m in each locality were done. At each 50 m, the nearest individual was sampled.

Molecular data

Leaves with no apparent damage were collected from 10 to 20 individuals from each allopatric (*Q. castanea*, *Q. crassifolia*, *Q. crassipes*, *Q. laurina*, and *Q. mexicana*) and sympatric population, respectively. Leaf tissue was frozen in liquid nitrogen and transported to the laboratory for DNA extraction. Total DNA was extracted and purified using the DNAeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). DNA quantification was done by fluorometric analysis (Eppendorf, Germany), and DNA quality was visualized by comparing the intensity of bands with known standards of lambda DNA on agarose gels at 0.8 %.

Genetic analyses were performed using six nuclear microsatellite primers (nSSRs): OC11, OE09, CO8, FO7 (Aldrich et al. 2002), QpZAG110 (Steinkellner et al. 1997), and QpZAG11 (Kampfer et al. 1998) that showed to be polymorphic in *Q. castanea*. PCR reactions were set-up as follows: 15 ng of DNA template, 50-mM KCl, 20-mM Tris–HCl (pH 8.4), 2-mM $MgCl_2$, 0.13 mM of each dNTP, 25 mM of each primer and 0.8 U of *Taq* polymerase, in a final volume of 15 µl. Reaction conditions were an initial denaturation step at 95 °C for 5 min, followed by 30 cycles at 94 °C for 1 min, 1 min at the appropriate annealing temperature, followed by 30 s at 72 °C, and a final extension at 72 °C for 8 min. Annealing temperature differed for each primer pair. 44 °C for ZAG110, 50 °C for CO8, 53 °C for OC11, ZAG11 and EO9, and 58 °C for FO7. PCR products were resolved on polyacrylamide gels at 6 % (7 M urea) at 60 W for 3 h to determine the polymorphic primers. We measured the length of the amplified microsatellites fragments by running an aliquot of each PCR product on an automatic sequencer ABI 3100 (Applied Biosystems, CA, USA) at 35 W for 80–90 min, using gene scan ROX-2500 (Applied Biosystems, CA, USA) as size standard. Alleles were scored using the Gene Mapper ver. 3.7 Software (Applied Biosystems, CA, USA).

Statistical analysis

Genetic assignment of allopatric and sympatric populations

To confirm the genetic identity of allopatric pure populations and to determine the proportions of ancestry of individuals from sympatric populations, we ran the program STRUCTURE 2.3 (Pritchard et al. 2000) with data obtained from six nSSRs. This program is based on a Bayesian clustering to infer population structure with genotype data. In this analysis, each population is characterized by a set of allele frequencies at each locus. Individuals are probabilistically assigned to K populations (species in our case), or to parental populations in the case of admixed ancestry. To determine the optimal number of genetic groups (K), we ran STRUCTURE with K varying from 1 to 10, with ten runs for each K value, to find the K value with the highest posterior probabilities. Also, we used the ΔK statistics to evaluate the change in likelihood (Evanno et al. 2005). Our parameters were 50,000 burn-in periods and 100,000 Markov chain Monte Carlo repetitions after burn-in. First, we did the genetic assignment analysis of the individuals of the allopatric populations, using the no admixture model with correlated frequencies without population information. Later, we used the mixed model with correlated frequencies to analyze all sympatric populations, including as reference populations the four red oak species potentially connected by gene flow with *Q. castanea* in each run. We classified each individual using the classification scheme proposed by Vähä and Primmer (2006) (e.g., Curtu et al. 2007; Burgarella et al. 2009; Lepais et al. 2009; Ortego and Bonal 2010), in which individuals with assignment coefficient: $Q \geq 0.90$ is considered as a purebred genotype and individuals with $Q < 0.90$ as a hybrid genotype. However, individuals with $Q < 0.90$ for one cluster, but $Q < 0.10$ for each of the remaining clusters was supposed to have the majority of their genome from one species without any significant influence from other species, and they were thus also classified as pure species. Introgressed forms are defined as those showing $Q < 0.90$ to belong their own species cluster and >0.10 probability of belonging to other species clusters. After this analysis, each individual was assigned to one genetic category based on Q value and species genome combination. Output from STRUCTURE was postprocessed for publication using the program DISTRUCT (Rosenberg 2004).

Results

Genetic assignment of allopatric and sympatric populations

In general, the results showed a clear correspondence between species designation and the inferred genetic cluster. According to the values of log likelihood, the highest posterior probability was obtained for five genetic clusters: $\ln P(D) = -6{,}007.56$. In consequence, STRUCTURE program determined that five genetic clusters best fit the data, which agrees with the existence of five phenotypically pure species. This result was also confirmed by the ΔK values, indicate that $K = 5$ is the most likely number of genetic groups (Fig. 2). Using the no admixture model in the program STRUCTURE, the allopatric reference populations had a high proportion of ancestry ($Q > 0.9$) from a single genetic group (Fig. 3).

Considering a threshold value of 0.90 to classify each individual as purebred or hybrid genotypes, the genetic analyses of 80 *Q. castanea* trees across four sympatric populations revealed the presence of 15 hybrid individuals (18.75 % of the total of individuals analyzed) with indications of genetic introgression between *Q. castanea* and three of four associated red oak species (Fig. 3). An exception was the Parque Nacional El Tepozteco (PNT) population (Table 1), where *Q. castanea* is coexisting with *Q. crassipes*, but we did not find individuals that showed a significant contribution of *Q. crassipes* or other genetic group, being practically a pure population. In the Parque Ecológico Ciudad de México (PECM) population, where *Q. castanea* coexists with *Q. crassipes* and *Q. laurina*, only the first species contributed to the *Q. castanea* genetic pool. Specifically, we found five individuals with evidence of admixture between *Q. castanea* and *Q. crassipes* genetic groups (*Q. castanea* and *Q. crassipes*, respectively: $Q = 0.791$ and 0.197; $Q = 0.297$ and 0.692; $Q = 0.405$ and 0.583; $Q = 0.448$ and 0.544; $Q = 0.502$ and 0.490). Similarly in the Parque Barranca de Tarango (PBT) population, we found three individuals showing admixture between *Q. castanea* and *Q. crassipes* genetic groups (*Q. castanea* and *Q. crassipes*, respectively: $Q = 0.617$ and 0.372; $Q = 0.729$ and 0.255; $Q = 0.540$ and 0.447). However, we did not detect hybrids with *Q. laurina* or *Q. mexicana* although both species were present in this site. In the Parque Ecológico Las Peñas (PLP) population gene flow occurred between *Q. castanea* and *Q. crassipes*, *Q. laurina* and *Q. crassifolia*. In this population, *Q. mexicana* was present as well, but hybrids between this species and *Q. castanea* were not found (Fig. 3). Specifically, we detected seven individuals with indication of introgression, two between *Q. castanea* and *Q. crassipes* (*Q. castanea* and *Q. crassipes*, respectively: $Q = 0.738$ and 0.255; $Q = 0.664$ and 0.315), four between *Q. castanea* and *Q. laurina* (*Q. castanea* and *Q. laurina*, respectively: $Q = 0.386$ and 0.581; $Q = 0.580$ and 0.409; $Q = 0.584$ and 0.406; $Q = 0.444$ and 0.546) and one individual that presents a combination between *Q. castanea*, *Q. crassifolia* and *Q. crassipes* genetic groups ($Q = 0.422$, 0.348, and 0.213, respectively). Finally, one individual from this population, showed high probability of belonging to the *Q. crassipes* genetic group (assignment coefficient: $Q > 0.90$; Fig. 3).

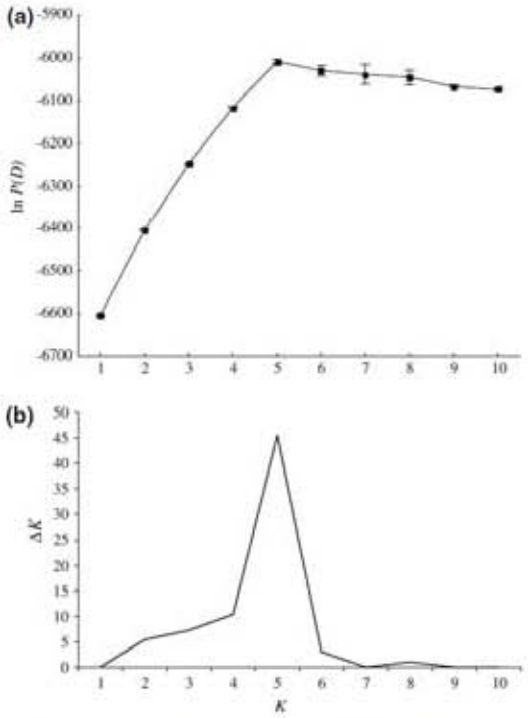
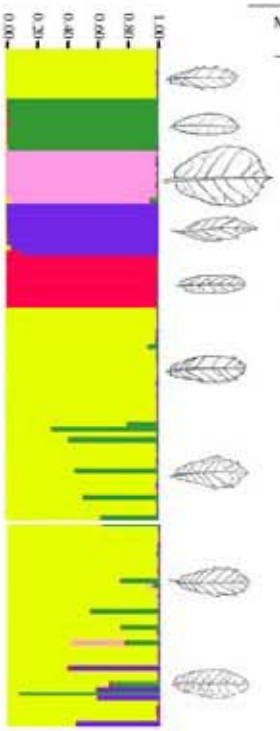


Fig. 2 Estimated genetic groups (K) from STRUCTURE clustering analysis: **a** mean and standard deviation of $\ln P(D)$ for ten independent runs of STRUCTURE, and **b** plot of statistics ΔK with respect to genetic clusters K (from 1 to 10). In both cases, the *peak* indicates the most probable number of genetic groups

Hybridization frequency and genetic combinations

We found that the occurrence and frequency of the different combinations between *Q. castanea* and red oak species varied among stands (Fig. 4). For example in the PNT population, we did not find evidence of a significant contribution of other genetic groups to the *Q. castanea* gene pool. In contrast, our analysis detected five *Q. castanea* × *Q. crassipes* hybrids (25 %) and 15 *Q. castanea*



Morphological group / Locality	Genetic assigment "*Q. castanea*"	"*Q. crassipes*"	"*Q. laurina*"	"*Q. mexicana*"	"*Q. crassifolia*"
Q. castanea	0.986	0.004	0.004	0.003	0.003
Q. crassipes	0.006	0.980	0.004	0.005	0.006
Q. crassifolia	0.003	0.004	0.003	0.003	0.986
Q. laurina	0.013	0.004	0.975	0.003	0.006
Q. mexicana	0.005	0.008	0.004	0.976	0.007
Sympatric PNT	0.981	0.004	0.004	0.008	0.003
Sympatric PECM	0.861	0.129	0.004	0.003	0.004
Sympatric PBT	0.927	0.060	0.004	0.006	0.003
Sympatric PLP	0.779	0.095	0.100	0.005	0.021

Fig. 3 Genetic assignment of five allopatric populations that represent pure species of: *Quercus castanea*, *Q. crassipes*, *Q. crassifolia*, *Q. laurina* and *Q. mexicana* and four sympatric populations based on the analysis of six nSSRs with a Bayesian method implemented in the program STRUCTURE. Each *colored horizontal line* represents the individual's probability of belonging to the cluster with that color. Values of the proportion of ancestry for each population are given in the table

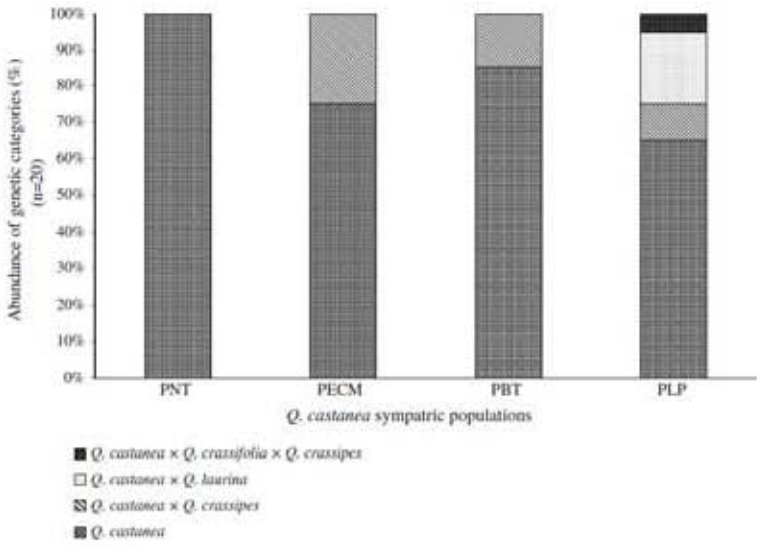


Fig. 4 Percentage of the different genetic category observed in each sympatric *Quercus castanea* population. Individuals were assigned to each category (*Q. castanea* pure genotype, *Q. castanea* × *Q. crassipes* hybrid genotype, *Q. castanea* × *Q. laurina* hybrid genotype, *Q. castanea* × *Q. crassifolia* × *Q. crassipes* hybrid genotype), depending on their individual coefficient of admixture derived from STRUCTURE. *PNT* Parque Nacional El Tepozteco, *PECM* Parque Ecológico de la Ciudad de México, *PBT* Parque Barranca de Tarango, *PLP* Parque Ecológico Las Peñas

genotypes (75 %) in the PECM population. Similarly, we found three *Q. castanea* × *Q. crassipes* hybrids (15 %) and 17 (85 %) *Q. castanea* genotypes in the PBT population. Finally, in the PEP population four *Q. castanea* × *Q. laurina* (20 %), two *Q. castanea* × *Q. crassipes* (10 %) and one *Q. castanea* × *Q. crassifolia* × *Q. crassipes* (5 %) hybrid individuals were detected. The rest of the individuals of this last population were *Q. castanea* genotypes (65 %).

Discussion

Our results provide genetic evidence that *Q. castanea* is involved in introgressive hybridization events with *Q. crassipes*, *Q. laurina*, and *Q. crassifolia*, three of the most common red oak species that coexist with *Q. castanea* in the temperate forests of The Transmexican Volcanic Belt (TVB). In contrast, hybrids between *Q. castanea* and *Q. mexicana* were not found, suggesting that differences in the reproductive barriers between species could be operating. Also, we found that the occurrence, frequency and admixture proportions of the different combinations between *Q. castanea* and red oaks change through the sites, probably due to influences of the variation in ecological factors across sympatric populations. Finally, our results suggest that the increase in the species richness of red oak local community favors the interspecific gene flow among *Q. castanea* and these associated species; however, the co-existence of different red oak species with *Q. castanea* in sympatric stands is not sufficient for hybridization. Our analyses provide evidences and new insights into hybridization and introgression dynamics within a Mexican red oak species complex, through a focal species, *Q. castanea*.

Genetic assignment of allopatric populations

The morphological identification of species was supported by genetic analyses based on six nSSRs that identified the same number of genetic groups, which agrees with the number of taxa involved in this complex. As suggested by Evanno et al. (2005), the intensity of sampling both individuals and markers plays a role in the correct detection of the number of genetic groups. In this sense, studies in closely related European (Valbuena-Carabana et al. 2005; Curtu et al. 2007; Salvini et al. 2009) and Mexican oaks (Peñaloza-Ramírez et al. 2010) have suggested that five or six microsatellite loci are sufficient to distinguish between pure species. On the other hand, an effect of the partial sampling of individuals on the correct detection of genetic groups has been detected (Evanno et al. 2005). However, in this study, ten individuals were sufficient to correctly assign to a species. This result, suggest that in the allopatric populations, each species remains distinct and has its own degree of genetic cohesiveness (Templeton 1989).

We found that the majority of the individuals from allopatric populations of each red oak species (including *Q. castanea*) were assigned to a single genetic group ($Q > 0.9$), a fact that suggests that these populations have not a significant genetic contribution from other species (Lepais et al. 2009); as a consequence they were useful as reference populations. Nevertheless, although these species are genetically isolated in allopatric conditions, the existence of genetically mixed individuals within *Q. castanea* populations indicates that the genetic isolation among *Q. castanea* and associated species is not complete.

