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TITULO DE LA TESIS

CAMBIOS EN LA ESTRUCTURA GENÉTICA, ATRIBUTOS FUNCIONALES FOLIARES, DEFENSA QUÍMICA Y DIVERSIDAD DE ARTRÓPODOS DEL DOSEL DE *QUERCUS LAURINA* A LO LARGO DE UN GRADIENTE DE DIVERSIDAD DE ENCINOS.

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A mis padres... Marcelina y Antonio que me han apoyado durante todo mi viaje.

Un viaje de mil millas comienza con un paso.

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associate to canopy of *Quercus laurina*: Importance of oak species diversity and foliar

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RESUMEN GENERAL

El género *Quercus* L. (encinos, robles) se caracteriza por tener una alta frecuencia de flujo génico interespecífico, generando potencialmente procesos de hibridación e introgresión (es decir, incorporación mediante hibridación y retrocruzamiento de alelos de un híbrido F1 en el acervo genético de una especie parental), siendo un proceso evolutivo clave para este grupo. Particularmente, las zonas del centro-oeste y sur de México presentan una alta diversidad de especies de encinos, coexistiendo en ciertas regiones geográficas desde dos hasta siete especies de encinos: Estas regiones representan gradientes de diversidad de encinos y zonas de alta actividad ecológica y evolutiva. Estudios previos han utilizado estos gradientes de diversidad de especies de encinos para evaluar la estructura y diversidad genética de especies focales de encinos, reportando una alta diversidad genética. Sin embargo, poco se conoce de los efectos de esta variación genética sobre la expresión y la variación fenotípica de las especies de encinos a nivel morfológico, fisiológico (i.e. defensa química) o fenológico. Además, la diversidad de las comunidades de encinos puede influenciar la estructura y composición de los ensamblajes de artrópodos e insectos fitófagos asociados al dosel de estos. En este trabajo de tesis se determinaron los siguientes objetivos: (i) los efectos de los cambios en la composición y estructura de la comunidad de encinos sobre la variación genética y atributos funcionales foliares; (ii) los cambios en la diversidad de artrópodos asociados al dosel de *Q. laurina* a lo largo de un gradiente de diversidad de especies de encinos y (iii) los patrones de ataque y composición de insectos fitófagos asociados al dosel de *Q. laurina* en el gradiente de diversidad de encinos. Dentro del Eje Neovolcánico Transversal de México, se seleccionaron cinco sitios de estudio, los cuales representan un gradiente de diversidad de encinos y donde ocurre *Q. laurina*. Para determinar los cambios en la estructura y diversidad genética de *Q. laurina* dentro del

gradiente de diversidad de encinos, se utilizaron microsatélites de núcleo y cloroplasto. De forma paralela, se cuantificaron siete atributos funcionales foliares para determinar la variación fenotípica de *Q. laurina* dentro del gradiente de diversidad de encinos y determinar si esta variación se debe a cambios en la estructura y diversidad genética de *Q.laurina* a lo largo del gradiente de diversidad de encinos. Se encontraron bajos niveles de diversidad genética de *Q. laurina* en el sitio de mayor riqueza de especies de encinos; lo cual sugiere que no están ocurriendo procesos de hibridación e introgresión en este sitio. Es posible que factores climáticos puedan ser las causas de la baja diversidad genética detectada en *Q. laurina* debido a que estos pueden afectar potencialmente los patrones fenológicos reproductivos. Por otra parte, se detectaron diferencias significativas en los atributos funcionales foliares entre los sitios de estudio, teniendo mayor variación en los sitios de mayor riqueza de especies de encinos; así como una asociación significativa con los factores climáticos, pero no con la estructura y diversidad genética de *Q. laurina*. Para el segundo objetivo, se encontró una mayor abundancia y riqueza de artrópodos en los sitios de mayor riqueza de especies de encinos rojos. Además, se encontraron diferencias entre el ensamblaje de las comunidades de artrópodos; teniendo los sitios de mayor riqueza de especies de encinos una estructura y composición de artrópodos del dosel totalmente distinta a los sitios de menor riqueza de especies de encinos. Esto puede atribuirse al hecho de las comunidades encinos representan una mayor disponibilidad de recursos y nichos para ser colonizados por un mayor número de especies de artrópodos (efectos bottom-up), aumentando conforme hay más especies de encinos. Este resultado concuerda con lo propuesto por la "Hipótesis de riqueza de especies de planta", la cual plantea que comunidades con mayor diversidad de especies de plantas representan zonas potenciales para ser colonizadas por más especies de insectos. Otra hipótesis que puede explicar estos

resultados es "La hipótesis del enemigo natural", la cual sugiere que la diversidad de enemigos naturales, incrementa en comunidades con una gran diversidad de plantas debido a la presencia de más presas potenciales y la disponibilidad de microambientes (efectos top-down). Por último, en el tercer objetivo, se encontró una mayor abundancia y riqueza de insectos fitófagos en los sitios de mayor riqueza de especies de encinos; así como diferencias en la composición de la comunidad de insectos fitófagos entre los sitios de estudios, donde el sitio de mayor riqueza de encinos presenta una comunidad de insectos fitófagos totalmente distinta al sitio de menor riqueza de encinos. Además, el gremio de los insectos masticadores de hojas fue el que presentó una mayor abundancia en todos los sitios de estudio; siendo particularmente más abundante en los sitios de mayor riqueza de especies de encinos (81.50%) en relación con los sitios de menor riqueza de encinos (47.63%); lo cual es consistente con la "Hipótesis de riqueza de especies de plantas". En el mismo sentido, los resultados indican mayores niveles de herbivoría y concentración de metabolitos secundarios asociados a defensa química, en los sitios de mayor riqueza de encinos en comparación con los sitios de menor riqueza. Esto es consistente con la idea de que la diversidad de las comunidades de plantas es un factor clave que puede aumentar o disminuir los porcentajes de herbivoría. Estos cambios en la comunidad de insectos fitófagos también pueden estar asociados a los cambios en la defensa química, asignando más recursos a la defensa química siendo la riqueza y abundancia de encinos un factor que influye sobre los niveles de herbivoría y defensa química en las comunidades de encinos.

INTRODUCCIÓN GENERAL

Los bosques templados mixtos se caracterizan por la ocurrencia de dos o más especies de árboles, los cuales presentan interacciones inter e intraespecíficas entre ellas (Bravo-Oviedo et al., 2014). Durante las últimas décadas, se ha resaltado la importancia de los bosques mixtos los cuales presentan niveles altos de productividad primaria (Chamagne et al., 2016), mayor estabilidad frente a cambios ambientales y antropogénicos (Morin et al. 2014; Van der Plas et al., 2016; Jactel et al., 2017) y soportan una gran diversidad de grupos de organismos (Tovar-Sánchez et al., 2013; García-Guzmán et al. 2017). Dentro de los sistemas templados, el género *Quercus* (encinos) representa uno de los grupos de plantas leñosas con mayor grado de riqueza y abundancia en el hemisferio norte, con alrededor de 500 especies en el mundo (Cavender-Bares, 2016); resaltando por una alta frecuencia de hibridación natural (Arnold 1997; 2006). Cuando dos especies pertenecientes a la misma sección de este género se encuentran en simpatría, con frecuencia ocurren eventos de hibridación (Tovar-Sánchez y Oyama 2004, González-Rodríguez & Oyama 2005, Riodan et al., 2016), aunque presenten diferencias morfológicas y fisiológicas muy marcadas. Resultado de este proceso, es común observar la ocurrencia de individuos híbridos entre dos o más especies cuando la fenología de éstas se sobrepone en una misma zona geográfica (Curtu et al. 2007a, Hipp & Weber 2008). Por lo cual, la hibridación se puede definir como la reproducción entre individuos de diferentes poblaciones de especies que son distinguibles en al menos un carácter heredable (Arnold 1997; Soltis & Soltis 2009).

Como consecuencia de la hibridación algunas poblaciones pueden presentar fenómenos de introgresión (i. e. flujo de genes entre dos formas diferenciadas, como producto repetido de eventos de hibridación y retrocruzamiento) (Harrison & Larson,

2014). Recientes estudios han encontrado que la hibridación interespecífica puede influir de forma considerable sobre la diversidad genética de las poblaciones involucradas (Arnold 2006), lo cual potencialmente puede dar lugar a la aparición de nuevos caracteres fenotípicos o atributos funcionales (i. e. bioquímicos, morfológicos, metabolitos de defensa) (Rieseberg, Archer y Wayne, 1999; Violle 2007; Lavorel et al. 2007) teniendo consecuencias en la adecuación de las plantas (Caseys et al. 2015). Los atributos funcionales se definen como caracteres observables o medibles (es decir, morfológicos, fisiológicos y fenológicos) que influyen en el rendimiento o la aptitud de un organismo, reflejando respuestas evolutivas a las condiciones ambientales. (McIntyre et al. 1999; Lavorel et al. 2007).

Por otra parte, se ha reportado que el dosel de los encinos soporta una gran diversidad de artrópodos (Tovar- Sánchez and Oyama 2006a, Maldonado-López et al. 2018). El hábitat del dosel proporciona recursos alimenticios, refugio y sitios de reproducción, oviposición y desarrollo para diversos gremios de artrópodos, como los herbívoros (incluidos los grupos masticadores, chupadores, inductores de agallas y minadores de hojas), depredadores, parasitoides y detritívoros, entre otros (Ulyshen, 2011; Maldonado-López et al. 2018); siendo lugares de una alta actividad ecológica. A escala local, la diversidad de la comunidad de plantas es un factor determinante que incide en la diversidad de artrópodos del dosel (Moreira et al. 2016). Por ejemplo, se ha registrado que la diversidad de insectos herbívoros aumenta de comunidades con pocas especies de plantas hasta comunidades de mayor diversidad de plantas (Cuevas-Reyes et al. 2004; Hertzog, 2017). Por lo cual, un incremento en la diversidad de recursos proporcionada por una alta diversidad de herbívoros presentes en comunidades de plantas muy diversas aumenta la diversidad de depredadores y otros niveles tróficos y afecta la estructura comunitaria de

artrópodos (Haddad et al. 2011; Moreira et al. 2016). En condiciones naturales, la calidad nutricional de las plantas puede verse afectada por la diversidad de plantas circundantes, influyendo en la biomasa, los atributos funcionales foliares (Kos et al., 2015) y en el metabolismo secundario asociado a defensa vegetal (Moreira et al., 2014), afectando las interacciones planta-insecto dentro de las comunidades de plantas. Por lo tanto, la estructura de las comunidades de encinos también puede ser un factor clave que determine la diversidad de artrópodos a lo largo de gradientes ecológicos (Haddad et al. 2011). El presente estudio se enfocó en determinar los efectos de los cambios en la composición y estructura de la comunidad de encinos sobre la variación genética, atributos foliares funcionales y la comunidad de artrópodos del dosel de *Quercus laurina*; así como evaluar si algunos factores abióticos influyen sobre la variación de atributos funcionales foliares y la comunidad de artrópodos del dosel dentro de un gradiente de diversidad de especies de encinos en México.

Sistema de estudio- En términos de número de especies y biomasa, el género *Quercus* (Fagaceae) es uno de los componentes más importantes de los bosques templados para América del Norte (Cavender-Bares 2019). México es un importante centro de diversificación y endemismo de los encinos con alrededor de 170 especies, de las cuales 100 son endémicas (Valencia 2004; Hipp et al. 2020). Particularmente, las zonas del centro-oeste y sur de México son muy ricas en especies de encinos pertenecientes a las secciones *Lobatae* (encinos rojos) y *Quercus* (encinos blancos) (Torres-Miranda et al. 2013; Rodríguez-Correa et al. 2015), donde pueden coexistir entre dos y seis especies a escala local (McCauley et al. 2019). Estos gradientes de riqueza de especies de encinos se han utilizado anteriormente para evaluar la estructura y diversidad genética de especies focales

de encinos que se encuentran a lo largo de estos gradientes. (Valencia-Cuevas et al. 2015).

Sin embargo, el flujo de genes interespecífico en las comunidades de encinos es un

fenómeno complejo, que se ve afectado por diferentes factores, como las condiciones

ambientales locales (Lepais et al. 2009, Lagache et al. 2013), la ubicación geográfica de la

zona de hibridación (Tovar-Sánchez and Oyama 2004), el establecimiento y sobrevivencia

de los híbridos, la estructura espacial de las especies de encinos (Salvini et al., 2009), la

proporción de polen conespecífico y la densidad de individuos reproductivamente

disponibles (Lagache et al., 2013). Por lo tanto, estos gradientes de riqueza resultan ser

excelentes modelos para evaluar diferentes aspectos ecológicos, genéticos y funcionales de

las comunidades de especies de encinos. Sin embargo, poco se conoce sobre los efectos de

la hibridación entre especies de encinos sobre la expresión de los atributos funcionales

foliares. Por ejemplo, Himrane et al. (2004) realizaron un estudio en la especie con origen

híbrido *Quercus subpyreneica* (*Q. faginea* × *Q. pubescens*), en el que los híbridos

presentaron mayor área foliar y conductividad hidráulica específica en comparación con sus

progenitores; lo cual indica que estos atributos funcionales podrían tener un valor

adaptativo (Schwarzbach et al., 2001). Esto indica que ciertos atributos funcionales foliares

podrían verse afectados por los procesos de hibridación presentes en los encinos.

Bajo este escenario de gradientes de diversidad de encinos, las comunidades de

artrópodos asociados al dosel de encinos pueden verse afectadas; ocasionando cambios en

su estructura y composición (Valencia-Cuevas et al. 2015; Maldonado-López et al. 2018).

Dos posibles hipótesis que no son excluyentes entre sí, pueden explicar estos cambios. La

primera se basa en la "Hipótesis de la riqueza de especies de plantas" (Fernades y

Price,1988), la cual propone que las comunidades con mayor diversidad de plantas

representan sitios potenciales para ser colonizados por un mayor número de especies de

insectos fitófagos ("efectos bottom-up") (Leroux y Loreau, 2015). La segunda se basa en "La hipótesis del enemigo natural" (Root 1973), la cual propone que la diversidad de depredadores y parasitoides (i.e enemigos naturales) aumenta en comunidades con una gran diversidad de plantas ya que estas representan sitios con más especies de presas y microambientes potenciales ("efectos de top-down") (Sobek et al. 2009).

En el mismo sentido, se ha reportado en la literatura que los árboles son generalmente más propensos a sufrir daños por insectos fitófagos cuando crecen en bosques mono-específicos que cuando se asocian con otras especies de árboles (Castagneyrol et al., 2014). Sin embargo, se ha reportado efectos neutrales de la diversidad de plantas sobre la incidencia del daño foliar causado por fitófagos generalistas (Rosado-Sánchez et al., 2018) e incluso un aumento en el daño foliar a medida que aumenta la diversidad de árboles (Schuldt et al., 2010). Por ejemplo, dos especies de plantas del mismo género tienden a sustentar comunidades de insectos fitófagos similares en comparación con las que se alimentan de especies de plantas de diferentes familias (Novotny et al., 2010). Por lo tanto, la diversidad de plantas puede reducir o aumentar los niveles de herbivoría en las plantas hospederas (Barbosa et al., 2009; Muiruri et al., 2019). Esto podría estar relacionado con cambios en la calidad nutricional y defensa química de la planta hospedera como consecuencia de los cambios de la composición de la comunidad de plantas (Abbas et al. 2013). Esto puede ser el resultado de la competencia entre la planta focal y la comunidad de plantas por la disponibilidad de recursos (e.g. luz, nutrientes del suelo, agua) (Kostenko et al. 2017). Por lo tanto, en este estudio se analizaron los efectos de la diversidad de encinos sobre aspectos genéticos y funcionales en una especie focal de encino (*Quercus laurina*) que ocurre en un gradiente de diversidad de encinos; así como la relación entre la riqueza

de especies de encinos y la diversidad de la comunidad de artrópodos e insectos fitófagos asociados al dosel de *Q. laurina.*

Estructura y objetivos del estudio- La presente tesis está conformada por tres capítulos. El primer capítulo tiene como objetivos: i) determinar si existen cambios en la diversidad genética poblacional de *Q. laurina* a lo largo de un gradiente de riqueza de especies de encinos; y ii) evaluar si los cambios en la diversidad genética poblacional de *Q. laurina* se correlacionan con variación expresada en los atributos funcionales foliares. Elegimos a *Q. laurina* porque tiene una amplia distribución en México (Valencia 2004), por lo que es factible encontrar comunidades con distinta diversidad de especies de encinos, donde esta especie focal está presente y es abundante. Nuestra hipótesis predice que un aumento en el número de especies de encinos en la comunidad modificará los niveles de diversidad genética como resultado de posibles eventos de flujo de génico, lo que resultará en un aumento en la variación de los atributos funcionales foliares en *Q laurina*.

En el segundo capítulo, evaluamos los cambios en la estructura y composición de la comunidad de artrópodos del dosel asociados a *Q. laurina* a lo largo del gradiente de riqueza de especies de encinos. Los objetivos para este capítulo fueron: i) Determinar si se incrementa la diversidad de artrópodos del dosel asociados a *Q. laurina* en las comunidades con mayor diversidad de especies de encinos, y ii) Evaluar los cambios de la estructura y composición de los gremios de artrópodos a lo largo del gradiente de riqueza de encinos

En el tercer capítulo se evaluaron los cambios en la estructura y composición de insectos fitófagos asociados con el dosel de *Q. laurina*, así como cambios en la defensa química foliar y niveles de herbivoría a lo largo del gradiente de riqueza de encinos. Para este capítulo los objetivos fueron: 1) Evaluar si el aumento en la diversidad de la

comunidad de encinos resulta en un incremento de la diversidad de la comunidad de insectos fitófagos asociados con el dosel de *Q. laurina*; y ii) Determinar si el incremento de la comunidad de insectos fitófagos genera mayores niveles de herbivoría, debido a una mayor disponibilidad de recursos para los insectos fitófagos y cambios en la concentración de compuestos químicos secundarios en *Q. laurina*.

CAPÍTULO I.

Genetic and functional leaf traits variability of *Quercus laurina* along an oak diversity gradient in Mexico

Marcela Sofía Vaca-Sánchez, Antonio González-Rodríguez, Yurixhi Maldonado-López, Ken Oyama, Maurício Lopes de Faria, Marcílio Fagundes and Pablo Cuevas-Reyes

Presentación

El en primer capítulo de este trabajo de tesis fue: i) determinar si existen cambios en la diversidad genética poblacional de *Q. laurina* a lo largo de un gradiente de riqueza de especies de encinos; y ii) evaluar si los cambios en la diversidad genética poblacional de *Q. laurina* se correlacionan con variación expresada en los atributos funcionales foliares. Nuestra hipótesis predice que un aumento en el número de especies de encinos en la comunidad modificará los niveles de diversidad genética como resultado de posibles eventos de flujo de génico, lo que resultará en un aumento en la variación de los atributos funcionales foliares en *Q laurina*. Se detectaron altos niveles de diversidad genética de *Q. laurina* en los sitios de mayor diversidad de encinos de la misma sección. Por otra parte, se encontraron diferencias significativas en los atributos funcionales foliares de *Q. laurina* entre los sitios de estudio, presentando mayor variación en los sitios con mayor riqueza de especies de encinos. Nuestros hallazgos concuerdan sugieren que un aumento en la diversidad funcional de *Q. laurina* (e.g. área foliar específica, área foliar y densidad foliar) está asociado con la riqueza de especies de encinos.

ORIGINAL PAPER

Genetic and functional leaf traits variability of *Quercus laurina* along an oak diversity gradient in Mexico

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Abstract

The ecological literature has documented the effects of plant hybridization on phenotypic variation, and dominant, intermediate, or novel morphological, chemical and physiological traits in hybrids. It is important to understand the ecological consequences of hybridization by evaluating their impact on phenotypic expression of functional traits. We evaluated the relationship between genetic diversity of *Quercus laurina* and functional foliar traits along an oak diversity gradient. We selected five study sites that represent an oak diversity gradient where *Q. laurina* is present. Using chloroplast and nuclear microsatellites, we evaluated genetic diversity, measured functional foliar traits of *Q. laurina* in each site and assessed the effects of local climate variables on the oak community and functional traits. We found a greater abundance of *Q. laurina* in all study sites. We did not find a relationship between the number of accompanying red oak species and the population genetic diversity in *Q. laurina*, but higher genetic diversity was found in all study sites in comparison with European oak species. Sites with more oak species had more variation of foliar functional traits. Our results do not support the hypothesis that predicts higher levels of genetic diversity of *Q. laurina* in communities with greater oak diversity from the same section, but we demonstrated an increase in the foliar functional traits of *Q. laurina* associated with oak richness and climate variables. We highlight the need to consider environmental and ecological variables linkages as regulatory mechanisms of the phenotypic plasticity expressed in changes of some functional attributes of oaks.

Keywords Oak species richness · Genetic diversity · Functional plant traits · *Q. laurina*

Introduction

The natural hybridization and introgression are important sources of evolutionary novelty in plant species, mainly because the recombination among parental species genomes

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results in a wide array of new genotypes upon which natural selection can act (Soltis and Soltis 2009). Several studies have documented the effects of plant hybridization on phenotypic variation and dominant, intermediate, transgressive or novel patterns of morphological, chemical and physiological traits (Arnold 1997, 2006; Whitham et al. 2012; Rieseberg et al. 1993, 1999). However, a critical aspect that has not been frequently assessed formally is the effect of plant hybridization and introgression (i.e. incorporation via hybridization and backcrossing of alleles from one F1 hybrids into the gene pool of one parental specie) (Harrison and Larson 2014) on the degree of functional trait variability. These processes can be shaped by climate factors in different ways: (i) limiting genetic exchange by abiotic selection between plant species that come into contact through environmental gradients (Gailing et al. 2012; Alberto et al. 2013; Riordan et al. 2016); (ii) favoring hybrid plants in areas of the environmental gradient under particular circumstances (i.e. selective environment in favor of hybrids) (Mallet 2005; Petit and Excoffier 2009) and (iii) promoting genetic exchange between plant species due to the presence of a strong environmental gradient where the greatest genetic exchange occurs in areas of intermediate climate, while the least genetic exchange is present at the extremes of the climatic gradient (Burge et al. 2019). In addition, genetic exchange also may be restricted by the presence of geographic or reproductive barriers, creating isolation by geographic distance (Ortego et al. 2015). Functional traits are defined as observable or measurable traits (i.e. morphological, physiological and phenological) that influence performance or fitness, which reflect evolutionary responses to environmental conditions (McIntyre et al. 1999; Lavorel et al. 2007). Therefore, it can be hypothesized that hybridization via the introgression should have an impact on both the population genetic diversity and the degree of functional trait variability of hybridizing populations (Cornelissen et al. 2003, 2004; Himrane 2002; Caseys et al. 2015). Additionally, some studies have documented that climate variables (i.e. temperature, precipitation) can modulate the responses of foliar functional traits of the oaks (Ramirez-Valiente et al. 2009; Arenas-Navarro et al. 2020a,b). Therefore, some of the relevant ecological and evolutionary consequences of hybridization and introgression cannot be understood without the evaluation of their impact on the phenotypic expression of the functional traits at both the individual and population level, which can be expressed as coefficients of variation (i.e. degree and the dispersion pattern around the mean) (Schwarzbach et al. 2001; Violle et al. 2007; Jung et al. 2010).

The genus *Quercus* is characterized by a high frequency of natural hybridization. When two species belonging to the same section of this genus are sympatric, hybridization events may occur, even if they are very different morphologically and physiologically (Tovar-Sánchez and Oyama 2004; González-Rodríguez et al. 2004, 2005; González-Rodríguez and Oyama 2005, Aldrich and Cavender-Bares 2011¸ Curtu et al. 2007a, b, c, Viscosi et al. 2012; Riodan et al. 2016). As a result of this process, it is common to observe the occurrence of hybrid individuals between two or more species when the flowering phenology overlaps in the same geographic zone (Curtu et al. 2007a, b, c, Hipp and Weber 2008). However, interspecific gene flow in oak communities is a complex phenomenon, which has been reported to be affected by several factors such as local environmental conditions (Williams and Ehleringer 2000, Williams et al. 2001, Lepais et al. 2009, Lagache et al. 2013, Valbuena-Carabana et al. 2007), geographical location of the hybridization zone (Tovar-Sánchez and Oyama 2004), the establishment of hybrid individuals and their survival, the spatial structure of oak species (Salvini et al. 2009), the proportion of conspecific pollen and the density of reproductively available individuals (Lagache et al. 2013).

Mexico is an important center of oak diversification and endemism (Hipp et al. 2018) with about 170 species, of which 100 are endemic (Valencia 2004; Hipp et al. 2020). Particularly, zones of central-western and southern Mexico are very rich in *Lobatae* (red oaks) and *Quercus* (white oaks) sections (Torres-Miranda et al. 2013; Rodríguez-Correa et al. 2015), where can co-occur between two and six species at the local scale (Cavender-Bares et al. 2018; McCauley et al. 2019). These species richness gradients in oak communities have been previously used to assess genetic diversity and structure in focal species that occur along the gradient (Valencia-Cuevas et al. 2014) as well as hybridization patterns (Valencia-Cuevas et al. 2015). Therefore, these richness gradients are excellent models to evaluate different ecological, genetic and functional aspects of oak species communities. However, the effects of hybridization among oak species on functional traits and their effects on the fitness of hybrids are not well known (Ehleringer and Smedley 1989; Williams and Ehleringer 2000). For example, in one of the few studies so far conducted, Himrane et al. (2004) found that the hybrid species *Quercus subpyreneica* (*Q. faginea* × *Q. pubescens*) presented greater leaf area and specific hydraulic conductivity compared with their parent species. Also, few studies have analyzed the relationship between genetic variation and foliar functional trait variability in communities with different richness of oak species.

Previously, it has been reported that *Q. laurina* hybridizes with several other Lobatae section species (red oaks) (Valencia 1994; González-Rodríguez et al. 2004, 2005; González-Rodríguez and Oyama 2005; Valencia-Cuevas et al. 2015; Ramos-Ortiz et al. 2015). The aims of this study were: (1) to determine if there are changes in the genetic population diversity of *Q. laurina* along a gradient of oak species richness; and (2) evaluate if changes in the population genetic

diversity of *Q. laurina* correlate with variations in functional foliar attributes. Our hypothesis predicts that an increase in the number of oak species in the community will modify the levels of genetic diversity as a result of possible gene flow events, which will result in an increase in the variability of functional foliar traits in *Q laurina*.

Material and methods

Study system and sample collection

The genus *Quercus* has two main sections according to its abundance and species richness, the *Quercus* (white oaks) and Lobatae (red oaks) sections (Valencia 2004). *Quercus laurina* Humboldt et Bonpland is a species of Mexican oak that is grouped in the section Lobatae of the genus (Nixon 1993). It is a tree between 10 and 30 m in height with a trunk diameter of 50 cm or more. Leaves are coriaceous, lanceolate or elliptic-oblanceolate, with a green and lustrous surface. The fruit is an ovoid acorn from 15 to 20 mm long and 15 to 17 mm in diameter (Arizaga 2009). The species is distributed at altitudes between 2440 and 3065 m in mixed oak forest or in pine-oak forest along the Sierra Madre del Sur and the Trans-Mexican Volcanic Belt (TMBV) (Valencia 1994).

For this study, five sites with presence of *Q. laurina* but with different richness of other oak species were selected (Table 1). Collection sites were located in the central-west portion of the TMBV, in the states of Michoacán and Jalisco. To describe the structure and richness of the oak communities, three independent transects of 100×40 m were randomly placed at each site, with at least 500 m of separation between each other. Within each transect, the species identity of all oak trees with a diameter at breast height (DBH) ≥ 10 cm was determined and the number of individuals per species was counted. From these data, species richness of oaks (SR), species richness of the Lobatae section

(SR_L), species richness of the *Quercus* section (SR_Q), the Shannon diversity index (H), Shannon diversity index of the *Lobatae* section (H'_L), Shannon diversity index of the *Quercus* section (H'_Q), density of all oak species individuals (D_S), density of individuals in the *Lobatae* section (D_L) and the density of individuals in the *Quercus* section (D_Q) were calculated. For the genetic and foliar functional traits analyses, the number of individuals of *Q. laurina* sampled in each study site was the following: Tequila Volcano ($N = 29$), Los Azufres ($N = 29$), Indaparapeo ($N = 30$), Carindapaz ($N = 29$) and Cerro Burro ($N = 29$). Each individual sampled was separated by at least 30 m from each other (Fig. 1). Of each individual, 10–15 young intact leaves were collected for genetic analysis and three branches exposed to the sun with mature leaves fully extended for the quantification of functional traits.