Genetic evidence for hybridization of *Q. castanea* across a natural gradient in red oaks

In this study, the Bayesian analysis showed the occurrence of 15 hybrid individuals that showed various degrees of admixture among *Q. castanea* and three of the four genetic groups involved in this complex, in sympatric populations across a natural gradient of red oak species richness (Fig. 3). These results are congruent with a significant increase in genetic diversity levels in *Q. castanea* populations (*He*) as the red oak species richness increases (Valencia-Cuevas et al. 2014). This suggests that the presence of admixed trees in the sympatric populations is the factor that promotes an increase in genetic diversity levels of *Q. castanea*. Similar results were obtained by Sánchez-Ortiz (2012) in a gradient of white oak species associated with *Q. glabrescens* Benth. (section *Quercus*) in Mexico. Moreover, the genetic diversity levels (Valencia-Cuevas et al. 2014) showed a positive and significant relationship with the number of hybrid individuals reported in this study ($r = 0.98$; $P < 0.002$). However, it is important to point out that the co-existence of other red oak species with *Q. castanea* not necessarily leads to hybridization, because this species did not show genetic evidence of hybridization with other red oak species present in the sympatric sites. Likewise, the genetic exchange that has occurred among *Q. castanea* and three of its associated red oak species suggests that the hybridization possibly has contributed to shape the patterns of atypical foliar morphological variation observed in sympatric populations of this species. Atypical morphological variation has been observed in other studies with oak species that occur in sympatry (González-Rodríguez et al. 2004; Tovar-Sánchez and Oyama 2004; Cavender-Bares and Pahlich 2009; Albarrán-Lara et al. 2010; Peñaloza-Ramírez et al. 2010) and this fact has usually been interpreted as supporting the hypothesis of interspecific gene flow. In the future, it would be interesting to study the influence of hybridization on

morphological features of *Q. castanea*, to test this hypothesis.

In particular, genetic data indicated that the major proportions of hybrid individuals were *Q. castanea* × *Q. crassipes*, and *Q. castanea* × *Q. laurina*. In contrast, hybrid individuals that seem to be an admixture among *Q. castanea* and two parental genomes were scarce. Our analysis only detected one hybrid individual *Q. castanea* × *Q. crassipes* × *Q. crassifolia* in PLP. Similar results have been reported in a white oak species complex in Europe (Lepais et al. 2009) and in two red oak species complexes, one in USA (Dodd and Afzal-Rafii 2004) and one in Mexico (Peñaloza-Ramírez et al. 2010). This deficit of tri-hybridizations has been explained on the basis that pre-zygotic reproductive barriers are not totally lost in hybrids, a condition that promotes that they remain reproductively isolated from non-parental species. In consequence, high fidelity towards their parental species and the production of numerous backcrosses in both directions results in the recovery of purebreds within a few generations, avoiding the complete species mixture in a hybrid swarm (Lepais and Gerber 2011). In spite of this, hybridization involving more than two species seems to happen in natural populations (Lepais et al. 2009; Peñaloza-Ramírez et al. 2010; this study). Kaplan and Fehrer (2007) explains that for a triple hybridization to occur it is required the production of fertile hybrid genotypes between at least two species, and then the crossing between hybrids and a third species, or between hybrids from different species combinations. In this sense, we suppose that the tree-hybrid individual that presented a combination between *Q. castanea*, *Q. crassifolia* and *Q. crassipes* genetic groups can be the result of the cross between a fertile hybrid genotype of *Q. crassipes* × *Q. crassifolia* and a *Q. castanea* genotype. This last possibility arises because *Q.* × *dysophylla* Benth. individuals (hybrid between *Q. crassipes* and *Q. crassifolia*; Tovar-Sánchez and Oyama 2004) are coexisting with *Q. castanea* in this locality and probably, a cross

ting with *Q. castanea* in this locality and probably, a cross

among this type of hybrid and *Q. castanea* has been the route of incorporation of *Q. crassifolia* genome into the genetic pool of this last species.

Hybridization rate between *Q. castanea* and red oaks across sympatric populations

The percentage of hybridization between *Q. castanea* and the associated red oak species varied from 15 to 35 % depending on the stand. These estimations are in accordance with previous studies on oak hybridization. For example, a study with three stands in Spain detected between 6 and 22 % of hybrids between *Q. petraea* and *Q. pyrenaica* (Valbuena-Carabana et al. 2007). Also, Lepais et al. (2009) found that the percentage of hybrid individuals ranged from 10.7 to 30.5 % in different stands (*Q. robur*, *Q. petraea*, *Q. pyrenaica*, and *Q. pubescens*).

Also, we found that the level of hybridization varies depending of the species combination: 12.6 % (*Q. castanea* × *Q. crassipes*) >5.1 % (*Q. castanea* × *Q. laurina*) >1.2 % (*Q. castanea* × *Q. crassipes* × *Q. crassifolia*); these values are similar to other studies. For example, analysis of interspecific gene flow between *Q. robur*, *Q. petraea*, *Q. pubescens*, and *Q. frainetto* showed that the level of hybridization varied from 1.7 to 16.2 % between pairs of species (Curtu et al. 2007). Burgarella et al. (2009) found that the hybrids between *Q. suber* and *Q. ilex*, comprised less than 2 % of adults in areas where their ranges overlap. In a study of *Q. virginiana* and *Q. geminata*, showed that 5.5 % of adults presented mixed ancestry (Cavender-Bares and Pahlich 2009). These last studies report that the percentage of hybrids found seems to vary between species pairs and stand investigated. Moreover, heterogeneous patterns of hybridization between the same species pair in different parts of its geographic distribution are not infrequent in oaks (Williams et al. 2001; Curtu et al. 2007; Jensen et al. 2009; Lepais et al. 2009). These differences in admixture rates between species pairs and stands have been explained due to differences in the reproductive barriers among species and environmental variation, respectively (Petit et al. 2002; Abadie et al. 2012; Lepais et al. 2013). For example, some studies report that particular oak species present differences in the strength of their reproductive barriers that limit the hybridization phenomenon (Abadie et al. 2012; Lepais et al. 2013). There is also evidence of asymmetric cross-compatibility between oak species (Steinhoff 1993), a fact that indicates that the success of hybridization events is preferentially directional and depends on the identity of the species that acted as maternal or paternal donor (Olrik and Kjær 2007; Lepais et al. 2013). In this context, it is probable that there might be some species more prone to hybridize with *Q. castanea* than others, a fact that might explain in part the

castanea than others, a fact that might explain in part the

higher frequency of *Q. castanea* × *Q. crassipes* hybrids or the absence of *Q. castanea* × *Q. mexicana* hybrids, at least for our study sites.

We found that the occurrence and hybridization level between *Q. castanea* and red oaks varied geographically. For example, we did not find hybrids of *Q. castanea* × *Q. crassipes* in PNT or *Q. castanea* × *Q. laurina* in the PBT and PECM populations, although *Q. crassipes* was present in the first site and *Q. laurina* was present in the last two sites. Also, both types of hybrids were the most common found in this work. These results acknowledge the importance of local environmental conditions on dynamics of interspecific gene flow between *Q. castanea* and associated red oaks. There are several studies that have documented that the levels of hybridization and introgression in

oaks vary with habitat conditions (Williams et al. 2001; Himrane et al. 2004; Lagache et al. 2013), geographic localization of the hybrid zone (Tovar-Sánchez and Oyama 2004), establishment and survivorship of hybrid individuals (Valbuena-Carabana et al. 2007), different rates of gene flow (Curtu et al. 2007), relative abundance and identity of species (Lepais et al. 2009), spatial structure of species (Salvini et al. 2009), or proportion of conspecific pollen and the density of individuals available for mating (Lagache et al. 2013). In this context, we suggest that the differences in the occurrence and frequency of hybrids could be influenced by the variation in ecological and geographical conditions along our study sites. The TVB is characterized by its complex topography, altitude and climatic diversity, which combined with its geographical position, provides a mosaic of environments, habitats and microhabitats (Challenger 1998). This ecological variation among stands may promote variation in isolating barriers between species (Buerkle and Rieseberg 2001; Lepais et al. 2009, 2013; Abadie et al. 2012). Previous work (Rieseberg and Willis 2007; Lexer and Widmer 2008; Lowry et al. 2008) has concluded that there is large diversity in barrier types and strength in different plant systems, consistent with different numbers, effects or types of genes potentially involved in reproductive isolation. Oaks are no exception, since recent studies report that several oak species present variation in the degree of reproductive isolation, which has been attributed to the plasticity in the expression of reproductive barriers as a consequence of variation in ecological conditions across mixed sites (Lepais and Gerber 2011; Abadie et al. 2012; Lepais et al. 2013). A good example is the contrast in the rate of hybridization that has been reported in different mixed stands of *Q. robur* and *Q. petraea* across Europe (Curtu et al. 2007; Lepais et al. 2009; Jensen et al. 2009). Under this scenario, it is possible that the variation in the occurrence and percentage of hybrids found between *Q. castanea* and red oaks in our different sympatric populations has been promoted by differences in the expression of reproductive barriers associated to environmental variation that characterizes the TVB.

The PLP population is of particular interest as in this population more species were coexisting with *Q. castanea*, and more hybrids were detected and different genetic combinations were found. This site is a natural protected area that presents an abrupt change in microclimatic conditions, combining mesic and xeric settings in a fine geographical scale. This environment has permitted the co-occurrence of *Q. castanea*, *Q. crassipes*, *Q. laurina*, *Q. mexicana* and *Q. crassifolia*, because the first two species have preferences for xeric conditions and the last three for mesic conditions. Under this scenario, it is probable that an increase in multi-species pollen as a result of floral overlapping between *Q. castanea* and the other red oaks may favor the occurrence of interspecific crosses (Lepais et al. 2009), promoting hybridization and subsequent introgression in this site. Similarly, several studies at the stand level have reported that hybridization and introgression in oaks may be facilitated when species co-occur in an area with smaller-scale environmental heterogeneity (Curtu et al. 2007; Valbuena-Carabana et al. 2007).

Management of genetic diversity in hybridizing oaks

Recent studies have reported high levels of nDNA and cpDNA diversity of *Q. castanea* populations located in TVB (Acosta 2008; Peñaloza-Ramírez 2011; Herrera-Arroyo 2013; Alvarado-Dávalos 2014; Valencia-Cuevas et al. 2014). These authors suggest that these results can be explained because *Q. castanea* is a species that probably has maintained high levels of gene flow through pollen, efficient dispersion of seeds, large and continuous populations during its evolution and a broad geographic range. Unfortunately, the temperate forests in Mexico are being cleared and fragmented for agriculture, cattle grazing and urban areas (Challenger 1998), a condition that puts under threat the maintenance of genetic diversity (Young et al. 1996; Lowe et al. 2005). Studies have shown that the levels of genetic diversity of *Q. castanea* are preserved in both nuclear and chloroplast genomes in adults and seedling populations of fragmented habitats located in the TVB (Herrera-Arroyo 2013; Alvarado-Dávalos 2014). These results are interesting, because studies on several tree species in fragmented landscapes have reported a reduction in genetic diversity levels, particularly in recently established individuals in comparison to older individuals due to reduction of gene flow, elevated inbreeding and genetic drift (Young et al. 1996; Lowe et al. 2005). These findings suggest that fragmentation is not a factor that influences the levels of genetic diversity of *Q. castanea* populations inside the TVB. In contrast, Valencia-Cuevas et al. (2014) found a positive and significant relationship between genetic diversity of *Q. castanea* (nDNA and cpDNA) and the number of red oak species growing in sympatry with it. These results suggest that interspecific hybridization might be responsible for the increase in genetic diversity levels of the sympatric populations of *Q. castanea*. Using a Bayesian clustering analyses, Alvarado-Dávalos (2014) found three different genetic groups through several *Q. castanea* populations in the TVB, whose proportions change with the fragmentation levels of the population. The author suggests that this condition is a consequence of the reduction in the proportion of conspecific pollen and loss of connectivity among individuals available to mating in the fragmented landscape. Therefore, the gene pool of the individuals in fragmented populations comes from external parents, a fact

that promotes the maintenance of the genetic diversity levels of *Q. castanea*. In addition, Valencia (1994) proposed that *Q. castanea* might probably exchange genes with *Q. affinis* Scheidw., *Q. crassifolia*, *Q. crassipes*, *Q. laurina* and *Q. mexicana*, forming a syngameon. This information along with the data of previous ecological and genetic studies about *Q. castanea* populations within the TVE suggest that interspecific gene flow is an important factor that promotes high genetic diversity levels in this red oak species.

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Anexo 2

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Effect of host-plant genetic diversity on oak canopy arthropod community structure in central Mexico

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Abstract

Background: Recently it has been proposed that the genetic diversity of foundation species influences the structure and function of the community by creating locally stable conditions for other species and modulating ecosystem dynamics. Oak species are an ideal system to test this hypothesis because many of them have a wide geographical distribution, and they are dominant elements of the forest canopy. In this study we explored the response of canopy arthropod community structure (diversity and biomass) to the level of genetic diversity of *Quercus crassipes* and *Q. rugosa*, two important canopy species. Also, we examined the effect of oak species and locality on some community structure parameters (diversity, biomass, rare species, and richness of arthropod fauna) of canopy arthropods. In total, 160 canopies were fogged in four localities at the Mexican Valley (ten trees per species per locality per season).

Results: *Q. crassipes* registered the highest number of rare species, diversity index, biomass, and richness in comparison with *Q. rugosa*. We found a positive and significant relationship between genetic diversity parameters and canopy arthropod diversity. However, canopy arthropod biomass registered an inverse pattern. Our results support the hypothesis that the genetic diversity of the host-plant species influences the assemblage of the canopy arthropod community.

Conclusions: The pattern found in our study provides a powerful tool when trying to predict the effects of the genetic diversity of the host-plant species on different community structure parameters, which permits assignment of a new conservation status to foundation species based on their genetic diversity.

Keywords: Arthropods community; Canopy; Foundation species; Genetic diversity; *Quercus*

Background

In the last decade, various studies have documented that genes can have an extended effect beyond the individual, leading to interactions with other species to produce community and ecosystem phenotypes (genetic diversity of foundation species, Whitham et al. 2006). Foundation species have been defined as 'species that structure a community by creating locally stable conditions for other species and by modulating and stabilizing fundamental ecosystem process' (Dayton 1972). This emphasis on foundation species, which are a small subset of the total species in an ecosystem, is because different studies have showed that the analysis of their genetic attributes can reveal strong and predictable effects on communities and ecosystems (Whitham et al. 2003, 2006). For example, studies in cottonwoods (Wimp et al. 2004), eucalyptus (Dungey et al. 2000), oaks (Tovar-Sánchez and Oyama 2006a,b), and willows (Hochwender and Fritz 2004) have evidenced that plant genetics can influence the associations and interactions of the communities associated with these species. The associated communities that have showed a response to the genetic differences within foundation species included taxa as diverse such as soil microbes (Schweitzer et al. 2008),

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aquatic invertebrates (Le Roy et al. 2006), mycorrizal fungi (Sthultz et al. 2009), understory plants (Adams et al. 2011), lichens (Lamit et al. 2011), and foliar arthropods (Wimp et al. 2004; Tovar-Sánchez and Oyama 2006b; Tovar-Sánchez et al. 2013). Likewise, ecosystem processes like nutrient cycling (Schweitzer et al. 2008), primary production (Crutsinger et al. 2006), and ecosystem stability (Keith et al. 2010) are affected by the genetics of foundation species.

Most of the evidence that indicates that the genetic diversity within the foundation species of terrestrial and aquatic habitats affecting the distributions of their associated species come from studies under experimental conditions (e.g., Wimp et al. 2007; Keith et al. 2010; Bangert et al. 2012). Nevertheless, it has been suggested that these studies do not show the potential consequences of different levels of genetic diversity in natural settings (Hughes et al. 2008) and may overestimate the importance of host-plant genetic attributes for structuring the communities (Tack et al. 2010, 2011). However, there are several studies in which the results obtained in experimental gardens have been corroborated in natural conditions [e.g., eucalyptus (Whitham et al. 1999; Dungey et al. 2000), and willows (Wimp et al. 2004, 2005)]. These results suggest that a genetic perspective of the community may be applicable, but there is still little understanding about the relative importance of a genetically-based trait variation within the foundation species and other factors for structuring communities in natural conditions (Wimp et al. 2007). These kinds of studies are valuable because they offer a realistic approach to processes that occur under natural conditions and the ability to span relatively large spatial or temporal scales, even when it is difficult to control variables related to the spatial location of host plants that can influence the abundance, distribution, and diversity of the species associated (Vellend and Geber 2005).

In general, both in natural and experimental conditions, the genetic diversity of the host plant has been analyzed under the assumption of the following gradient of genetic diversity [parental < F1 < backcrosses (Whitham et al. 1994; Wimp et al. 2005, 2007; Tovar-Sánchez and Oyama 2006b; Adams et al. 2011)] or considering that genetic diversity increases when more than one genotype is present (Bailey et al. 2006). In contrast, few studies have evaluated the relationship between some measures of host-plant genetic diversity on community metrics (Wimp et al. 2004; Tovar-Sánchez and Oyama 2006b; Tovar-Sánchez et al. 2013).