Genetic analysis

The leaves were placed on ice immediately since collection and afterwards stored in the laboratory at -80 °C. DNA extraction was performed from 100 mg of leaf tissue using the protocol of Lefort and Douglas (1999). To evaluate the patterns of genetic diversity in the five sampled populations, we used both nuclear and chloroplast DNA microsatellites (nSSRs and cpSSRs, respectively). Seven nSSRs previously characterized in *Q. rubra* (Aldrich et al. 2002) were chosen on the basis of reproducibility and polymorphism assessed in preliminary trials: quru-GA-OI01, quru-GA-OM05, quru-GA-OM07, quru-GA-IC08, quru-GA-2F05, quru-GA-2M04 and quru-GA-IF07) as well as three cpSSRs: cmcs6, cmcs7 and udt1. All primers were multiplexed in single PCR reactions using the QIAGEN Multiplex PCR kit (QIAGEN). The final volume of each reaction was 5 μL, containing 1 μL of Multiplex PCR Master Mix, 2 mM of each primer, deionized water, and 20 ng of DNA. The thermal cycling program was run on the Applied Biosystems thermal cycler. The program consisted of one cycle at 95 °C for 15 min and

Table 1 The diversity of the oak community per study site

Study sites	SR	H'	SR_L	H'_L	SR_Q	H'_Q	D_S	D_L	D_Q	Altitude (m.a.s.l)	AP (mm)	AMT(°C)	TWQ(°C)	TCQ(°C)
Tequila Volcano	4	1.08	3	0.94	1	0	930	890	40	2734	849	12.4	13.9	11.6
Los Azufres	3	0.95	2	0.47	1	0	485	275	210	2875	1118	14.9	19.2	17.7
Indaparapeo	3	0.74	2	0.49	1	0	415	380	35	2710	717	17.9	12.7	10.3
Carindapaz	3	0.44	2	0.21	1	0	470	445	25	2343	1425	11.9	15.6	15.1
Cerro Burro	2	0.17	1	0	1	0	465	445	20	3084	1034	15.1	15.7	14.7

The total density of individuals is given in individuals per hectare (Ind/Ha)

Table shows species richness of oaks (SR), species richness of *Lobatae* section (SR_L), species richness of *Quercus* section (SR_Q), the Shannon diversity index (H), Shannon diversity index of the *Lobatae* section (H'_L), Shannon diversity index of the *Quercus* section (H'_Q), density of all oak species individuals (D_S), density of individuals in the *Lobatae* section (D_L) and density of individuals in the *Quercus* section (D_Q), altitude, annual precipitation (AP), annual mean temperature (AMT), mean temperature of the warmest quarter and mean temperature of the coldest quarter per site along the gradient of oak diversity
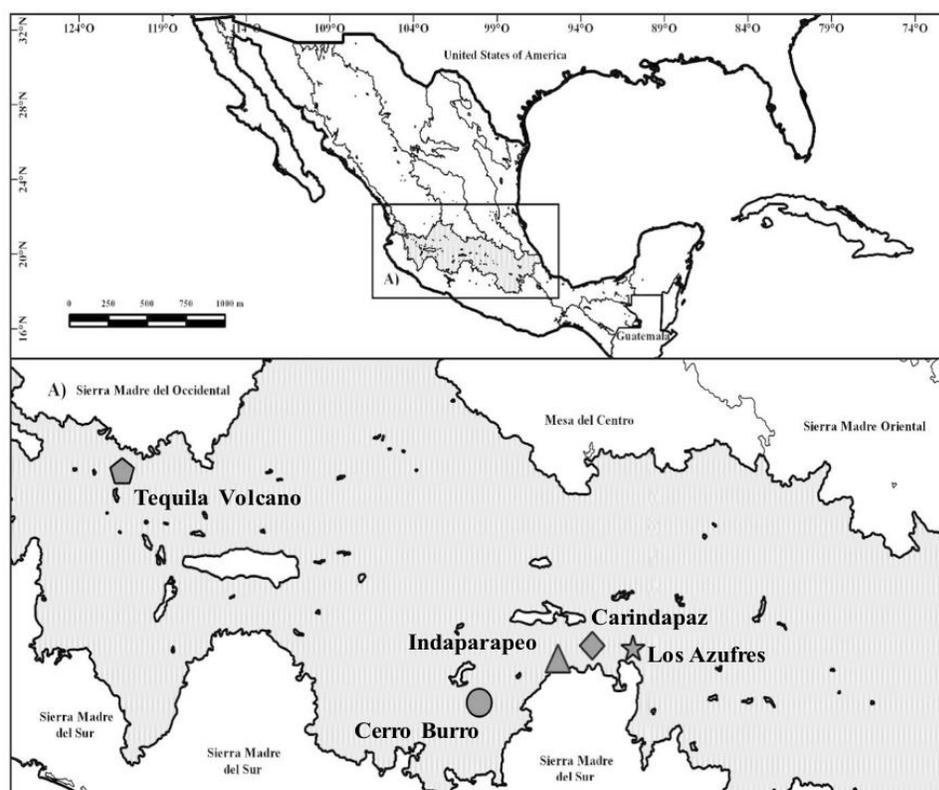
Fig. 1 Geographical distribution map of the study sites, which are located in the states of Michoacán and Jalisco in the central-western part of the Trans-Mexican Volcanic Belt (TMBV). The Tequila Vol-cano site is represented by the pentagon, Carindapaz by the rhombus, Indaparapeo by the triangle, Los azufres by the star and Cerro Burro by the circle

then 40 cycles, each with denaturation at 95 ℃ for 30 s, annealing at 55 ℃ for 90 s, and extension at 72 ℃ for 60 s. A final extension at 60 ℃ for 30 min was included. Multiplex PCR products were diluted 1:1 in deionized water and run in an ABI-PRISM 3100-Avant sequencer with the GeneScan-500 LIZ size standard included (Applied Biosystems). DNA fragment final sizing was performed using Peak Scanner software, version 1.0 (Applied Biosystems) (Sebastiani et al. 2004; Deguilloux et al. 2003; Ramos-Ortiz et al. 2016).

Plant functional traits analysis

We analyzed the following plant functional traits: diameter at breast height (DBH), chlorophyll content (Chl), leaf area (LA), leaf dry weight (LDW), leaf thickness (LT), specific leaf area (SLA) and leaf density (LD). The diameter at breast height (DBH) was measured in the field for each individual. The content of chlorophyll was measured in three leaves per individual using a portable chlorophyll meter SPAD 502 (Minolta). To determine leaf area (cm^2), leaf dry weight (g), leaf thickness (mm), specific leaf area ($cm^2 g^{-1}$) and leaf density ($g cm^{-3}$), we collected three branches per individual and placed them in botanical presses; then, we selected 30 complete leaves without apparent damage by insect herbivores and they were placed in an oven to dry. Dry weight was obtained by weighing the leaves on an analytical balance. Leaf thickness was obtained with a Vernier caliper. To measure the leaf area, a digital analysis of the scanned leaves was performed using the ImageJ program (Schneider et al. 2012). The specific leaf area was obtained by dividing the leaf area (cm^2) between the dry weight (g), and the leaf density was obtained by dividing the specific leaf area ($cm^2 g^{-1}$) between the thickness (mm).

Statistical analysis

We performed a generalized linear model (GLM) analysis using a Poisson error distribution and a log link function to determine the differences in the abundance of oak species, as well the differences between the two oak sections in the study sites along the gradient of species richness. The study sites were used as the independent variable and the total abundance of oaks and abundance per section as the response variables (SAS, Stokes et al. 2000).

Multivariate analyses were used to evaluate the differences in the oak community composition between the five study sites. The five communities of oak species were ordered by non-metric multidimensional scaling (NMDS) using an abundance similitude matrix and the Bray–Curtis index as a distance metric. Later, a nonparametric permutation procedure (ANOSIM) was used to check whether oaks species composition differed among the five study sites. Again, the ANOSIM was based on a binary matrix of oak species occurrence in each study site, using the Bray–Curtis index as a distance metric and 5000 permutations (Hammer et al. 2001). Pairwise ANOSIMs were performed between all pairs of sites as a post hoc test. These analyses were developed using PAST software (Hammer et al. 2001).

The patterns of genetic diversity in the study sites were assessed by estimating the mean number for the nSSR data set of alleles per locus (N_a), mean of the effective number of alleles (N_e), mean observed heterozygosity (H_O), mean expected heterozygosity (H_E), mean fixation index (F), and their respective standard errors with GenAlEx 6.5 (Peakall and Smouse 2012). For the cpSSRs data set we calculated the number of haplotypes in each population (A), the number of private haplotypes (P), the effective number of haplotypes (N_{he}), the rarefacted haplotypic richness (R_h), the haplotype diversity (h_S), and the mean genetic distance between individuals (D^2_sh) with the Haplotype Analysis program version 1.05 (Eliades and Eliades 2009).

For the functional traits measured, the average and the coefficient of variation (CV; defined as the ratio of the standard deviation to the mean) (Albert et al. 2011; Wellstein et al. 2013) as a relative measure of phenotypic variability were obtained for each population for all the functional traits measured. The significant differences among populations for each functional trait were evaluated through a one-way analyses of variance (ANOVA), using average values of individual trees. A stepwise canonical discriminant analysis (CDA) using individual tree averages for each functional trait was performed to determine which functional traits had the highest variation among the five study sites. These analyses were made with the JMP 9 program (SAS Institute 2005).

We also assessed the climatic variables that may potential have an influence on the variability of functional characters and on genetic variation. Climatic data were obtained for each of the 19 bioclimatic variables available in WorldClim (http://worldclim.org/version2) for each study site and subsequently eliminated redundant variables on the basis of the results of a paired correlation analysis. (The criterion was to eliminate one variable from each pair with r > 0.80 retaining the more general variable.) The variables selected were mean annual temperature (MAT), mean annual precipitation (MAP), mean temperature of the warmest quarter (TWQ) and mean temperature of the coldest quarter (TCQ).

We used Spearman's correlation (r_S) to determine if there is a relationship between the richness and diversity of oak species in the local communities, with the values of the CV of the functional characters measured by population, the measures of genetic diversity based on nSSRs and cpSSRs, and the four selected climatic variables. These analyses were made with the JMP 9 program (SAS Institute 2005).

Finally, to evaluate the effects of the oak community on genetic variation, we conducted a redundancy analysis (RDA) using the data matrix obtained from the oak community of the five study sites and the data matrix of the genetic variation of the five populations studied of *Q. laurina*. To determine the effects of the genetic variation and climatic variables on each foliar functional trait, we performed three different redundancy analyses. The first was a full model (FRDA model) that included the genetic variation matrix of *Q. laurina*, the climatic variables of each study site and the foliar functional traits of *Q. laurina*. The second RDA model only included the matrix of the climatic variables and the foliar functional traits of *Q. laurina* of the five study sites. Finally, the third RDA model only included the data matrices of the genetic variation and the foliar functional traits of *Q. laurina* of all study sites. For all genetic variation analyses performed, we first transformed the genotypes of each single-locus into allelic variables assigning a score of 0, 0.5 and 1, depending on whether the individual presented homozygous or heterozygous alleles at that locus (Westfall and Conkle 1992). RDA is an ordering method multivariate used to evaluate loci and predictors of genetic variables simultaneously such as phenotype traits and environment variables (Riordan et al. 2016).

Results

Composition of the oak community

Along the diversity gradient, five oaks species were found, three belonging to the *Lobatae* section (*Q. laurina*, *Q. crassifolia* and *Q. calophylla*) and 2 belonging to the section *Quercus* (*Q. obtusata* and *Q. rugosa*). Significant differences were found in the abundance of oak species between the study sites ($\chi^2 = 409.9$, df = 4, $P = 0.0001$), as well as between the oak sections ($\chi^2 = 358.3$, df = 1, $P = 0.0001$).

The study sites with lowest diversity levels of oaks were Cerro Burro and Carindapaz, and the sites with the highest diversity levels were Indaparapeo, Los Azufres and the Tequila Volcano (Table 1). The results of the similarity of oak community showed that NMDS ordination explained 84% of the variance between sampling points (axis $1 = 70\%$ and axis $2 = 14\%$), where each point is a two-dimensional representation of the composition of oak species based on global NMDS. The composition of oak species varied between the study sites (ANOSIM $r = 0.47$, $n = 15$, $P = 0.0001$) (Fig. 2). The results of post hoc pairwise ANOSIM tests showed differences in the oak species composition

between all study sites. Particularly, Tequila Volcano and Cerro Burro had totally different assemblages in comparison with the other three study sites ($P < 0.05$).

Genetic diversity of *Q. laurina* along the diversity gradient of oak species

The seven nSSRs revealed high levels of genetic diversity for *Q. laurina* in all study sites (Table 2). N_a ranged from 10.42 to 12.42, with population Carindapaz showing the highest value and Tequila the lowest (Table 2), and N_e ranged from 5.87 to 8.07, with a similar pattern to N_a. High H_O (ranging

Fig. 2 Non-metric dimensional scaling (NMDS) ordinations illustrating similarity of oak species composition between the study sites along oak diversity gradient. Each point is a two-dimensional (axis 1 and axis 2) representation of oaks species composition on global, non-metric multidimensional scaling (NMDS)
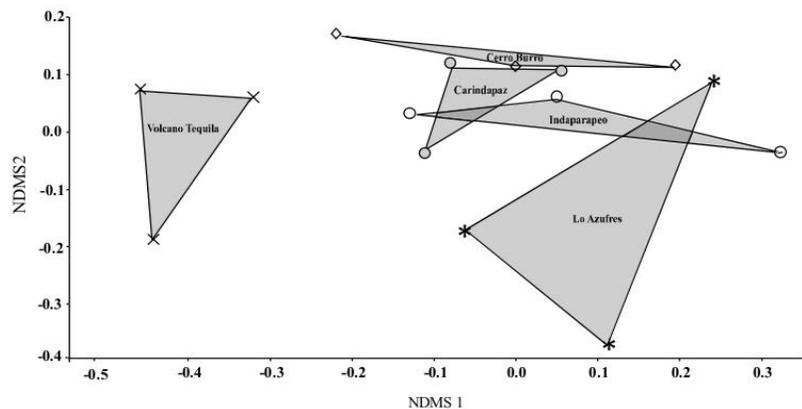
Table 2 Measures of genetic diversity based on SSRs

Study sites					
(a) Nuclear SSRs					
	N_a	N_e	H_o	H_e	F
Tequila Volcano	10.42 (0.36)	5.87 (0.69)	0.55 (0.07)	0.81 (0.01)	0.32 (0.10)
Los Azufres	11.42 (0.97)	6.32 (0.95)	0.51 (0.06)	0.81 (0.03)	0.36 (0.07)
Indaparapeo	10.57 (1.41)	6.07 (0.55)	0.60 (0.04)	0.82 (0.02)	0.27 (0.02)
Carindapaz	12.42 (1.25)	8.07 (1.15)	0.54 (0.07)	0.86 (0.01)	0.36 (0.08)
Cerro Burro	11.57 (2.41)	7.13 (1.70)	0.52 (0.07)	0.81 (0.03)	0.36 (0.07)
(b) Chloroplast SSRs					
	A	P	N_e	R_h	H_e	D^2_sh
Tequila Volcano	1	0	1	0	0	0
Los Azufres	9	6	8	8	0.95	0.97
Indaparapeo	6	4	3.18	3.44	0.71	0.14
Carindapaz	2	1	1.55	1	0.38	0.51
Cerro Burro	3	1	1.21	1.14	0.18	0.14

Table shows (*a*): mean number of different alleles (N_a), mean effective number of alleles (N_e), observed heterozygosity (H_O), expected heterozygosity (H_E), fixation index (F); and chloroplast haplotype diversity (*b*): number of haplotypes (A), number of private haplotypes (P), effective number of haplotypes (N_e); haplotypic richness (R_h), haplotype diversity (H_e), mean genetic distance between individuals (D^2_sh). Standard error in parenthesis

from 0.51 to 0.60) and H_E (ranging from 0.81 to 0.86) values were observed. Positive F values suggest a deficiency of heterozygotes probably related to the presence of null alleles previously observed in this species (Ramos-Ortiz et al. 2015).

According to cpSSRs, we identified 15 different haplotypes in all study sites. Of these, 9 haplotypes were detected in Los Azufres, 6 in Indaparapeo, 3 in Cerro Burro, 2 in Carindapaz and only one haplotype was identified in the Tequila Volcano. Finally, only three haplotypes were shared among the all study sites (Table 2).

Functional foliar traits along diversity gradient of oaks

The ANOVA test showed significant differences in the functional traits among the study sites (Table 3). The functional traits that showed significant differences were LA, LDW, SLA, LD and Chl (Table 3). In the five study sites, the plant functional traits that showed higher CV were LD, SLA, LDW and LA, while LT and Chl showed lower variation (Table 4). Among the five populations, the one from Tequila Volcano showed the higher CV for LD (94.32%), SLA (64.05%), LDW (49.42%) and LT (28.56%). The stepwise canonical discriminant analysis indicated that the variable that contribute more importantly to differentiate populations was LD, followed by LDW, SLA, LA, Chl and LT. The two first canonical discriminant functions allowed highly significant discrimination among populations (Wilks' lambda = 0.13; $P = 0.0001$) and explained 80.26% and 14.04% of the variation, respectively (Fig. 3). In particular, oak trees in the Tequila Volcano were more associated with the values of

Table 3 Comparison of foliar functional traits between study sites according to ANOVA test. Equal letters represent equal means between populations

Response variables	Tequila Volcano	Los Azufres	Indaparapeo	Carindapaz	Cerro Burro
(a) Mean ± SE					
Leaf area	12.26 ± 0.41[(A)]	10.75 ± 0.69[(A)]	22.85 ± 1.05[(B)]	13.04 ± 0.61[(A)]	17.15 ± 1.02[(C)]
Leaf dry weight	0.30 ± 0.01[(A)]	0.16 ± 0.01[(A)]	0.16 ± 0.007[(B)]	0.29 ± 0.09[(A)]	0.27 ± 0.01[(C)]
Foliar thickness	0.60 ± 0.01[(A)]	0.41 ± 0.01[(A)]	0.43 ± 0.05[(A)]	0.52 ± 0.07[(A)]	0.61 ± 0.07[(A)]
Specific leaf area	50.02 ± 3.32[(A)]	73.24 ± 1.75[(B)]	172.21 ± 11.37[(C)]	70.99 ± 1.68[(B)]	66.43 ± 1.56[(A)]
Leaf density	142.72 ± 14.39[(A)]	185.95 ± 6.79[(A)]	545.12 ± 55.45[(B)]	173.33 ± 9.41[(A)]	139.83 ± 6.19[(A)]
Chlorophyll Content	42.34 ± 0.55[(A)]	40.27 ± 0.57[(AB)]	38.77 ± 0.50[(B)]	40.54 ± 0.54[(AB)]	41.92 ± 0.43[(A)]

Source	Response variables	F	df	p	
(b) One-way ANOVA tests between-subjects effects					
Sites	Leaf area	36.1549	4	<0.0001*	
	Leaf dry weight	29.9014	4	<0.0001*	
	Leaf thickness	2.7642	4	<0.0303*	
	Specific leaf area	68.1107	4	<0.0001*	
	Leaf density	38.3666	4	<0.0001*	
	Chlorophyll Content	6.8227	4	<0.0001*	

The units for each functional attribute are: leaf area (LA) (cm^2), leaf dry weight (LDW) (mg), leaf thickness (LT) (mm), specific leaf area (SLA) (cm^2 g^{-1}) and leaf density (LD) (g cm^{-3}), chlorophyll content (SPAD units)

Table 4 Coefficients of variation (CV) of foliar functional traits between study sites

Study sites	LDW	LT	LA	SLA	LD	Chl	DBH
Tequila Volcano	49.42	28.57	38.95	64.05	94.42	7.15	75.36
Los Azufres	45.93	19.22	42.16	28.36	38.32	7.12	47.92
Indaparapeo	46.57	18.47	44.38	61.76	81.83	7.12	36.78
Carindapaz	41.07	24.85	37.87	36.21	72.16	7.20	70.18
Cerro Burro	45.83	26.20	43.32	29.78	40.91	5.45	53.69

Values are given in percentages.

The abbreviations for each trait are the following: *LA* leaf area, *LDW* leaf dry weight, *LT leaf* thickness, *SLA* specific leaf area, *LD* leaf density, chlorophyll content (SPAD units) and diameter at breast height (DBH) (cm)

Fig. 3 Canonical discriminant analysis (CDA) of the functional traits of *Q. laurina* between study sites along the oak diversity gradient. Each point is an individual two-dimensional representation (axis 1 and axis 2) of the individuals of *Q. laurina* present at each study sites, where Tequila Volcano is represented by crosses, Los Azufres by asterisks, Indaparapeo by circles, Carindapaz by gray dots and Cerro Burro by diamonds
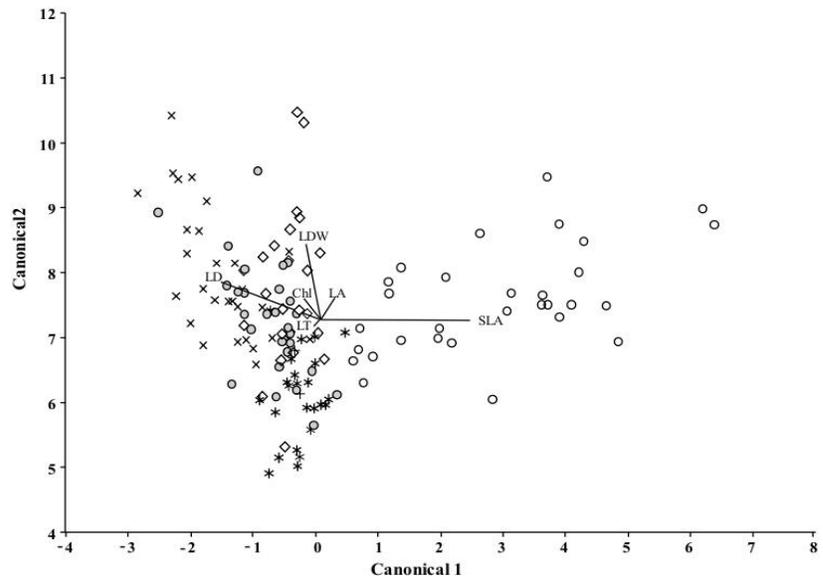
LD, while oak trees in Indaparapeo and Cerro Burro were more associated with the values of SLA and LDW.

Correlations between genetic diversity, foliar functional traits and the structure of the oak community

We found significant relationships between the community structure of oaks and the measures of genetic diversity. Particularly, for the nSSR data set, *Na* and *Ne* showed a significant negative correlation with H'L ($r_S = -0.90$; $P = 0.03$, in both cases) and a positive correlation with DQ ($r_S = 0.90$; $P = 0.03$). For the cpSSRs A, N_e, H_e, and R_h showed a negative correlation with the density of oak individuals belonging to the *Lobatae* section (DL) ($r_S = -0.97$; $P = 0.004$). For the CV of foliar functional traits and the oak community structure, Spearman's correlations showed significant positive relationships between the CV of LDW and H'L ($r_S = 0.90$; $P = 0.03$) and a negative correlation between the CV of LDW and DQ ($r_S = -0.90$; $P = 0.03$). The CV of LDW was also negatively correlated with the measurements of genetic diversity *Na* and *Ne* ($r_S = -1.0$; $P = 0.001$). Similarly, the CV of LT presented a negative correlation with N_e and H_e ($r_S = -0.90$; $P = 0.03$) (Table 5). On the contrary, the CV of SLA and LD were positively correlated with H_O ($r_S = 0.90$; $P = 0.03$) (Table 5).

For the case of climatic variables, we observed positive correlations between the CV of LA with MAT, TWQ and TCQ ($r_S = 0.90$; $P = 0.03$). We only detected a negative

correlation between total density of oak individuals (DS) and TCQ ($r_S = -0.90$; $P = 0.03$) (Table 5). The measures of genetic diversity were not correlated with climatic variables.

Effects of oak community on genetic variation of *Q. laurina*

According to the redundancy analysis, we did not find a significant association between the oak community parameters and the genetic variation of *Q. laurina* along the oak diversity gradient (RDA; $R^2_{adj} = 2.6\%$, $P = 0.28$).