Canopy arthropod communities have been widely used to evaluate the influence of the genetic diversity of host plants on their associated communities (Whitham et al. 1999; Hochwender and Fritz 2004; Wimp et al. 2004, 2007; Bangert et al. 2006; Tovar-Sánchez and Oyama 2006b; Keith et al. 2010; Tack et al. 2010; Castagneyrol et al. 2012; Tovar-Sánchez et al. 2013). This preference is probably because the canopy is a habitat that can be physically delimited as their arthropod communities are considered the main component in terms of abundance and species diversity (Stork and Hammond 1997). Recently made estimates suggest that the global average richness of this group is of 6.1 million species (Hamilton et al. 2013). Additionally, arthropods play an important role in ecological terms, acting as pollinators, prey, parasites, parasitoids, herbivores, and detritivores (McIntyre et al. 2001).

The effects of the foundation species' genetic characteristics on the arthropod community structure have been detected in metrics as a composition (Bangert et al. 2005; Wimp et al. 2005; Bailey et al. 2006), richness (Dungey et al. 2000; Bangert et al. 2005, 2006, 2008; Crawford and Rudgers 2013) and species diversity (Wimp et al. 2004; Tovar-Sánchez and Oyama 2006b; Ferrier et al. 2012; Tovar-Sánchez et al. 2013). In general, the studies have reported that unique arthropod communities were associated with different genotypes of the host plant (Bangert et al. 2006; Ferrier et al. 2012) and that the richness and species diversity increases as the genotype number also increases [e.g., genotypic diversity (Wimp et al. 2005; Ferrier et al. 2012)] when the genetic diversity of the population increases (Wimp et al. 2004; Tovar-Sánchez and Oyama 2006b; Tovar-Sánchez et al. 2013), or when the individual genetic diversity level increases (Tovar-Sánchez et al. 2013). These patterns have been explained considering that an increase in the host-plant genetic diversity can generate changes in their morphological (Lambert et al. 1995; González-Rodríguez et al. 2004; Tovar-Sánchez and Oyama 2004), phenological (Hunter et al. 1997), and plant architecture (Martinsen and Whitham 1994; Whitham et al. 1999; Bangert et al. 2005), as well as in their secondary chemistry (Fritz 1999; Wimp et al. 2004). These characters constitute a wide array of resources and conditions that can be exploited by their associated herbivores. These results suggest that the effects of genetic diversity on community function can be equal or greater in magnitude compared to species diversity (Hughes et al. 2008), emphasizing the important role that genetic diversity can play in ecological processes. The incorporation of these types of studies into the field of biodiversity research is a logical extension of the theory underlying previous diversity studies, recognizing that genetic diversity is one of the fundamental levels of biodiversity (Hughes et al. 2008).

Knowledge of mechanisms that may be driving the associations between arthropods and plants plays a key role in our understanding of the impact of plant genetic diversity on dependent arthropod communities; however, these mechanisms remain poorly understood

(Wimp et al. 2007). It has been suggested that phenotypic traits that affect arthropod communities as phenology, physical defenses, and foliar chemistry are features that have a genetic basis (Johnson and Agrawal 2005; Bangert et al. 2006) but have only rarely been linked to both plant genetics and arthropod community structure (Wimp et al. 2007). Also, these attributes can vary between host-plant species (Foss and Rieske 2003; Forkner et al. 2004; Marquis and Lill 2010), affecting both the quantity and quality of resources available to arthropods (Murakami et al. 2007). Understanding the strength of these associations is important as they provide a mechanistic approach to comprehend the relationship between plant genetic diversity, environment, and arthropod community structure.

Oaks (Fagaceae, *Quercus*) are an ideal system to study the effects of host-plant species genetic diversity on their associated canopy communities because of their high levels of genetic variation (e.g., Tovar-Sánchez et al. 2008; Valencia-Cuevas et al. 2014, 2015); many of their species show a wide geographical distribution and canopy dominance (Valencia 2004), and constitute the habitat of different species. Therefore, some of them can be considered as foundation species. Unfortunately, there are a few studies that have analyzed the influence of the oak host genetic diversity on their canopy arthropods community. In addition, the results of these studies have been contrasting. For example Tovar-Sánchez and Oyama (2006b), reported a positive and significant relationship between population genetic diversity of seven hybrid zones from the *Q. crassipes* × *Q. crassifolia* complex in Mexico and the canopy endophagous insect community diversity. Similarly, the *Q. castanea* and *Q. crassipes* plants that were genetically more diverse supported higher richness, diversity, and species density of the canopy ectophagous insects (Tovar-Sánchez et al. 2013) in central Mexico. In contrast, Tack et al. (2010, 2011) found that genetic diversity has little influence on the endophaguos insect community associated to *Q. robur* in Finland. Similar results were reported by Castagneyrol et al. (2012), who found that the host-plant genetic attributes (genetic diversity, relatedness, and genetic identity) did not have a significant effect on the phytophagous insect community structure (endophagous and ectophagous) associated to *Q. robur* canopy in France. The contrasting results of these investigations show the need for further studies that help us understand the importance of the genetic diversity of oak populations on canopy arthropod communities.

The aims of this study were to analyze the canopy arthropod community structure of *Quercus crassipes* and *Q. rugosa* from a genetic perspective, to answer the following questions: 1) Does the genetic diversity of host-plant species affect the arthropod community structure in terms of species diversity and biomass? 2) Does the canopy arthropod-community structure vary between oak host species and localities? We predict that more genetically diverse host plants should support more diverse communities because they offer a wider array of resources and conditions to be exploited.

Methods

Study sites and oak species

The Mexican Valley has a well delimitated biogeographical area of 7500 km^2 covering several states of Central Mexico surrounded by the main Mexican Sierras. Altitude ranges from 2,230 m to 2,500 m at the bottom and 3000 m to 5450 m in mountain areas. The most important vegetation types in the Mexican Valley are *Abies*, *Pinus* and *Quercus* forests (Rzedowski and Rzedowski 2001). To minimize geological historic and environmental site effects, we chose four localities [Parque Nacional El Chico (PNECh) in Hidalgo State, Parque Ecológico de la Ciudad de México (PECM) in Mexico City, and Jilotepec and Juchitepec in Mexico State] (Figure 1) that have the following common traits: it has the same geological history [the Mexican Valley is part of the Trans-Mexican Volcanic Belt (Rzedowski and Rzedowski 2001), and its formation process began during the Quaternary-Pliocene (Ferrusquía-Villafranca 1998)], weather (temperate subhumid), altitude (between 2540 m to 2720 m), vegetation type (mature oak), tree age (between 10 m to 13 m), and soil type (volcanic origin or derived from igneous and sedimentary rocks). These areas present almost no local disturbance inside the forest because they are under protection standards or because its rocky substrate prevents agriculture and livestock (Table 1).

Quercus crassipes Humb. & Bonpl. (*Lobatae*) and *Q. rugosa* Née (*Quercus*) are abundant species in the four study sites. Both can be recognized easily in the field from its leaf characteristics such as shape, size, coloration, and pubescence. *Q. crassipes* include trees up to 17 m tall and 1 m in trunk diameter. Leaves are deciduous, coriaceous, narrowly elliptic, and lanceolate. It flowers in May and bears fruits from September to January. It is distributed within the southeast part of the Sierra Madre Oriental and the Trans-Mexican Volcanic Belt (TVB), between 1900 m to 3500 m a.s.l. *Q. rugosa* includes large trees of up to 20 m in height with a trunk diameter of 1 m. Leaves are evergreen or semi-deciduous at maturity; they are thick and rigid, strongly rugose, and obovate to elliptic-obovate. The flowering season is in August. Fruits are produced annually (November to March). This species is distributed in the major Mexican mountain ranges [SMOr, Sierra Madre Occidental (SMOc), Sierra Madre del Sur (SMS), Sierra Norte de Oaxaca (SNO), Sierra de Chiapas (SCh), and TVB], at an altitude of 1800 m to 2900 m (Rangel et al. 2002).

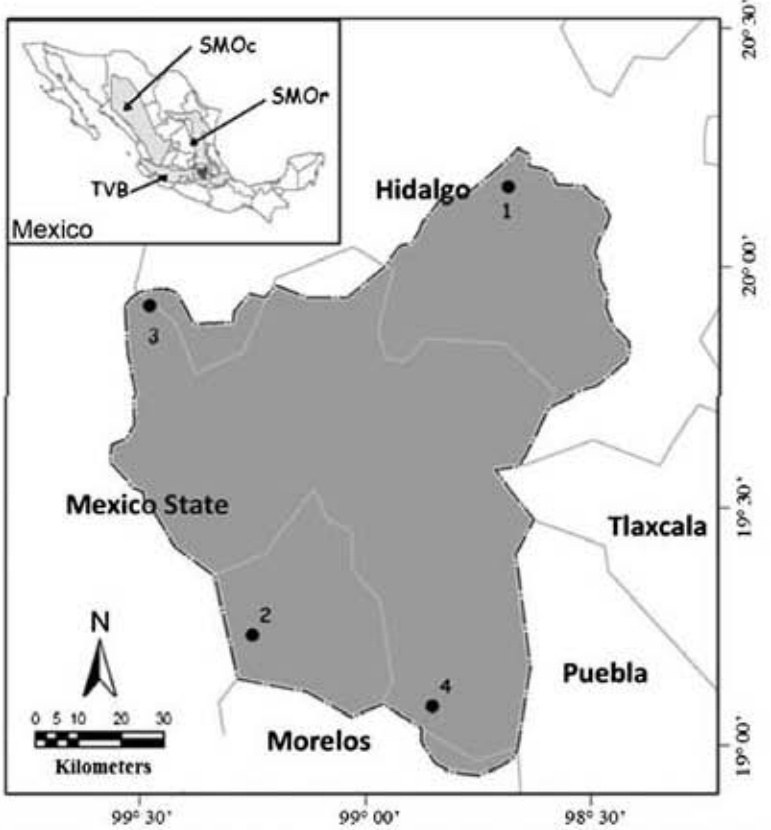

Figure 1 Geographical distribution of the four study sites along the Mexican Valley: 1) Parque Nacional El Chico (PNECh), 2) Parque Ecológico Ciudad de México (PECM), 3) Jilotepec, and 4) Juchitepec. (*Gray circle symbol*) = Mexican Valley, SMOr = Sierra Madre Oriental, SMOc = Sierra Madre Occidental, TVB = Trans-Mexican Volcanic Belt.

Molecular data

Leaves with no apparent damage were collected from twenty individuals per species in each study site [*Q. crassipes* (n = 80) and *Q. rugosa* (n = 80)]. Leaf tissue was frozen in liquid nitrogen and transported to the laboratory for DNA extraction. Total DNA was extracted and purified by using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA). DNA quantification was done by fluorometric analysis, and DNA quality was visualized by comparing the intensity of bands with known standards of lambda DNA on agarose gels at 0.8%. Genetic analyses were performed using randomly amplified

Table 1 Locality name, state, geographic coordinates, altitude, annual precipitation, slope, and *Quercus* species

Locality	State	Latitude (N), longitude (W)	Altitude (m)	Annual precipitation (mm)	Slope (°)	*Quercus* species
PNECh	Hidalgo	20°10', 98°41'	2540	1, 200.2	14	*Q. crassipes*, *Q. rugosa*, *Q. mexicana*, *Q. laurina*, *Q. crassifolia*, *Q. desericola*, and *Q. greggii*.
PECM	Mexico City	19°15', 99°11'	2620	1,084.9	11	*Q. crassipes*, *Q. rugosa*, *Q. castanea*, *Q. laeta*, and *Q. laurina*.
Jilotepec	Mexico State	19°55', 99°29'	2570	754.3	8	*Q. crassipes*, *Q. rugosa*, *Q. laeta*, and *Q. crassifolia*
Juchitepec	Mexico State	19°05', 98°51'	2720	729.9	9	*Q. crassipes*, *Q. rugosa*, and *Q. gregii*.

polymorphic DNA (RAPDs) and microsatellite markers (SSRs).

For RAPDs, sixty 10-base pair (pb) primers of random sequence (Kits A, B, C; Operon Technologies, Alameda, California, USA) were tested. Eighteen of them were selected based on the amplification results and reproducibility. The selected primers produced a total of 121 polymorphic bands. PCR reactions were done in a PTC-100 Programmable Thermal Controller (MJ Research Inc.) as follows: 10 ng of DNA templete, 50 mM KCl, 10 mM Tris-HCl (pH 8.4), 2 mM $MgCl_2$, 0.1 mM of each dNTP, 0.2 mM of each primer, and 1 U of *Taq* polymerase in a final volume of 25 μl. Reaction conditions were the following: an initial 2 min denaturation step at 94°C, followed by 45 cycles at 94°C for 1 min, 1 min at 36°C, followed by an annealing temperature at 72°C for 30 s and a final extension at 72°C for 7 min. DNA fragments were separated through electrophoresis on agarose gels at 2.8%, stained with ethidium bromide, and developed on an UV light table. The molecular weight of the DNA fragments was estimated by comparison with a 1 kb DNA ladder.

Microsatellites primers (Ccmp3, Ccmp4, and Ccmp41) were obtained from Weising and Gardner (1999). PCR reactions were done as follows: 15 ng of DNA template, 50 mM KCl, 20 mM Tris-HCl (pH 8.4), 2 mM $MgCl_2$, 0.13 mM of each dNTP, 25 mM of each primer, and 0.8 U of *Taq* polymerase in a final volume of 25 μl. Reaction conditions were an initial denaturation step at 95°C for 5 min, followed by 30 cycles at 94°C for 1 min, 1 min at the appropriate annealing temperature, followed by 30s at 72°C, and a final extension at 72°C for 8 min. Annealing temperature differed for each primer pair. 50°C for Ccmp3, 48°C for Ccmp4, and 55°C for Ccmp41. PCR products were resolved on polyacrilamide gels at 6% (7 M urea) at 60 W for 3 h in order to determine the polymorphic primers. We measured the length of the amplified microsatellites fragments by running an aliquot of each PCR product on an automatic sequencer ABI 3100 (Applied Biosystems CA, USA) at 35 W for 80 min to 90 min using gene scan ROX-2500 (Applied Biosystems, CA, USA) as size standard. Alleles were scored using the Gene Mapper ver. 3.7 Software (Applied Biosystems, CA, USA).

Canopy arthropod communities

The arthropod community structure was surveyed in forty trees of both species. Ten individuals per species were vouchered and fogged during rainy (August 2005) and dry (February 2005) seasons on each locality. Sampling was done seasonally, which allowed having a representative annual sample of the canopy arthropodofauna, as suggested by previous studies, which have demonstrated that seasonality modifies both composition and richness in oaks (Tovar-Sánchez and Oyama 2006a; Tovar-Sánchez 2009). The individual trees sampled in this study had a height between 10 m and 13 m (mean ± d.e., 11.0 ± 0.13 m).

Arthropods were collected by fogging the entire canopy of a single tree with 750 ml of non-persistent insecticide (AqualPy, AgrEvo, Mexico). This insecticide is composed of 30 g pyrethrine/l and 150 g piperonyl-butoxide/lL at a concentration of 30% v/v. Fallen arthropods from each fogged tree were collected in ten plastic trays (each 0.32 m^2 area) located randomly under the crowns. Canopies of trees selected for fogging were isolated from other trees as far as possible, by avoiding overlapping. A measure of the exploited canopy volume was estimated by multiplying the difference between the total height and the height to the lowest branch with denser leaf cover of each tree by 3.2, which is the area of collecting trays (Tovar-Sánchez 2009). The arthropods were separated into morphospecies and after sorted to major orders. All samples were sent to arthropod specialists for taxonomic identification. Abundance of each morphospecies was also counted.

The biomass of canopy arthropods associated to *Q. crassipes* and *Q. rugosa* was calculated using the model proposed by Tovar-Sánchez (2009) for oaks in the Mexican Valley. A sample of six individuals/taxa was chosen and then put in a drier at 40°C until constant weight. Weight was determined on an analytical scale.

Statistical analysis

Genetic diversity of oak host species

Genetic diversity of *Q. crassipes* and *Q. rugosa* was estimated for SSRs and RAPDs molecular markers as the average expected heterozygosity (*He*). We used this parameter of genetic diversity in order to compare the results with others studies in oaks. Genetic data were analyzed with TFPGA v. 1.3 and POPGENE v. 1.31. The data were transformed as $\sqrt{x}$ (Zar 2010), and we used a *t*-student test to examine differences in genetic diversity between species. A Kruskal-Wallis analysis of variance was used to determine differences in oak-species genetic diversity among sites. Thereafter, a Tukey test was conducted to determine significant differences (Zar 2010). Statistical analyses were conducted using STATISTICA for Windows v. 8.0 software (StatSoft 2007).

Canopy arthropods

The diversity of the canopy arthropod community was estimated at the morphospecies level by using the Shannon-Wiener index (*H'*). This index was then compared between pairs of localities with a randomization test as described by Solow (1993). This test re-samples 10,000 times from a distribution of species abundances produced by the sum of the two samples. In addition,

rare species number (*RS*) was analyzed. Rare species were defined as those species represented by fewer than four individuals in the samples (Tovar-Sánchez and Oyama 2006a).