Effects of genetic variation of *Q. laurina* and climate variables on foliar functional traits

The full RDA model that included both climate variables and the genetic variation showed a significant association only between climatic variables and foliar functional traits (RDA; $R^2_{adj} = 75\%$, $P = 0.0001$). Of the total climate variables analyzed ($N = 19$), only four were significantly associated with all foliar functional traits (i.e. mean annual temperature, mean annual precipitation, mean temperature of the warmest quarter and mean temperature of the coldest quarter). The second RDA model (only climate variables) exhibited that the climate variables and foliar functional traits had a significant association along oak diversity gradient (RDA; $R^2_{adj} = 73\%$, $P = 0.0001$). Particularly, the mean annual precipitation (MAP) was significantly associated with leaf thickness (LT) and leaf density (LD) in the Indaparapeo

Table 5 Spearman's correlations between the diversity of oak species in local communities, coefficient of variation (CV) of functional traits, genetic diversity (nuclear and chloroplast) and selected climatic variables

|	Index of community Oak structure				Foliar functional traits coefficient of variation						Measures of genetic diversity (nSSRs and cpSSRs)							Climate variables		
	H'_L	D_Q	D_S	D_L	LDW	LA	SLA	DBH	LD	LT	N_a	N_e	H_o	A	N_e	H_e	R_h	ATM	TWQ	TCQ
H'_L	–																			
D_Q	−0.80	–																		
D_S	0.70	−0.60	–																	
D_L	0.34	−0.05	0.35	–																
LDW	0.90*	−0.90*	0.30	0.15	–															
LA	0.10	−0.10	−0.70	−0.46	−0.50	–														
SLA	0.70	−0.30	0.10	0.66	−0.30	−0.10	–													
DBH	0.10	−0.10	0.70	0.87	–	−0.80	0.40	–												
LD	0.10	−0.20	0.60	0.87	0.10	−0.10	−0.10	0.40	–											
LT	0.70	−0.30	0.10	0.66	0.60	−0.50	0.30	0.90*	0.30	–										
N_a	−0.90*	0.90*	−0.30	−0.15	−1.0*	−0.30	−0.60	–	−0.60	−0.10	–									
N_e	−0.90*	0.90*	−0.30	−0.15	−1.0*	−0.30	−0.60	–	−0.60	−0.10	0.50	–								
H_o	0.60	−0.10	−0.30	0.35	0.50	0.20	0.90*	–	0.90*	−0.10	−0.50	0.50	–							
A	−0.20	−0.15	−0.40	−0.97*	−0.10	0.60	−0.70	−0.90*	−0.70	−0.80	0.10	0.10	0.10	–						
N_e	−0.10	−0.20	−0.30	−0.97*	−0.20	0.30	−0.60	−0.80	−0.60	−0.90*	0.20	0.20	0.30	–	–					
H_e	−0.10	0.10	−0.40	−0.97*	−0.20	0.30	−0.60	−0.80	−0.60	−0.90*	0.20	0.20	0.30	–	–	–				
R_h	−0.20	0.10	−0.30	−0.97*	−0.10	0.60	−0.70	−0.90*	−0.70	−0.80	0.10	0.10	0.40	–	–	–	–			
ATM	−0.44	−0.10	−0.70	−0.46	−0.50	1.0*	−0.10	−0.80	0.30	−0.50	−0.30	0.30	0.20	0.60	0.30	0.30	0.60	–		
TWQ	−0.10	−0.10	−0.70	−0.10	−0.50	0.90*	−0.10	−0.80	−0.10	−0.50	−0.30	0.30	0.20	0.60	0.30	0.30	0.60	–	–	
TCQ	−0.30	−0.10	−0.90*	−0.61	−0.10	0.90*	−0.30	−0.90*	−0.30	−0.70	0.10	0.10	0.10	0.70	0.50	0.50	0.70	0.90	0.90	–

Index of community structure: Shannon diversity index of the *Lobatae* section (H'_L), density of individuals in the *Quercus* section (D_Q), density of all oak species individuals (D_S) and density of individuals in the *Lobatae* section (D_L). Genetic measures: Number of different alleles (N_a), Effective number of alleles (N_e); Shannon index (I), observed heterozygosity (H_o); Shannon index (I); Number of haplotypes detected in each population (A), Effective number of haplotypes (N_e); Haplotypic richness (R_h) and Haplotype diversity (H_e). The units for each functional trait are: leaf area (LA) (cm²), leaf dry weight (LDW) (mg), leaf thickness (LT) (mm), specific leaf area (SLA) (cm² g⁻¹) and leaf density (LD) (g cm⁻³). Climate variables: mean average temperature (AMT), mean temperature of warmest quarter (TWQ) and mean temperature of coldest quarter (TCQ)

*Significant values $p < 0.05$ and the non-correlations are shown in (—)

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site. For Tequila Volcano, mean annual temperature (MAT) and mean temperature of the warmest quarter (TWQ) were significantly associated with leaf dry weight (LDW) and chlorophyll content. Mean temperature of the coldest quarter (TCQ) was significantly associated with specific leaf area (SLA) and leaf area (LA) in Carindapaz, Cerro Burro and Los Azufres (Fig. 4a). Finally, the third RDA model (only the genetic diversity) did not detect a significant association between the genetic diversity and foliar functional traits of

Q. laurina along oak diversity gradient (RDA; $R^2_{adj}=0.28\%$, $P=0.12$) (Fig. 4b).

Fig. 4 Redundancy analysis (RDA) of genetic diversity, climatic variables on foliar functional traits of *Q. laurina* between study sites along the oak diversity gradient. **a** Biplot showing the result of RDA for genetic variation and foliar functional traits. **b** Biplot showing the result of RDA for climatic variables and foliar functional traits. Tequila Volcano is represented by crosses, Los Azufres by asterisks, Indaparapeo by circles, Carindapaz by gray dots and Cerro Burro by diamonds
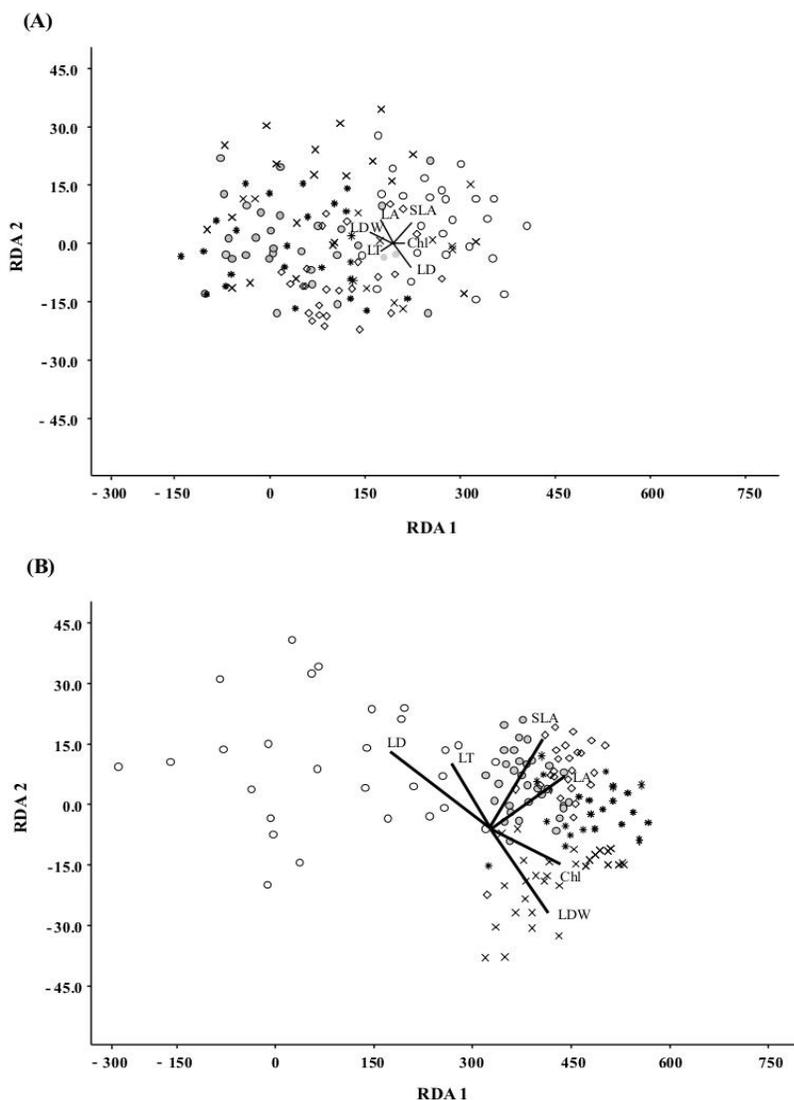
Discussion

Relationship between the oak community structure and the genetic diversity of *Q. laurina*

Oaks represent one of the groups of woody plants with the highest degree of richness and abundance in the Northern Hemisphere (Cavender-Bares 2016). In our study, we observed oak communities with species richness ranging from two to four oak species coexisting at the stand level. In terms of the number of species in the *Lobatae* section, we observed the coexistence of *Q. laurina* with either one or two other red oaks (*Q. crassifolia* and *Q. calophylla*). In addition, in all study sites, the individual trees belonging to the *Lobatae* section were dominant in relation to the abundance of the individuals of section *Quercus*, except in Los Azufres site, where a high number of individuals of *Q. rugosa* were observed. In particular, *Q. laurina* was the dominant species; representing on average 88% of the individuals present in the study sites. Occasional hybridization between *Q. laurina* and *Q. crassifolia* has been previously described (Valencia 1994). On the contrary, we are not aware of reports of morphological indications of introgression between *Q. calophylla* and the other two species.

We found high levels of genetic diversity for *Q. laurina* in all study sites. The number of alleles per locus (N_a) and the number of effectives alleles per locus (N_e) ranged from 10.42 to 12.42 and from 5.87 to 8.07, respectively. These values are similar and even higher than those reported by Ramos-Ortiz et al. (2015) for a hybridization zone between *Q. laurina* x *Q. affinis*. In the case of heterozygosity levels, we found high H_O (ranging from 0.51 to 0.60) and H_E values (ranging from 0.81 to 0.86). Particularly, the Carindapaz site was the one with the highest levels of genetic diversity. In contrast, the site with the greatest richness and abundance of oak species (Tequila Volcano) was the site that presented the lowest levels of genetic diversity. These results do not support our hypothesis that predicts higher levels of genetic diversity of *Q. laurina* in sites with greater occurrence of oak species from the same section. This fact suggests that in the Tequila Volcano, hybridization and introgression processes are not happening. It is possible that climate factors could be the causes of the low genetic diversity detected in *Q. laurina* in the Tequila Volcano due these factors can potentially affect the reproductive phenological patterns generating interspecific reproductive asynchrony (Zeng et al. 2011; Kim et al. 2018). For example, Soularue and Kremer (2014) found that climatic variables directly affected the reproductive phenological patterns in *Quercus petrea* along an altitudinal gradient, causing a temporary asynchrony in flower production, which in turn, reduced the genetic diversity.

An alternative explanation of this result is the fact that in this study site, the relative abundance of each oak species was very low in comparison with the abundance of *Q. laurina*. Some studies have shown that the differences in the local abundance or occurrence frequency of each oak species and local environmental conditions may determine hybridization rates between different taxa and even reduce hybridization (Lepais et al. 2009).

In the case of the cpDNA, we identified in total 15 different haplotypes and 12 private haplotypes. Only three haplotypes were present in the five study sites. In the Azufres site, two other red oak species occurred together with *Q. laurina*, and we found higher haplotype diversity in this species, with nine haplotypes in total and six private haplotypes. In contrast, the population with less haplotypes was the Tequila Volcano with only one, which was shared with other populations. These results might be suggesting that cpDNA haplotype diversity could be influenced by the number of sympatric red oak species occurring with *Q. laurina*, since it has been shown that the introgression of cpDNA occurs frequently in oaks, even without any sign of introgression at morphological or nuclear markers (Dumolin-Lapegue et al. 1999; Belahbib et al. 2001; Ramos-Ortiz et al. 2015).

Functional foliar traits and genetic diversity of *Q. laurina*

The adjustment of functional foliar traits of plants allows them to achieve greater reproductive success (Sandquist and Ehleringer 1997; Violle, 2014). It has been documented that functional traits such as specific leaf area, leaf thickness, stomatal density and pigment content, can vary in space (Körner and Cochrane 1985; Reich et al. 1997; Filella and Peñuelas 1999), and over time (Ehleringer 1982; Abrams et al. 1994; Wagner 2004) as result of changes in environmental conditions (e.g. temperature, precipitation, incidence of light and humidity, and genetic factors (e.g. gene flow, hybridization and introgression (Violle et al. 2007). In our study, we found significant differences in functional traits among sites. The traits that showed significant differences among the sites were LA, LDW, SLA, LD and Chl. The coefficient of variation (CV) of some of these functional foliar traits, such as SLA, LD, LA, LT and LDW showed higher levels in sites with more oak species richness. In particular, specific leaf area (SLA), leaf area (LA) and leaf density (LD) are part of the leaf economic spectrum (LES) traits associated with leaf lifespan, resource acquisition, and nutrient use (Wright et al. 2004; Reich, 2014). This agrees with some studies that have shown that plant communities with high diversity have a greater functional diversity or greater coefficients of variation in some functional traits, which in turn, favors the access of more resources for plants (i.e. nutrients,

water, pollinators and symbiotic interactions) (Tilman 1999; Maherali and Klironomos 2007; Fornara and Tilman 2008). Therefore, the differences between plants in functional traits and/or the presence of higher coefficients of variation represent the way in which they acquire, process and invest their resources affecting the composition, structure, diversity and functioning of ecosystems (Loreau et al. 2001; Díaz et al. 2004). Our findings agree with this idea and suggest that an increase in functional diversity of *Q. laurina* (i.e. specific leaf area, leaf area and leaf density) is associated with oak species richness.

For the case of climatic variables, we found that the variation of leaf area had a significant relationship with TMA, TWQ and TCQ. We only detected a negative relationship between oak individuals' density (DS) and the mean temperature of coldest quarter (TCQ). This indicated that the variation in temperature can be an important abiotic factor that affects leaf area suggesting the presence of phenotypic plasticity in *Q. laurina*. It has been proposed that brevideciduous oaks such as *Q. laurina* are characterized by having small leaves with low specific leaf area to avoid water loss and increase the photoprotection against high temperatures and reduce the drought stress in the summer months (Cavenderes-Bares et al. 2018). Our results in accord with this idea because along of environmental gradient, leaves of *Q. laurina* had a great variation associated with differences in temperature between study sites, and in sites with higher temperature the individuals of *Q. laurina* presented smaller leaves. Finally, the RDA analysis showed a significant association of the foliar functional traits with climatic variables along the oak diversity gradient. This is accord with Ramirez-Valiente et al. (2009) that detected a relationship between foliar functional traits and water availability and temperature among populations of *Quercus suber* along a climatic gradient.

Our findings support the idea that the variation of foliar functional traits of *Q. laurina* could be more related to both environmental (i.e. temperature and precipitation) and ecological factors (community structure of oak species). Additionally, our results do not support the hypothesis that predicts higher levels of genetic diversity of *Q. laurina* in communities with greater occurrence of oak species from the same section. This fact could be related to the differences detected in the climate variables along the oak diversity gradient that potentially reduce the genetic exchange between oak species, being more evident in the Tequila Volcano. We highlight the need to consider environmental and ecological variables linkages as a regulatory mechanism of the phenotypic plasticity expressed in changes of foliar functional attributes of oaks that can affect individual tree performance and demographic traits of populations.

References

Abrams MD, Kubiske ME, Mostoller SA (1994) Relating wet and dry year ecophysiology to leaf structure in contrasting temperate tree species. Ecol 75:123–133. https://doi.org/10.2307/1939389

Albert CH, Grassein F, Schurr FM, Vieilledent G, Violle C (2011) When and how should intraspecific variability be considered in trait-based plant ecology? Perspect Plant Ecol Syst 13:217–225. https://doi.org/10.1016/j.ppees.2011.04.003

Alberto FJ, Derory J, Boury C, Frigerio JM, Zimmermann NE, Kremer A (2013) Imprints of natural selection along environmental gradients in phenology related genes of *Quercus petraea*. Genetics 195:495–512. https://doi.org/10.1534/genetics.113.153783

Aldrich PR, Michler CH, Sun W, Romero-Severson J (2002) Microsatellite markers for northern red oak (Fagaceae: *Quercus rubra*). Mol Ecol 2:472–474. https://doi.org/10.1046/j.1471-8286.2002.00282.x

Aldrich PR, Cavender-Bares J (2011) Quercus. In: Kole C (ed) Wild crop relatives: genomic and breeding resources. Springer, Berlin, pp 89–129. https://doi.org/10.1007/978-3-642-21250-5_6

Arenas-Navarro M, García-Oliva F, Terrazas T, Torres-Miranda A, Oyama K (2020a) Leaf habit and stem hydraulic traits determine functional segregation of multiple oak species along a water availability gradient. Forests 11:894. https://doi.org/10.3390/f11080894

Arenas-Navarro M, García-Oliva F, Torres-Miranda A, Téllez-Valdés O, Oyama K (2020b) Environmental filters determine the distribution of tree species in a threatened biodiversity hotspot in western Mexico. Bot Sci 98:219–237. https://doi.org/10.17129/botsci.2398

Arizaga S, Martínez J, Salcedo M y Bello M (2009) Manual de la biodiversidad de encinos michoacanos. Instituto Nacional de Ecología ed, México

Arnold ML (1997) Natural hybridization and evolution. Oxford University Press, New York, USA

Arnold ML (2006) Evolution through genetic exchange. Oxford University Press, New York, USA

Belahbib N, Pemonge M-H, Ouassou A, Sbay H, Kremer A, Petit RJ (2001) Frequent cytoplasmic exchanges between oak species that are not closely related: *Quercus suber* and *Q. ilex* in Morocco. Mol Ecol 10:2003–2012. https://doi.org/10.1046/j.0962-1083.2001.01330.x

Burge DO, Parker VT, Mulligan M, Sork VL (2019) Influence of a climatic gradient on genetic exchange between two oak species. Am J Bot 106:864–878. https://doi.org/10.1002/ajb2.1315

Caseys C, Stritt C, Glauser G, Blanchard T, Lexer C (2015) Effects of hybridization and evolutionary constraints on secondary metabolites: the genetic architecture of phenylpropanoids in European *Populus* species. PLoS ONE 10:128–200. https://doi.org/10.1371/journal.pone.0128200

Cavender-Bares J (2016) Diversity, distribution and ecosystem services of the North American oaks. Int Oaks 27:37–49

Cavender-Bares J, Kothari S, Meireles JE, Kaproth MA, Manos PS, Hipp AL (2018) The role of diversification in community assembly of the oaks (*Quercus L.*) across the continental US. Am J Bot 105:565–586. https://doi.org/10.1002/ajb2.1049

Cornelissen JHC et al (2003) A handbook of protocols for standardized and easy measurement of plant functional traits worldwide. Aust J Bot 51:335–380. https://doi.org/10.1071/BT02124

Curtu AL, Gailing O, Finkeldey R (2007a) Evidence for hybridization and introgression within a species-rich oak (*Quercus* spp.) community. Evol Biol 7:1–15. https://doi.org/10.1186/1471-2148-7-218

Curtu AL, Gailing O, Leinemann L, Finkeldey R (2007b) Genetic variation and differentiation within a natural community of five

oak species (*Quercus* spp.). Plant Biol 9:116–126. https://doi.org/10.1186/1471-2148-7-218

Curtu AL, Gailing O, Leinemann L, Finkeldey R (2007c) Genetic variation, differentiation within a natural community of five oak species (*Quercus* spp.). Plant Biol 9:116–126. https://doi.org/10.1055/s-2006-924542

Deguilloux MF, Dumolin-Lapègue S, Gielly L, Grivet D, Petit RJ (2003) A set of primers for the amplification of chloroplast microsatellites in *Quercus*. Mol Ecol Notes 3:24–27. https://doi.org/10.1046/j.1471-8286.2003.00339.x

Diaz S et al (2004) The plant traits that drive ecosystems: evidence from three continents. J Veg Sci 15:295–304. https://doi.org/10.1111/j.1654-1103.2004.tb02266.x

Dumolin-Lapegue S, Kremer A, Petit RJ (1999) Are chloroplast and mitochondrial DNA variation species independent in oaks? Evolution 53:1406–1413. https://doi.org/10.2307/2640887

Ehleringer J (1982) The influence of water stress and temperature on leaf pubescence development in *Encelia farinosa*. Am J Bot 69:670–675. https://doi.org/10.2307/2442956

Ehleringer JR, Smedley MP (1989) Stomatal sensitivity and water-use efficiency in oaks and their hybrids. In: Wallace A, McArthur ED, Haferkamp MR (eds): In: Symposium on shrub ecophysiology and biotechnology. USDA Forest Service Gen Tech Rep INT-256, USDA, Washington, pp 98–102

Eliades N-G, Eliades DG (2009) Haplotype analysis: Software for analysis of haplotypes data. Forest genetics and forest tree breeding. Georg-August University Göttingen, Göttingen

Filella I, Peñuelas J (1999) Altitudinal differences in UV absorbance, UV reflectance and related morphological traits of *Quercus ilex* and *Rhododendron ferrugineum* in the Mediterranean region. Plant Ecol 145:157–165

Fornara DA, Tilman D (2008) Plant functional composition influences rates of soil carbon and nitrogen accumulation. J Ecol 96:314–322. https://doi.org/10.1111/j.1365-2745.2007.01345.x

Gailing O, Lind J, Lilleskov E (2012) Leaf morphological and genetic differentiation between Quercus rubra L. and Q. ellipsoidalis E.J. Hill populations in contrasting environments. Plant Syst Evol 298(8):1533–1545. https://doi.org/10.1007/s00606-012-0656-y

González-Rodríguez A, Arias DM, Valencia S, Oyama K (2004) Morphological and RAPD analysis of hybridization between *Quercus laurina* and *Quercus affinis* (Fagaceae), two Mexican red oaks. Am J Bot 91:401–409. https://doi.org/10.3732/ajb.91.3.401

González-Rodríguez A, Arías DM, Oyama K (2005) Genetic variation of populations within the *Quercus affinis–Quercus laurina* (Fagaceae) complex analyzed with RAPD markers. Can J Bot 83:155–162. https://doi.org/10.1139/b04-162

González-Rodríguez A, Ken O (2005) Leaf morphometric variation in Quercus affinis and Q. laurina (Fagaceae), two hybridizing Mexican red oaks. Bot J Linn Soc 147(4):427–435. https://doi.org/10.1111/j.1095-8339.2004.00394.x

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: Paleontological statistics software package for education and data analysis. Paleontol electron, 4:1–9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm.

Harrison RG, Larson EL (2014) Hybridization, introgression, and the nature of species boundaries. J Hered 105:795–809. https://doi.org/10.1093/jhered/esu033

Himrane H (2002) Etude de l'hétérogeneité de la progéniture de l'hybride *Quercus subpyrenaica*. E.H. del Villar. CIHEAM, Saragosa

Himrane H, Camarero JJ, Gil-Pelegrín E (2004) Morphological and ecophysiological variation of the hybrid oak *Quercus subpyrenaica* (*Q. faginea x Q. pubescens*). Trees 18:566–575. https://doi.org/10.1007/s00468-004-0340-0

Hipp AL, Weber JA (2008) Taxonomy of Hill's oak (*Quercus ellipsoidalis*: Fagaceae): evidence from AFLP data. Syst Bot 33:148–158

Hipp AL, Manos PS, González-Rodríguez A, Hahn M, Kaproth M, McVay JD, Avalos SV, Cavender-Bares J (2018) Sympatric parallel diversification of major oak clades in the Americas and the origins of Mexican species diversity. New Phytol 217:439–452. https://doi.org/10.1111/nph.14773

Hipp Andrew L et al (2020) Genomic landscape of the global oak phylogeny. New Phytol 316(5832):1746–1748. https://doi.org/10.1111/nph.16162

Jung V, Violle C, Mondy C, Hoffmann L, Muller S (2010) Intraspecific variability and trait-based community assembly. J Ecol 98(5):1134–1140. https://doi.org/10.1111/j.1365-2745.2010.01687.x

Kim BY, Wei X, Fitz-Gibbon S, Lohmueller KE, Ortego J, Gugger PF, Sork VL (2018) RADseq data reveal ancient, but not pervasive, introgression between Californian tree and scrub oak species (*Quercus* sect. Quercus: Fagaceae). Mol Ecol 22:4556–4571. https://doi.org/10.1111/mec.14869

Körner C, Cochrane P (1985) Stomatal responses and water relations of *Eucalyptus pauciflora* in summer along an elevational gradient. Oecologia 66:443–455. https://doi.org/10.1007/BF00378313

Lagache L, Klein EK, Guichoux E, Petit R (2013) Fine-scale environmental control of hybridization in oaks. Mol Ecol 22:423–436. https://doi.org/10.1111/mec.12121

Lavorel S, Díaz S, Cornelissen JHC, Garnier E, Harrison SP, McIntyre S, Urcelay C (2007) Plant functional types: are we getting any closer to the Holy Grail? Terrestrial ecosystems in a changing world. Springer, Heidelberg, pp 149–164

Lefort F, Douglas GC (1999) An efficient micro-method of DNA isolation from mature leaves of four hardwood tree species *Acer, Fraxinus Prunus and Quercus*. Ann for Sci 56(3):259–263. https://doi.org/10.1051/forest:19990308

Lepais O, Petit RJ, Guichoux E, Lavabre JE, Alberto F, Kremer A, Gerber S (2009) Species relative abundance and direction of introgression in oaks. Mol Ecol 18:2228–2242. https://doi.org/10.1111/j.1365-294X.2009.04137.x

Loreau M, Naeem S, Inchausti P, Bengtsson J, Grime JP, Hector A, Hooper DU, Huston MA, Raffaelli D, Schmid B, Tilman D, Wardle DA (2001) Biodiversity and ecosystem functioning: current knowledge and future challenges. Science 294:804–808. https://doi.org/10.1126/science.1064088

Maherali H, Klironomos JN (2007) Influence of phylogeny on fungal community assembly and ecosystem functioning. Science 316:1746–1748. https://doi.org/10.1126/science.1143082

Mallet J (2005) Hybridization as an invasion of the genome. Trends Ecol Evol 20:229–237. https://doi.org/10.1016/j.tree.2005.02.010

McCauley RA, Cortés-Palomec AC, Oyama K (2019) Species diversification in a lineage of Mexican red oak (*Quercus* section *Lobatae* subsection *Racemiflorae*) he interplay between distance, habitat, and hybridization. Tree Genet Genomes 15:2–27. https://doi.org/10.1007/s11295-019-1333-x

McIntyre S, Lavorel S, Landsberg J, Forbes TDA (1999) Disturbance response in vegetation–towards a global perspective on functional traits. J Veg Sci 10(5):621–630. https://doi.org/10.2307/3237077

Nixon KC (1993) The genus *Quercus* in Mexico. In: Ramammoorthy TP, Bye R, Lot A, Fa J (eds) Biological diversity of Mexico: origins and distribution. Oxford University Press, USA

Ortego J, Gugger PF, Sork VL (2015) Climatically stable landscapes predict patterns of genetic structure and admixture in the Californian canyon live oak. J Biogeogr 42(2):328–338. https://doi.org/10.1111/jbi.12419

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537–2539. https://doi.org/10.1111/j.1471-8286.2005.01155.x

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Petit RJ, Excoffier L (2009) Gene flow and species delimitation. Trends Ecol Evol 24(7):386–393. https://doi.org/10.1016/j.tree.2009.02.011

Ramírez-Valiente JA, Lorenzo Z, Soto A, Valladares F, Gil L, Aranda I (2009) Elucidating the role of genetic drift and natural selection in cork oak differentiation regarding drought tolerance. Mol Ecol 18:3803–3815. https://doi.org/10.1111/j.1365-294X.2009.04317.x

Ramos-Ortiz S, Oyama K, Rodríguez-Correa H, González-Rodríguez A (2015) Geographic structure of genetic and phenotypic variation in the hybrid zone between Quercus affinis and Q. laurina in Mexico. Plant Species Biol 31:219–232. https://doi.org/10.1111/1442-1984.12109

Reich PB, Walters MB, Ellsworth DS (1997) From tropics to tundra: global convergence in plant functioning. Proc Natl Acad Sci 94:13730–13734. https://doi.org/10.1073/pnas.94.25.13730

Reich PB (2014) The world-wide 'fast-slow' plant economics spectrum: a traits manifesto. J Ecol 102:275–301. https://doi.org/10.1111/1365-2745.12211

Rieseberg LH, Ellstrand NC, Arnold M (1993) What can molecular and morphological markers tell us about plant hybridization? Crit Rev Plant Sci 12(3):213–241. https://doi.org/10.1080/07352689309701902

Rieseberg LH, Archer MA, Wayne RK (1999) Transgressive segregation, adaptation and speciation. Heredity 83(4):363–372. https://doi.org/10.1038/sj.hdy.6886170

Riordan EC, Gugger PF, Ortego J, Smith C, Gaddis K, Thompson P, Sork VL (2016) Association of genetic and phenotypic variability with geography and climate in three southern California oaks. Am J Bot 103(1):73–85. https://doi.org/10.3732/ajb.1500135

Rodríguez-Correa H, Oyama K, MacGregor-Fors I, González-Rodríguez A (2015) How are oaks distributed in the neotropics? A perspective from species turnover, areas of endemism, and climatic niches. Int J Plant Sci 176:222–231. https://doi.org/10.1086/679904

Salvini D, Bruschi P, Fineschi S, Grossoni P, Kjaer ED, Vendramin GG (2009) Natural hybridization between Quercus petraea (Matt.) Liebl. and Quercus pubescens (Willd.) within an Italian stand as revealed by microsatellite fingerprinting. Plant Biol 11(5):758–765. https://doi.org/10.1111/j.1438-8677.2008.00158.x

Sandquist DR, Ehleringer JR (1997) Intraspecific variation of leaf pubescence and drought response in *Encelia farinosa* associated with contrasting desert environments. New Phytol 135:635–644. https://doi.org/10.1046/j.1469-8137.1997.00697.x

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9(7):671–675. https://doi.org/10.1038/nmeth.2089

Schwarzbach AE, Donovan LA, Rieseberg LH (2001) Transgressive character expression in a hybrid sunflower species. Am J Bot 88(2):270–277. https://doi.org/10.2307/2657018

Sebastiani F, Carnevale C, Vendramin GG (2004) A set of mono- and dinucleotide chloroplast microsatellites in Fagaceae. Mol Ecol Notes 4:259–261. https://doi.org/10.1111/j.1471-8286.2004.00635.x

Soltis PS, Soltis DE (2009) The role of hybridization in plant speciation. Annu Rev Plant Biol 60:561–588. https://doi.org/10.1146/annurev.arplant.043008.092039

Soularue JP, Kremer A (2014) Evolutionary responses of tree phenology to the combined effects of assortative mating, gene flow

and divergent selection. Heredity 113:485–494. https://doi.org/10.1038/hdy.2014.51

Stokes ME, Davis CS, Koch GG (2000) Categorical data analysis using the SAS system, 2nd edn. SAS, Cary

Tilman D (1999) The ecological consequences of changes in biodiversity: a search for general principles. Ecology 80:1455–1474. https://doi.org/10.1890/0012-9658

Torres-Miranda A, Luna-Vega I, Oyama K (2013) New approaches to the biogeography and areas of endemism of red oaks (Quercus L., Section Lobatae). Syst Biol 62(4):555–573. https://doi.org/10.1093/sysbio/syt021

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

Valbuena-Carabaña M, González-Martínez SC, Hardy OJ, Gil L (2007) Fine-scale spatial genetic structure in mixed oak stands with different levels of hybridization. Mol Ecol 16:1207–1219. https://doi.org/10.1111/j.1365-294X.2007.03231.x

Valencia S (1994) Contribución a la delimitación taxonómica de tres especies del género *Quercus* subgénero *Erythrobalanus*: *Q. laurina* Humboldt et Bonpland, *Q. affinis* Scheidweiler y *Q. ghiesbreghtii* Martens et Galeotti. MsC Dissertation. Universidad Nacional Autónoma de México

Valencia S (2004) Diversidad del género *Quercus* en México. Bol Soc Bot Méx 75:33–53. https://doi.org/10.17129/botsci.1692

Valencia-Cuevas L, Mussali-Galante P, Piñero D, Castillo-Mendoza E, Rangel-Altamirano G, Tovar-Sánchez E (2015) Hybridization of *Quercus castanea* (Fagaceae) across a red oak species gradient in Mexico. Plant Syst Evol 301:1085–1097. https://doi.org/10.1007/s00606-014-1151-4

Valencia-Cuevas L, Piñero D, Mussali-Galante P, Valencia-Ávalos S, Tovar-Sánchez E (2014) Effect of a red oak species gradient on genetic structure and diversity of *Quercus castanea* (Fagaceae) in Mexico. Tree Genet Genomes 10:641–652. https://doi.org/10.1007/s11295-014-0710-8

Violle C, Navas ML, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E (2007) Let the concept of trait be functional. Oikos 116:882–892. https://doi.org/10.1111/j.2007.0030-1299.15559.x

Violle C et al (2014) The emergence and promise of functional biogeography. Proc Natl Acad Sci 111:13690–13696. https://doi.org/10.1073/pnas.1415442111

Viscosi V, Antonecchia G, Lepais O, Fortini P, Gerber S, Loy A (2012) Leaf shape and size differentiation in white oaks: assessment of allometric relationships among three sympatric species and their hybrids. Int J Plant Sci 173(8):875–884. https://doi.org/10.1086/667234

Wagner D et al (2004) The genetic basis of singlet oxygen-induced stress responses of *Arabidopsis thaliana*. Science 306:1183–1185

Wellstein C, Chelli S, Campetella G, Bartha S, Galiè M, Spada F, Canullo R (2013) Intraspecific phenotypic variability of plant functional traits in contrasting mountain grasslands habitats. Biodivers Conserv 22(10):2353–2374. https://doi.org/10.1007/s10531-013-0484-6

Westfall RD, Conkle MT (1992) Allozyme markers in breeding zone designation. New for 6:279–309. https://doi.org/10.1007/978-94-011-2815-5_15

Whitham TG, Gehring CA, Lamit LJ, Wojtowicz T, Evans LM, Keith AR, Smith DS (2012) Community specificity: life and afterlife

effects of genes. Trends Plant Sci 17(5):271–281. https://doi.org/10.1016/j.tplants.2012.01.005

Williams DG, Ehleringer JR (2000) Carbon isotope discrimination and water relations of oak hybrid populations in southwestern Utah. Am Nat 60:121–129. https://doi.org/10.1007/s00442-003-1437-7

Williams JH, Boecklen WJ, Howard DJ (2001) Reproductive processes in two oak (*Quercus*) contact zones with different levels of hybridization. J Hered 87:680–690. https://doi.org/10.1046/j.1365-2540.2001.00968.x

Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F et al (2004) The worldwide leaf economics spectrum. Nature 428:821–827. https://doi.org/10.1038/nature12872

Zeng YF, Liao WJ, Petit RJ, Zhang DY (2011) Geographic variation in the structure of oak hybrid zones provides insights into the dynamics of speciation. Mol Ecol 20:4995–5011. https://doi.org/10.1111/j.1365-294X.2011.053x

CAPÍTULO II.