The arthropods biomass (*W*) was calculated according to Tovar-Sánchez (2009):

$$W = \left(e^{-10.644}\right)\left(L^{2.587}\right)$$

where *W* is the biomass in mg (dry weight) and *L* is the body length in millimeters. A mean size and aggregate biomass of the morphospecies population in the sample was estimated from the number of individuals, the mean size of all others measured, and the number of individuals of the same morphospecies. This estimation was calculated for oak canopy arthropod biomass in temperate forests from the Mexican Valley.

Two-Factor Analysis of Variance (Model I fixed effects, Zar, 2010) was conducted to test differences in canopy arthropod biomass, species richness, number of rare species among localities (L), species (S), and interaction L × S. Data were transformed as follows: X' = log X - 1 (Zar 2010). To determine significant differences in species richness, number of rare species, and biomass between localities, a posterior Tukey test was conducted (Zar 2010). Statistical analyses were conducted using STATISTICA for Windows v. 8.0 software (StatSoft 2007).

General Linear Model (GLM) Analysis of Covariance (Model I fixed effects; Zar 2010) was performed to determine the effect of the locality (L), oak species (S), Genetic diversity, and interaction locality × oak species (L × S) on canopy arthropod biomass and Shannon-Wiener diversity index.

Diversity (*H'*) and biomass (*W*) variables were not correlated with each other. In order to determine the effects of locality, oak species (*Q. crassipes*, *Q. rugosa*) and host-plant genetic diversity (expected heterozygosity estimated with microsatellite and RAPDs data) on canopy arthropods diversity index (*H'*) and biomass, we performed a GLM. The model used a Poisson error distribution and log link function. GLM describes the effects of variables in a multivariate-model setting. This analysis has the advantages that even if a variable has a non-significant effect on a variable when subjected to univariate analysis, it may still be a significant variable in a multivariate-model setting when accounting for covariance with other factors (Hillebrand et al. 2008). We pooled the following genetic data from 20 trees within each locality: the community was quantified at the stand level and the occurrence of individual SSRs and RAPDs markers present in each locality, resulting in a unique genetic diversity value for each locality. Locality and oak species were considered as categorical fixed factors and genetic diversity a continuous factor. Statistical analyses were conducted using species diversity and richness version 3.03. and the General Linear Model platform within STATISTICA for Windows v. 8.0 software (StatSoft 2007).

Results

Genetic diversity of *Quercus crassipes* and *Q. rugosa*

Genetic diversity analyses revealed that the expected heterozygosity was significantly higher in *Q. crassipes* than *Q. rugosa* populations [RAPDs (t = 3.59, P < 0.05); SSRs (t = 3.45, P < 0.05)] (Table 2). A Kruskal-Wallis analysis of variance showed significant differences in genetic diversity indexes (*He*) among populations of *Q. crassipes* and among populations of *Q. rugosa* (SSR's: H = 11.29, P = 0.002; RAPDs: H = 9.87, P = 0.009). A multiple comparison Tukey test (RAPDs) showed that *Q. rugosa* and *Q. crassipes* present the following *He* gradient PNECh = PECM > Jilotepec = Juchitepec. While SSRs registered the next *He* pattern *Q. rugosa*: PNECh > PECM > Jilotepec = Juchitepec, and *Q. crassipes*: PNECh < PECM > Jilotepec > Juchitepec.

Arthropods composition (abundance)

Canopy arthropod communities were represent by a total of 44,627 arthropods included in 614 morphospecies belonging to the following 24 orders: Araneae, Astigmata, Coleoptera, Cryptostigmata, Dermaptera, Diptera, Entomobryomorpha, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, Mecoptera, Mesostigmata, Neuroptera, Opilionida, Oribatida, Orthoptera, Poduromorpha, Pseudoscorpiones, Psocoptera, Prostigmata, Symphypleona, Thysanoptera, and Trichoptera (nomenclature based on Evans 1992;

Table 2 Genetic diversity parameters for three chloroplast microsatellite loci and 18 RAPD loci, in *Quercus crassipes* and *Q. rugosa* populations

Population	N	Average expected heterozygosity	
		RAPDs	SSRs
Q. crassipes			
PNECh	20	0.43	0.51
PECM	20	0.46	0.58
Jilotepec	20	0.30	0.32
Juchitepec	20	0.28	0.26
Average	*20*	*0.37 (0.03)**	*0.42 (0.02)**
Q. rugosa			
PNECh	20	0.33	0.40
PECM	20	0.32	0.35
Jilotepec	20	0.25	0.21
Juchitepec	20	0.23	0.20
Average	*20*	*0.28 (0.02)**	*0.48 (0.07)**

Numbers in parenthesis are standard error. *Significant differences (P < 0.05) (t- student test).
N, sample size; (standard errors).

Hopkin 1997; Deharveng 2004; Triplehorn and Johnson 2005).

Community structure of canopy arthropods associated to *Q. crassipes* and *Q. rugosa*

Shannon-Wiener diversity (H'), species richness (S), number of rare species (RS), and biomass (W) total values were significantly different ($P < 0.05$) between *Q. crassipes* ($H' = 5.2$, $S = 569$, $RS = 575$, $W = 560.20$) and *Q. rugosa* ($H' = 4.4$, $S = 450$, $RS = 438$, $W = 313.25$). In addition, in all localities these parameters were higher in *Q. crassipes* than in *Q. rugosa* ($P < 0.05$) (Table 3). PNECh and Juchitepec consistently showed significant differences for H', S, RS, and W values in both oak species. In contrast, PECM and Jilotepec had similar values, excepting H' in *Q. crassipes*, and RS for both oak species (Table 3). For *Q. crassipes* and *Q. rugosa*, the Shannon-Wiener diversity index (H') differed significantly between localities ($P < 0.05$), except from PECM and Juchitepec for *Q. rugosa*. However, some oak host individuals presented the same diversity values within and among localities for both species. In general, a statistically significant effect of the locality ($F_{3,152} = 8.151$, $P < 0.001$), the species ($F_{1,152} = 23.902$, $P < 0.001$) and interaction L × S ($F_{2,152} = 3.205$, $P < 0.001$) was detected on rare species. *Q. crassipes* had more number of rare species (less than four individuals) than *Q. rugosa*. Between localities, PNECh showed the highest number of rare species, followed by PECM, Jilotepec, and Juchitepec (Figure 2). Similar results were registered in arthropod species richness, a statistically significant effect of locality ($F_{3,152} = 16.023$, d.f. = 3, $P < 0.001$), species ($F_{1,152} = 32.007$, $P < 0.001$), and interaction L × S ($F_{2,152} = 3.283$, $P < 0.05$) was detected. For arthropod biomass, a statistically significant effect of the locality ($F_{3,152} = 12.952$, $P < 0.001$), the species ($F_{1,152} = 30.741$, $P < 0.001$), and interaction L × S ($F_{2,152} = 9.708$, $P < 0.001$) was registered.

Table 3 Shannon-Wiener diversity index (H'), species richness (S), rare species (RS) and coefficient of variation (CV) of S and RS (in parentheses); and biomass (W) mg DW/m^2 (standard error in parentheses) of canopy arthropods associated to *Quercus crassipes* and *Q. rugosa* in four localities in the Mexican Valley

Locality	H'	S (CV)	RS (CV)	W
			Q. crassipes	
PNECh	5.0^{A}	224 (10.11)^{a}	173 (10.20)^{a}	427.63 (0.042)^{a}
PECM	4.4^{B}	202 (6.17)^{a}	165 (7.39)^{a}	338.15 (0.054)^{ab}
Jilotepec	3.8^{C}	183 (9.43)^{a}	124 (12.48)^{b}	490.29 (0.020)^{b}
Juchitepec	3.0^{D}	162 (6.57)^{b}	113 (12.26)^{c}	984.75 (0.040)^{c}
			Q. rugosa	
PNECh	4.6^{A}	165 (8.71)^{a}	127 (8.38)^{a}	200.92 (0.053)^{a}
PECM	4.0^{B}	158 (6.09)^{ab}	119 (9.86)^{b}	282.89 (0.027)^{b}
Jilotepec	3.5^{B}	153 (5.05)^{b}	105 (12.68)^{c}	354.19 (0.042)^{bc}
Juchitepec	2.7^{C}	117 (9.43)^{c}	87 (13.36)^{c}	415.61 (0.027)^{c}

Same letters show that the mean values for each locality did not differ at $\alpha = 0.05$ (capital letters = Solow test; lower case letters = Tukey's test).

Effect of genetic diversity of oak host species on canopy arthropod community

In general, the diversity (H') and biomass of canopy arthropod species differ significantly among localities, oak species and genetic diversity (He). Also, the interaction locality × oak species was significant, independently of molecular marker used (SSRs and RAPDs). The only variable that had not a significant effect on canopy arthropod diversity was oak species (S) using both molecular markers, and the interaction locality × oak species on arthropod biomass (Table 4).

Discussion

The hypothesis that genetic diversity of foundation species affects the community structure of the canopy arthropods was supported by our results. Also we found that the arthropod community structure was significantly different between host oak species and localities.

Genetic diversity of *Quercus crassipes* and *Q. rugosa*

In general, our study demonstrates that *Q. crassipes* had higher levels of genetic diversity than *Q. rugosa*. These high genetic diversity levels in *Q. crassipes* may be due to incipient reproductive barriers, which facilitate interspecific crosses with closely related species. For example, Valencia (1994) proposed that a group of oaks conformed by *Q. affinis*, *Q. crassipes*, *Q. crassifolia*, *Q. laurina*, *Q. mexicana*, and *Q. rubramenta* may experience genetic exchange when they occur in sympatric/mixed stands. This last scenario has been corroborated by Tovar-Sánchez and Oyama (2004) for the *Q. crassipes* × *Q. crassifolia* complex, González-Rodríguez et al. (2004) for the *Q. laurina* × *Q. affinis* complex, and Valencia-Cuevas et al. (2015) for *Q. castanea*, *Q. laurina*, and *Q. crassifolia*. The species mentioned above are distributed along the Mexican Valley, a fact that may facilitate the genetic exchange with *Q. crassipes*.

Moreover, when the study sites are classified by their number of red oak species, the following pattern is observed PNECh > PECM > Jilotepec > Juchitepec, which is congruent with the genetic diversity pattern for both species (Table 2). Therefore, we suggest a possible relationship between the number of red oak species and their genetic diversity levels. This is supported by the work of Valencia-Cuevas et al. (2014), who reported an increase on the levels of *Q. castanea* genetic diversity as the local richness of the red oak community also increases.

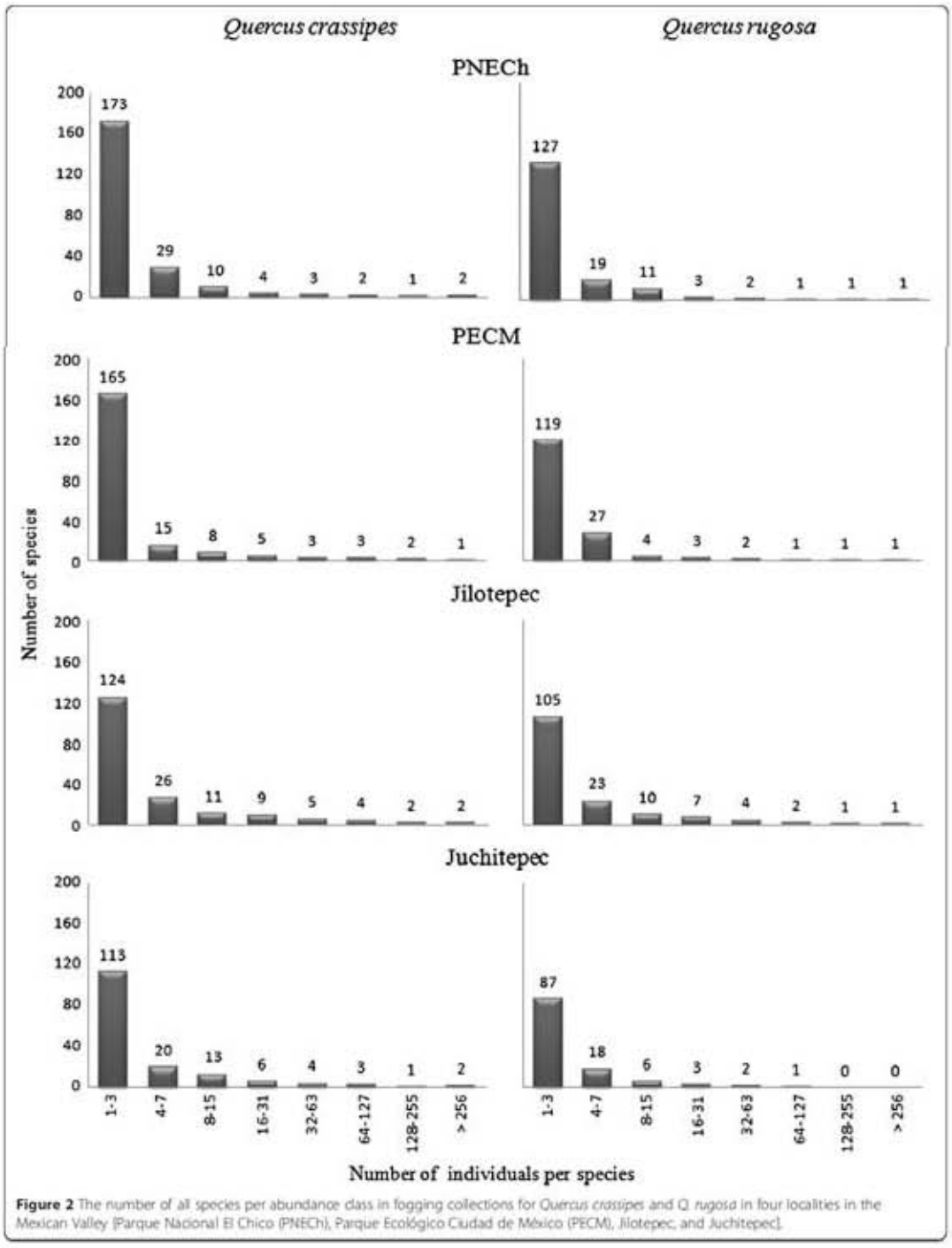

Figure 2 The number of all species per abundance class in fogging collections for *Quercus crassipes* and *Q. rugosa* in four localities in the Mexican Valley (Parque Nacional El Chico (PNECh), Parque Ecológico Ciudad de México (PECM), Jilotepec, and Juchitepec).

Table 4 Results from the General Lineal Model (GLM Analysis of Covariance) testing the effects of locality (PNECh, PECM, Jilotepec, and Juchitepec), oak species (*Quercus crassipes* and *Q. rugosa*), host-plant genetic diversity (expected heterozigosity, estimated with microsatellites and RAPDs data), and the interaction L × S on canopy arthropod diversity and biomass

	Arthropod community responses							
	Shannon-Wiener diversity				Biomass			
	df	MS	F	P	df	MS	F	P
				Microsatellites				
Locality (L)	3	1.86	23.17	<0.000	3	0.46	29.01	<0.000
Oak species (S)	1	0.10	1.23	0.271	1	0.96	59.58	<0.000
Genetic diversity	1	4.50	56.18	<0.000	1	0.44	27.59	<0.000
L × S	2	1.23	15.36	<0.000	2	0.04	2.56	0.084
Residual	72	0.08			72	0.02		
				RAPDs				
Locality (L)	3	0.71	8.31	<0.000	3	0.28	17.32	<0.000
Oak species (S)	1	0.13	1.48	0.227	1	0.87	54.49	<0.000
Genetic diversity	1	9.42	109.82	<0.000	1	0.85	52.74	<0.000
L × S	2	0.73	8.53	<0.000	2	0.16	10.08	<0.000
Residual	72	0.09			72	0.02		

Particularly, the hybridization phenomenon has been documented between *Q. crassipes* and *Q. crassifolia* in Jilotepec (Tovar-Sánchez and Oyama 2006a), and possible hybrids have been observed in PNECh between *Q. crassipes* and *Q. crassifolia* (S. Valencia, Science Faculty Herbarium, Universidad Nacional Autónoma de México). In addition, there is evidence that *Q. rugosa* hybridizes with *Q. glabrescens* at the PNECh (Núñez-Castillo et al. 2011). The above statements support that *Q. crassipes* and *Q. rugosa* presents higher genetic diversity levels at the PNECh as a result of interspecific hybridization, since genetic combinations produced by introgression exceeds the possible combinations resulting from mutational processes (Anderson 1949). This may increase the genetic diversity levels.