Canopy arthropod diversity associated with *Quercus laurina*: importance of an oak species diversity gradient on abundance, species richness and guild composition

Marcela Sofía Vaca-Sánchez, Yurixhi Maldonado-López, Antonio González-Rodríguez, Ken Oyama, G. Wilson Fernandes, Marcílio Fagundes, Edmundo Carlos López-Barbosa, Joan Sebastian Aguilar-Peralta and Pablo Cuevas-Reyes

Presentación

En este segundo capítulo, evaluamos los cambios en la estructura y composición de la comunidad de artrópodos del dosel asociados a *Q. laurina* a lo largo del gradiente de riqueza de especies de encinos. Los objetivos para este capítulo fueron: i) Determinar si se incrementa la diversidad de artrópodos del dosel asociados a *Q. laurina* en las comunidades con mayor diversidad de especies de encinos, y ii) Evaluar los cambios de la estructura y composición de los gremios de artrópodos a lo largo del gradiente de riqueza de encinos. Encontramos que los sitios con mayor diversidad de encinos presentaron la mayor riqueza, abundancia, así como diferencias en la composición de la comunidad de artrópodos, en comparación con los sitios de menor diversidad de encinos. Los resultados de este estudio son consistentes con la premisa de que, para los bosques templados, las comunidades de árboles representan un elemento clave para dar forma a la estructura física ambiental; razón por la que se ha sugerido que la diversidad de especies de árboles podría influir en un aumento en la riqueza y abundancia de artrópodos del dosel.

ORIGINAL PAPER

Canopy arthropod diversity associated with *Quercus laurina*: importance of an oak species diversity gradient on abundance, species richness and guild composition

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Abstract

Central-west and south of Mexico are particularly rich in oak species, coexisting from two to six species and generating gradients of oak diversity that could potentially affect community structure of canopy arthropods. We evaluated changes in canopy arthropod diversity of the *Quercus laurina* along a gradient of oak diversity in the central-west portion of the Trans-Mexican Volcanic Belt. Five study sites that represent an oak diversity gradient and, where *Q. laurina* is also widespread were selected. At each site, five mature trees of *Q. laurina* were randomly selected to collect canopy arthropods using fogging techniques. We assessed the effects of local climate variables on the oak community and the canopy arthropod diversity. We collected 7479 arthropods representing 1154 morphospecies grouped into 15 different orders. Differences in arthropod diversity, abundance and guild composition between oak communities were detected. The general pattern showed a highest richness and abundance of arthropods in Tequila volcano, which represented the site with the greatest diversity of oaks. A significant positive relationship between arthropod abundance and oak species diversity was found. Arthropod guild composition differed along the gradient of oak diversity. Local environmental variables were related with oak community, but did not explained canopy arthropod diversity. At the local scale, plant species richness is the main factor that determines the canopy arthropod abundance and richness of *Q. laurina*, affecting the structure and composition of arthropod guilds. We highlight the importance of conserving oak species because they represent key elements of temperate forest that harbor high arthropod diversity.

Implications for insect conservation Our findings confirm that at the local scale, plant species richness is the main factor that determines the arthropod diversity associated with the canopy of *Quercus laurina*, affecting trophic levels and the composition of arthropod guilds. Therefore, the oak species diversity in temperate forests is a key factor to harbor and preserving the diversity of canopy arthropods in temperate ecosystems.

Keywords Biotic interactions · Canopy arthropods · Oak diversity · Bottom-up effects · Top-down effects

Introduction

Tree canopies perhaps maintain the largest portion of arthropod diversity, which in total with terrestrial arthropods vary from 5 to 12 million species (Stork et al. 2008; Hamilton et al. 2013), of which 20 to 25% are canopy exclusive (Stork 2018). The canopy habitat provides food resources, shelter and sites for reproduction, oviposition and development for diverse arthropod guilds, such as herbivores (including chewing, sucking, gall-inducing and leaf-mining groups), predators, parasitoids and detritivores, among others (Ulyshen 2011; Maldonado-López et al. 2018).

Different factors could be associated with arthropod diversity on tree canopies. Environmental factors such as temperature, humidity, light incidence and wind speed, as well as aspects of the structure and composition of plant communities, including species richness, plant density and age, have been proposed as drivers of canopy arthropod diversity at different spatial scales (Tal et al. 2008; Ulyshen

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2011). Additionally, the physical structure of tree canopies, comprising forest height, vertical foliage complexity and the abundance of tree cavities and other structural aspects that may enhance habitat heterogeneity, has been proposed as one of the main factors that explain arthropod diversity (Basset et al. 2003; Ulyshen 2011).

At the local scale, plant diversity is a determinant factor that influences canopy arthropod diversity (Moreira et al. 2016). For example, the diversity of herbivorous insects increases from communities with few plant species to more diverse communities (Cuevas-Reyes et al. 2004; Ulyshen et al. 2011; Hertzog 2017). This pattern is due to the fact that a more diverse plant community represents a higher number of resources and niches to be used and colonized by herbivorous arthropod species (Cuevas-Reyes et al. 2004; Schmidl et al. 2008). Similarly, a greater diversity of resources provided by a high herbivore diversity present in highly diverse plant communities increases the diversity of predators and other trophic levels, affecting the whole community structure of arthropods (Randlkofer et al. 2010; Haddad et al. 2011; Moreira et al. 2016;). Therefore, the structure of plant communities can be a key factor that determines arthropod diversity along ecological gradients (Haddad et al. 2011).

Two possible hypothesis that are not mutually exclusive can explain these patterns. The first is based on "the natural enemy hypothesis" (Root 1973) that proposes that the diversity of predators and parasitoids (natural enemies) increases in communities with a great diversity of plants because these represent sites with more potential prey species and microenvironments, stressing the importance of "top-down effects" (Wilby and Thomas 2002; Sobek et al. 2009). This hypothesis implies an indirect mechanism in the interaction between plants and herbivorous insects, according to which the presence of their natural enemies such as spiders, ants, wasps, ground-beetles, etc., exerts a top-down control on herbivore abundance (Pearce et al. 2003). For example, Sobek et al. (2009) showed an increase in herbivorous insects and predator arthropod diversity as the diversity of trees increases in a temperate forest. The second, called "the resource specialization hypothesis" (Novotny et al. 2002) predicts a greater abundance of specialized arthropods in communities with a greater diversity of plants, with herbivore insect diversity being modulated by the diversity and quality of plants, thus giving preponderance to bottom-up effects (Cook-Patton et al. 2011; Leroux and Loreau 2015).

One of the most important components of temperate forests in the Northern Hemisphere, both in terms of species number and biomass, is the genus *Quercus* (Fagaceae) (Cavender-Bares 2019). This genus represents a large and ecologically diverse group of woody plants with temperate origin (Hipp et al. 2020). Oaks are also known to support a great diversity of organismal groups, such as ectomycorrhizal fungi (García-Guzmán et al. 2017), epiphytic plants

(Maclean et al. 2017) and canopy arthropods (Tovar-Sánchez and Oyama 2006a, b; Valencia 2015; Maldonado-López et al. 2018). Several areas in central-western and southern Mexico are particularly rich in oak species, particularly of the *Lobatae* (red oaks) and *Quercus* (white oaks) sections (Torres-Miranda et al. 2013; Rodríguez-Correa et al. 2015), where between two and up to six species can occur in sympatry at the local scale, with species of the two sections often coexisting (Cavender-Bares et al., 2018; McCauley et al. 2019). Thus, oak community diversity gradients are an excellent model to evaluate the changes in canopy arthropod diversity (Root 1973; Sobek et al. 2009). To our knowledge, the existence of this relationship has not been tested in tree canopies in temperate forests and neither along a gradient of tree diversity. Therefore, in this study we evaluated the changes in the structure and composition of canopy arthropods associated to a focal species (*Quercus laurina*) along a gradient of diversity along oak communities. We chose *Q. laurina* because it has a wide distribution in Mexico (Valencia 2004), making it feasible to find communities with varying oak species diversity where this focal species is present and abundant. The specific questions addressed were: (i) Does canopy arthropod diversity associated to *Q. laurina* increase in communities with a greater diversity of oak species? and (ii) How do the structure and composition of arthropod guilds vary along the oak species diversity gradient?

Materials and methods

Study species

Quercus laurina Humb et Bonpl belongs to the section *Lobatae* (red oaks) of the genus *Quercus* and is endemic species of oak for Mexico (Valencia 2004). It is a tree that grows up to 30 m, has coriaceous, lanceolate or elliptic-oblanceolate leaves, with a green and lustrous surface; the fruit is an ovoid acorn, with an average size of 15 to 20 mm long and 15 to 17 mm in diameter. It is distributed between 2440 and 3065 m.a.s.l. and occurs in mixed oak and pine-oak forests, in the Sierra Madre del Sur and the Trans-Mexican Volcanic Belt (TMBV) (Valencia 1994).

Oak diversity gradient

Field work was performed in five sites in the central-west portion of the Trans-Mexican Volcanic Belt in which the diversity and composition of oak communities has been previously analyzed (McCauley et al. 2019) and represent an oak diversity gradient with the presence of *Q. laurina* in all cases (Fig. 1). However, for this study, the oak communities were described again because it was not possible
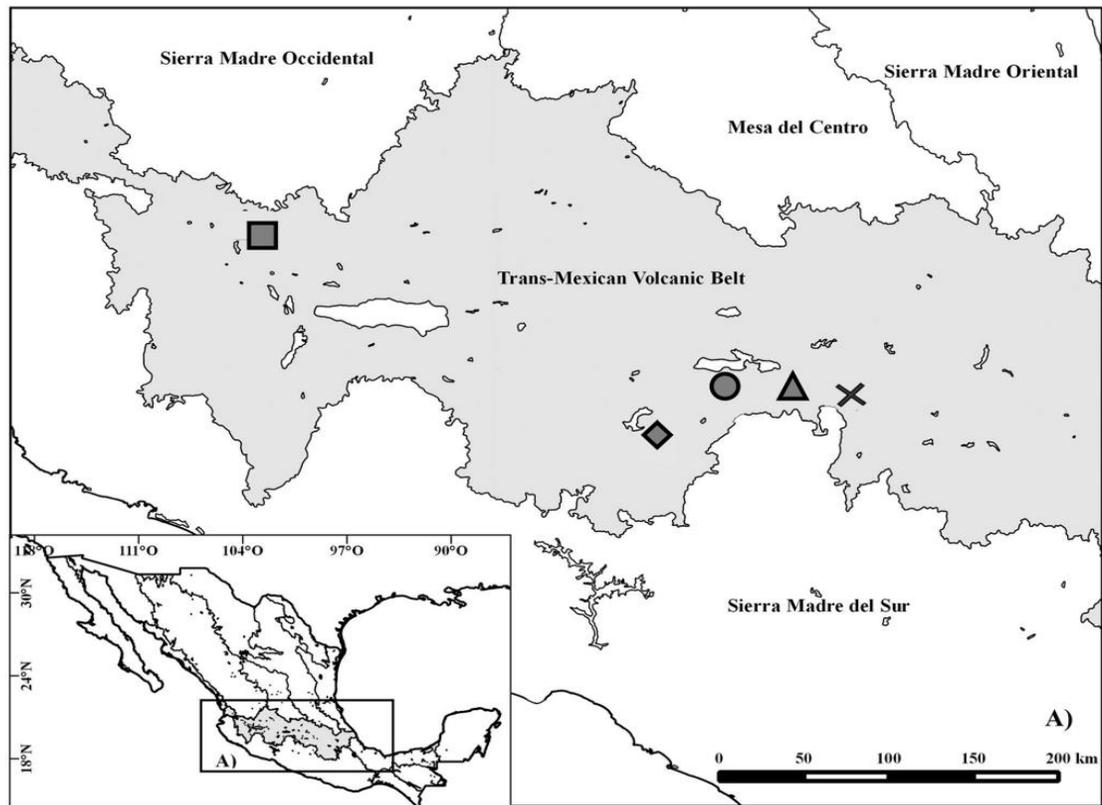
Fig. 1 Geographical distribution map of the study sites, which are located in the states of Michoacán and Jalisco, in the central-western zone of the Trans-Mexican Volcanic Belt (TMBV). The Tequila Vol-cano site is represented by the square, Carindapaz by the triangle, Indaparapeo by the circle, Los Azufres by the cross and Cerro Burro by the rhombus

to locate precisely the same transects previously analyzed. Therefore, at each site we sampled three independent transects of 100×40 m with at least 500 m of separation from each other. Within each transect, we recorded the number of individuals with a diameter at breast height (DBH) ≥ 10 cm of each of the oak species present. From the summed data of the three transects per site, we calculated the oak species richness (OSR) Shannon diversity index (H′), as well as the total density of oak individuals (TDOI; trees per hectare, including all species), the density of individuals belonging to the *Lobatae* section (DILS) and the density of individuals belonging to the *Quercus* section (DIQS). All sites were almost pure oak stands (i.e. with oaks representing at least 90% of the trees), with moderate levels of anthropogenic disturbance. None of the sites was within a natural protected area.

Canopy arthropod diversity

Sampling of canopy arthropods was performed at the end of the rainy season (September) of 2014. At each site, five mature medium-sized trees (DBH 30–40 cm) of *Q. laurina* were randomly selected to collect canopy arthropods using fogging techniques (Erwin and Geraci 2009). We chose these size class of trees because larger trees are more difficult to sample with this technique. Fogging was applied in all trees in the morning between 05:00 and 6:00 A.M. Each tree was nebulized for a period of 10 min using a Swingfog SN-50 Thermal Fogger to disperse a mix of synergized pyrethrins (30 g/l) and piperonyl butoxide (150 g/l). We placed 8 funnel-shaped trays of $1m^2$ under the crown of each fogged tree, and after 50 min, we performed the first arthropod collection using ropes to shake the canopy (Marques et al. 2006;

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Barringer et al. 2019). After 2 h of the first collection, a second collection was performed to collect further arthropods. Arthropods were stored in 95% ethanol and transported to the Agroecology Laboratory of Universidad Michoacana de San Nicolás de Hidalgo (UMSNH) for taxonomic identification. Arthropods were identified to family level with the aid of one of the coauthors (ELB) a specialist taxonomist and using specialized texts. Additionally, each arthropod individual was assigned to a trophic guild (phytophages, predators, detritivores-saprophages, parasitoids, hematophages, mycophages) (Triplehorn and Johnson 2005; Ubick et al. 2017). Arthropod abundance and species richness were estimated using morphospecies criteria, which are recognized as taxonomic units (Majer et al. 2000; Stiegel and Mantilla-Contreras 2018).

Statistical analysis

Oak community composition

To determine the differences in the abundance of oak species and abundance per oak taxonomic section (*Lobatae* vs *Quercus*) between study sites along the diversity gradient, we performed generalized linear models (GLM) analyses respectively, using a Poisson error distribution and a log link function. Study sites were used as the independent variable and the total abundance of oaks and oak abundance per taxonomic section were considered as the response variables (SAS, Stokes et al. 2000).

Canopy arthropod composition

Canopy arthropod abundance and species richness were compared between study sites using a GLM analysis with a Poisson error distribution and a log link function. The study sites were used as the independent variable and arthropod abundance and species richness as the response variables. Furthermore, we performed a logistic regression analysis with the CATMOD procedure (SAS 2000) that is a general procedure for modelling categorical data, to evaluate the differences in the frequency of arthropod orders between study sites. The frequency of arthropod orders was considered as the response variable and study sites as independent variable. Because the distribution of the dependent variable did not follow a normal distribution, we used a Poisson distribution with a logarithmic link function (Stokes et al. 2000).

The Pielou-evenness index was used at the order level to calculated arthropod diversity in each study site. This measure of equitability compares the observed Shannon–Wiener index against the distribution of individuals between the observed species which would maximize diversity (Magurran 1988; Wan et al. 2014).

The richness of the canopy arthropod families of each study site was obtained by rarefaction curves using the program EstimateS 9.1.0 (Colwell 2013). The scale of the independent variable (X) was represented by the number of estimated individuals of arthropods in the canopy. For the application of the rarefaction method, a data set was standardized and compared using the number of individuals as the sampling effort (Gotelli and Colwell 2001). A 95% confidence intervals (CI) were used in each rarefaction curve to determine if the differences in richness between localities was a result of true richness or due to abundance.

To determine the influence of the sites and the parameters of the oak community on the canopy arthropod diversity, as well on the diversity of each guild, we perform multiple GLMs analyses. In the first two GLMs analyses, the response variables were the abundance and richness of canopy arthropods, while the explanatory variables were the study sites and the following parameters of the oak community: (i) oak species richness (OSR), total density of all oak species (TDOS), density of oaks Lobatae section (DOLS) and density of oaks *Quercus* section (DOQS). For the GLMs analyses of the diversity of arthropod guilds, the abundance and richness of each guild (i.e. phytophagous, predators, detritivorous-saprophagous and parasitoids) were considered as the response variables. The study sites and the same parameters of the oak community used for the first GLMs were considered as the explanatory variables. The mycophagous and hematophagous guilds were excluded from the analyses because they presented a very low abundance and richness in each study site. In addition, Shannon's diversity index also was excluded from the models and did not show significant effects on any of the GLMs performed. For all GLMs a Poisson error distribution and a logarithmic link function were used (Stokes et al. 2000).

A principal component analysis on correlations was carried out on the parameters of oak community and the abundance and richness of canopy arthropod at the five study sites along the gradient. The purpose of this analysis was to create a multidimensional representation of the parameters of the oak community of the study sites and their probably correlation with the abundance and richness of arthropods along the gradient.

Multivariate analyses were used to evaluate differences in arthropod composition between the five study sites. Thus, the five communities of arthropods were ordered by non-metric multidimensional scaling (NMDS) using an abundance similitude matrix and the Bray–Curtis index as a distance metric. Afterwards, a non-parametric permutation procedure (ANOSIM) was used to check whether arthropod species composition differed among the five study sites. Again, the ANOSIM was based on a binary matrix arthropod species occurrence in each study site, using the Bray–Curtis index as a distance metric and 5000 permutations (Hammer

et al. 2001). Pairwise ANOSIMs were performed between all pairs of sites as a post-hoc test. These analyses were developed using PAST software (Hammer et al. 2001).

Relationships between local climate variables, oak community and canopy arthropod diversity

We also assessed the effects of climate variables on the composition of the oak community and the canopy arthropod community of *Q. laurina* between all study sites. Climatic data were obtained for each of the 19 bioclimatic variables available in WorldClim (http://worldclim.org/version2) for each study site, and then, we eliminated redundant variables on the basis of the results of a paired correlation analysis (the criterion was to eliminate one variable from each pair with $R > 0.80$ retaining the more general variable). The variables selected were mean annual temperature (MAT), mean annual precipitation (MAP), mean temperature of the warmest quarter (TWQ) and mean temperature of the coldest quarter (TCQ). Hence, we performed a canonical correspondence analysis (CCA) to determine whether there is any correlation between climate variables and the oak community composition and the canopy arthropod community in the study sites. CCA has proved a valuable tool for exploring species-environment correlations (Jin et al. 2016).

For this analysis, we used the statistical package PAST 3.23 (Hammer et al. 2001).

Results

Composition of the oak community

In total, we found five oak species in the study sites, three grouped in the *Lobatae* section (*Q. laurina, Q. crassifolia* and *Q. calophylla*) and two in the *Quercus* section (*Q. obtusata* and *Q. rugosa*). *Quercus laurina* was the most abundant species at all sites, representing from 50% of the individuals in the Tequila Volcano and to 95% in Cerro Burro. The second most abundant species was *Q. rugosa* which was present in four sites, while *Q. calophylla* was found in three sites and finally *Q. crassifolia* in two sites (Fig. 2). The Tequila Volcano presented the highest oak species abundance and richness, followed by Los Azufres, Indaparapeo, Carindapaz and Cerro Burro (Table 1). The oak community with the highest density (930 trees/ha considering all species) was also Tequila Volcano, and then Los Azufres, Cerro Burro, Indaparapeo and Carindapaz (Table 2). In all sites, species belonging
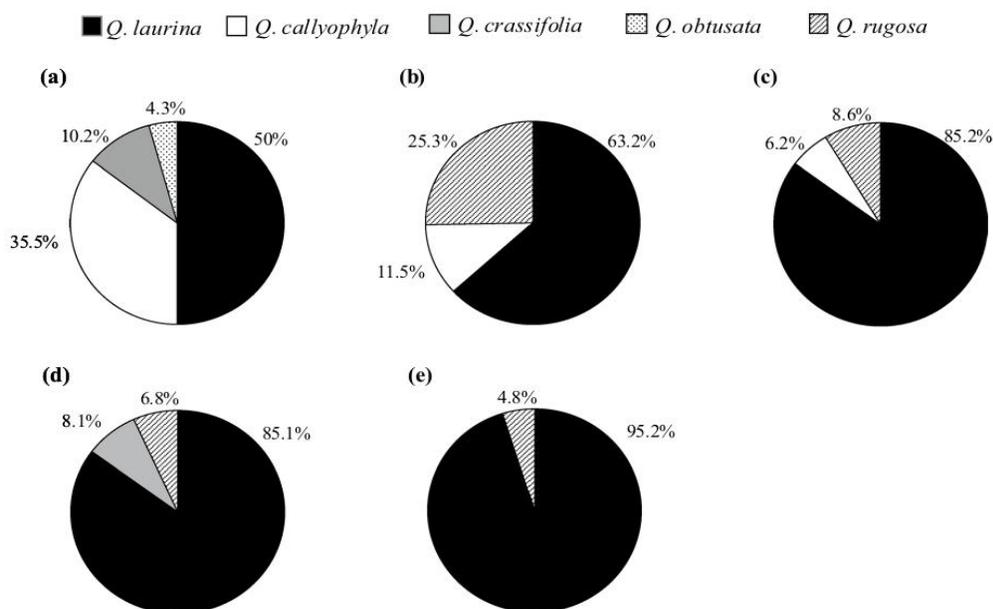

Fig. 2 Frequency of oak species present in the five study sites along the oak diversity gradient. The frequency of oak species per site is given in percentage and they appear from highest to lowest oak spe-cies richness, being: **a** Volcano Tequila, **b** Los Azufres, **c** Indapara-peo, **d** Carindapaz and **e** Cerro Burro

to the *Lobatae* section were more abundant, ranging from 96% of the individuals (Tequila Volcano) to 75% (Los Azufres) (Table 2).

We found significant differences in the abundance of oaks species between the study sites ($\chi^2 = 409.9$, df = 4, $P = 0.0001$), as well as between the oak taxonomic sections ($\chi^2 = 358.3$, df = 1, $P = 0.0001$) (Fig. 2).

Arthropod community along the oak diversity gradient

We collected a total of 7479 arthropods associated to *Q. laurina* in all study sites, distributed as follows: 3408 in Tequila Volcano, 1393 in Los Azufres, 1120 in Indaparapeo, 747 in Carindapaz and 811 in Cerro Burro. These arthropods were separated into a total of 1154 morphospecies (Online Appendix 1) which grouped in 15 orders (Fig. 3). In general, the frequency of arthropod orders

Table 1 The community composition of oak species, geographical characteristics along the oak species diversity gradient

Study sites	OSR	H'	TDOS	DOLS	DOQS	Latitude	Longitude	MAT (°C)	Altitude (masl)	AP (mm)
Tequila Volcano	4	1.1	930	890	40	20.79	− 103.84	12.4	2734	849
Los Azufres	3	0.9	435	325	110	19.79	− 100.68	14.9	2875	1118
Indaparapeo	3	0.7	405	370	35	19.67	− 100.85	17.9	2710	717
Carindapaz	3	0.4	370	345	25	19.69	− 100.91	11.9	2343	1425
Cerro burro	2	0.2	415	395	20	19.44	− 101.51	15.1	3084	1034

Oak species richness (OSR), Shannon diversity index (H'), total density of all oak species (TDOS), density of oaks *Lobatae* section (DOLS), density of oaks *Quercus* section (DOQS), mean annual temperature (MAT), altitude and annual precipitation (AP). The total density of individuals is given in individuals per hectare (Ind/Ha)

Table 2 Composition and density of oak species of each taxonomic section present at each study site along the diversity gradient. The density of individuals is given in individuals per hectare (Ind/Ha)

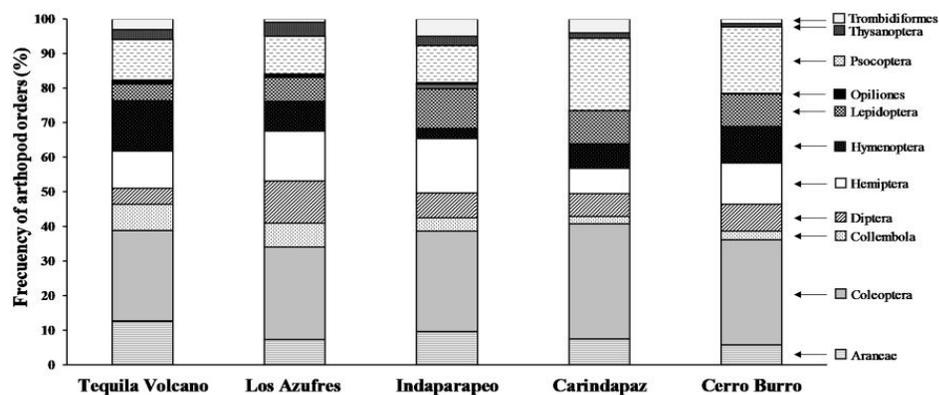| Study sites	Lobatae section			Quercus section		Total
	Q. laurina	*Q. callophyla*	*Q. crassifolia*	*Q. rugosa*	*Q. obtusata*	
Tequila Volcano	465	330	95	0	40	930
Los Azufres	275	50	0	110	0	435
Indaparapeo	345	25	0	35	0	405
Carindapaz	315	0	30	25	0	370
Cerro Burro	395	0	0	20	0	415

Fig. 3 Differences in the frequency of the arthropod orders associated to canopy of *Q. laurina* along the oak diversity gradient. Blattodea, Neuroptera Orthoptera and Pseudoscorpionida orders were present at a very low frequency only in a one particular study site

was different between study sites ($\chi^2 = 35.1$; d.f. $= 4$; $P < 0.0001$) (Fig. 3). Per site, the number of arthropod orders found varied between 12 (Tequila Volcano) and 9 (Carindapaz) (Fig. 3). The most abundant order (in terms of number of individuals) at all study sites was Coleoptera, followed by Psocoptera and Hemiptera. Particularly, Tequila Volcano, which was the site with higher oak species richness had a higher frequency of the Coleoptera (26.1%), Hymenoptera (14.7%) and Araneae (12.5%), while the site with lowest richness of oaks (i.e. Cerro Burro) had 30.6% of Coleoptera, 19.4% of Psocoptera and (12.1%) of Hemiptera (Fig. 3).

Shannon's diversity index values per site at the order level were 2.14, 2.13, 2.11, 1.78 and 1.93 for Tequila Volcano, Los Azufres, Indaparapeo, Carindapaz and Cerro Burro, respectively. All study sites had low values of evenness in the abundance of orders according to Pielou-evenness index, which ranged from $J = 0.77$ in Carindapaz to $J = 0.88$ in Tequila Volcano. The rarefaction analysis showed that the richness of arthropod families was significantly higher in the Volcano Tequila, followed of Indaparapeo, Los Azufres, Cerro Burro and Carindapaz (Fig. 4).

Arthropod abundance (measured as the mean number of individual arthropods per tree) associated to the canopy of *Q. laurina* was significantly different along the oak diversity gradient ($\chi^2 = 274.1$; d.f. $= 4$, $P < 0.0001$) (Fig. 5a). Trees of *Q. laurina* in Tequila Volcano harbored higher arthropod abundance (681.6 ± 13.6) in comparison with trees of Los Azufres (348 ± 5.24), Indaparapeo (224 ± 18.5), Carindapaz (149.4 ± 6.72) and Cerro Burro (162.2 ± 19.32) (Fig. 4a). Similarly, the mean richness of arthropod morphospecies per tree was greater in the Tequila Volcano (65.6 ± 7.62) than in Los Azufres (53 ± 5.1) and Indaparapeo (51.2 ± 9.67 SE) and lower in Carindapaz (28.2 ± 3.8) and Cerro Burro (34.4 ± 5.3) ($\chi^2 = 72.9$; d.f. $= 4$, $P < 0.0001$) (Fig. 5b).

In total, we recorded six different guilds distributed as follows, in terms of the total number of individual arthropods in each guild: phytophages (55.5%), detritivores-saprophages (16.5%), predators (14.5%), parasitoids (12.6%), hematophages (0.5%), and microphages (0.5%). The relative abundance of these arthropod guilds also differed significantly among the study sites. Phytophages represented a higher proportion of the arthropod community at Tequila Volcano (51.7%) and a lower proportion at Los Azufres (37.3%), while the other sites showed intermediate values ($\chi^2 = 269.31$; d.f. $= 4$, $P < 0.0001$) (Fig. 6a). In contrast, predators were more abundant in Indaparapeo (26.7%) and least abundant in the Tequila Volcano (11.1%), with intermediate values in the other three sites ($\chi^2 = 5.51$; d.f. $= 4$, $P < 0.018$). In turn, parasitoids showed a higher relative abundance in Los Azufres and Cerro Burro (around 19%), while they were least abundant in Indaparapeo (6.9%). The frequency of detritivores varied less across sites, being between 18.7 and 21.6%.

In terms of the number of morphospecies, the higher proportion corresponded to the phytophagous guild (37.6% of the total), while 26.5, 17.9, 17.12, 1 and 0.4% of the morphospecies corresponded to detritivorous-saprophagous, predators, parasitoids, hematophagous and mycophagous, respectively. The proportion of morphospecies belonging to each guild also varied among sites. Phytophagous morphospecies represented 44.9% of the total in Tequila Volcano, while they represented 30.7% in Los Azufres, with intermediate values in the other three sites ($\chi^2 = 435.69$; d.f. $= 4$, $P < 0.0001$). Significant differences were also observed for predators ($\chi^2 = 32.25$; d.f. $= 4$, $P < 0.018$), parasitoids ($\chi^2 = 63.65$; d.f. $= 4$, $P < 0.0001$) and detritivorous-saprophagous ($\chi^2 = 63.65$; d.f. $= 4$, $P < 0.0001$) (Fig. 6b).

The GLM models indicated that parameters of the oak community and the study sites had significant effects on the

Fig. 4 Rarefaction curves of canopy arthropods of *Q. laurina* for each of the sites along the oak diversity gradient. The sites were rarefied considering the number of individuals observed (white circles) to allow a valid comparison of arthropod family richness between the study sites
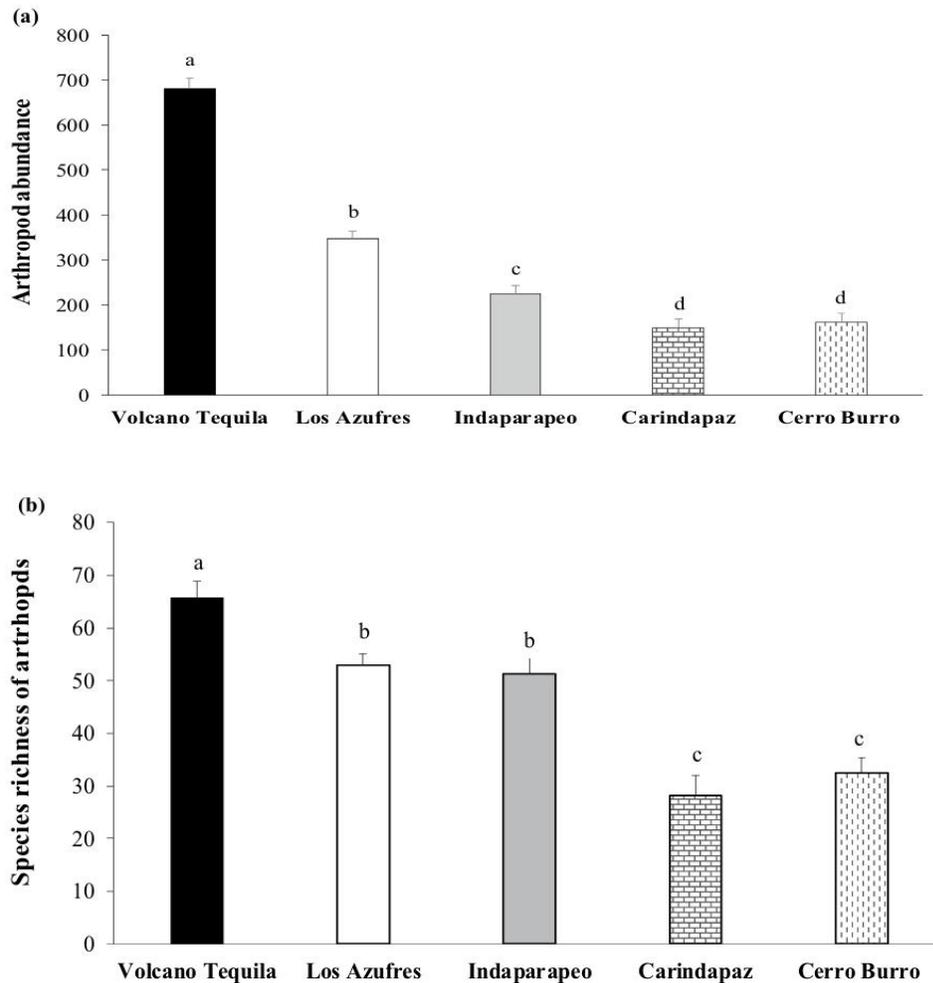
(a)

(b)
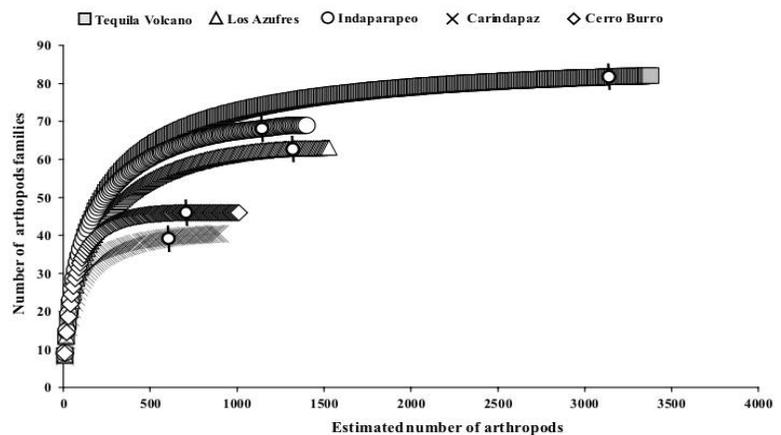
Fig. 5 Diversity patterns of canopy arthropods species of *Q. laurina* along the oak diversity gradient. **a** Differences in arthropod abundance between study sites; **b** Comparison of arthropod richness. Different letters indicate statistically significant differences according to LSMeans test ($P < 0.05$)

canopy arthropod abundance and richness, as well on the diversity of arthropod guilds associated to the canopy of *Q. laurina*. The parameters of oak community OSR ($F = 2197$; d.f. $= 22$; $P < 0.0001$), TDOS ($F = 304.78$; d.f. $= 21$; $P < 0.0001$), DOLS ($F = 180.75$; d.f. $= 20$; $P < 0.0001$), DOQS ($F = 33.02$; d.f. $= 19$; $P < 0.0001$) had significant effects on the arthropod abundance and richness; as well the study sites ($F = 31.05$; d.f. $= 18$; $P < 0.0001$) (Table 3). For the case of arthropod guilds, the phytophagous abundance was affected only by three parameters of the oak

community: OSR ($F = 182.43$; d.f. $= 22$; $P < 0.0001$), TDOS ($F = 734.16$; d.f. $= 21$; $P < 0.0001$) and DOLS ($F = 227.53$; d.f. $= 20$; $P < 0.0001$). The phytophagous richness was influenced for OSR ($F = 12.14$; d.f. $= 22$; $P < 0.0001$), TDOS ($F = 26.91$; d.f. $= 21$; $P < 0.0001$) and DOQS ($F = 6.61$; d.f. $= 19$; $P < 0.0001$) (Table 3). The predator abundance was significant influenced for two parameters of the oak community: OSR ($F = 26.76$; d.f. $= 22$; $P < 0.0001$) and DOLS ($F = 43.37$; d.f. $= 20$; $P < 0.0001$). We did not find significant effects on the richness of predators (Table 3).
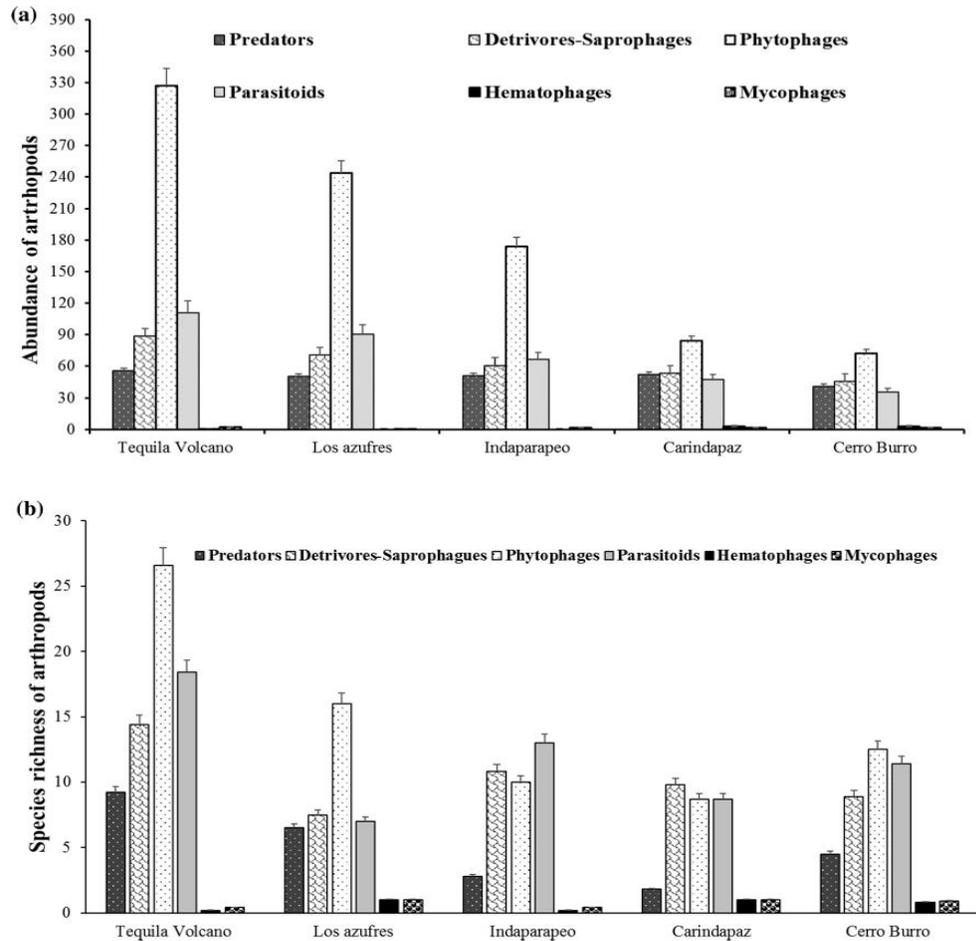
(a)

(b)

Fig. 6 Diversity patterns of arthropod guilds associated to canopies of *Q. laurina*. **a** Abundance of arthropod guilds, **b** species richness of arthropod guilds. Common letters identify means that were not significantly different according to LSMeans test ($P < 0.05$)

We found a similar pattern for the abundance of detritivores–saprophagous and parasitoids. The abundance of the detritivores–saprophagous was significant affected by OSR ($F = 64.45$; d.f. = 22; $P < 0.0001$) and TDOS ($F = 118.75$; d.f. = 20; $P < 0.0001$). The parasitoids abundance was influenced by OSR ($F = 349.66$; d.f. = 22; $P < 0.0001$), TDOS ($F = 256.44$; d.f. = 21; $P < 0.0001$) and DOLS ($F = 19.45$; d.f. = 20; $P < 0.0001$) (Table 3).

In turn, the principal components analysis revealed that the PC1 was positively correlated with parameters of the oak community as OSR, TDOI, DILS and arthropods abundance. The sites of Tequila Volcano and Indaparapeo were high on PC1. PC2 was positively correlated with DIQS, H' and the richness of arthropods. The site of Los Azufres was high on PC2; and for the other sites we did not find a clear correlation between the oak community and the abundance and richness of arthropods (Fig. 7).

The results of the similarity of arthropod community showed that NMDS ordination explained 62% of the variance between sampling points (axis 1 = 52% and axis 2 = 10%), where each point is a two-dimensional representation of the composition of arthropod species in a single tree based on global NMDS. The composition of arthropod species associated to *Q. laurina* varied between the study sites

Table 3 Results of the GLMs for the parameters of oak community and study sites on the arthropod abundance, arthropod species richness and abundance and richness of phytophagous, predators, detritivorous-Saprophagous and parasitoids associated to the canopy of *Q. laurina* along the oak diversity gradient

Response variables	Explanatory variables	Deviance	Residual DF	Residual deviance	F	P
Arthropod abundance	OSR	2197.6	22	643.2	2197.6	<0.001
	TDOS	304.7	21	338.4	304.7	<0.001
	DOLS	180.7	20	157.7	180.8	<0.001
	DOQS	33.0	19	124.7	33.0	<0.001
	Sites	31.0	18	93.7	31.1	<0.001
Arthropod richness	OSR	7.7	22	152.4	7.74	<0.001
	TDOS	21.4	21	130.9	21.4	<0.001
	DOLS	5.7	20	125.3	5.7	<0.001
	DOQS	11.2	19	114.1	11.2	<0.001
	Sites	23.2	18	90.9	23.2	<0.001
Phytophagous abundance	OSR	182.4	22	2405.7	182.4	<0.001
	TDOS	734.2	21	1671.6	734.2	<0.001
	DOLS	227.5	20	1444.0	227.5	<0.001
	DOQS	0.78	19	1443.2	0.82	0.3
	Sites	178.4	18	1138.2	160.3	<0.001
Phytophagous richness	OSR	12.1	22	79.6	12.1	<0.001
	TDOS	26.9	21	52.7	26.9	<0.001
	DOLS	2.1	20	50.5	2.18	0.13
	DOQS	6.6	19	43.9	6.61	<0.001
	Sites	435.7	18	63.9	435.7	<0.001
Predators abundance	OSR	26.8	22	201.5	26.8	<0.001
	TDOS	1.9	21	199.5	1.9	0.15
	DOLS	43.3	20	156.2	43.3	<0.001
	DOQS	3.6	19	152.6	3.6	0.05
	Sites	25.7	18	126.9	25.7	<0.001
Predators Richness	OSR	0.97	22	45.8	0.93	0.32
	TDOS	0.39	21	45.0	0.44	0.52
	DOLS	3.2	20	42.2	3.2	0.07
	DOQS	2.3	19	39.9	2.34	0.12
	Sites	32.3	18	63.9	32.2	<0.001
Detritivorous-Saprophagous abundance	OSR	64.7	22	221.7	64.6	<0.001
	TDOS	118.8	21	102.9	118.8	<0.001
	DOLS	2.9	20	100.1	2.9	0.09
	DOQS	0.95	19	99.1	0.92	0.32
	Sites	6.7	18	92.0	6.7	<0.001
Detritivorous-Saprophagous richness	OSR	1.4	22	53.2	1.5	0.22
	TDOS	0.002	21	53.2	0.002	0.98
	DOLS	1.4	20	51.8	1.4	0.24
	DOQS	2.6	19	49.1	2.6	0.10
	Sites	11.7	18	37.40	11.7	<0.001
Parasitoids abundance	OSR	349.8	22	377.6	349.9	<0.001
	TDOS	256.4	21	121.2	256.4	<0.001
	DOLS	19.4	20	101.7	19.5	<0.001
	DOQS	0.44	19	101.3	0.44	0.50
	Sites	14.6	18	86.7	14.5	<0.001
Parasitoids richness	OSR	2.2	22	54.4	2.2	0.13
	TDOS	4.8	21	49.6	4.8	0.02
	DOLS	0.22	20	49.3	0.22	0.63

Table 3 (continued)

Response variables	Explanatory variables	Deviance	Residual DF	Residual deviance	F	P
	DOQS	0.41	19	48.9	0.41	0.52
	Sites	13.9	18	35.0	19.9	<0.001

The parameters of the oak community are: Oak species richness (OSR), total density of all oak species (TDOS), density of oaks Lobatae section (DOLS) and density of oaks *Quercus* section (DOQS)

Fig. 7 Principal component analysis of the parameters of the oak community and the abundance (AA) and richness (AR) of arthropods in the canopies of *Q. laurina* at the five sites presents along the oak's diversity gradient. Vectors show the strength and direction of the relationship between the parameters of the oak community and axes. The parameters of the oak community are: Oak species richness (OSR), Shannon diversity index (H′), total density of all oak individuals (TDOI), density of individuals in the *Lobatae* section (DILS), density of individuals in the *Quercus* section (DIQS)
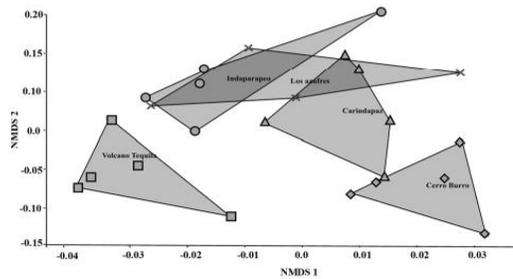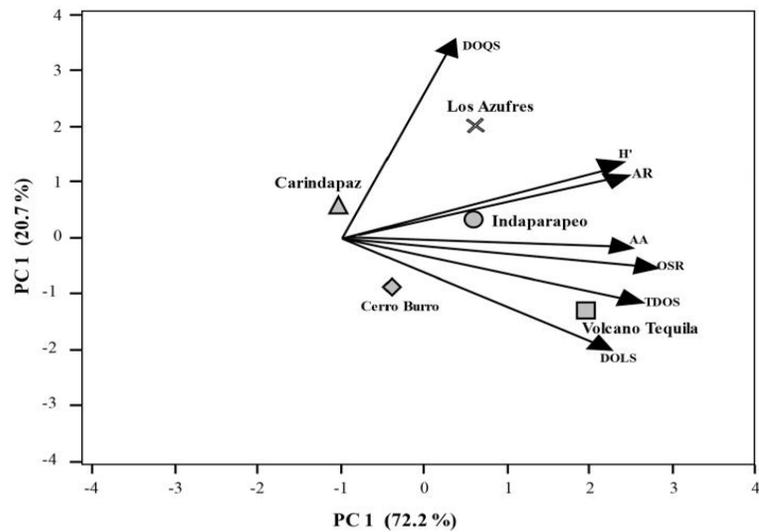

Fig. 8 Non-metric multidimensional scaling (NMDS) ordinations illustrating similarity of arthropod taxonomic composition between the study sites along the oak diversity gradient. Each point is a two-dimensional (axis 1 and axis 2) representation of arthropod species composition on an individual tree based on global, non-metric multidimensional scaling (NMDS)

(ANOSIM $r=0.52$, n=25, $P<0.001$) (Fig. 8). The results of post-hoc pairwise ANOSIM tests showed different composition of arthropod species between all study sites. Particularly, Tequila Volcano and Cerro Burro had totally different assemblages in comparison with the other three study sites ($P<0.05$). Moreover, the arthropod species composition of Carindapaz also differed from Los Azufres ($P<0.05$).

Local climate variables, oak community and canopy arthropod diversity

Canonical correspondence analyses (CCA) between climate variables and the oak community composition along the oak diversity gradient showed significant correlations in the Permutation test of all canonical axes (Trace=0.22, $P=0.004$). The first two axes explained 85.15% (axis 1=65.41% and axis 2=19.74%) of the spatial variation of the oak community relative to climate variables, where MAP, TCQ and TWQ had a strong correlation with the oak community composition. Particularly, Tequila Volcano, Los Azufres and Indaparapeo showed a greater correlation between climatic variables and the composition of the oak community.

The results of the canonical correspondence analysis (CCA) between the climate variables and arthropod community did not show significant correlations in the Permutation test of all canonical axes (Trace=0.1294, P=0.34). This indicates that

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the composition of the canopy arthropod community associated to *Q. laurina* was not influenced by local environmental variables of each study site.

Discussion

Oaks represent one of the most diverse groups of woody plants in the Northern Hemisphere, with Mexico being a center of oak diversity and endemism (Cavender-Bares 2016). Our results agree with this idea, since at the local scale, we detected a diversity gradient of four species, ranging from localities with only two species to localities with oak species coexisting. In terms of the number of species of the *Lobatae* section, we observed the coexistence of *Q. laurina* with up to two other species of red oaks, such as *Q. crassifolia* and *Q. calophylla*. In general, we found a greater abundance of oaks from the *Lobatae* section, with *Q. laurina* being the dominant species with an average 88% of the individuals present in the study sites. In addition, we found correlations between climatic variables (MAP, TCQ and TWQ) and oak community composition in study sites with greater richness and abundance of oaks. These results can be explained by the high climatic variation present along the Sierra Madre del Sur and the Trans-Mexican Volcanic Belt (Currie and Francis 2004). Some environmental variables such as temperature, precipitation, habitat heterogeneity, and past climate change have been proposed as the drivers of contemporary species richness patterns of oaks (Hipp et al. 2019). Since Mexico is a diversity center for the genus *Quercus*, the coexistence among different species is expected, and particularly, the co-occurrence of the two main sections seem to be the norm rather than the exception (Rodríguez-Correa et al. 2015; Cavender-Bares et al. 2018; McCauley et al. 2019).

In total, we detected 1154 morphospecies of arthropods grouped in 15 orders associated to the canopy of *Q. laurina*. The abundance and richness of the arthropod community associated to *Q. laurina* was different along the gradient of diversity of oak species. Trees of *Q. laurina* that occurred in the site with highest oak species diversity (i.e. Tequila volcano) had the highest richness and abundance of arthropods. Additionally, the NMDS showed differences in the composition of the arthropod community along the oak diversity gradient, agreeing with the fact that Tequila Volcano had a significantly different composition of the arthropod community compared with the other study sites. Two hypotheses that are not mutually exclusive can explain our results. "The plant species richness hypothesis" (Fernandes and Price 1988) that proposes that communities with greater plant diversity represent more potential sites to be colonized by a greater number of insect species ("bottom-up effects") (Hertzog 2017). Some

studies have shown that plant diversity is a factor that contributes to the maintenance of herbivorous insect diversity (Haddad et al. 2011). A meta-analysis, involving 52 independent comparisons, confirmed that diversity of herbivorous insects increases with a greater diversity of plants (i.e. 18 correlations derived from galling herbivores and 34 from non-galling herbivores) (Araújo 2013). In our case, oak communities represent to arthropods a higher resource and niches availability to be colonized by a greater number of species (bottom-up forces: plants-herbivores), where species richness of arthropods in the canopy of *Q. laurina* increases as more oak species are available. Additionally, it has been proposed that plant diversity is a key driver for increasing herbivory by insects because heterospecific neighbors can decrease a plant resistance (i.e. associative resistance) or increase herbivory susceptibility (i.e. associative susceptibility), increasing the likelihood of focal plants being attacked by herbivorous insects. (Root 1973; Tahvanainen and Root 1972; White and Whitham 2000). This fact has been confirmed by meta-analyses suggesting that associative resistance is the most common pattern in forest ecosystems communities (Jactel & Brockerhoff 2007; Castagneyrol et al. 2014, 2017). In either of the two ideas raised, our results support the "plant species richness hypothesis" (Fernandes and Price 1988) where an increase in the richness of oak species generates a greater diversity of arthropods in the canopy of *Q. laurina*.

A second hypothesis "The natural enemy hypothesis" (Root 1973) suggests that the diversity of natural enemies (i.e. predators and parasitoids) increases in communities with a great plant diversity because of the presence of more potential prey and availability of microenvironments ("top-down effects") (Wilby and Thomas 2002; Sobek et al. 2009). This hypothesis implies an indirect mechanism between plants and herbivorous insects, where the presence of natural enemies exerts a top-down control on herbivore abundance (Pearce et al. 2003). Therefore, a greater diversity of resources provided by a high herbivore diversity present in highly diverse oak community can increase the diversity of predators (bottom-up forces: arthropod-arthropod) affecting the community structure of arthropods (Randlkofer et al. 2010; Haddad et al. 2011; Moreira et al. 2016; Hertzog 2017).