Effect of genetic diversity of oak host species on canopy arthropod community

We found a significant effect of the host genetic diversity on parameters of arthropod community structure [Shannon-Wiener diversity (H') and biomass (W)]. These results are consistent with those reported by Wimp et al. (2004), who found that the cottonwood's genetic diversity (heterozygosity) (*Populus fremontii* × *P. angustifolia*) has a significant influence on the diversity (H') of their associated gall-forming insects, explaining about 60% of the variability in the community. Similarly, Tovar-Sánchez and Oyama (2006b) reported that the oak genetic diversity (Shannon-Wiener) (*Quercus crassipes* × *Q. crassifolia*) explained about 78% of the diversity (H') of associated gall-forming insects. This could be explained due to the high level of specialization of gall-forming insects, since they have been considered as species-organ-tissue specific (Stone et al. 2002). This high level of specialization along with their tight relationship with host species may account for their high level of response to host species in comparison to canopy epiphyte insects.

Host-plant genetic diversity not only has direct impact on the associated community of herbivores, yet, its effects can be extended to the following trophic levels indirectly, by promoting a cascade effect throughout the community (Whitham et al. 2006). For example, an increase in host-plant genetic diversity can promote an increase in their architectural complexity and nutritional quality (Bailey et al. 2004). This may favor a greater density of herbivores (Bailey et al. 2006), depredation intensity, and parasitism degree (Sarfraz et al. 2008).

Canopy arthropod community structure (H', S, RS, and W) differed significantly between host species. *Q. crassipes* had the highest values in all the parameters mentioned. This pattern may be explained by the higher dominance and genetic diversity of *Q. crassipes* in all localities. In general, this species dominates oak forests, and its great abundance and genetic diversity may be favoring the availability of resources and conditions, resulting in a more complex arthropod assemblage. These results are supported by several studies that have showed that the increase in genetic variation in plants can generate a large amount of variation in morphological (González-Rodríguez et al. 2004; Tovar-Sánchez and Oyama 2004, López-Caamal et al. 2013), phenological (Hunter et al. 1997), architectural (Bangert et al.

2005), and chemical traits (Fritz 1999). All these features are genetically controlled given that arthropods are sensitive to these host-plant traits; it is not surprising that they would closely track the plant genetic *via* these traits (Bangert et al. 2008). A similar response has been reported in canopy cottonwoods (Wimp et al. 2004), willows (Hochwender and Fritz 2004), and eucalyptus (Dungey et al. 2000).

In general, the canopy arthropod community associated with *Q. crassipes* and *Q. rugosa* was represented by few abundant species and many rare species, which agrees with that reported in other studies (e.g., Tovar-Sánchez 2009). Particularly, the results showed that the canopy of *Q. crassipes* supports a greater number of rare species than *Q. rugosa*. Probably because the first species offers a wider range of resources and conditions as a result of their genetic diversity as already explained. This is supported by the work of Tovar-Sánchez and Oyama (2006a), who reported a greater number of rare species in hybrids of *Q. crassipes* × *Q. crassifolia* complex, where genetic diversity is increased.

These studies have suggested that the areas with more genetically diverse hosts can be considered as centers of diversity and species richness (Tovar-Sánchez and Oyama 2006a), areas of great ecological and evolutionary activity, providing new habitats for associated communities. Our results showed that the arthropod diversity (H') for *Q. crassipes* and *Q. rugosa* presents the following gradient: PNECh > PECM > Jilotepec > Juchitepec. In general, this pattern is consistent with the level of genetic diversity among localities. In addition, this pattern could be related to the number of arboreal species growing in simpatry with *Q. crassipes* and *Q. rugosa* in each locality, a phenomenon that is known as "associational susceptibility" (White and Whitham 2000), in which plant species present greater diversity of herbivores when spatially associated with heterospecific neighbors (White and Whitham 2000).

Implications for conservation

Mexico is one of the centers of diversification of the genus *Quercus* with more than 161 species (Valencia 2004). Oak and pine trees are the dominant species in most of the temperate forests of Mexico and they provide fundamental ecosystem services. In particular, some oak species can be considered foundation species. Unfortunately, deforestation rates are increasing in Mexican forests (≈314 thousand ha/year, FAO 2006) with potentially serious implications. From a conservation perspective, this study suggests that the maintenance of the genetic diversity of the host plants is crucial for the preservation of associated species. Also, it is a priority to assign a new conservation status for foundation species and propose strategies to safeguard mechanism to maintain their genetic diversity. When the foundation species are the habitat, a loss of genetic diversity will result in a loss of habitat that could have a potential effect on species across multiple trophic levels and major taxonomic groups (Bangert et al. 2005). This serves as a guide for future conservation efforts and provides a mechanism for why conservation efforts may fail if they do not consider the community consequences of genetic variation in foundation species, because their extended phenotypes affect the rest of the community.

Conclusions

In order to understand the assembly of natural communities, some factors such as interactions, degree of disturbance, type and quality of resources and environmental conditions have been widely studied. Recently, a genetic approach has revealed that the influence of genetic diversity extends to the community level. In this study, we found a genetic diversity effect of oak host species on canopy arthropod community, regardless of the molecular marker used as well as the host plant species type. Since oaks represent dominant trees in Mexican temperate forest, these findings may be important locally and at a landscape level. The consideration of the genetic diversity of the foundation species can be a general and efficient approach to conserving processes and diverse assemblages in nature. The development of this community genetic perspective should help us to understand the natural world, its complex interactions, and the effects of anthropogenic change.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors participated in the review, topic design, in the data analyses, and in the manuscript writing. Also, all authors read and approved the final version of the manuscript.

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Anexo 3

ANIMAL SCIENCE, ISSUES AND RESEARCH

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CONTENTS

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Chapter 1

PROXIMAL AND EVOLUTIONARY FACTORS THAT INFLUENCE ARTHROPOD COMMUNITY STRUCTURE ASSOCIATED TO VASCULAR PLANTS

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ABSTRACT

Plant-insect interaction has maintained stable for more than 300 million years, a fact that has been related with these groups capacity to escape or associate with their counterparts for reproduction, protection and feeding, among others. Arthropod communities are influenced by various factors; however, some studies suggest that genetic, chemical and morphological variability of host plants are the factors which influence the most on arthropod community structure. In contrast, intrinsic insect characteristics, such as the exoskeleton, wings, and feeding preferences can explain their evolutionary success. In general, it has been proposed that proximal (ecological) and distal (evolutionary) factors are responsible for population abundance and distribution and for the community structure and functioning. Among proximal factors, we can mention: diversity (genetic and specific), interactions, disturbances, geographical and seasonal variations and edaphic factors. Among distal causes we can point out: natural selection, coevolution and adaptive radiation. For all the aforementioned, in this chapter we will describe and discuss factors the influence plant-insect interactions which are very important to elucidate the reasons for the ecological and evolutionary success of both groups.

Keywords: arthropod communities, distal factors, proximal factors

1. INTRODUCTION

Studying the interactions between living beings and their environment has interested human beings since they appeared on earth, even though the formal study of ecology started less than 200 years ago (Egerton, 2001). Recently, ecological studies acquired great importance due to the increasing degradation of ecosystems all around the world (Brehm et al., 2005), a development that has altered the interactions between different biological groups and endangers the dynamics that sustain most ecosystems (Díaz and Cabido, 2001).

One of the main challenges of studying ecology is to recognize and understand the mechanisms that enable species to coexist with each other, and to understand how their interactions affect the structure and functioning of biological communities. One of the approaches that have been used to understand these mechanisms is the grouping of species by ecological similarities (Sheley and James, 2010). Lindeman (1942) proposes the classification of species based on their position in the trophic chain: producers, consumers and decomposers. This classification is directly based on the interaction between animals and the plants from which they obtain resources. This chapter will use the definition of functional groups of De Bello et al. (2010), who grouped organisms according to the characteristics that are similar between them and that lead them to respond in a similar way to changes in the environment and/or to have a similar impact on ecosystem processes. Numerous studies support this type of classification (e.g., Hochwender and Fritz, 2004; Franks et al., 2009; Morais and Cianciaruso, 2014). This definition of functional group allows researchers to contextualize, in a broader way, their understanding of how the presence of different species of arthropods and their intra and interspecific interactions influence the structure and functioning of arthropod communities.

This chapter describes how proximate (ecological) and distal (evolutionary) causation factors have influenced the distribution and abundance of arthropod populations and the structure and functioning of arthropod communities. The proximate causes that will be analyzed include plant diversity, associational susceptibility, plant productivity, plant genetic diversity, chemical composition, structural complexity, environmental gradients, seasonality and disturbances. The distal causes include coevolution, hybridization and adaptive radiation. The chapter will focus on examples of arthropod species associated with oak species and will describe the main factors that influence the interaction between plants and insects and how this relationship contributes and/or has contributed to the ecological and evolutionary success of both groups.

2. Biotic Factors

2.1. Plant Diversity and Associational Susceptibility

Arthropods maintain a close relationship with their host plants, from which they obtain a wide range of benefits (e.g., food, shelter from predators or adverse conditions, places for sexual display, etc.) (Strong et al. 1984; Schoonoven et al., 2005). It has been proposed that plant diversity may be important in determining the diversity of the associated fauna (Hunter and Price, 1992; Siemman et al., 1998; Scherber et al., 2010). The argument is that a greater diversity of plants provides a broader range of resources and conditions that can sustain a greater number of associated species (Siemann et al., 1998; Haddad et al., 2001; Crutsinger et al., 2006; Vehviläinen et al., 2008). Field and experimental studies have confirmed this hypothesis by reporting an increase in the diversity of phytophagous arthropods as a result of the increase in diversity of the associated plants (Siemman, 1998; Knops et al., 1999; Hawkins and Porter, 2003). This increase in the spectrum of resources and conditions associated with plant diversity not only reduces the competition between arthropod species with similar requirements but can also facilitate the arrival and colonization by new species that exploit other available resources and conditions (Schowalter et al., 2011), which results in greater diversification.

Moreover, it has been reported that the diversity of plant species can have a positive influence on the richness of parasitoid arthropod species and their predators (Hunter and Price 1992; Siemman et al., 1998; Wojtowicz et al., 2014; Valencia-Cuevas et al., 2017). For example, Brown and Ewel (1987) proposed the phenomenon known as "associational susceptibility," which suggests that host plants spatially associated with heterospecific neighbors can sustain a community of herbivorous arthropods with greater abundance and diversity. This phenomenon is expected to occur when generalist arthropods benefit from the wide range of resources and conditions provided by diverse plant communities (Unsicker et al., 2008). It would also occur when the focal plant is the least preferred host but grows near the preferred host plant (Atsatt and O'Dowd, 1976); this promotes the

mobility of arthropod species to neighboring plants after using the preferred host plant (White and Whitham, 2000).

The greater diversity of herbivores associated with host plants that coexist in floristically complex plant communities has been reported in temperate Mexican forests with respect to communities of ectophagous arthropods associated with the canopy of *Quercus crassifolia* (Tovar-Sánchez et al., 2015a), *Quercus crassipes* and *Q. rugosa* (Tovar-Sánchez et al., 2015b), and with respect to the community of gall-inducing endophagous insects (Cynipidae) associated with the canopy of *Q. castanea* through a gradient of species richness of red oak (section: *Lobatae*) in temperate forests of the center of Mexico (Valencia-Cuevas et al., 2017).

This suggests that a diverse community of plant species favors a greater diversity of the associated arthropod communities by making available for them a broader range of resources and conditions. This information should be considered when planning the management and conservation of diverse ecosystems.

2.2. Plant Productivity: Biomass

All organisms need energy to synthesize the molecules required to perform survival, growth and reproduction processes. The ability to obtain energy is an essential factor for individuals that is associated with their level of adaptation (Schowalter, 2011). In terrestrial ecosystems, plants are the main responsible for transforming solar energy into chemical energy through photosynthesis. Part of this energy is stored in plants in the form of organic matter or biomass; the rate at which this biomass is produced is known as primary productivity. Vegetable biomass can be used for energy by heterotrophic organisms, including arthropods. Thus, a higher primary productivity leads to a greater availability of resources for consumer species, increasing their abundance and the number of species associated with plants (Srivastava and Lawton, 1998).

Herbivore arthropods obtain the matter and energy they need to carry out their vital functions directly from plants. Predatory or parasitoid

arthropods benefit indirectly from plant biomass because they use herbivores as a resource and can even respond directly to changes in vegetation (Siemman, 1998; Begon et al., 2006; Haddad et al., 2009). This dynamic should be considered when trying to understand the close relationship between plants and arthropods from different trophic levels, as well as the influence of plant biomass on the functioning and structure of arthropod communities (Strong et al., 1984).

In general, the biomass of plant communities can vary spatially and temporally (Begon et al., 2006), which has been explained as a result of the heterogeneity of environmental conditions and biotic processes (Valencia-Cuevas and Tovar-Sánchez, 2015). In turn, the characteristics of arthropod communities such as the abundance, richness and diversity of species respond to variations in plant biomass, that is, variations in conditions and in the availability of resources (Crutsinger et al., 2006; Haddad et al., 2009; Tomas et al., 2011; McArt et al., 2012). An example of the influence of plant biomass on arthropod communities can be seen in temperate ecosystems, where the phenology of plants is influenced by precipitation patterns. Plant biomass increases (production of branches, leaves and fruits) during the rainy season, which also creates the necessary conditions for the growth of various species of epiphytic plants in the canopy (Valencia-Cuevas and Tovar-Sánchez, 2015). Arthropod communities associated with the oak canopy (Quercus) of temperate forests have shown an increase in species abundance, richness and diversity when the range of resources and conditions becomes broader as a result of the increase in the biomass of host plants hosts during the rainy season (Forkner et al., 2004; Southwood et al., 2005; Tovar-Sánchez, 2009; Tovar-Sánchez et al., 2013; Tovar-Sánchez, 2015a).

At a spatial level, differences in biotic or abiotic factors may create differences in the amount of biomass generated by plant communities (Valencia-Cuevas and Tovar-Sánchez, 2015). Changes in biomass and plant productivity have been reported as a result of the changes in temperature and precipitation that occur along altitudinal gradients (Sundqvist et al., 2013). This has consequences for the abundance, diversity and richness of

arthropod species (Körner, 2007; Lessar et al., 2011; Sundqvist et al., 2013; Bernardou et al., 2015).

Biological processes can also affect the production of plant biomass and, in turn, the arthropod communities associated with plants. Some studies have found that the diversity of plant species (Tilman et al., 1996; Cardinale et al., 2007; Haddad et al., 2009) and genotypes (Crutsinger et al., 2006; 2008 a, b) favors plant productivity [due to niche complementarity or facilitation (Hooper et al., 2005)], which benefits species richness and abundance of arthropods (Johnson and Agrawal, 2005; Johnson et al., 2006; Crutsinger et al., 2006; 2008a, b).

2.3. Plant Genetic Diversity

Genetic diversity is defined as the magnitude of genetic variability at the individual, population or species level (Nason, 2002). It is considered the raw material for evolution through natural selection (Fisher, 1930) and a fundamental source of biodiversity (Huges et al., 2008). In the last 20 years, different studies have found evidence that the genetic diversity of host plants is an important ecological factor that can influence the structure of the animal communities associated with them (Whitham et al., 2012; Crustinger et al., 2016). This evidence is associated with the recognition that the phenotypic characteristics of plant populations present substantial genetic diversity (Geber and Griffen, 2003). High-impact genes with influence at the community level have already been identified using QTLs (quantitative trait loci); they include genes that are responsible for the phenological pulses involved in the formation of buds (Frewen et al., 2000), tree growth and architecture, (Bradshaw and Stettler, 1995), resistance to pathogens (Newcombe and Bradshaw, 1996), and production of secondary metabolites (Shepherd et al., 1999; Freeman et al., 2008).

The study of plant-arthropod interactions has made it possible to demonstrate the influence of genes at the community level. The preferred study systems include founding species and their associated phytophages (herbivores) (Whitham et al., 2003, 2006, 2012). Founding species are plant

species that structure communities by creating locally stable conditions, providing resources for other species and contributing to the modulation and stabilization of ecosystem processes (Ellison et al., 2005). In general, the genetic diversity of host plants is positively and significantly related to the diversity, richness and relative abundance of the associated herbivore communities. An example of this pattern can be found in poplars (Wimp et al., 2004; Bangert et al., 2005, 2006, 2008; Compson et al., 2016), oaks (Tovar-Sánchez and Oyama, 2006b; Tovar-Sánchez et al., 2013, 2015a, b; Valencia-Cuevas et al., 2017), eucalyptus (Dungey et al., 2000), and willows (Hochwender and Fritz, 2004). Even in poplars and oaks, the response of phytophagous organisms to the genetic diversity of host plants has been consistent regardless of the host species, type of forest and geographic scale (population and region). It has been suggested that an increase in the genetic diversity of host plants can generate changes in their morphological (Lambert et al., 1995; González-Rodríguez et al., 2004; Tovar-Sánchez and Oyama, 2004), phenological (Hunter et al., 1997), architectural (Martinsen and Whitham, 1994; Whitham et al., 1999; Bangert et al., 2005), and chemical (Fritz, 1999) characteristics, broadening the range of resources and conditions that can be exploited by herbivores.