Another important result is the fact that trees of *Q. laurina* growing in Tequila Volcano harbored higher abundance and species richness of phytophages, predators and parasitoids. Similar results have been shown in other ecological studies supporting the idea that communities with high plant diversity can represent more availability of resources, niches and microenvironments to be colonized by more arthropod guilds (Sobek et al. 2009; Hertzog 2017). However, the great diversity of predators and parasitoids registered in the most diverse oak community also can suggests that

natural enemies can be regulating arthropod diversity (top-down forces). Some studies suggest that top-down forces in communities increase with resource availability to primary producers (Root 1973; Boyer et al. 2003). Therefore, we propose that our results can be explained by a combination of both factors, where bottom-up forces will set the stage for top-down forces to act. For example, plant diversity can increase the abundance of herbivores through greater availability and/or quality of food, niches, microenvironments and shelter, which in turn will determine the abundance and diversity of predators, affecting the predation pressure (Forkner and Hunter 2000). The role of top-down and bottom-up forces in terrestrial communities has been a subject of debate in the ecological literature (Schmitz et al. 2000; Halaj and Wise 2001). Particularly, in oak communities, some studies have suggested bottom-up forces as the main regulatory mechanism of the distribution and abundance of herbivorous insects through leaf nutritional quality and phenology (Feeny 1970; Schultz and Baldwin 1982; West 1985; Faeth and Bultman 1986; Rossiter et al. 1988). However, more recent studies suggest that bottom-up and top-down forces interact together by molding and structuring arthropod communities (Forkner and Hunter 2000; Castagneyrol et al. 2014, 2017). For example, a meta-analysis including 172 studies showed that top-down forces were stronger than bottom-up forces to control herbivorous insects, where, chewing, sucking and gall-making herbivores were more affected by top-down than bottom-up forces, and parasitoids and predators had equally strong top-down effects on insect herbivores (Vidal and Murphy 2018).

Additionally, the presence of geographic barriers such as mountainous terrains that are characteristic of the Trans-Mexican Volcanic Belt (TMBV), also could be affecting the canopy arthropod community, limiting their distribution and reducing the arthropod migration between localities (Novotny and Weiblen 2005; Arriaga-Jiménez et al. 2018). Furthermore, given that some arthropod groups exhibit high levels of intraspecific aggregation as a result of oviposition behavior, it is possible to expect a limited spatial dispersion between communities (Caballero et al. 2011), mainly in the Tequila volcano, which was the locality most separated from the other study sites and with highest arthropod diversity.

Our results show significant differences in the composition of the arthropod community associated with the canopy of *Q. laurina* along the gradient of diversity of oak species. The sites with more diversity of oak species presented higher levels of arthropod abundance and richness, thus a greater presence of arthropod guilds (phytophages, predators, parasitoids) and a greater presence of adult arthropods and nymphs. These results are consistent with the premise that for habitats in temperate forests, tree communities are a key element for shaping the environmental physical structure; reason that has been suggested that tree species

diversity could influence an increase in richness and abundance of canopy arthropod (Lassau et al. 2005; Affeld 2008; Moreira et al. 2016). This fact is consistent with the absence of significant relationships between environmental variables (i.e. temperature, precipitation) and the canopy arthropod community in all study sites, suggesting that the differences in the oak community structure between localities are the main factor that is modulating the community of arthropods associated to the canopy of *Q. laurina* along the oak diversity gradient.

Finally, one little discussed factor in the ecological literature that potentially affect the insect abundance on a tree is the problem associated with the assignment of arthropod species to guilds (Stork 1987). It has been shown that the incorrect assignment of the food guilds of only a few insect species can result in important differences in the composition and diversity of the arthropod communities of the canopy (Stork 2018). For example, for many groups of canopy insects, the larval and adult stages have the same feeding habits. However, in some groups of hymenopterans, the larvae act as parasites and the adults may feed on honey, nectar, or not feed, which affects the estimates of insect diversity patterns (Stork 1987).

In conclusion, our study showed changes in arthropod diversity of the canopy of *Q. laurina* along an oak species diversity gradient. We detected differences in arthropod diversity, abundance and guild composition between oak communities. The general pattern showed higher arthropod diversity in Tequila volcano, which represented the site with the greatest diversity of oaks. Our findings confirm that at the local scale, plant species richness is the main factor that determines the arthropod diversity associated to canopy of *Q. laurina*, affecting trophic levels and the structure and composition of arthropod guilds. Mexico is the main center of richness, diversification and endemism of oaks in the northern hemisphere. Thus, we highlight the importance of conserving oak species because they represent key elements of temperate forest that harbor high diversity of animals.

Declarations

Conflict of interest All authors declare that they have no conflict of interest.

References

Affeld K (2008) Spatial complexity and microclimatic responses of epiphyte communities and their invertebrate fauna in the canopy of northern rata (*Metrosideros robusta* A. Cunn.: Myrtaceae) on the West Coast of the South Island, New Zealand. Dissertation, Lincoln University

Arriaga-Jiménez A, Rös M, Halffter G (2018) High variability of dung beetle diversity patterns at four mountains of the Trans-Mexican Volcanic Belt. PeerJ 6:e4468. https://doi.org/10.7717/peerj.4468

Barringer LE, Bartlett CR, Erwin TL (2019) Canopy assemblages and species richness of planthoppers (Hemiptera: Fulgoroidea) in the Ecuadorian Amazon. Insecta Mundi 0726:1–16

Basset Y, Hammond PM, Barrios H, Holloway JD, Miller SE (2003) Vertical stratification of arthropod assemblages. In: Basset Y, Novotny V, Miller SE, Kitching RL (eds) Arthropods of tropical forests: spatio-temporal dynamics and resource use in the canopy. Cambridge University Press, Cambridge, pp 17–27

Boyer AG, Swearingen RE, Blaha MA, Fortson CT, Gremillion SK, Osborn KA, Moran MD (2003) Seasonal variation in top-down and bottom-up processes in a grassland arthropod community. Oecologia 136(2):309–316. https://doi.org/10.1007/s00442-003-1272-x

Caballero M, Lozano-García S, Vázquez-Selem L, Ortega B (2011) Evidencias de cambio climático y ambiental en registros glaciales y en cuencas lacustres del centro de México durante el último máximo glacial. Boletín De La Sociedad Geológica Mexicana 62(3):359–377

Castagneyrol B, Régolini M, Jactel H (2014) Tree species composition rather than diversity triggers associational resistance to the pine processionary moth. Basic Appl Ecol 15(6):516–523. https://doi.org/10.1016/j.baae.2014.06.008

Castagneyrol B, Bonal D, Damien M, Jactel H, Meredieu C, Muiruri EW, Barbaro L (2017) Bottom-up and top-down effects of tree species diversity on leaf insect herbivory. Ecol Evol 7(10):3520–3531. https://doi.org/10.1002/ece3.2950

Cavender-Bares J (2016) Diversity, distribution and ecosystem services of the North American oaks. Int Oaks 27:37–49

Cavender-Bares J (2019) Diversification, adaptation, and community assembly of the American oaks (Quercus), a model clade for integrating ecology and evolution. New Phytol 221(2):669–692. https://doi.org/10.1111/nph.15450

Cavender-Bares J, Kothari S, Meireles JE, Kaproth MA, Manos PS, Hipp AL (2018) The role of diversification in community assembly of the oaks (*Quercus* L.) across the continental US. Am J Bot 105(3):565–586. https://doi.org/10.1002/ajb2.1049

Colwell RK (2013) Estimates: statistical estimation of species richness and shared species from samples. Version 9. User's Guide and application. http://purl.oclc.org/estimates

Cook-Patton SC, McArt SH, Parachnowitsch AL, Thaler JS, Agrawal AA (2011) A direct comparison of the consequences of plant genotypic and species diversity on communities and ecosystem function. Ecology 92:915–923. https://doi.org/10.1890/10-0999.1

Cuevas-Reyes P, Quesada M, Hanson P, Dirzo R, Oyama K (2004) Diversity of gall-inducing insects in a Mexican tropical dry forest: the importance of plant species richness, life-forms, host plant age and plant density. J Ecol. https://doi.org/10.1111/j.0022-0477.2004.00896.x

Currie DJ, Francis AP (2004) Taxon richness and climate in angiosperms: is there a globally consistent relationship that precludes region effects? Am Nat 163:780–785

de Araújo WS (2013) Different relationships between galling and non-galling herbivore richness and plant species richness: a meta-analysis. Arthropod Plant Interact 7(4):373–377. https://doi.org/10.1007/s11829-013-9259-y

Erwin TL, Geraci CJ (2009) Chapter 4. Amazonian rainforests and their richness of Coleoptera, a dominant life form in the critical zone of the Neotropics. In: Foottit RG, Adler PH (eds) Insect biodiversity: science and society. Wiley-Blackwell, West Sussex

Faeth SH, Bultman TL (1986) Interacting effects of increased tannin levels on leaf-mining insects. Entomology 40(3):297–301. https://doi.org/10.1111/j.1570-7458.1986.tb00515.x

Feeny P (1970) Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology 51(4):565–581. https://doi.org/10.2307/1934037

Fernandes GW, Price PW (1988) Biogeographical gradients in galling species richness. Oecologia 76(2):161–167. https://doi.org/10.1007/BF00379948

Forkner RE, Hunter MD (2000) What goes up must come down? Nutrient addition and predation pressure on oak herbivores. Ecology 81(6):1588–1600. https://doi.org/10.1890/0012-9658(2000)081

García-Guzmán OM, Garibay-Orijel R, Hernández E, Arellano-Torres E, Oyama K (2017) Word-wide meta-analysis of *Quercus forests* ectomycorrhizal fungal diversity reveals southwestern Mexico as a hotspot. Mycorrhiza 27(8):811–822. https://doi.org/10.1007/s00572-017-0793-9

Gotelli N, Colwell RK (2001) Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecol Lett 4:379–391. https://doi.org/10.1046/j.1461-0248.2001.00230.x

Haddad NM, Crutsinger GM, Gross K, Haarstad J, Tilman D (2011) Plant diversity and the stability of foodwebs. Ecol Lett 14:42–46. https://doi.org/10.1111/j.1461-0248.2010.01548.x

Halaj J, Wise DH (2001) Terrestrial trophic cascades: how much do they trickle? Am Nat 157:262–281. https://doi.org/10.1086/319190

Hamilton AJ, Novotny VWEK, Bassset Y, Benke KK, Grimbacher PS, Samuelson GA, Weiblen GD, Yen JDL, Stork NE, Weiblen GD, Yen JDL (2013) Estimating global arthropod species richness: refining probabilistic models using probability bounds analysis. Oecologia 171:357–365. https://doi.org/10.1007/s00442-012-2434-5

Hammer Ø, Harper DA, Ryan PD (2001) PAST: Paleontological statistics software package for education and data analysis. Palaeontol Electron 4(1):9

Hertzog L (2017) Plant diversity impacts on arthropod communities and arthropod-mediated processes. Dissertation, Technische Universität München

Hipp Andrew L, Manos PS et al (2019) Genomic landscape of the global oak phylogeny. New Phytol 226:1198–1212. https://doi.org/10.1111/nph.16162

Hipp Andrew L et al (2020) Sympatric parallel diversification of major oak clades in the Americas and the origins of Mexican species diversity. New Phytol 217:439–452

Jactel H, Brockerhoff EG (2007) Tree diversity reduces herbivory by forest insects. Ecol Letters 10(9):835–848. https://doi.org/10.1111/j.1461-0248.2007.01073.x

Jin J, Wang Y, Jiang H, Kong Y, Zhang X (2016) Improvement of ecological geographic regionalization based on remote sensing and canonical correspondence analysis: A case study in China. Sci China Earth Sci 59(9):1745–1753

JMP®, Version .2008, SAS Institute Inc., Cary, NC, USA ISBN 978-1-59994-924-6

Lassau SA, Hochuli DF, Cassis G, Reid CA (2005) Effects of habitat complexity on forest beetle diversity: do functional groups respond consistently. Divers 1:73–82. https://doi.org/10.1111/j.1366-9516.2005.00124.x

Leroux SJ, Loreau M (2015) Theoretical perspectives on bottom-up and top-down interactions across ecosystems. Trophic Ecol 3:28. https://doi.org/10.1017/CBO9781139924856.002

Maclean JE, Mitchell RJ, Burslem DF, Genney D, Hall J, Pakeman J (2017) The epiphytic bryophyte community of Atlantic oak woodlands shows clear signs of recovery following the removal of invasive *Rhododendron ponticum*. Biol Conserv 212:96–104. https://doi.org/10.1016/j.biocon.2017.06.003

Magurran AE (1988) Ecological diversity and its measurement. Princeton University Press, Princeton

Majer JD, Recher HF, Ganesh S (2000) Diversity patterns of eucalypt canopy arthropods in eastern and western of Australian. Ecol Entomol 25:295–306. https://doi.org/10.1046/j.1365-2311.2000.00259.x

Maldonado-López Y, Vaca-Sánchez MS, González-Rodríguez A, Oyama K, López-Barbosa, Fagundes M, Cuevas-Reyes P (2018) Hybridization increases canopy arthropod diversity in the *Quercus affinis× Quercus laurina* complex. J Insect Conserv 22(5–6):781–793. https://doi.org/10.1007/s10841-018-0103-7

Marques MI, Adis J, dos Santos GB, Battirola LD (2006) Terrestrial arthropods from tree canopies in the Pantanal of Mato Grosso, Brazil. Rev Bras Entomol 50:257–267. https://doi.org/10.1590/S0085-56262006000200007

McCauley RA, Cortés-Palomec AC, Oyama K (2019) Species diversification in a lineage of Mexican red oak (Quercus section Lobatae subsection Racemiflorae) the interplay between distance, habitat, and hybridization. Tree Genet 15:27. https://doi.org/10.1007/s11295-019-1333-x

Moreira X, Abdala-Roberts L, Rasmann S, Castagneyrol B, Mooney KA (2016) Plant diversity effects on insect herbivores and their natural enemies: current thinking, recent findings, and future directions. Curr Opin Insect 14:1–7. https://doi.org/10.1016/j.cois.2015.10.003

Novotny V, Weiblen GD (2005) From communities to continents: beta diversity of herbivorous insects. In: Annales Zoologici Fennici. Finnish Zoological and Botanical Publishing Board. pp 463–475

Novotny V, Basset Y, Miller SE, Weiblen GD, Bremer B, Cizek L, Drozd P (2002) Low host specificity of herbivorous insects in a tropical forest. Nature 416(6883):841–844

Pearce JL, Venier LA, McKee J, Pedlar J, McKenney D (2003) Influence of habitat and microhabitat on carabid (Coleoptera: Carabidae) assemblages in four stand types Influence of habitat and microhabitat on carabid (Coleoptera: *Carabidae*) assemblages in four stand types. Can Entomol 135(3):337–357. https://doi.org/10.4039/n02-031

Randlkofer B, Obermaier E, Hilker M, Meiners T (2010) Vegetation complexity: the influence of plant species diversity and plant structures on plant chemical complexity and arthropods. Basic Appl Ecol 11:383–395. https://doi.org/10.1016/j.baae.2010.03.003

Rodríguez-Correa H, Oyama K, MacGregor-Fors I, González-Rodríguez A (2015) How are oaks distributed in the neotropics? A perspective from species turnover, areas of endemism, and climatic niches. Int J Plant Sci 176:222–231. https://doi.org/10.1086/679904

Root RB (1973) Organization of a plant–arthropod association in simple and diverse habitats: the fauna of collards (*Brassica oleracea*). Ecol Monogr 43:95–124. https://doi.org/10.2307/1942161

Rossiter M, Schultz JC, Baldwin IT (1988) Relationships among defoliation, red oak phenolics, and gypsy moth growth and reproduction. Ecology 69(1):267–277. https://doi.org/10.2307/1943182

SAS (2000) Categorical data analysis using the SAS system. SAS Institute, Cary

Schmidl J, Bussler H (2008) Xylobiontic beetle guild composition and diversity driven by forest canopy structure and management. In: Floren A, Schmidl J (eds) Canopy arthropod research in Europe. Bioform Entomology, Nuremberg, pp 299–323

Schmitz OJ, Hamback PA, Beckerman AP (2000) Trophic cascades in terrestrial systems: a review of the effects on carnivore removals on plants. Am Nat 155:141–153. https://doi.org/10.1086/303311

Schultz JC, Baldwin IT (1982) Oak leaf quality declines in response to defoliation by gypsy moth larvae. Science 217(4555):149–151. https://doi.org/10.1126/science.217.4555.149

Sobek S, Scherber C, Steffan-Dewenter I, Tscharntke T (2009) Sapling herbivory, invertebrate herbivores and predators across a natural tree diversity gradient in Germany's largest connected deciduous forest. Oecologia 160:279–288. https://doi.org/10.1007/s00442-009-1304-2

Stiegel S, Mantilla-Contreras J (2018) Environment vs. plant ontogeny: arthropod herbivory patterns on European beech leaves along the vertical gradient of temperate forests in Central Germany. Insects 9:9–28. https://doi.org/10.3390/insects9010009

Stokes ME, Davis CS, Koch GG (2000) Categorical data analysis using the SAS system. Wiley, Hoboken, p 626

Stork NS (1987) Guild structure of arthropods from Bornean rain forest trees. Ecol Entomol 12:69–80. https://doi.org/10.1111/j.1365-2311.1987.tb00986.x

Stork NS (2018) How many species of insects and other terrestrial arthropods are there on Earth? Annu Rev Entomol 63:31–45. https://doi.org/10.1146/annurev-ento-020117-043348

Stork NS, Grimbacher PS, Storey R, Oberprieler RG, Reid C, Slipinski C (2008) What determines whether a species of insect is described? Evidence from a study of tropical forest beetles. Insect Conserv Divers 1:114–119. https://doi.org/10.1111/j.1752-4598.2008.00016.x

Tahvanainen JO, Root RB (1972) The influence of vegetational diversity on the population ecology of a specialized herbivore, *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae). Oecologia 10(4):321–346. https://doi.org/10.1007/bf00345736

Tal O, Freiberg M, Morawetz W (2008) Micro-climatic variability in the canopy of a temperate forest. In: Floren A, Schmidl J (eds) Canopy arthropod research in Europe. Bioform Entomology, Nuremberg, pp 49–59

Torres-Miranda A, Luna-Vega I, Oyama K (2013) New approaches to the biogeography and areas of endemism of red oaks (*Quercus* L., Section Lobatae). Syst Biol 62(4):555–573. https://doi.org/10.1093/sysbio/syt021

Tovar-Sánchez E, Oyama K (2006a) Community structure of canopy arthropods associated to *Quercus crassifolia* x *Quercus crassipes* complex. Oikos 112:370–381. https://doi.org/10.1111/j.0030-1299.2006.14029.x

Tovar-Sánchez E, Oyama K (2006b) Effect of hybridization of the *Quercus crassifolia* x *Quercus crassipes* complex on the community structure on endophagous insects. Oecologia 147:702–713. https://doi.org/10.1007/s00442-005-0328-5

Triplehorn CA, Johnson NF, Borror DJ (2005) An introduction to the study of insects. Thompson Brooks/Cole, Belmont

Ubick, D, P Paquin, P, Cushing, Roth VD (2017) Spiders of North America. An Identification Manual. Second Edition. American Arachnological Society. Keene, New Hampshire USA 377pp.

Ulyshen MD (2011) Arthropod vertical stratification in temperate deciduous forests: implications for conservation-oriented management. For Ecol Manag 261:1479–1489. https://doi.org/10.1016/j.foreco.2011.01.033

Valencia S (1994) Contribución a la delimitación taxonómica de tres especies del género Quercus subgénero Erythrobalanus. Tesis de

Maestría, Facultad de Ciencias, Universidad Nacional Autónoma de México, Mexico

Valencia S (2004) Diversidad del género Quercus (Fagaceae) en México. Bol Soc Bot México 75:33–53. https://doi.org/10.17129/botsci.1692

Valencia-Cuevas L, Mussali-Galante P, Piñero D, Castillo-Mendoza E, Rangel-Altamirano G, Tovar-Sánchez E (2015) Hybridization of *Quercus castanea* (Fagaceae) across a red oak species gradient in Mexico. Plant Syst Evol 301:1085–1097. https://doi.org/10.1007/s00606-014-1151-4

Vidal MC, Murphy SM (2018) Bottom-up vs top-down effects on terrestrial insect herbivores: a meta-analysis. Ecol Lett 21(1):138–150. https://doi.org/10.1111/ele.12874

Wan NF, Ji XY, Jiang JX (2014) Testing the enemies hypothesis in peach orchards in two different geographic areas in eastern China: the role of ground cover vegetation. PLoS ONE 9(6):e99850. https://doi.org/10.1371/journal.pone.0099850

West C (1985) Factors underlying the late seasonal appearance of the lepidopterous leaf-mining guild on oak. Ecol Entomol 10(1):111–120. https://doi.org/10.1111/j.1365-2311.1985.tb00540.x

White JA, Whitham TG (2000) Associational susceptibility of cottonwood to a box elder herbivore. Ecology 81(7):1795–1803. https://doi.org/10.1890/0012-9658(2000)081

Wilby A, Thomas MB (2002) Natural enemy diversity and pest control: patters of pest emergence with agricultural intensification. Ecol Letters 5:353–360. https://doi.org/10.1046/j.1461-0248.2002.00331.x

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CAPÍTULO III.

Changes in herbivory levels and insect herbivore assemblages associate to canopy of *Quercus laurina*: Importance of oak species diversity and foliar chemical defense

Marcela Sofía Vaca-Sánchez, Yurixhi Maldonado-Lopez, Ken Oyama , Guillermo Delgado-Lamas , Joan Sebastian Aguilar-Peralta , Magno Augusto Zazá, Mauricio Lopes de Faria, Marcílio Fagundes, Ma. Carmen López-Maldonado and Pablo Cuevas-Reyes[1]

Presentación

El tercer capítulo comprende evaluar los cambios en la estructura y composición de insectos fitófagos asociados con el dosel de *Q. laurina*, así como cambios en la defensa química foliar y niveles de herbivoría a lo largo del gradiente de riqueza de encinos. Para este capítulo los objetivos fueron: 1) Evaluar si el aumento en la diversidad de la comunidad de encinos resulta en un incremento de la diversidad de la comunidad de insectos fitófagos asociados con el dosel de *Q. laurina*; y ii) Determinar si el incremento de la comunidad de insectos fitófagos genera mayores niveles de herbivoría, debido a una mayor disponibilidad de recursos para los insectos fitófagos y cambios en la concentración de compuestos químicos secundarios en *Q. laurina*. Los resultados indican mayor abundancia y riqueza de insectos fitófagos, mayor porcentaje de herbivoría por insectos fitófagos y las concentraciones más altas de metabolitos asociados a defensa vegetal se encontraron en los sitios con mayor diversidad de especies de encinos. En nuestro caso, las comunidades de encino representan para los insectos fitófagos una mayor disponibilidad de recursos y nichos para ser localizados y colonizados por un mayor número de insectos fitófagos.

1   **Changes in herbivory levels and insect herbivore assemblages associate to canopy of**

2   *Quercus laurina*: **Importance of oak species diversity and foliar chemical defense**

3

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23

24   **Running Head**: **Phytophagous assemblages in oak diversity gradient**

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2

29   **Abstract**

30   The role of tree diversity on the composition of the canopy insect herbivore community in

31   temperate forests has received little attention. Some areas in central-western and southern

32   Mexico are particularly rich in oak species, where between two and up to six species can

33   co-occur at the local level. We evaluated whether more diverse oak species communities

34   harbor a greater canopy insect herbivore diversity and produce higher rates of herbivory

35   through changes in foliar secondary metabolites on *Quercus laurina* along oak diversity

36   gradient. Five study sites that represent a gradient of oak diversity and, where *Q. laurina* is

37   also widespread were selected. Five mature *Q. laurina* trees were randomly selected per

38   site to collect canopy insect herbivores using fogging techniques. Foliar secondary

39   metabolites and herbivory were measured collecting leaves from different canopy strata.

40   We also assessed the effects of local climate variables on the canopy phytophagous

41   community of *Q. laurina* along oak diversity gradient. We found a total of 3,693 insect

42   herbivores associated to *Q. laurina* trees along the oak diversity gradient. The general

43   pattern showed higher insect herbivore diversity in Tequila volcano, which represented the

44   site with the greatest diversity of oaks. We also detected a increment of the herbivory levels

45   in more diverse oak communities, as well significant relationships between chemical

46   defense and the composition of the insect herbivore community, but not with environmental

47   variables. Our findings indicate that on a local scale, oak diversity is the main factor

48   determining the diversity of insect herbivores; as well the herbivory levels and foliar

49   chemical composition of *Q. laurina* trees. We highlight the importance of conservation of

50   oak communities, because they represent key elements of temperate forest that harbor high

51   diversity of phytophagous insects and drives the plant-insect interactions.

52   **Key words**: Phytophagous insect assemblages, oak richness, bottom-up effects, secondary

3

53   metabolites.

54

4

55 **Introduction**

56 Mixed temperate forests are characterized by the co-occurrence of two or more tree species

57 that are immediate neighbours and maintain inter- and intraspecific interactions between

58 them (Bravo-Oviedo et al., 2014), resulting in an important source of ecological services

59 due to their high primary productivity (Chamagne et al., 2016; Jactel et al., 2018), stability

60 against environmental and anthropogenic changes (Morin et al., 2014; Van der Plas et al.,

61 2016) and resistance to abiotic (i.e. drought and fire) and biotic stressors (i.e. insect

62 herbivores and pathogens) (Jactel et al., 2017). Consequently, it has been suggested that

63 tree diversity is an important factor that confers resistance and resilience to forest in

64 relation to the incidence of insect herbivores (Bauhus et al., 2017). For example, mixed

65 species forests have low herbivoy levels by insects compared to monospecific forests,

66 according to a meta-analyses of planted and natural forests and biodiversity-ecosystem

67 functioning experiments (Jactel & Brockerhoff, 2007 Castagneyrol et al., 2014).

68 Additionally, it has been corroborated that trees are generally more prone to suffering

69 higher levels of damage by insect herbivores when grow in monospecific stands than when

70 are associated with other tree species (Vehviläinen et al., 2007; Castagneyrol et al., 2014;).

71 Conversely, other studies have shown neutral effects of plant diversity on the incidence of

72 generalist insect herbivores that cause foliar damage (Rosado-Sánchez et al., 2018) and

73 even an increase in foliar damage as tree diversity increases (Novotny et al., 2010) in the

74 case of host plant species of the same genus that tend to support similar insect herbivore

75 communities compared to insect communities that feed on host plant species of different

76 taxa (Ødegaard et al., 2005). Thus, plant diversity can reduce or increase the insect

77 herbivore diversity and the herbivory levels on their host plants (Barbosa et al., 2009;

78 Muiruri el al., 2019).

5

79   In this way, the vegetation complexity and characteristics of neighboring plants can

80   greatly influence the interactions among insect herbivores and the focal host plant, affecting

81   host plant selection and plant suceptibility (Plath et al., 2012; Kostenko et al., 2017). This

82   fact is known as associative effects (Root 1973, Barbosa et al., 2009) which involve direct

83   and indirect interactions between nearby plants according of two distinct hypotheses: i)

84   "The associational resistance hypothesis" (ARH) that proposes that the increase of plant

85   diversity generates heterespecific neighborhoods of plant species that can reduce the

86   damage by specialist insect herbivores on a particular plant species (Field et al., 2020). This

87   prediction is based in which a focal plant and its morphological, nutritional quality, and

88   chemical defense characteristics may experience associative resistance against insect

89   herbivores when growing in a neighborhood in close proximity to other plant species

90   (Coley & Barone, 1996; Plath et al., 2012), and ii) "The associated susceptibility

91   hypothesis" (ASH) that state that an increase in plant diversity increases the damage by

92   generalist insect herbivores due to a wider range of food resources availability to insects in

93   more diverse plant communities (Unsicker et al., 2008; Plath et al., 2011). Associative

94   susceptibility can also occur in the case when a focal plant is less preferred by insects

95   herbivores, but growing in close proximity and surrounded by a extremely preferred host

96   plants, which favors the incidence of generalist insect herbivores after depletion of the

97   preferred host plant (White & Whitham, 2000).

98   Under natural conditions, plant nutritional quality and chemical defenses may be

99   influenced by the diversity of the neighboring plant community as result of competition

100   between the host plant and its neighbours for the availability of resources (i.e. light, soil

101   nutrients, water) (Abbas et al., 2013; Kos et al., 2015; Moreira et al., 2014; Kostenko et al.

102   2017). For example, Mraja et al., (2011) documented that the catalpol concentrations (a

6

103   iridoid glycoside associated to herbivory defense) increased as the plant species richness

104   was higher in the experimental grassland plots of *Plantago lanceolata*. Some theories

105   predict that the concentration of plant secondary metabolites can be influenced by the

106   diversity of the plant community. For example, "The growth-defense trade-off hypothesis"

107   (Coley et al., 1985; Endara & Coley, 2010; Eisenhauer et al., 2009), that states that plants

108   will allocate more resources for defense in more diverse plant communities, where a greater

109   plant diversity increases the competition for nutrients, water and light, reducing growth of

110   the focal plants and the levels of herbivore attack.

111   In the Northern Hemisphere, oak (*Quercus* spp.) forests host rich communities of

112   canopy arthropods compared to other kind of forests (Valencia-Cuevas et al., 2015;

113   Maldonado-López et al., 2018). High arthropod diversity often results in a complex

114   community structure of herbivores, predators and parasites (Mitchell et al., 2019; Sierzega

115   & Eichholz, 2019). Mexico is an important center of oak diversification and endemism

116   (Hipp et al., 2018) with a total number of species close to 170 and 100 endemics (Valencia

117   2004; Hipp et al., 2018). Furthermore, a wide range of oak communities with different

118   species diversity and composition can be found (Torres-Miranda et al., 2013; Rodríguez-

119   Correa et al., 2015). Some areas in central-western and southern Mexico are particularly

120   rich in oak species, where between two and up to six species can co-occur at the local level.

121   These diversity gradients are an excellent model to evaluate different ecological aspects of

122   oak communities such as canopy arthropods composition. Our main objective was to

123   evaluate whether more diverse oak species communities harbor a greater canopy insect

124   herbivore diversity and produce higher rates of herbivory on the focal plant *Quercus*

125   *laurina* along the oak diversity gradient. The specific questions addressed were: i) Does the

126   oak diversity gradient increase the diversity of the canopy insect herbivore community in

7

127   *Q. laurina*? 2) Are *Q. laurina* chemical defense compounds and herbivory levels affected

128   by oak diversity ? and 3) Is there a relationship between leaf chemical defense, herbivory

129   levels and the insect herbivore diversity of *Q. laurina* along oak diversity gradient?

130

131   **Material and methods**

132   **Study system**

133   *Quercus laurina* Humb et Bonpl is an endemic oak species from México which belongs to

134   the section *Lobatae* (red oaks) of the genus *Quercus* (Nixon, 1993). It is a tree with a height

135   between 10 and 30 m that occurs in mixed oak and pine forests in the Sierra Madre del Sur

136   and the Trans-Mexican Volcanic Belt (TMBV) (Valencia, 1994). The leaves are

137   coriaceous, lanceolate or elliptic-oblanceolate, with a green and lustrous surface and its

138   fruit is an ovoid acorn, with an average size of 15 to 20 mm long and 15 to 17 mm in

139   diameter (Arizaga 2009). Based on data from a previous study (Vaca-Sánchez et al., 2021),

140   we selected five study sites which represent a gradient of oak diversity and where *Q.*

141   *laurina* is present in all sites. The study sites are located in the central-western portion of

142   the Trans-Mexican Volcanic Belt: 1) Tequila Volcano, 2) Los Azufres, 3) Indaparapeo, 4)

143   Carindapaz and 5) Cerro Burro (Table 1). A full characterization of oak community

144   composition is given in Supplementary Materials (Appendix 1).

145

146   **Canopy insect herbivores sampling**

147   Sampling of canopy insect herbivores was performed at the end of the rainy season

148   (September) of 2014. In each study site, five mature medium-sized trees (DBH 30-40 cm)

149   of *Q. laurina* were randomly selected to collect canopy phytophagous insects using fogging

150   techniques (Erwin & Geraci, 2009). We chose these size class of trees because larger trees

8

151   are more difficult to sample with this technique. Fogging was applied in all trees during the

152   morning, between 05:00 and 6:00 A.M. Each tree was nebulized for a period of 10 minutes

153   using a Swingfog SN-50 Thermal Fogger to disperse a mix of synergized pyrethrins (30

154   g/l) and piperonyl butoxide (150 g/l). Under each tree canopy, we place 8 funnel-shaped

155   trays of 1m^2 each one under tree canopy, and after 50 minutes, we performed the first insect

156   collection (Barringer et al., 2019). The remaining insects were captured using ropes to

157   shake the canopy after 2 h from the first insect collection (Marques et al. 2006). The insect

158   herbivores collected were stored in 70% ethanol and transported to the Agroecology

159   Laboratory of Universidad Michoacana de San Nicolás de Hidalgo, for taxonomic

160   identification. Insect herbivores were separated and identified to the family level, and were

161   assigned to feeding group: (i) exophagous (i.e. leaf-chewing and sap-sucking) and

162   endophagous insects (i.e. leaf-miners and gall-inducing insects) (Triplehorn et al., 2005,

163   Ubick et al., 2017). Abundance and species richness was estimated using morphospecies

164   criteria (recognizable taxonomic units) (Majer et al., 2000; Stiegel & Mantilla-Contreras,

165   2018).

166

167   **Foliar chemical analysis**

168   For the quantification of the secondary metabolites, in the same adult trees of *Q.*

169   *laurina s*elected to analyze the canopy insect herbivores, we collected 30 intact leaves and

170   immediately stored in liquid nitrogen. The chemical metabolites quantified were: total

171   soluble phenols, total flavonoids, proanthocyanidins and galotannines. The total content of

172   soluble phenolics was estimated using a modification of the Folin–Ciocalteau method

173   (Torres et al., 1987; Maldonado-López et al., 2015). In the case of flavonoids, the samples

174   were extracted with 80% ethanol. 0.1 ml of supernatant was diluted in 0.9 ml 80% ethanol.

9

175   A 0.5 ml aliquot was placed in a tube, and 0.3 ml $NaNO_2$ (1:20) were added. After 5 min, 3

176   ml $AlCl_3$ (1:10) was added. Six min later, the solution was mixed and the absorbance was

177   measured at 510 nm (Zhuang et al., 1992; Maldonado-López et al., 2015). The content of

178   total flavonoids was calculated from a standard curve of quercitin.

179   For tannin quantification samples were extracted with 70 % aqueous acetone,

180   allowed to stand for 1 h at room temperature with continuous stirring and centrifuged for 10

181   min at 3000 rpm. The acetone extract was reduced to the aqueous phase, and then frozen

182   and lyophilized. 0.1 % ascorbic acid was used as conservative (Hagerman 1987;

183   Maldonado-López et al., 2015). To quantify gallotannins, a modification of the method by

184   Inoue and Hagerman (1988) was used. One ml of the samples suspended in 0.2N H_2SO_4

185   were placed in a 2 ml tube and dissolved in 1 ml 1 M H_2SO_4. The samples were hydrolyzed

186   for 4 h at 100°C. A 0.1 ml aliquot of the hydrolysate was mixed with 0.3 ml of 0.667 %

187   methanlicrhodanine solution and 0.2 ml of 0.5 M KOH solution. After 2.5 min, the mixture

188   was diluted to 5 ml with distilled water. The absorbance at 520 nm was read. The rhodanine

189   assay was standardized with gallic acid) (Cuevas-Reyes et al., 2017). Soluble

190   proanthocyanidins (PAS) were measured using a modification of the method of Watterson

191   and Butler (1983). Samples were resuspended in 50 % methanol. 0.1 ml of extract and 0.6

192   ml of water were added to 6 ml 1-butanol/concentrated HCl (95:5, v/v). After mixing, the

193   tubes were placed in a bath for 50 min at 95 C. The absorbance at 555 nm was measured.

194   The standard curve was prepared with known concentrations of (+) catechin hydrate

195   (Maldonado-López et al., 2015).

196

197   **Herbivory measurements**

198   To determine the herbivory levels by leaf-chewing insects, ten adult trees of *Q. laurina*

                                                                                          10

199   were selected in each study site (five trees not previously sampled and the same five used

200   for the analysis of the canopy insect herbivores). We used a stratified sampling in the

201   canopy of the each tree , collecting three branches at each canopy stratum (lower, medium

202   and high). From each branch, 10 leaves were randomly selected (N= 30 leaves per

203   individual and N= 300 leaves per site). We took a digital image of each leaf to calculate the

204   total leaf area and the leaf area removed by insect herbivores using the program ImageJ

205   1.51j87 (https ://image j.nih.gov/ij/) (Aguilar-Peralta et al. 2020). The percentage of leaf

206   area removed by leaf-chewing insects was calculated for each leaf by (leaf area

207   consumed/total leaf area) * 100. Herbivory data was transformed as arc-sine square root to

208   meet normality (Cuevas-Reyes et al., 2018a).

209

210   **Statistical analysis**

211   *Canopy insect herbivores composition analysis*

212   Canopy insect herbivore abundance and species richness were compared between

213   study sites using a GLM analysis with a Poisson error distribution and a log link function.

214   The study sites were used as the independent variable and insect herbivore abundance and

215   species richness was considered as the response variables. Furthermore, to evaluate the

216   differences in the frequency of the insect herbivore orders between study sites, we

217   performed a logistic regression analysis using the CATMOD procedure (SAS 2008), that is

218   a general procedure for modelling categorical data. The frequency of insect herbivore

219   orders was considered as the response variable and the study sites as independent variable.

220   Because the distribution of the dependent variable data did not follow a normal distribution,

221   a Poisson distribution with a logarithmic link function was used (Stokes et al. 2000). A

222   Pielou-evenness index was used at the insect herbivore order level to estimate

11

223   phytophagous diversity in each study site. This measure of equitability compares the

224   observed Shannon-Wiener index against the distribution of individuals between the

225   observed species which would maximize diversity (Wan et al., 2014).The richness of the

226   canopy insect herbivore families of each study site was obtained by rarefaction curves

227   using the program EstimateS 9.1.0 (Colwell 2013). The scale of the independent variable (X

228   axis) was represented by the number of estimated individuals of insect herbivores in the

229   canopy. For the application of the rarefaction method, a data set was standardized and

230   compared using the number of individuals as the sampling effort (Gotelli & Colwell, 2001).

231   A 95% confidence intervals (CI) were used in each rarefaction curve to determine if the

232   differences in richness between localities was a result of true richness or due to abundance.

233   A GLM analysis was applied to determine the differences in canopy insect

234   herbivore abundance and species richness between study sites for each trophic guilds

235   groups of phytophagous insects. A Poisson error distribution and a log link function were

236   used (Stokes et al., 2000). Multivariate analyses were used to evaluate differences in

237   phytophagous composition between the five study sites. Thus, the five communities of

238   phytophagous insects were ordered by non-metric multidimensional scaling (NMDS) using

239   an abundance similitude matrix and the Bray-Curtis index as a distance metric. After, a

240   non-parametric permutation procedure (ANOSIM) was used to check whether

241   phytophagous insect species composition differed among the five study sites. The

242   ANOSIM was based on a binary matrix insect species occurrence in each study site, using

243   the Bray-Curtis index as a distance metric and 5000 permutations (Hammer et al., 2001).

244   Pairwise ANOSIMs were performed between all pairs of sites as a post-hoc test.

245

246   *Foliar secondary metabolites and herbivory levels analysis*

12

247   To analyze the differences in secondary metabolites concentration as well the

248   herbivory levels between study sites along the oak diversity gradient, we performed a one-

249   way analysis of variance (One-way ANOVA) respectively using the average values of each

250   tree. Study sites were considered as explanatory variable and the secondary metabolites

251   concentration was considered as the response variable. A LSMeans test was used for post-

252   comparison between study sites (SAS, Stokes et al. 2000). In adittion, a series of analyses

253   of covariance (ANCOVA tests) were performed to evaluate whether secondary compounds

254   covariate with the herbivory in all study sites (Maldonado-López et al. 2014). In the model,

255   study sites were considered as explanatory variable, herbivory as response variable and

256   total phenols, flavonoids, PAS and gallotanins as covariates (JMP statistical software

257   version 15.1.0) (SAS Institute Inc., Cary, NC, USA).

258

259   *Relationships between the oak community parameters and the insect herbivore community*

260   Based on multiple regression models, we used ordinary least squares (OLS) between the

261   oak community parameters and the insect herbivore community to test the effects the oak

262   community (i.e. oak species richness, Shannon diversity index (H'), total density of all oak

263   species, density of oaks Lobatae section and density of oaks Quercus section) over the

264   abundance and richness of insect herbivores, as well on the foliar secondary metabolites

265   along the oak diversity gradient. OLS is a commonly used analysis to minimize the

266   distances in the observed responses with those predicted in the linear approximations.

267   (Bystriakova et al., 2018).

268

269   *Relationships between local climate variables, leaf secondary metabolites and the insect*

270   *herbivore community*

13

271   We assessed both the effects of local climate variables and the leaf secondary metabolites

272   on the canopy insect herbivore community associated to *Q. laurina* along oak diversity

273   gradient. First, a canonical correspondence analysis (CCA) was performed to determine

274   whether there is any correlation between the local climate variables and the canopy insect

275   herbivore community in each study site. The climatic data was obtained for each of the 19

276   bioclimatic variables available in WorldClim (http://worldclim.org/version2). The climatic

277   data was downloaded at a spatial resolution of 30 arc seconds from the WorldClim dataset

278   (Global Climate Data, http://www.worldclim.org/) (Hijmans, Cameron, Parra, Jones, &

279   Jarvis, 2005) for each study site. We eliminated the redundant variables on the basis of the

280   results of a paired correlation analysis (the criterion was to eliminate one variable from

281   each pair with R > 0.80 retaining the more general variable). The variables selected were

282   mean annual temperature (MAT), mean annual precipitation (MAP), mean temperature of

283   the warmest quarter (TWQ) and mean temperature of the coldest quarter (TCQ). Second, a

284   CCA was performed to determine the relationships between foliar secondary metabolites

285   and the insect herbivore community along the oak diversity gradient. For this, we used the

286   statistical package PAST 3.23 (Hammer et al, 2001). CCA has proved valuable tool for

287   exploring species-environment correlations (Jin et al., 2016).

288

289   **Results**

290   *Insect herbivore community along the oak diversity gradient*

291   We registered a total of 3,693 insect herbivores associated to *Q. laurina* individuals

292   growing along the oak diversity gradient distributed as follows: 1,909 in Tequila Volcano,

293   223 in Los Azufres, 544 in Indaparapeo, 488 in Carindapaz and 529 in Cerro Burro. All

294   insect herbivores were separated in 485 different morphospecies grouped in 7 orders

14

295   (Figure 2) (For more details, see Appendix 1). Overall, the frequency of insect herbivore

296   orders was different between study sites (χ^2 = 928.38; d.f. = 4; P < 0.0001). The most

297   frequent orders in all study sites was Coleoptera, followed by Hemiptera and Lepidoptera.

298   Particularly, Tequila Volcano, which was the site with higher oak species richness had a

299   higher frequency of the Coleoptera (58.87%), Lepidoptera (16.34%), Hymenoptera (

300   6.75%) and Thysanoptera (5.76 %), while the site with lowest richness of oaks (i.e. Cerro

301   Burro) had 36.95 % of Coleoptera, 8.55 % of Lepidoptera and (2.92%) of Hymenoptera

302   (Figure 1).

303   Shannon's diversity index values per site at the order level were for Tequila

304   Volcano = 2.41, Los Azufres = 2.19, Indaparapeo = 2.21, Carindapaz = 2.32 and Cerro

305   Burro =2.03. All study sites differed in the evenness of the abundance of orders according

306   to Pielou-evenness index, which ranged from J = 0.62 in Tequila Volcano to J = 0.93 in

307   Cerro Burro. The rarefaction analysis showed that the richness of the insect herbivore

308   families was significantly higher in the Volcano Tequila, followed by Indaparapeo, Cerro

309   Burro, Carindapaz and Los Azufres (Figure 2).

310   Insect herbivore abundance (measured as the mean number of insect herbivores per

311   tree) associated to the canopy of *Q. laurina* was different along the oak diversity gradient

312   (χ^2 = 799.03; d.f. = 4, P < 0.0001). Trees of *Q. laurina* in the Tequila Volcano harbored

313   higher insect herbivore abundance (381.8 ± 1.73) in comparison with trees growing in Los

314   Azufres (55.75 ± 0.30), Indaparapeo (108.8 ± 0.63), Carindapaz (97.7 ± 1.40) and Cerro

315   Burro (105.8 ± 1.22). Similarly, the mean richness of morphospecies of insect herbivores

316   per tree was greater in the Tequila Volcano (60.8 ± 2.62) than in Los Azufres (32 ± 1.03)

317   and Indaparapeo (43.6± 5.67 SE) and lower in Carindapaz (27.4 ± 3.8) and Cerro Burro

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318   (29.2 ± 1.3) $(\chi^2 = 104.5$; d.f. = 4, $P < 0.0001)$.

319   Overall, the insect herbivore guilds were represented as follows: leaf-chewing

320   (69.72 %), sap-sucking (14.02 %), gall-inducing (9.72 %) and leaf-miners (6.52 %) (For

321   more details, see Appendix 2). The abundance of insect herbivore guilds differed

322   significantly between the study sites $(\chi^2 = 460.04$; d.f. = 4, $P < 0.0001)$. Leaf-chewing

323   insects had the higher abundance along the oak diversity gradient in the Tequila Volcano

324   (311.2 ± 2.33) followed by Indaparapeo (85.8 ± 0.89) while the lowest abundance was

325   detected in the site with less oak diversity (Los Azufres) (28.25 ± 0.38). Gall-Inducing

326   insects were the second most abundant guild in Tequila Volcano (38.6 ± 2.24). Contrary,

327   Cerro Burro had the higher abundance of the sap-sucking insects in comparison with the

328   other study sites $(\chi^2 = 6.84$; d.f. = 4, $P < 0.018)$ (Figure 3a).

329   The proportion of morphospecies belonging to each insect herbivore guild also

330   varied among study sites. Leaf-chewing morphospecies represented 71.05% in the Tequila

331   Volcano, while they represented 33.57% in Carindapaz, with intermediate values in the

332   other three sites $(\chi^2 = 102.78$; d.f. = 4, $P < 0.0001)$. Significant differences were also

333   observed for leaf-miner insects $(\chi^2 = 8.95$; d.f. = 4, $P < 0.008)$, gall-inducing insects $(\chi^2 =$

334   6.71; d.f. = 4, $P < 0.0009)$ and sap-sucking insects $(\chi^2 = 20.13$; d.f. = 4, $P < 0.0001)$ (Figure

335   3b).

336   The results of the similarity of insect herbivores community showed that NMDS

337   ordination explained 90.5 % of the variance between sampling points (axis 1 = 90 % and

338   axis 2 = 0.5 %), where each point is a two-dimensional representation of the composition of

339   insect herbivore species in a single tree based on global NMDS. The composition of insect

340   herbivores associated to *Q. laurina* varied between the study sites (ANOSIM r = 0.28, n =

16

341   24, *P*< 0.002) (Figure 4). The results of post-hoc pairwise ANOSIM tests showed different

342   composition of insect herbivore species between all study sites. Particularly, the Tequila

343   Volcano and Carindpaz had totally different assemblages in comparison with the other

344   three study sites (*P* < 0.05).

345

346   *Chemical composition and foliar herbivory levels*

347   We found statistically significant differences in the leaf secondary metabolites

348   concentration between individuals of *Q. laurina* along the oak diversity gradient: total

349   phenols concentration (*F* = 2.70; d.f = 4; *P* < 0.04), flavonoids (*F* = 5.17; d.f = 4; *P* <

350   0.001; proanthocyanidins (PAS) (*F* = 3.50; d.f = 4; *P* < 0.01) and gallotanins (*F* = 2.84; d.f

351   = 4; *P* < 0.03) (Table 2). A general pattern was detected, *Q. laurina* trees growing in the

352   site with the greatest oak species richness (i.e. Tequila volcano) had higher total phenols

353   concentration, flavonoids, proanthocyanidins (PAS) and gallotanins concentration in

354   comparison with *Q. laurina* individuals occurring in sites with less oak diversity (see Table

355   2).

356   The One-way ANOVA test for herbivory showed that the amount of leaf area

357   consumed by leaf-chewing insects differed significantly between the five study sites (*F* =

358   3.71; d.f = 4; *P* < 0.01). Along the oak diversity gradient, *Q. laurina* trees growing in

359   theTequila volcano had higher herbivory levels in comparison to the other study sites

360   (Figure 5). Aditionally, a significant effects of gallotanins and PAS on herbivory levels

361   were detected (Table 3). Contrary, according to ANCOVA test, no significant effects of

362   total phenols and flavonoids on herbivory was found (Table 3).

363   Our results indicated that the abundance and species richness of insect herbivores

364   associated to the canopy of *Q. laurina* were significantly correlated to the oak community

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365   parameters. In particular, we found significant positive relationships between insect

366   herbivores abundance and the oak species richness (R^2=0.32, F= 3.20, P< 0.03) and

367   Shannon diversity index (H') (R^2 =0.38, F= 2.26, P<0.03), as well as with the total density

368   of oak individuals (R^2 =0.66, F= 5.37, P<0.001) and the density of oak individuals of the

369   *Lobatae* section (R^2 =0.65, F= 5.48, P<0.001) (Table 4). For the case of the insect

370   herbivore richness, we found a significant relationship with the Shannon diversity index (R^2

371   =0.31, F= 2.47, P<0.02) as well with the density of oak individuals of the *Lobatae* section

372   (R^2 = 0.65, F= 5.48, P<0.001) (Table 4).

373   Foliar secondary metabolites showed significant relationships with some oak

374   community parameters: 1) the total phenols concentration were negative related with the

375   oak species richness (R^2=0.24, F=2.66, P< 0.01), the total density of oak individuals (R^2

376   =0.28, F=2.94, P<0.001) as well with the density of oak individuals of the *Lobatae* section

377   (R^2 = 0.44, F= 3.54, P<0.001). Similarly, gallotanins concentration were positively related

378   with four oak community parameters, except the density of oaks of the Quercus section (see

379   Table 4). In contrast, a positive relationship was detected between proanthocyanidins and

380   the total density of oak individuals (R^2 =0.28, F= 2.21, P<0.03).

381   Finally, the Canonical correspondence analyses (CCA) between foliar chemical

382   defense (i.e. secondary metabolites) and the insect herbivore community along the oak

383   diversity gradient showed significant correlations according to the Permutation test of all

384   canonical axes (Trace = 0.11, P= 0.01). The first two axes explained 89.84% (axis 1=

385   79.13% and axis 2= 10.71%) of the spatial variation of the insect herbivore community

386   relative to secondary metabolites, where total phenols, flavonoids, proanthocyanidins

387   (PAS) and gallotannis had a strong correlation with the insect herbivore composition.

388   Particularly, the insect herbivore community of the Tequila Volcano was more influenced

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389 by the flavonoids, proanthocyanidins (PAS) and gallotannis; being the leaf-chewing insects

390 the most influenced by the presence of these secondary metabolites(Figure 6a). Contrary,

391 the Canonical correspondence analysis (CCA) between local climatic variables and the

392 insect herbivore community along the oak diversity gradient did not show significant

393 correlations according to the Permutation test that included all canonical axes (Trace =

394 0.1494, *P* = 0.5). This result indicates that the composition of the phytophagous insect

395 community associated to *Q. laurina* was not influenced by local environmental variables

396 present in the studys sites, (Figure 6b).

397

398 **Discussion**

399   Plant diversity effects on chemical defense composition and its potential links with

400 insect herbivore community might be regulate plant-insect interactions along the plant

401 communities (Koricheva et al., 2017, Ebeling et al., 2019). Our study is one of the firsts to

402 analyze the effects of the oak diversity on both leaf chemical defenses and the assembling

403 of insect herbivore community. Overall, we found 485 different morphospecies of insect

404 herbivores grouped in 7 orders that were associated to the canopy of *Q. laurina* along oak

405 diversity gradient. Additionally, we detected that insect herbivore species composition was

406 different along the oak diversity gradient, as well as a greater insect herbivore species

407 diversity on trees growing in sites with higher oak diversity such as the Volcano Tequila.

408 These results can be explained by "The plant species richness hypothesis" proposed by

409 Fernandes and Price (1988), that suggest that communities with greater plant diversity

410 represent potential resources and niches available to be colonized by a greater number of

411 insect herbivore species ("bottom-up effects") (Ebeling et al.2018). Our results agree with

412 others studies at the local scale that have shown a positive relationship between plant

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413   species richness and herbivores insect richness (Novotny et al., 2002; Cuevas-Reyes et al.,

414   2004). Similarly, a greater diversity of insect herbivores present in highly diverse plant

415   communities can increase the higher trophic levels as predators, affecting the community

416   structure of arthropods (Haddad et al., 2011; Moreira et al., 2016; Randlkofer et al., 2018).

417   Therefore, plant diversity can be a key factor that determine the arthropod diversity along

418   diversity gradients (Knops et al., 1999; Haddad et al., 2011). We also found that the

419   abundance and richness of insect herbivores of the canopy of *Q. laurina* were significantly

420   correlated to some parameters of the oak community such as the abundance and the oak

421   species richness, Shannon diversity index, as well as with the total density of oaks and the

422   density of oak individuals of the *Lobatae* section. These results confirm the fact that the oak

423   communities with higher species richness represent to insect herbivores a higher resources

424   and niches availability to be colonized by a greater number of insects, where species

425   richness of insect herbivores of the canopy of *Q. laurina* increases as more oak species are

426   available. Our results accord with a meta-analysis involving 52 independent comparison

427   (i.e 18 correlations derived from gall-inducing insects and 34 from non-galling herbivores)

428   (Araújo 2013), confirming that insect herbivore diversity increase as the diversity of plants

429   increases.

430   In the same way, our results showed that the herbivory levels by leaf-chewing

431   insects were higher in the Tequila volcano, that was the site with highest diversity of oak

432   species. This result can be explained by the higher abundance of leaf-chewing insects

433   registered in sites with highest oak diversity and that belonging to families of generalist

434   insects (i.e. Pyrallidae, Lymantridae, Geometridae, Arctiidae). This fact is consistent with

435   the idea that plant diversity is a key factor that may increase the plant susceptibility to

436   insect herbivore attack according to the associative susceptibility hypothesis (ASH) (Brown

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437   and Ewel 1987; Barbosa et al. 2009) that propose an increase in plant diversity increases

438   the damage by generalist insect herbivores due to a wider range of food resources

439   availability to insects in more diverse plant communities (Unsicker et al., 2008; Plath et al.,

440   2011). An alternative explanation is that *Q. laurina* trees were less preferred by this

441   generalist insect herbivores, but growing in close proximity and surrounded by a extremely

442   preferred host plants (other oak species), which favors the incidence of generalist insect

443   herbivores after depletion of the preferred host plant (Unsicker et al., 2008; Karban et al.,

444   2010; Castagneyrol et al., 2014; Welti et al., 2017). For example, Castagneyrol et al. (2014)

445   found that phylogenetic distance between the focal plant and alternative host plant species

446   that occur in the same neighborhood is a important factor that determinate de percentages

447   of foliar damage caused by generalist insect herbivores, where more related host plants are

448   more likely to share functional traits involved in host recognition and exploitation by

449   insects (Gómez et al., 2010; Wiens et al., 2010) and therefore, are more prone to share the

450   same herbivores (Ødegaard et al., 2005; Weiblen et al., 2006).