Likewise, it has been observed that the genetic identity of host plants is an important regulator of the structure of arthropod communities, since genetically similar hosts sustain associated communities of genetically similar arthropods (Bangert et al., 2006; Compson et al., 2016)This fact has been explained by considering that genetically more similar populations have greater similarity in their physical, chemical and phenological characteristics, which will favor the establishment of more similar arthropod communities (Bangert and Whitham, 2007). The ability of herbivorous arthropods to discriminate between plant genotypes has been observed in different plant species, including poplars (Wimp et al., 2005; Compson et al., 2016), eucalyptus (Dungey et al., 2000), willows (Hochwender and Fritz, 2004) and oaks (Tovar-Sánchez and Oyama, 2006b).

The aforementioned studies have analyzed communities of specialized (endophagus) and generalist (ectophagous) herbivores, showing that the first group is more sensitive to the effects of the genetic diversity of the host

plants (Bangert et al., 2005, 2006, 2008; Shuster et al., 2006; Tovar-Sánchez and Oyama, 2006b; Valencia-Cuevas et al., 2017). Endophagous herbivores are an important functional group that inhabits the canopy of trees, and includes insects such as gall-formers, leaf miners and leaf-rollers, which are characterized by living within the tissues of leaves and feeding on the mesophyll tissue (Cornell, 1990). It has been proposed that the heritable phenological and chemical signals that are produced by host plants determine the selection of oviposition and gall formation sites by endophagous herbivores (Abrahamson et al., 1993). For example, when choosing oak trees, gall-forming wasps of the Cynipidae family choose specific species, organs and tissues (Stone, 2002). This high degree of specialization and the close relationship of these insects with the host plant could be the reason why this group is so sensitive to the genetic diversity of the host (Tovar-Sánchez and Oyama, 2006b).

The genetic diversity of host plants not only has a direct effect on the associated herbivore communities; its influence can extend indirectly to the following trophic levels, creating a cascading effect through the ecosystem (Whitham et al., 2003, 2012). For example, an increase in the genetic diversity of the host plant may promote an increase in its architectural complexity and nutritional quality (Bailey et al., 2004; Glynn et al., 2004), which will favor an increase in the diversity, abundance and quality of the associated herbivores (Bailey et al., 2006; Valencia-Cuevas et al., 2017) which, in turn, will increase the intensity of predation and the degree of parasitism (Sarfraz et al., 2008).

In the coming years, a major challenge will be to understand the connections between evolutionary and ecological processes, given the continuing loss of genetic diversity throughout the world (Butchart et al., 2010) and the potential consequences on biological communities of the changes in the patterns of genetic variation caused by large selective events (Genung et al., 2011). Recognizing the influence of the genetic diversity of host plants on ecological processes constitutes a valuable contribution to ecological theory; the study of arthropods and their host plants has undoubtedly made a significant contribution to this theoretical advance.

2.4. Chemical Composition: Nutritional Quality and Secondary Metabolites

The plant chemicals that can influence the associated arthropod communities can be divided in two categories: food and defense (Strong, 1984). An example of food chemicals is foliar nitrogen, a critical component for phytophagous insects (Strong et al., 1984); its concentration is positively and significantly associated with the growth, reproduction and survival rate of herbivorous insects (Mattson Jr., 1980). The content of nitrogen in the leaves can vary due to leaf ontogeny or between plant species (Jeffries et al., 2006), altering the feeding preferences of herbivorous insects (Coley and Barone, 1996; Marquis and Lill, 2010). For example, low nitrogen content has been associated with a low preference and reduced performance of herbivorous insects, as the palatability of a plant depends on the carbon/nitrogen ratio of the leaves (Schädler et al., 2003).

From a community-focused perspective, there are several studies that show the importance of the nitrogen content of host plants on their associated insect communities. Some studies have reported a positive and significant relationship between the concentration of foliar nitrogen and the density of herbivorous insects (e.g., leaf miners, chewing insects, gall-formers and leaf rollers) in various species of oak: *Quercus alba* (Wold and Marquis, 1997), *Q. prinus*, *Q. rubra* (Forkner and Hunter, 2000), *Q. dentata* (Nakamura et al., 2008), *Q. germinata*, *Q. laevis* (Cornelissen and Stiling, 2006, 2008), *Q. alba*, *Q. coccinea* and *Q. velutina* (Marquis and Lill, 2010). Likewise, a greater richness of species of chewing insects (Lepidoptera) has been reported when there is a greater amount of foliar nitrogen in *Q. crispula* (Murakami et al., 2005, 2007, 2008).

It has also been well documented that plants produce other chemical substances (e.g., oxalic acid, alkaloids, phenolic compounds, toxic lipids, flavonoids, tannins and lignins) that act as defenses or insect attractants (Becerra et al., 2001). In response to these signals or chemical defenses, there can be changes in the structure of arthropod communities associated with the canopy of trees (Inoue et al., 2003).

Tannins stand out among secondary metabolites for their defensive role and their effect on herbivorous insects and on the structure of their communities (Feeny, 1970). It has been reported that tannins reduce the growth and survival of phytophagous insects (Kause et al., 1999; Lill and Marquis, 2001), produce lethal deformities (Barbenhenn and Martin, 1994) and increase parasitism rates (Faeth and Bultman, 1986). At the community level, a negative relationship has been reported between the concentration of tannins in host plants and the abundance and richness of herbivorous insects (e.g., chewing insects, leaf miners and gall-formers) that inhabit the canopy of the following oak species: *Q. alba*, *Q. velutina* (Le Corff and Marquis, 1999; Forkner et al., 2004), *Q. crispula* (Murakami et al., 2005, 2007, 2008), *Q. germinata*, *Q. laevis* (Cornelissen and Stiling, 2006, 2008) and *Q. gambelii* × *Q. grisea* (Yarnes et al., 2008).

There are reports that the species of individual plants has a significant effect on the concentration of nitrogen and secondary metabolites (Suomela and Ayres, 1994), which suggests that species variability affects the foraging activity and spatial distribution of arthropods. The concentration of nitrogen and secondary metabolites may depend on the following factors: 1) the genotype of the host plant (Glynn et al., 2004), 2) the environmental conditions (Larsson et al., 1986), and 3) the resources of the host plant (Ricklefs, 2008).

Gall-formers (Cynipidae) are a group of insects that is sensitive to the differences in leaf chemistry between species of oaks. Abrahamson et al. (1998, 2003) found that the structure of gall-wasp communities was different and particular in each of six different species of oak (*Q. laevis*, *Q. myrtifolia*, *Q. inopina*, *Q. chapmanii*, *Q. geminata* and *Q. minima*). Similar results were reported for the complex *Q. crassipes* × *Q. crassifolia* in Mexico (Tovar-Sánchez and Oyama, 2006b), for *Q. infectoria* and *Q. brantii* (Nazemi et al., 2008) in Iran, and for *Q. castanea* and *Q. crassipes* in Mexico (Tovar-Sánchez et al., 2013). Researchers suggest that this sensitivity is explained by the close relationship between plants and insects, which is behind the high degree of specialization of these insects with respect to the chemicals of their host oaks.

Moreover, oaks have been observed to show seasonal variations in foliar chemistry. Some examples have been documented in *Q. robur* (Feeny, 1970; Salminen et al., 2004), *Q. alba*, *Q. velutina* (Le Corff and Marquis, 1999), *Q. alba* (Lill and Marquis, 2001), *Q. crispula* (Murakami et al., 2005, 2007, 2008), *Q. germinata* and *Q. laevis* (Cornelissen and Stiling, 2006, 2008). These studies found temporal variations in the nutritional quality of the leaves; as the ontogenetic development of leaves progressed, the content of tannins and lignins increased and the content of water and nitrogen decreased (Feeny, 1970). Several studies have shown that the richness, diversity, abundance and biomass of the arthropods associated with the canopy of oak trees decrease as the season progresses, while the structure of the communities changes in response to variations in the chemistry of the host plants (e.g., Forkner et al., 2004; Southwood et al., 2004; Yarnes et al., 2008).

Studying the chemistry of host plants and of its influence on plant-insect interactions is crucial for understanding the structure of arthropod communities and the preferences of individual arthropods regarding oviposition site, feeding habits, ontogenetic performance and change of host.

2.5. Structural Complexity

Since plant communities determine the physical structure of different environments, they have a great influence on the structure of the associated animal communities (Strong et al., 1984; Halaj et al., 2000; Tews et al., 2004). It has been suggested that structurally more complex environments offer a wider range of available habitats and shelters and create the conditions for the occurrence of speciation events as a result of the adaptation of species to various environmental conditions (Halaj et al., 2000; Tews et al., 2004; Kallimanis et al., 2008; Antonelli and Sanmartın, 2011), all of which promotes the coexistence, persistence and diversification of species (Stein et al., 2014). In the case of arthropods, the abundance and architecture of the plants with which they are associated (e.g., shape and size of leaves, shoots, branches and epiphytic plants, as well as the texture of the

stem or bark; Halaj et al., 2000; Sobek et al., 2009) constitute a very important element of the structure and complexity of their habitat. Its importance lies in the fact that the heterogeneity of the habitat is related to the availability of basic resources for herbivores such as: food, shelter and foraging, oviposition and sexual display sites (Halaj et al., 2000; Novotny et al., 2006)For example, some studies have shown that the presence of epiphytic plants (e.g., orchids, bromeliads, ferns, mosses, lichens, etc.), which differ substantially between them in their structure, growth habit and function, increases the structural complexity of the tree canopy, offering a great diversity of microhabitats and resources (Ishii et al., 2004) that can be used by the arthropods associated with the trees.

Another factor that increases the complexity of the habitat of arthropods associated with plants is the richness of species in plant communities. Plant communities rich in species have also greater richness, diversity and abundance of arthropod species (Sobek et al., 2009). The positive relationship between the richness of plant species and the richness of arthropod species has been widely documented (Gaston, 1991; Siemman, 1998; Knops et al., 1999; Hawkins and Porter, 2003; Vehviläinen et al., 2008). An increase in plant diversity represents an increase in the diversity of resources available for herbivores, which allows more consumer species to coexist (Hutchinson, 1959), since it is more likely that a particular resource is available to a particular consumer. Under this scenario, the diversity of herbivores is promoted by plant diversity (Chown et al., 1998; Novotny et al., 2006; Kumar et al., 2009). Moreover, plant diversity can indirectly influence predator communities through its effect on the diversity, abundance and quality of their prey (herbivores) (Chown et al., 1998; Scherber et al., 2010; Valencia-Cuevas et al., 2017).

Another scenario in which the effect of habitat complexity becomes evident is of the succession of plant communities. For example, the structural complexity of forests increases with their age, that is, mature forests are structurally more complex than young forests or plantations (Schowalter, 1995; Hardiman et al., 2011), since the former tend to have a greater number of large in terms of height and biomass, more tree species and different strata of vegetation (herbaceous, shrubs, trees) (Bazzaz, 1975;

Fernandes et al., 2010). Mature forests also contain trees of different ages, which gives them greater structural complexity (Ishii et al., 2004). In short, the presence of tree species with diverse physiognomy and growth patterns, as well as individuals of different sizes, generates horizontal and vertical variation, which promotes microenvironmental heterogeneity (Stein et al., 2014) and provides more diverse resources and conditions that can be exploited by arthropods.

An example of the positive effect of the structural complexity that age gives to a forest habitat on the richness of the arthropod fauna associated with the tree canopy was observed in the community of chewing insects associated with the canopy of *Q. alba* and *Q. velutina* (Marquis and Le Corff, 1997) and the community of lepidoptera associated with the canopy of *Quercus* spp. (Summerville and Crist, 2002, 2003). Furthermore, Marquis et al. (2002) showed that the abundance of shelter-building caterpillars (Lepidoptera) is related to the architecture of their host oaks (*Q. alba*). Their results showed a positive and significant relationship between the structural complexity of the canopy of this tree species, measured as the proportion of overlapping leaves, and the abundance of caterpillars, which indicates the importance of tree architecture for these herbivores.

The results of the different studies included here suggest that habitat complexity is a crucial factor favoring the diversity of arthropod species, which means that management and conservation plans aimed at the preservation of these organisms should contemplate the inclusion of this factor when designing strategies for the maintenance of biodiversity.

2.6. Interactions

Galls are considered a "hot spot" because, throughout the different stages of their development, they are the place where several species interact. Gall-inducing insects (Cynipini) are found there during the formation stage (Pujade-Villar, 2013). Galls are induced by the chemical action caused by the secretions and excretions of insect larvae, which control

the development of galls; if the larvae die the development of galls stops (Folliot, 1977; Pujade-Villar, 2013).

Synergini insects also appear in galls, as inquilines or commensals. The synergini lost their ability to induce their own galls (Pénzes et al., 2012), but they can still induce their own larval chambers and create their own nutritional tissue; furthermore, they can kill inducer organisms by competing with them for space and/or food (Ronquist, 1999; Maldonado-López, et al., 2013). Chalcidoid insects (Chalcidoidea) can also be found during the formation of galls. They belong to six families (Eulophidae, Eupelmidae, Eurytomidae, Ormyridae, Pteromalidae and Torymidae), and their ecological function in galls is not yet completely understood, since they can act as phytophagous insects, predators, parasitoids and hyperparasitoids. It is thought that chalcidoids regulate the populations of gall-inducing insects, since they can kill between 40 and 100% of inducer insects (Gibson, 2006).

There are few studies on the primary fauna of galls; however, it is already possible to associate some genera of synergini and chalcidoids to certain gall-inducing insects. Examples of this have been documented by Serrano-Muñoz (2016), who mentions that the insects found in the galls induced by Trigonaspis oscura in *Q. rugosa* include the inquiline *Synergus* sp. and the chalcidoids *Baryscapus*, *Brasema*, *Eurytoma*, *Sycophila* and *Ormyrus*. Furthermore, the inquiline *Synergus* and the chalcidoids *Galeopsomyia*, *Eurytoma*, *Ormyrus* and *Sycophila* have been found in galls induced by *Atrusca pictor* in *Q. frutex*, while the inquiline *Synerus* and the chalcidoids *Baryscapus*, *Eurytoma*, *Galeopsomyia*, *Ormyrus* and *Torymus* were found in galls induced by *Disholcaspis potosina* in *Q. obtusata*. Once a gall is established, secondary fauna can be found in them, constituted by small arthropods that use the gall as a refuge and/or food (Pujade-Villar, 2013). Valencia-Cuevas et al. (2017) collected Diptera (Cecidomyiidae and Chloropidae), Lepidoptera (Bedellinae and Gelechiidae) and Hymenoptera (Apidea, Bethylidae, Braconidae, Figitidae and Sphecidae) from galls of *Q. castanea*. That was the first study carried out in Mexico that mentioned the secondary fauna that can be found in plant galls, providing a background for future studies. After a gall falls to the ground, it is consumed by soil organisms such as fungi and small arthropods. However, sometimes the galls

are consumed by birds or mammals. It is expected that future studies expand our knowledge of the biological interactions centered around galls, and of the ecological function of each of their inhabitants.

3. Abiotic Factors

3.1. Environmental Gradients

The ability of arthropods to establish themselves in a given habitat is limited in part by variations in the physical environment (Menke and Holway, 2006). Factors such as temperature, humidity, precipitation, solar radiation and wind speed directly affect the survival, reproductive capacity and longevity of arthropods (Willmer, 1982). For example, the desiccation of insects in immature stages increases in environments with high temperatures and lower humidity (Willmer, 1982). In fact, it has been reported that insect larvae survive and grow better under conditions of higher humidity (Hunter and Willmer, 1989; Larsson et al., 1997). Likewise, it has been observed that herbivorous arthropods can be directly affected by solar radiation (light and heat), but also indirectly by affecting the nutritional quality of their host plants (Fukui, 2001). Finally, radiation and wind cause changes in the temperature of the air, which affects the survival and growth of arthropods (Porter and Gates, 1969). These changes in the physical environment can modify the behavior, physiology and morphology of individual arthropods, inducing alterations in the distribution and abundance of populations (McKinney, 2008) and, consequently, in the structure of arthropod communities (Valencia-Cuevas and Tovar-Sánchez, 2015).

Abiotic factors can also affect plant communities along environmental gradients, inducing changes in species richness, genetic diversity, abundance and total biomass (Begon et al., 2006); this creates heterogeneous habitats (in terms of conditions and resources), which in turn induce changes in the associated arthropod communities (Valencia-Cuevas and Tovar-Sánchez, 2015). If the composition and diversity of plant communities vary predictably across habitats and biogeographical zones (Gurevitch et al., 2002), it is reasonable to assume that the strength of plant-herbivore interactions can also vary. Two of the most recognized and studied environmental gradients are those

associated with latitudinal and altitudinal changes (Gaston, 2000; Lomolino et al., 2006b). In general, climatic variables (temperature, humidity, precipitation), solar radiation and edaphic parameters (pH, availability of nutrients), among others, vary with latitude and altitude (Körner, 2007). Several studies have reported the response of various biological groups, including arthropods and their host plants, to environmental gradients (Hodkinson, 2005; Sundqvist et al., 2013; Bernadou et al., 2015). Changes in the abiotic factors described above affect the phenology, morphology, physiology and chemistry of host plants (Hodkinson, 2005), altering their ability to defend against herbivorous arthropods. (Pellisier et al., 2012). Thus, changes in altitude (Rodríguez-Castañeda et al., 2010; Beck et al., 2011; Sundqvist et al., 2013; Bernadou et al., 2015) and latitude (Gaston and Lawton 1988; Hillebrand 2004; Dyer et al., 2007; Kraft et al. 2011) correlate with changes in the diversity, composition and abundance of plant-associated arthropods. One of the most frequently observed patterns along altitudinal gradients is the decrease in species richness as altitude increases (Rabhek, 2005; Grytnes and McCain, 2007; McCain et al., 2011). However, some studies have found peaks of species richness at intermediate altitudes (Rahbek, 2005; Kessler et al., 2011). Both patterns have been documented in arthropod communities (Olson, 1994; Sanders et al., 2003).

A study carried out in Mexico along an altitudinal gradient in a temperate forest (*Abies-Quercus*) evidenced a decrease in the abundance and diversity of species of the arthropod community associated with the mulch forest, as well as changes in the composition of species along the gradient (Rodríguez-Domínguez, 2010). The author suggests that this response from the arthropod community can be partly explained by changes in factors such as temperature

The study of the influence of abiotic factors along environmental gradients has served to understand the limits imposed by environmental conditions on the distribution of arthropods, which, in turn, helps to understand their biology and abundance (Andrew and Hughes, 2005; Sundqvist et al., 2013). Furthermore, the response of arthropod species and communities to changes in abiotic factors associated with environmental gradients can be used as a predictive tool to understand the potential impact of climate change (Hodkinson, 2005) and the future of arthropod biodiversity (Fukami and Wardle, 2005; McCain and Colwell, 2011).

3.2. Seasonality

The abundance and distribution of arthropods can change across space and time. An arthropod species will only be present in a given habitat when it has the capacity to reach it (dispersion), when the resources and conditions necessary for its establishment become available and when its competitors, predators or parasitoids allow it (Begon et al., 2006). Thus, the temporal sequence of the appearance and disappearance of an arthropod species in a given habitat depends on how the influence of resources, conditions and enemies changes over time. Needless to say, the factors that affect vegetation will have an effect on arthropod communities (Valencia-Cuevas and Tovar-Sánchez, 2015).

Highly synchronized phenological events are characteristic of deciduous temperate forests, where the foliage of most tree species regrows in spring and falls in the autumn (Strong et al., 1984). These seasonal cyclical changes in plant species change the distribution and abundance of the arthropod species associated with the tree canopy. Different studies have documented the seasonal changes in the composition of arthropod communities and the decrease in the relative abundance and richness of arthropod species associated with the canopy of temperate forests (Gering et al., 2003; Tovar-Sánchez, 2009; Tovar-Sánchez et al., 2013). These responses have been explained by considering that the nutritional quality of the leaf tissue found in temperate forests decreases as the season progresses (Feeny, 1970); leaves become harder, their content of water and nitrogen content decreases and the concentration of tannins and fiber increases (Feeny, 1970). These changes in foliage characteristics contribute to the appearance of arthropod species with different feeding preferences (e.g., leaf-chewing insects at the beginning of the season, sucking insects at the end of the season; Strong et al., 1984; Southwood et al., 2004, 2005), changing the composition of arthropod communities.

Another characteristic of temperate forests is the seasonal pattern of the rainy season, which lasts six to seven months and is followed by a dry season that can last from five to six months (Rzedowski, 1978). These annual variations in the rainfall pattern affect the phenology of vegetation and its

associated arthropod communities. The formation of branches, foliage and fruits, and the growth of epiphytic plants, increases during the rainy season, broadening the range of resources and conditions that can be exploited by the arthropods inhabiting the canopy. These events can create microclimatic changes (Basset and Novotny, 1999) and increase the heterogeneity of the habitats used by arthropods (Yarnes and Boecklen, 2005). Moreover, young leaves, which are less hard, of higher nutritional quality and with a smaller amount of chemical defenses, are more abundant during the rainy season (Kursar and Coley, 2003; Forkner and Marquis, 2004). Finally, the increase in plant biomass during the rainy season in temperate forests can create for foliage arthropods to colonize new trees (Basset et al., 1992).

Oaks are one of the most representative genera of temperate forests. Studies of the arthropod communities associated with the canopy of this plant group have documented the effect of their phenological changes on the communities associated with them. For example, as the seasons progress, a decrease in density, richness, diversity and biomass has been reported in the community of leaf-chewing insects associated with the canopy of *Q. alba* and *Q. velutina* in Missouri (Forkner et al., 2004), the community of herbivorous insects that inhabit the canopy of *Q. cerris*, *Q. ilex*, *Q. petraea* and *Q. robur* in France (Southwood et al., 2004, 2005), and the community of beetles associated with the canopy of *Quercus* spp. in Turkey (Şen and Gök, 2009).

Likewise, greater diversity, species richness, density and biomass have been reported during the rainy season in the collembola community living in Tillandsia spp., which grows associated with the canopy of *Quercus* spp. in a temperate forest in central Mexico (Palacios-Vargas and Castaño-Meneses, 2003). These changes have also been observed in the community of ectophagous insects associated with the canopy of *Q. laurina* and *Q. rugosa* (Tovar-Sánchez, 2009), and the canopy of *Q. castanea* and *Q. crassipes* (Tovar-Sánchez et al., 2013) in a temperate forest in central Mexico.

In general, the high sensitivity of arthropods to changes in biotic and abiotic parameters suggests its usefulness as bioindicators; they can be an

important tool to understand and predict the effect of environmental change on biodiversity.

3.3. Disturbances

Disturbances are discrete events that alter the structure of populations, communities and ecosystems by changing the availability of resources and the prevailing conditions (White and Pickett, 1985). Succession is the process of recovery after a disturbance (Harper, 1977) in which biological communities experience changes in their composition, complexity, diversity and habits (Currano et al., 2011). In plant communities, disturbances act as promoters of succession, giving rise to vegetation mosaics with different degrees of structural complexity (White and Pickett, 1985; Siemann et al., 1998; Fernandes et al., 2010) and inducing the turnover of species. In sum, they affect the dynamics of biological communities (Hughes et al., 2007; Ilg et al., 2008; Gerisch et al., 2012). In the case of arthropods, they are affected, directly and indirectly, by the intensity, frequency, duration, and area of the disturbances (Currano et al., 2011). Directly when frequent disturbances maintain the diversity of biological communities at low levels by causing local extinction events and limiting the dispersion of species (Hanski, 1994). Indirectly by causing changes in the structure of plant communities, which affects the spatial and temporal patterns of the diversity of the arthropods associated with them (Fagan et al., 1999; Jeffries et al., 2006).

In general, biological communities are subject to the effects of disturbances of natural [e.g., fire, storms, hurricanes, floods, etc. (Dziock et al., 2006)] and anthropogenic [e.g., deforestation, agriculture, urbanization, etc. (Hirao et al., 2007)] origin. This section addresses only the effects of anthropogenic disturbances, the frequency and intensity of which have increased dramatically in recent years.

Several studies have documented that anthropogenic disturbances affect arthropod communities in several ways and with different intensity (Hill et al., 1995; Floren and Linsenmair, 2001; Currano et al., 2011); there is no unique pattern. Disturbance events can have negative or positive effects, or

simply have no effect, on the structure of arthropod communities (Mackey and Currie, 2001). This range of responses can be explained by differences in the habitat requirements, dispersal abilities and distribution patterns of different arthropod species (Cooke and Roland, 2000; Gibb et al., 2013), as well as by differences in the scale and degree of the disturbances (Lewis, 2001; Niemelä et al., 2002), historical factors and heterogeneity of the sites (Bruno et al., 2003; Hamer et al., 2003). For example, the abundance and diversity of arthropod species that depend on resources that are only available after a disturbance (e.g., dead wood) could increase rapidly after the event, compared to those species that depend on microhabitats that are not regularly affected by disturbances (Gibb et al., 2013). In consequence, undisturbed sites can make biological communities more stable but less diverse.

The canopy of oaks and the arthropods associated with it constitute a useful system to illustrate the diversity of responses to disturbances of arthropod communities. A study by Tovar-Sánchez et al. (2003) compared three forests with different degrees of disturbance in the Valley of Mexico and found significant differences in the abundance and diversity of ectophagous arthropods associated with the canopy of *Q. castanea*, *Q. crassipes*, *Q. crassifolia*, *Q. greggii*, *Q. laeta* and *Q. rugosa*. Similar responses were reported regarding the richness of herbivorous insects associated with the canopy of *Q. alba* and the richness and abundance of leaf-chewing insects associated with the canopy of *Q. alba* and *Q. velutina* in Missouri (Forkner et al., 2006, 2008). Moreover, Summerville and Crist (2002, 2003) found changes in the composition of species, and a decrease in the richness thereof, in the lepidopteran community associated with the canopy of *Quercus* ssp. in recently felled forests compared to non-felled ones.

Other studies have reported that the arthropod fauna associated with the canopy of oaks does not respond to disturbances. For example, the composition and species richness of beetles associated with the canopy of *Quercus* spp. in forest fragments in Bulgaria with different degrees of urbanization (rural/suburban/urban) did not present differences between them (Niemelä et al., 2002). The authors suggest that local factors such as

temperature, humidity or edaphic conditions may have played a more important role in the structure of the beetle community, considering that the degree of habitat disturbance that occurred in the three groups of forest fragments was moderate.

Some studies have reported that arthropods benefit from disturbances (Chust et al., 2007; Maldonado-López et al., 2015). Maldonado-López et al. (2015) reported greater abundance and diversity of gall-inducing insects of the Cynipidae family as the fragmentation of oak forests increased. The authors explain that these results can be explained by the dispersal capacity of these arthropods and by the increase in the quality of the host plants, measured in terms of the amount of leaves, buds and petioles, which are the sites where cynipids induce the formation of galls.

The future of biodiversity depends to a large extent on the generation of knowledge that is useful for managing ecosystems that have been altered by human activities. One of the most important things is to identify the species or groups of species that are sensitive to these alterations (Gardner, 2010). The sensitivity of arthropods to changes in their habitat is a very valuable attribute that makes this animal group a potential ecological indicator that can provide information on the conservation state of different ecosystems (Valencia-Cuevas and Tovar-Sánchez, 2015).

4. Evolutionary Factors

4.1. Coevolution

Since the publication of the study by Ehrlich and Raven (1964) on the coevolution between plants and animals, the scientific community became greatly interested in the study of ecological interactions, their possible evolutionary histories and their role in the structuring of biological communities (Oyama, 1999). The study by Ehrlich and Raven proposed the concept of coevolution as a mechanism that could explain the joint evolution of butterflies of the superfamily Papilionoidea and the plant species with which they were associated. They used this concept to explain the process

by which plants develop new chemical defenses and insects evolve resistance or tolerance to these new defenses. The authors suggested that this type of reciprocal responses between plants and insects could be considered one of the most important regulating factors of diversity in terrestrial communities, one in which a crucial role is played by the biochemical innovation that takes place in plants (Ehrlich and Raven, 1964; Becerra et al., 2009; Futuyma and Agrawal, 2009; Thompson, 2013). To study this mechanism, Berenbaum (1983) analyzed the chemical composition of plants of the family Umbelliferae and the herbivores associated with plant species with different chemical composition. A characteristic of this group of plants is that they contain a chemical group known as coumarins, which have at least four derivative groups that differ in a single chemical radical. This study showed that the degree of toxicity of each derived chemical group increases with its degree of complexity, which suggests that this progressive increase in toxicity corresponds to a biosynthetic advance against herbivorous insects. Another contribution of the study carried out by Berenbaum was to show that the plant taxa with the most advanced coumarins were richer in species compared to those with less advanced chemicals. It also showed that the insect taxa that fed on plants with more complex coumarins were richer in species than other taxa that fed on plants with simpler coumarins. These results provided the first evidence of the important association between the evolution of plant-insect interactions and diversification events. Furthermore, the results obtained by this study inspired decades of work that aimed to document this type of evolutionary scenarios (Berenbaum, 2001).

Coevolution is a process that defines and redefines the interactions between different species (Thompson, 2001). Gall-inducing insects and their host plants constitute a good model to illustrate coevolution events. It has been suggested that gall formation emerged as a defense mechanism in plants to isolate potentially harmful insects and restrict their development (Ananthakrishan, 1984; Stone et al., 2002). However, insects evolved in response to this strategy, inducing changes in plant growth and living within its tissues (Ananthakrishnan, 1984). They managed to use galls to obtain food, protection against predators, drying and shelter for reproduction

(Fernandes and Price, 1988). This has led some researchers to suggest that the formation of galls is an evolutionary step towards a stronger relationship between genetic changes and the exploitation of plant species (Ananthakrishnan, 1984).

An example of this type of interaction can be observed in cynipid wasps (Hymenoptera: Cynipidae), which form galls in oak trees (Fagaceae: *Quercus*). The interaction between cynipid wasps and oaks has a long history of at least 30 million years (starting in the Oligocene or Miocene; Kinsey, 1930). An important characteristic of this group of insects is its specificity regarding the genus of the host plant and even the organs that are attacked by them, so that a certain species of cynipidae is associated only with a certain species or related group of plant species and induces galls constantly and exclusively in a single organ of the plant (Stone et al., 2002). Any organ and part of the plant can be attacked by arthropods, including roots, stems, buds, leaves, flowers and fruits (Ronquist and Liljeblad, 2001; Stone et al., 2002). Tannins are part of the defensive chemistry oaks. These chemical compounds stand out for their role as herbivore repellents (Feeny, 1970) and for their influence on the structuring of phytophagous insect communities (Inoue et al., 2003). Tannins have been reported to affect insect growth and survival (Kause et al., 1999), reducing their biomass (Lill and Marquis, 2001), producing lethal deformities (Barbenhenn and Martin, 1994) and increasing parasitism rates (Faeth and Bultman, 1986). However, this chemical barrier has not been effective in controlling the growth and development of gall-inducing cynipids. Considering that the generation time of oaks is much longer than that of gall wasps, it is not surprising that the latter have evolved different strategies to overcome the tannin barrier. Some insect species have developed powerful phenoloxidase systems, which are enzymes that have the ability to oxidize tannins (Nierenstein, 1930). Beside tannins, the defense mechanisms of oaks against the attack of cynipids include seasonal changes in their nutritional quality and in the turgidity of their leaves (Strong et al., 1984; Forkner et al., 2004). However, these insects have managed to evade these defenses by manipulating the levels of tannins and nutrients in the gall tissues (Fay and Hartnett, 1991; Bagatto et al., 1996; Hartley, 1998, Schönrogge et al., 2000). This scenario shows the way in

which these two groups of organisms have coevolved over millions of years (Nieves-Aldrey, 1987).

Another model that can be used to identify reciprocal adaptive responses are the interactions between parasites and hosts (Beranbaum, 2001). The communities associated with the galls induced by cynipids (Hymenoptera, Cynipidae) belong to several trophic levels, forming complex networks composed of inquiline, parasitoid and successor insects (Askew, 1984). Galls constitute extended phenotypes of the genes of the wasps that induce these structures in the tissue of their host oak (Dawkins, 1982; Stone and Cook, 1998; Stone and Schönrogge, 2003). These structures have evolved into increasingly complex morphologies, the purpose of which, in part, has been to exclude the natural predators of these wasps, mainly parasitoid insects. It has been suggested that the relationship between parasitoid insects and their host wasps has been maintained through coevolution processes that have given rise to diverse communities that include a third of all animal species (Bailey et al., 2009). Parasitoid insects cause high mortality in gall wasps (Stone et al., 2002; Stone and Schönrogge, 2003); thus, natural selection could have favored adaptive responses by the host insects to reduce the rate of parasitism (Abrahamson and Weis, 1997; Stone and Schönrogge, 2003). Because the attack of parasitoids involves oviposition through the gall tissues, selection could have favored gall-forming insects with genes that induce the formation of structures in the galls that reduce or prevent such attacks (Stone and Schönrogge, 2003; Singer and Sireman, 2005; Abrahamson and Blair, 2008). The defensive phenotypes acquired by gall wasps have probably stimulated reciprocal evolutionary changes (Abrahamson and Weis, 1997; Agrawal, 2001) in the characteristics of parasitoid insects, such as the length of the ovipositor (Askew, 1965). Bailey et al. (2009) studied the effect of different host characteristics on 48 communities of parasitoids that attack gall-inducing wasps of the family Cynipidae in oak trees. The authors showed that gall attributes such as turgor, hairiness and the presence of sticky substances, as well as their position in the host plant, had a significant effect on the composition of the parasitoid insect community. It has been suggested that these changes in parasitoid insect communities reflect the action of different species; small

parasitoid species attack during the early development of the galls, while larger ones with longer ovipositors attack during the later stages of gall development (Briggs and Latto, 1996; Abrahamson and Weis, 1997; Stone et al., 2002). These results support the hypothesis that the evolution of gall morphology has been an adaptive response of wasps to minimize the attacks from their associated parasitoids (Abrahamson and Weis, 1997; Stone and Schönrogge, 2003; Abrahamson and Blair, 2008). However, the results also suggest that parasitoids are still searching how to counteract these defenses (Wiebes-Rijks and Shorthouse, 1992; Stone et al., 2002).

4.2. Hybridization

Natural hybridization is a common phenomenon in plant species (Whitney et al., 2010) that has been recognized as a substantial evolutionary force that favors the process of species diversification and increases intraspecific genetic diversity (Rieseberg and Ellstrand, 1993; Whitham et al., 1999). In the last decades, hybrid zones have allowed to study the effect of the interspecific genetic flow on plant-insect interactions (Whitham, 1989; Dungey et al., 2000; Tovar-Sánchez and Oyama, 2006a, b; Yarnes et al., 2008; Valencia-Cuevas et al., 2017). Several studies have focused on the response of arthropods, particularly phytophagous ones, to the variations found in these areas (Boecklen and Spellenberg, 1990; Aguilar and Boecklen, 1992; Prezsler and Boecklen, 1994; Tovar-Sánchez and Oyama, 2006a, b; Yarnes et al., 2008). The host plants of arthropods have unique combinations of genetically based characteristics that could be associated with the oviposition preferences of the associated insects and the resistance characteristics of the plants (Boecklen and Spellenberg, 1990; Aguilar and Boecklen, 1992; Fritz, 1999). Thus, genetic variations that occur as a result of hybridization events may affect the distribution of herbivorous and pathogenic arthropods (Whitham et al., 1994; Fritz, 1999). For example, differences in the abundance or composition of arthropod communities in hybrid zones may be the result of the presence of susceptible hybrid genotypes, while in other areas the hybrids may be resistant, resulting in

hybrid zones containing plants with greater or lesser resistance (Martinsen et al., 2000), which affects the structure of the arthropod community.

In general, phytophagous arthropods show four response patterns to the hybridization of their host plants: 1) Susceptibility: more insect species in hybrid hosts than in the parent species (Fritz et al., 1994; Whitham et al., 1994); 2) Dominance: hybrids sustain as many species of herbivores as some of the parent species (Fritz et al., 1994, 1996; Fritz, 1999); 3) Resistance: hybrids sustain less herbivores than the parent species (Boecklen and Spellenberg, 1990; Fritz et al., 1994, 1996; Fritz, 1999); 4) Additivity: hybrids sustain an intermediate number of insects compared to the parent species (Boecklen and Spellenberg, 1990; Fritz et al., 1994, 1996; Fritz, 1999). The presence, in phytophagous arthropods, of different response patterns to the hybridization of their host plants has been attributed to the extension, in time and space, of the geographical distribution of hybrid zones, environmental gradients, the genetic status of hybrids, morphological and chemical similarities between parent species, and the genetic mechanisms that determine the inheritance of resistance mechanisms in hybrids (Boecklen and Spellenberg, 1990; Strauss, 1994; Fritz, 1999; Whitham et al., 2003).

In the reviews carried out by Strauss (1994) and Whitham et al. (1999), the authors found that, in 152 cases analyzed, 79% of the taxa showed a significant response to the hybridization of the host plant, and the most frequent response was susceptibility of the hybrids, with 28% of the cases (43 studies). They also found that herbivores of both parent species accumulate in hybrids and hybrid zones.

Other factors that may affect the response of arthropods to the hybridization of their host plants are: 1) the level of genetic variation in the host plants present in hybrid zones, and 2) the pattern of introgression (Whitham et al., 1999). The highest genetic variation is expected to appear when all hybrid classes are present within a hybrid zone, so that any factor that eliminates one or more classes may have negative consequences on the levels of variation (Whitham et al., 1999). For example, it has been proposed that the greatest genetic diversity occurs as a result of bidirectional introgression, that is, when the first generation of hybrids (F1) are fertile,

reproduce and form backcrosses with both parent species. A continuum of genotypes between both species would be expected as a result of the combinations and permutations of the genome of the two parental species (Tovar-Sánchez and Oyama, 2004). Nevertheless, it is also possible that fertile F1 hybrids cross with only one of the parent species (Keim et al., 1989), a process known as unidirectional introgression. Under this hybridization scenario, the continuum of hybrid genotypes exists only between the hybrids and one of the parent species, while a morphological and genetic vacuum is left between the hybrids and the other parent species, which results in a reduction of the genetic diversity of the hybrid zone. In the last scenario, F1 hybrids are sterile, survive by cloning, and genetic diversity is at its lowest level (Whitham et al., 1999). An example that illustrates the influence of introgression and genetic diversity in hybrid zones on arthropod communities is the study by Tovar-Sánchez and Oyama (2006b) in seven hybrid zones of the hybrid complex *Q. crassipes* × *Q. crassifolia* in Mexico. Their results showed that in the hybrid zone where bidirectional introgression was detected, the hybrids sustained the greatest richness of endophagous insects (Hymenoptera and Lepidoptera), compared to the hybrids in the other six hybrid zones where introgression was unidirectional. The authors also reported a positive and significant relationship between the genetic diversity of the hybrid zone and the diversity of endophagous arthropods associated with the same oak complex. They found the greatest genetic diversity in the hybrid zone where bidirectional introgression took place (Tovar-Sánchez and Oyama, 2004), which favors the species diversity of the community of endophagous insects. Whitham et al. (1999) proposed that the richness of arthropod species is highest in hybrid zones with bidirectional introgression, intermediate in hybrid zones formed by unidirectional introgression, and lowest in hybrid zones formed by sterile F1 hybrids, where no backcrossing with parent species occurs. This pattern has been explained by considering that hybrid genotypes are eliminated in the hybridization scenarios with unidirectional introgression and sterile hybrids, while phytophagous arthropods of the two parental species accumulate in the hybrid zones, affecting the diversity pattern of arthropod species (Whitham et al., 1999).

Hybridization is a mechanism that has allowed species of arthropods to change to a new species of host plant. It has been suggested that for this host change to occur, herbivores must be preadapted to switch to a new host species, but they do not because the new host is not present (Pre-adaptation hypothesis; Thomas et al., 1987). When herbivores are not pre-adapted to a new host plant, one or more key mutations must occur for phytophagous arthropods to recognize it as a new and better host (Mutation hypothesis; Jermy, 1984). Floate and Whitham (1993) proposed the hybrid bridge hypothesis that predicts that intermediate hybrid plants facilitate switching hosts from one species to another, since organisms associated with a particular plant can experiment and adapt gradually to the genome of another plant species. If a host species has an allopatric distribution with respect to another potential host, this creates a barrier that prevents phytophagous arthropods from switching hosts (Keim et al., 1989). The pre-adaptation hypothesis suggests that arthropods will not switch host species unless the hosts have a sympatric distribution (Thomas et al., 1987). Therefore, if two species hybridize, hybrid intermediaries become "spatial bridges" through which arthropods can switch to a new host plant species, even though the parent species have an allopatric distribution.

A study that supports the hypothesis of the hybrid bridge was carried out by Tovar-Sánchez and Oyama (2006b); they found that the hybrids that resulted from the cross of *Q. crassipes* and *Q. crassifolia* (*Q.* × *dysophylla*) hosted nine species of insects that usually inhabit the canopy of the two parental species.

Considering the frequency with which the phenomenon of hybridization occurs in plants, several studies suggest that hybrid zones are the centers of arthropod biodiversity (Whitham, 1989; Dungey et al., 2000; Tovar-Sánchez and Oyama, 2006a, b; Valencia-Cuevas et al., 2017). They also suggest that these areas are useful for exploring ecological and evolutionary processes at multiple levels (Strauss, 1994; Whitham et al., 1999). Conserving these areas becomes important because they have positive effects on the arthropod communities associated with species involved in hybridization events.

4.3. Adaptive Radiation

Arthropods represent more than half of the known species on earth (Hamilton et al., 2010); they have experienced several important ecological and evolutionary radiations (Condamine et al., 2016). Their evolution is associated with the great diversity of life forms and development strategies that can be found among them; this diversity has allowed them to occupy almost every ecological niche (Grimaldi and Engel, 2005). Their great diversity reflects the variety of adaptive transformations they have undergone under similarly varied environmental conditions (Schowalter, 2000). Thanks to the fossil record, it has been possible to determine that arthropods were able to survive the most severe mass extinction events, adapting to radical changes in terrestrial vegetation, continental rearrangements and changes in environmental parameters (Condamine et al., 2016). The most common hypotheses that have tried to explain the diversification of arthropods through their evolutionary history mention low rates of extinction and resilience to mass extinctions, as well as the acquisition of novel abilities that allowed them to occupy new niches (Labandeira et al., 1993; Grimaldi and Engel, 2005; Mayhew, 2007; Rainford et al., 2014).

It has been suggested that the diversity of morphological characters in the buccal apparatus, the appearance of wings, their small size, the presence of an exoskeleton and the process of complete metamorphosis constituted novelties that allowed insects to adapt to different environments and, consequently, to diversify (Strong et al., 1984; Labandeira et al., 1994; Condamine et al., 2016). An analysis of the fossil record of insects at the family level revealed that the appearance of wings is associated with a high rate of species origination, while the process of complete metamorphosis is associated with a higher rate of diversification (Labandeira et al., 1994). These results are consistent with those of a phylogenetic study involving 82% of all insect families that identified 45 changes in the diversification rate corresponding to the tree of life of these organisms. The authors mention that two of these changes are major ones and are related to the origin of flight and the emergence of the process of complete metamorphosis (Rainford et

al., 2014). It has also been suggested that environmental and physical factors resulting from climatic or geological events, and even the emergence of new ecological niches, could have induced adaptive responses in arthropods (Condamine et al., 2016).

The case of herbivorous insects and their host plants can illustrate some of the scenarios mentioned above. Insects are the most dominant group on Earth in terms of species richness and ecological function (Wilson, 1992). Within this group, those with phytophagous habits have the highest species richness (Rivera, 1991; Schowalter, 2000); of these, the most important orders are Lepidoptera and Orthoptera, since close to 99% of their species are phytophagous (Strong et al., 1984). According to the fossil record, terrestrial plants and insects appeared in the Devonian period about 380-400 million years ago (Rohdendorf and Raznitsin, 1989; Wooton, 1981). Furthermore, it is believed that the periods of greatest increase in plant diversity are the Devonian period (mid Cretaceous), the Upper Cretaceous and the Tertiary (Knoll et al., 1979 These periods of diversification of plant species coincide with the increase in the diversity of phytophagous insects, probably as a response to the emergence of new niches as a result of increased plant diversity (Strong et al., 1984). Comparing the diversity of herbivorous insects with that of their non-herbivorous relatives suggest that the acquisition of a plant-based diet is associated with speciation and diversification (Mitter et al., 1988). Recent phylogenetic-molecular studies support the above hypothesis by indicating that orders such as Hymenoptera, Lepidoptera and Orthoptera (Hunt et al., 2007; Moreau et al., 2006; Ahrens et al., 2014) became widely diversified during the Cretaceous in response to newly formed niches (Mayhew, 2007). It has also been reported that an important part of the phytophagous beetle fauna emerged during radiation events caused by the appearance of new angiosperm lineages (Farrell, 1998). Furthermore, the appearance in the fossil record of gall-inducing wasps coincides with the origin of the main lineages of oaks that constitute their host plants (Ronquist and Liljeblad, 2001). Their diversification process was probably stimulated by the acquisition of novel traits that allowed them to exploit available resources (Strong et al., 1984; Cornell, 1989; Hespenheide, 1991; Labandeira et al., 1993; Grimaldi and Engel, 2005).

Moreover, it has been suggested that the Devonian and Carboniferous plant communities were structurally more complex than their predecessors (Sporne, 1975). Some researchers speculate that the increasing complexity of plants could have contributed to the increasing insect diversity (Strong et al., 1984). It has been suggested that the appearance of giant arborescent plants at the end of the Devonian period had two important consequences for the evolution of insects: a) it favored the evolution of winged insects and b) it stimulated the diversification of insects during the Carboniferous period (Strong et al., 1984). These hypotheses have been supported by contemporary studies that have shown that habitat complexity influences the diversity of insect communities (Halaj et al., 2000; Tews et al., 2004; Kallimanis et al., 2008; Antonelli and Sanmartin, 2011).

There are also studies that suggest that changes in global temperature and fluctuations in the content of atmospheric O_2 and CO_2 could be associated with diversification events of herbivorous insects (Labandeira, 2006). These possible associations could have been mediated by the effect of environmental factors on the vegetation that constituted the main resource for herbivorous insect communities.

Evolutionary novelties promoted by competition are considered one of the most important causes of adaptive radiation (Schluter, 2001). This may be due to the fact that the struggle for resources modifies the morphological and ethological characteristics that favor the use of unexploited resources (Abrams, 2000), resulting in genetic and phenotypic divergence, which would eventually cause species radiation. However, it has been suggested that interspecific competition has had limited influence on the diversification of arthropods (Strong et al., 1984). This hypothesis has been proposed with respect to insects. Information from the fossil record and contemporary insect communities suggest that interspecific competition has probably had little weight in the diversification of this group (Lawton and Strong, 1981; Strong et al., 1984; Denno et al., 1995), since their natural enemies and other factors keep their population low compared to the availability of resources, reducing the need for competition (Hairston et al., 1960). Several studies have reported that some species of herbivorous insects facilitate the presence of other species of insects by creating entry points, shelters or other

modifications in the host plant (Waltz and Whitham, 1997; Martinsen et al., 2000; Lill and Marquis, 2003). However, Ronquist and Liljeblad (2001) suggest that inquiline insects associated with the galls induced by wasps of the family Cynipidae may have emerged as a result of a competitive interaction with the latter, losing the ability to induce galls and becoming obligatory inquilines (Ronquist, 1994).

The development of adaptations that allowed them to maintain feeding relationships with plants is another important factor in the diversification of phytophagous (Janz, 2011; Condamine et al., 2016). Plants are the most important food resource in terrestrial ecosystems (Begon et al., 2006). However, the consumption of plant biomass by herbivores represents a loss of energy for the plant. Under this scenario, plants seek to break their interaction with herbivorous organisms. At the same time, the herbivores that depend on certain plants seek to maintain their interaction with them by adapting to the changes of their host plants. In this process, herbivores have managed to diversify. In Europe, for example, the species *Quercus robur* has been reported to have 20 different types of galls formed by 20 different species of gall-forming wasps of the family Cynipidae (Crawley, 1997), possibly due to the existence of very specific defense mechanisms that have been suppressed in various forms by wasps, leading to radiation events. The insects associated with a single plant species represent an extreme case of adaptive radiation through the differential use of resources (Oyama, 2012). A study conducted by Abrahamson et al. (2003) reported that the community of gall-inducing wasps associated with six species of oak showed different assemblages in response to the presence of different chemical compounds in each host oak. This suggests that the presence of different defense chemicals in the host oaks may have contributed to the adaptive radiation of their associated herbivores.

CONCLUSION

When we talk about loss of species, we refer to those species that have already been taxonomically identified. According to recent estimates,

arthropods are the most diverse biological group on the planet, but there are not many researchers to study them. This creates the urgent need to train human resources interested in the study, description and analysis of arthropods.

In general, studies aimed at the conservation of species are often focused on preserving plant species and "charismatic" animal species. Thus, arthropods are usually not given much attention.

There should be more studies focused on characterizing the structure of arthropod communities, not only because of the high levels of species richness and abundance they contain, but also because of the important ecological role they play (as functional groups). In natural conditions, all interactions (biotic, chemical, genetic and environmental) affect arthropods; however, their most important interaction is with host plants (whether they are specialists or generalists), since their survival, adaptation, evolution and diversity seem to be directly related to the range of resources and conditions that plants "offer" them directly (e.g., herbivores) or indirectly (e.g., parasites, parasitoids, decomposers, etc.). Further studies should aim towards the following objectives: first, to describe and make an inventory of the diversity of arthropods. Second, to study the factors that modify the structure of their communities. Third, to document the interactions between species of arthropods belonging to different trophic levels. Fourth, to describe the ecological role of arthropod species. These academic efforts should go hand-in-hand with efforts to conserve as many ecosystems as possible in order to preserve the greatest possible number of species. The data obtained from studies such as this one suggests that arthropods can be used as bioindicator species of environmental quality.

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