451   In the other hand, it has been also proposed that the changes in foliar chemical

452   composition affects the insect herbivore community to be the direct link between plants and

453   insect herbivores (Zunjarrao et al., 2019). In our study, the foliar secondary metabolites

454   concentration varied along the oak diversity gradient, where *Q. laurina* trees growing in

455   sites with the highest diversity of oaks (i.e. Tequila Volcano and Los Azufres) had higher

456   concentration of total phenols, flavonoids, PAS and gallotannins in comparison with less

457   diverse oak sites such as Cerro Burro and Carindapaz. Aditionally, we detected three

458   significant relationships between the oak community parameters and foliar secondary

459   metabolites: proanthocyanidins and gallotanins concentration showed a positive

460   relationship with the oak diversity, as well the total phenols concentration had a negative

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461   relationship to oak diversity. These results can be explained by "The growth-defense trade-

462   off hypothesis" (Eisenhauer et al., 2009) state that plants will allocate more resources for

463   chemical defense in more diverse plant communities, due greater plant diversity increases

464   the competition for nutrients, water and light reducing plant growth. In our case, it is

465   possible that some compounds such as total phenols and flavonoids may be more associated

466   with other physiological functions such as leaf photoprotection and not act as a efectively

467   defenses against generalist herbivores because individuals of *Q. laurina* that occurred in

468   sites of greater oak diversity had higher levels of herbivory and higher concentration of this

469   secondary metabolites (Barbehenn et al., 2011). For example, Rosado-Sánchez et al. (2018)

470   showed a negative effects of the tree diversity on total phenols and phenolic compounds in

471   *Swietenia macrophylla* and *Tabebuia rosea* between two types of conditions (i.e

472   monoculture vs polyculture).

473   Another important result is the relationship detected between secondary metabolites

474   concentration and the insect herbivory community along the oak diversity gradient. We

475   detected that total phenols, flavonoids, PAS and Gallotannis concentration had a strong

476   correlation with the insect herbovore composition; particularly with the leaf-chewing insect

477   guild. This relationship could be explain by "The hypothesis of the specialist-generalist

478   dilemma" (Van der Meijden 1996) that proposes that the concentration of secondary plant

479   compounds in a plant depends on the proportion of generalist and specialists herbivorous.

480   In our case, the different proportion of generalist phytophagous (i.e. leaf-chewing insects)

481   between the study sites may be indirecting driven by changes in concentration of chemical

482   compounds associated with the defense against generalist herbivores, as a consecuences of

483   changes in the diversity of oaks along the diversity gradient. This result match with Moreira

484   et al. 2014, which reported increased production of anti-herbivores, such as phenolic

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485   compounds (i.e. total phenols) chemical defense and changes in herbivory levels on

486   *Swietenia macrophylla* in sites with highest plant diversity

487   In conclusion, our study showed changes in insect herbivore diversity of the canopy

488   of *Q. laurina* along the oak diversity gradient. The general pattern showed higher insect

489   herbivore diversity in the Tequila volcano, which represented the site with the greatest

490   diversity of oaks. Also, we detected a increment of the herbivory levels in more diverse oak

491   communities, as well a changes in the composition of the insect herbivore community. Our

492   findings confirm that at the local scale, oak species richness is the main factor that

493   determines the phytophagous insects diversity associated to canopy of *Q. laurina*, as well

494   the herbivory levels and foliar chemical composition of *Q. laurina*. We highlight the

495   relevance of conserving oak species, because they are key elements of temperate forest in

496   the North Hemisphere that harbor high diversity of insect herbivores and are important for

497   the maintenance of biotic interactions in this ecosystems.

498

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505   **Data availability:** Data will be made available upon request.

506   **Authors' contributions:** All authors contributed to the study conception and design.

507   Material preparation, data collection and analysis were performed by Marcela Sofía Vaca-

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509   Mauricio Lopes de Faria, Marcílio Fagundes and Pablo Cuevas-Reyes. The first draft of the

510   manuscript was written by [Marcela SofíaVaca-Sánchez], [Yurixhi Maldonado-Lopez],

511   [Ken Oyama], [Guillermo Delgado] ,[Pablo Cuevas-Reyes] and all authors commented on

512   previous versions of the manuscript. All authors read and approved the final manuscript.

513

514   **References**

515   Abbas M, Ebeling A, Oelmann Y, Ptacnik R, Roscher C, Weigelt A, Weisser WW, Wilcke

516   W, Hillebrand H (2013) Biodiversity effects on plant stoichiometry. PLoS ONE 8:

517   e58179. doi:10.1371/journal.pone.0058179

518   Aguilar-Peralta JS, González-Rodríguez A, Maldonado-López, Y, Fagundes M, Faria ML,

519   Ávila-Cabadilla LD, …Cuevas-Reyes, P (2020) Contrasting patterns of

520   morphology, fluctuating asymmetry and leaf herbivory in three plant species of

521   different successional stages of a tropical dry forest. Trees. doi:10.1007/s00468-

522   020-01982-z

523   Arizaga S, Martínez-Cruz J, Salcedo-Cabrales M y Bello-González MA (2009) Manual de

524   la biodiversidad de encinos michoacanos. Instituto Nacional de Ecología ed.

525   México, D.F.

526   Barbehenn, RV & Constabel PC (2011) Tannins in plant–herbivore interactions.

527   Phytochemistry 72(13), 1551–1565. doi:10.1016/j.phytochem.2011.01.040

528   Barbosa P, Hines J, Kaplan I, Martinson H, Szczepaniec A, Szendrei Z (2009)

529   Associational resistance and associational susceptibility: having right or wrong

530   neighbors. Annual Review of Ecology, Evolution, and Systematics 40: 1–20.

531   doi:10.1146/annurev.ecolsys.110308.120242

532   Barringer, LE, Bartlett CR, & Erwin TL (2019) Canopy assemblages and species richness

533   of planthoppers (Hemiptera: Fulgoroidea) in the Ecuadorian Amazon. Insecta

534   Mundi.

535   Bauhus J, Forrester DI, Gardiner B, Jactel H, Vallejo R & Pretzsch H (2017) Ecological

536   Stability of Mixed-Species Forests. Mixed-Species Forests 337–382.

537   doi:10.1007/978-3-662-54553-9_7

25

538   Bravo-Oviedo, Andres et al. (2014) European Mixed Forests: definition and research

539   perspectives. Forest Systems 23(3), 518-533.

540   Bystriakova N, Griswold T, Ascher JS et al. (2018) Key environmental determinants of

541   global and regional richness and endemism patterns for a wild bee subfamily.

542   Biodiversity and Conservation 27(2),287–309.doi.org/10.1007/s10531-017-1432-7

543   Castagneyrol B, Giffard B, Pere C, Jactel H (2013) Plant apparency, an overlooked driver

544   of associational resistance to insect herbivory. Journal of Ecology 101: 418–429.

545   doi:doi: 10.1111/1365-2745.12055

546   Castagneyrol B, Jactel H, Vacher C, Brockerhoff EG & Koricheva, J (2014) Effects of

547   plant phylogenetic diversity on herbivory depend on herbivore specialization.

548   Journal of Applied Ecology, 51(1), 134-141. doi:10.1111/1365-2664.12175

549   Chamagne J, Tanadini, M, Frank D, Matula R, Paine CT, Philipson CD, ... & Hector A

550   (2017) Forest diversity promotes individual tree growth in central European forest

551   stands. Journal of Applied Ecology, 54(1), 71-79. doi: 10.1111/1365-266412783

552   Coley PD & Barone JA (1996) Herbivory and plant defenses in tropical forests. Annual

553   review of ecology and systematics, 27(1), 305-335.

554   doi:10.1146/annurev.ecolsys.27.1

555   Coley PD, Bryant JP & Chapin FS (1985) Resource Availability and Plant Anti herbivore

556   Defense. Science, 230(4728), 895-899. doi:10.1126/science.230.4728.895

557   Colwell RK (2013) EstimateS: Statistical estimation of species richness and shared species

558   from samples. Version 9. User's Guide and application published at:

559   http://purl.oclc.org/estimates.

560   Cuevas-Reyes P, Canché-Delgado A, Maldonado-López Y, Fernandes GW, Oyama K, &

561   González-Rodríguez A (2018) Patterns of herbivory and leaf morphology in two

26

562   Mexican hybrid oak complexes: Importance of fluctuating asymmetry as indicator

563   of environmental stress in hybrid plants. Ecological Indicators, 90, 164–170.

564   doi:10.1016/j.ecolind.2018.03.009

565 Cuevas-Reyes P, Oyama K, González-Rodríguez A, Fernandes GW & Mendoza-Cuenca, L

566   (2011) Contrasting herbivory patterns and leaf fluctuating asymmetry in

567   *Heliocarpus pallidus* between different habitat types within a Mexican tropical dry

568   forest. Journal of Tropical Ecology, 27(4), 383-391.

569   doi:10.1017/s026646741100006x

570 Cuevas-Reyes P, Pérez-López G, Maldonado-López Y & González-Rodríguez A (2017)

571   Effects of herbivory and mistletoe infection by *Psittacanthus calyculatus* on

572   nutritional quality and chemical defense of *Quercus deserticola* along Mexican

573   forest fragments. Plant Ecology, 218(6), 687–697. doi:10.1007/s11258-017-0721-2

574 Cuevas-Reyes P, Canché-Delgado A, Maldonado-López Y, Fernandes GW, Oyama K,

575   González-Rodríguez A (2018a) Patterns of herbivory and leaf morphology in two

576   Mexican hybrid oak complexes: Importance of fluctuating asymmetry as indicator

577   of environmental stress in hybrid plants. Ecological Indicators 90,164–170.

578   doi.org/10.1016/j.ecolind.2018.03.009

579 De Araújo WS (2013) Different relationships between galling and non-galling herbivore

580   richness and plant species richness: a meta-analysis. Arthropod-Plant Interactions,

581   7(4), 373–377. doi:10.1007/s11829-013-9259-y

582   doi.org/10.1016/j.biocon.2019.03.040

583 Ebeling A, Hines J, Hertzog LR, Lange M, Meyer ST, Simons NK & Weisser WW (2018)

584   Plant diversity effects on arthropods and arthropod-dependent ecosystem functions

585   in a biodiversity experiment. Basic and Applied Ecology, 26, 50–63.

27

586   doi:10.1016/j.baae.2017.09.014

587   Eisenhauer N, Milcu A, Nitschke N, Sabais ACW, Scherber C & Scheu S (2009)

588   Earthworm and belowground competition effects on plant productivity in a plant

589   diversity gradient. Oecologia, 161(2), 291–301. doi:10.1007/s00442-009-1374-1

590   Endara & Coley (2010) The resource availability hypothesis revisited: a meta-analysis.

591   Functional Ecology, 25(2), 389-398.doi.org/10.1111/j.1365-2435.2010.01803.x

592   Erwin TL, Geraci CJ (2009) Chapter 4. Amazonian rainforests and their richness of

593   Coleoptera, a dominant life form in the critical zone of the Neotropics. p. 49–67.

594   Field E, Castagneyrol B, Gibbs M, Jactel H, Barsoum N, Schönrogge K & Hector A (2020)

595   Associational resistance to both insect and pathogen damage in mixed forests is

596   modulated by tree neighbour identity and drought. Journal of Ecology, 108(4),

597   1511-1522. doi: 10.1111/1365-2745.13397

598   Garibaldi LA, Kitzberger T, Ruggiero A (2011) Latitudinal decrease in folivory within

599   Nothofagus pumilio forests: dual effect of climate on insect density and leaf traits?

600   Global Ecology and Biogeography 20:609-619.

601   Gómez JM, Verdú M & Perfectti, F (2010) Ecological interactions are evolutionarily

602   conserved across the entire tree of life. Nature, 465(7300), 918–921.

603   doi:10.1038/nature09113

604   Gotelli NJ & Colwell RK (2001) Quantifying biodiversity: procedures and pitfalls in the

605   measurement and comparison of species richness. Ecology letters, 4(4), 379-391.

606   Haddad NM, Crutsinger GM, Gross K, Haarstad J, Tilman D (2011) Plant diversity and the

607   stability of foodwebs. Ecology Letters 14:42-46. doi.org/10.1111/j.1461-

608   0248.2010.01548.x

609   Hagerman AE (1987) Radial diffusion method for determining tannin in plant extracts.

28

610   Journal of Chemical Ecology 13:437–449. doi: 10.1007/BF01880091

611   Hammer Ø, Harper DA, Ryan PD (2001) PAST: Paleontological statistics software

612   package for education and data analysis. Palaeontol electron, 4(1), 9. http://palaeo-

613   electronica.org/2001_1/past/issue1_01.htm.

614   Hipp AL, Manos PS, González-Rodríguez A, Hahn M, Kaproth M, McVay JD, Avalos SV

615   and Cavender-Bares J (2018) Sympatric parallel diversification of major oak clades

616   in the Americas and the origins of Mexican species diversity. New Phytologist

617   217:439–452. doi: 10.1111/nph.14773

618   Hijmans RJ, Cameron SE, Parra JL, Jones PG & Jarvis A (2005) Very high resolution

619   interpolated climate surfaces for global land areas. International Journal of

620   Climatology: A Journal of the Royal Meteorological Society 25(15), 1965–

621   1978.doi.org/10.1002/joc.1276

622   Inoue KH, Hagerman AE (1988) Determination of gallotannin with rhodanine. Analytical

623   Biochemistry 169:363–369

624   Jactel H, Bauhus J, Boberg J, Bonal D, Castagneyrol B, Gardiner B & Brockerhoff, EG

625   (2017) Tree diversity drives forest stand resistance to natural disturbances. Current

626   Forestry Reports, 3(3), 223-243.doi:10.1007/s40725-017-0064-1

627   Jactel H, Brockerhoff EG (2007) Tree diversity reduces herbivory by forest insects.

628   Ecology Letters, 10(9), 835-848. doi:10.1111/j.1461-0248.2007.01073.x

629   Jactel H, Gritti ES, Drössler L, Forrester DI, Mason WL, Morin X, et al. (2018) Positive

630   biodiversity–productivity relationships in forests: climate matters. Biology Letters,

631   14(4), 20170747. doi:10.1098/rsbl.2017.0747

632   Jin J, Wang Y, Jiang H, Kong Y, Lu X & Zhang X (2016) Improvement of ecological

633   geographic regionalization based on remote sensing and canonical correspondence

29

634 analysis: A case study in China. Science China Earth Sciences, 59(9), 1745–1753.

635 doi:10.1007/s11430-016-5297-5

636 Karban R, Karban C, Huntzinger M, Pearse I & Crutsinger G (2010) Diet mixing enhances

637 the performance of a generalist caterpillar, *Platyprepia virginalis*. Ecological

638 Entomology, 35(1), 92–99. doi:10.1111/j.1365-2311.2009.01162.x

639 Koricheva J, Barantal S & Iason G (2017) Relative importance of tree species and

640 genotypic diversity for above- and below ground processes in boreal forest. Impact,

641 2017(1), 58–60. doi:10.21820/23987073.2017.1.58

642 Kos M, Bukovinszky T, Mulder PPJ & Bezemer TM (2015) Disentangling above- and

643 belowground neighbor effects on the growth, chemistry, and arthropod community

644 on a focal plant. Ecology, 96(1), 164–175. doi:10.1890/14-0563.1

645 Kostenko O, Mulder PPJ, Courbois M & Bezemer TM (2017) Effects of plant diversity on

646 the concentration of secondary plant metabolites and the density of arthropods on

647 focal plants in the field. Journal of Ecology, 105(3), 647–660. doi:10.1111/1365-

648 2745.12700

649 Majer JD, Recher HF, Ganesh S (2000) Diversity patterns of eucalypt canopy arthropods in

650 eastern and western of Australian. Ecological Entomology 25:295–306.

651 doi.org/10.1046/j.1365-2311.2000.00259.x

652 Maldonado-López Y, Cuevas-Reyes P, González-Rodríguez A, Pérez-López G, Acosta-

653 Gómez C & Oyama K (2015) Relationships among plant genetics, phytochemistry

654 and herbivory patterns in *Quercus castanea* across a fragmented landscape.

655 Ecological Research, 30(1), 133–143. doi:10.1007/s11284-014-1218-2

656 Maldonado-López Y, Vaca-Sánchez MS, González-Rodríguez A, Oyama K, López-

657 Barbosa, Fagundes M, Cuevas-Reyes P (2018). Hybridization increases canopy

30

658   arthropod diversity in the *Quercus affinis × Quercus laurina* complex. Journal of

659   Insect Conservation, 22(5), 781-793. doi.org/10.1007/s10841-018-0103-7

660   Marques MI, Adis J, dos Santos GB, Battirola LD (2006) Terrestrial arthropods from tree

661   canopies in the Pantanal of Mato Grosso, Brazil. The Revista Brasileira de

662   Entomologia ,50:257–267. doi.org/10.1590/S0085-56262006000200007

663   Mitchell RJ, Bellamy PE, Ellis CJ, Hewison RL, Hodgetts NG, Iason GR, ... & Taylor AF

664   S (2019) Collapsing foundations: The ecology of the British oak, implications of its

665   decline and mitigation options. Biological Conservation, 233, 316-327.

666   Moreira X, Abdala-Roberts L, Parra-Tabla V & Mooney KA (2014) Positive effects of

667   plant genotypic and species diversity on anti-herbivore defenses in a tropical tree

668   species. PLoS ONE 9(8)e105438. doi:10.1371/journal.pone.0105438

669   Morin X, Fahse L, de Mazancourt C, Scherer-Lorenzen M & Bugmann H (2014) Temporal

670   stability in forest productivity increases with tree diversity due to asynchrony in

671   species dynamics. Ecology letters, 17(12), 1526-1535. doi: 10.1111/ele.12357

672   Mraja A, Unsicker SB, Reichelt M, Gershenzon J & Roscher C (2011) Plant community

673   diversity influences allocation to direct chemical defence in *Plantago lanceolata*.

674   PLoS ONE, 6(12),e28055.doi:10.1371/journal.pone.0028055

675   Muiruri EW, Barantal S, Iason GR, Salminen JP, Perez-Fernandez E & Koricheva J (2019)

676   Forest diversity effects on insect herbivores: do leaf traits matter?. New Phytologist,

677   221(4), 2250-2260.doi: 10.1111/nph.15558

678   Nixon KC (1993) The genus *Quercus* in Mexico. In: Ramammoorthy TP, Bye R, Lot A, Fa

679   J (eds) Biological diversity of Mexico: origins and distribution. Oxford University

680   Press, New York,USA.

681   Novotny V, Miller SE, Baje L, Balagawi S, Basset Y, Cizek L ... & Weiblen GD (2010)

31

682   Guild-specific patterns of species richness and host specialization in plant–herbivore

683   food webs from a tropical forest. Journal of Animal Ecology, 79(6), 1193-1203.

684   doi.org/10.1111/j.1365-2656.2010.01728.x

685   Ødegaard F, Diserud OH & Østbye K (2005) The importance of plant relatedness for host

686   utilization among phytophagous insects. Ecology Letters, 8(6), 612-617.doi:

687   10.1111/j.1461-0248.2005.00758.x

688   Plath M, Mody K, Potvin C & Dorn S (2011) Establishment of native tropical timber trees

689   in monoculture and mixed-species plantations: small-scale effects on tree

690   performance and insect herbivory. Forest Ecology and Management, 261(3), 741-

691   750.doi:10.1016/j.foreco.2010.12.004

692   Rodríguez-Correa H, Oyama K, MacGregor-Fors I, González-Rodríguez A (2015) How are

693   oaks distributed in the neotropics? A perspective from species turnover, areas of

694   endemism, and climatic niches. International Journal of Plant Sciences 176:222–

695   231.doi.org/10.1086/679904

696   Root RB (1973) Organization of a plant–arthropod association in simple and diverse

697   habitats: the fauna of collards (*Brassica oleracea*). Ecological Monographs 43:95-

698   doi.org/10.2307/1942161

699   Rosado-Sánchez S, Parra-Tabla V, Betancur-Ancona D, Moreira X & Abdala-Roberts L

700   (2018) Effects of tree species diversity on insect herbivory and leaf defenses in

701   *Cordia dodecandra*. Ecological Entomology, 43(6), 703-711.

702   doi.org/10.1111/een.12648

703   SAS Institute JMP®, Version 2008, Inc., Cary, NC, USA ISBN 978-1-59994-924-6

704   Sierzega KP, Eichholz MW (2019) Linking conservation implications of modified

705   disturbance regimes, plant communities, plant associations, and arthropod

32

706   communities. Oecologia 189, 267–277.doi.org/10.1007/s00442-018-4292-2

707   Stiegel S, Mantilla-Contreras J (2018) Environment vs. plant ontogeny: Arthropod

708   herbivory patterns on European beech leaves along the vertical gradient of

709   temperate forests in Central Germany. Insects 9:9-28.

710   doi.org/10.3390/insects9010009

711   Stokes ME, Davis CS and Koch GG (2000) Categorical Data Analysis Using the SAS

712   System. John and Wiley and Sons, Inc., USA., pp: 626.

713   Torres AM, Mau-Lastovicka T, Rezaaiyan R (1987) Total phenolics and high-performance

714   liquid chromatography of phenolic acids of avocado. Journal of Agricultural and

715   Food Chemistry 35:921–925.doi: 10.1021/jf00078a018

716   Torres-Miranda A, Luna-Vega I, Oyama K (2013) New approaches to the biogeography

717   and areas of endemism of red oaks (Quercus L., Section Lobatae). Systematic

718   Biology 62(4), 555-573.doi.org/10.1093/sysbio/syt021

719   Triplehorn CA, Johnson NF, Borror DJ (2005) An introduction to the study of insects.

720   Thompson Brooks/Cole, Belmont

721   Ubick DP, Paquin P, Cushing-Roth VD (2017) Spiders of North America. An Identification

722   Manual. Second Edition. (Eds) American Arachnological Society. Keene, New

723   Hampshire U.S.A. 377pp.

724   Unsicker SB, Oswald A, Köhler G & Weisser WW (2008) Complementarity effects

725   through dietary mixing enhance the performance of a generalist insect herbivore.

726   Oecologia, 156(2), 313-324. doi:10.1007/s00442-008-0973-6

727   Vaca-Sánchez MS, González-Rodríguez A, Maldonado-López Y, Oyama K, de Faria ML,

728   Fagundes M & Cuevas-Reyes P (2021) Genetic and functional leaf traits variability

729   of *Quercus laurina* along an oak diversity gradient in Mexico. European Journal of

33

730   Forest Research, 1-15.doi.org/0.1007/s10342-021-01401-z

731   Valencia S (1994) Contribución a la delimitación taxonómica de tres especies del género

732   Quercus subgénero *Erythrobalanus*. Tesis de Maestría, Facultad de Ciencias,

733   Universidad Nacional Autónoma de México, México, D.F., México.

734   Valencia-Cuevas L, Mussali-Galante P, Piñero D, Castillo-Mendoza E, Rangel-Altamirano

735   G, Tovar-Sánchez E (2015) Hybridization of *Quercus castanea* (Fagaceae) across a

736   red oak species gradient in Mexico. Plant Systematics and Evolution 301:1085–

737   1097.doi.org/10.1007/s00606-014-1151-4

738   Van der Meijden E (1996) Plant defence, an evolutionary dilemma: contrasting effects of

739   (specialist and generalist) herbivores and natural enemies. Proceedings of the 9th

740   International Symposium on Insect-Plant Relationships, 307–310. doi:10.1007/978-

741   94-009-1720-0_69

742   Van der Plas F, Manning P, Soliveres S, Allan E, Scherer-Lorenzen M, Verheyen K, ... &

743   Fischer M (2016) Biotic homogenization can decrease landscape-scale forest

744   multifunctionality. Proceedings of the National Academy of Sciences, 113(13),

745   3557-3562. doi:10.1073/pnas.1517903113

746   Vehviläinen H, Koricheva J, & Ruohomäki K (2007) Tree species diversity influences

747   herbivore abundance and damage: meta-analysis of long-term forest experiments.

748   Oecologia, 152(2), 287-298. doi.org/10.1007/s00442-007-0673-7

749   Wan NF, Ji XY, Jiang JX (2014) Testing the enemies hypothesis in peach orchards in two

750   different geographic areas in eastern China: the role of ground cover vegetation.

751   PloS one, 9(6), e99850. doi.org/10.1371/journal.pone.0099850

752   Watterson JJ, Butler LG (1983) Occurrence of an unusual leucoanthocyanidin and absence

753   of proanthocyanidins in sorghum leaves. Journal of Agricultural and Food

34

754   Chemistry 31:41–45. doi:10.1021/jf00115a011

755   Weiblen GD, Webb CO, Novotny V, Basset Y & Miller SE (2006) Phylogenetic dispersion

756   of host use in a tropical insect herbivore community. Ecology, 87(sp7), S62-S75.

757   doi:10.1890/0012-9658(2006)87[62:pdohui]2.0.co;2

758   Welti E, Helzer C & Joern A (2017) Impacts of plant diversity on arthropod communities

759   and plant-herbivore network architecture. Ecosphere, 8(10), e01983.

760   doi:10.1002/ecs2.1983

761   White JA, Whitham TG (2000) Associational susceptibility of cottonwood to a box elder

762   herbivore. Ecology, 81(7), 1795-1803. doi.org/10.1890/0012-9658(2000)081

763   Wiens JJ, Ackerly DD, Allen AP, Anacker BL, Buckley LB, Cornell HV, Damschen EI,

764   Jonathan Davies T, Grytnes JA, Harrison SP, Hawkins BA, Holt RD, McCain CM

765   & Stephens PR (2010) Niche conservatism as an emerging principle in ecology and

766   conservation biology. Ecology Letters, 13, 1310–1324. doi:10.1111/j.1461-

767   0248.2010.01515.x

768   Zhuang XP, Lu YY, Yang GS (1992) Extraction and determination of flavonoids in ginkgo.

769   Chinese Herbal Medicines 23:122–1

770   Zunjarrao SS, Tellis MB, Joshi SN & Joshi RS (2020) Plant-insect interaction: the saga of

771   molecular coevolution. Co-evolution of secondary metabolites, 19-45.

772   doi/10.1007/978-3-319-96397-6_42.

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775   **Table 1.** Geographical characteristics and community composition of oak species along the diversity gradient. Geographical

776   coordinates, Annual Temperature (AT), Annual Precipitation (AP), Oak species richness (OSR), Shannon diversity index (H'), total

777   density of all oak species (TDOS), density of oaks *Lobatae* section (DOLS), density of oaks *Quercus* section (DOQS), per site. The

778   total density of individuals is given in individuals per hectare (Ind/Ha).

Study sites	Latitude	Longitude	AT (°C)	AP (mm)	OSR	H'	TDOS	DOLS	IDQS
Tequila Volcano	20.79	-103.84	12.4	849	4	1.1	930	890	40
Los Azufres	19.79	-100.68	14.9	1118	3	0.9	435	325	110
Indaparapeo	19.67	-100.85	17.9	717	3	0.7	405	370	35
Carindapaz	19.69	-100.91	11.9	1425	3	0.4	370	345	25
Cerro burro	19.44	-101.51	15.1	1034	2	0.2	415	395	20

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787 **Table 2**. Differences in the foliar secondary metabolites concentration between *Q. laurina* individuals growing along the oak diversity

788 gradiente according to One-way ANOVA test. Different letters indicate the means statistically different according to LSMeans test

789 (*P*< 0.05). The abbreviation PAS corresponds to Proanthocyanidins.

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(a) Mean ± SE					
Response variables	**Tequila Volcano**	**Los Azufres**	**Indaparapeo**	**Carindapaz**	**Cerro Burro**
Total phenols (mg g^{-1})	10.61±0.15[a]	9.65±0.19[b]	8.12±0.16[c]	7.13±0.15[d]	7.42±0.18[d]
Flavonoids (mg g^{-1})	1.38±0.05[a]	0.78±0.07[b]	0.89±0.08[b]	0.70±0.05[c]	0.60±0.06[c]
PAS (mg g^{-1})	4.44±0.12[a]	3.62±0.15[b]	1.83±0.10[c]	3.61±0.14[b]	2.94±0.12[d]
Gallotannins (mg g^{-1})	3.51±0.10[a]	2.93±0.14[b]	2.25±0.09[b]	2.38±0.10[b]	1.99±0.08[c]

(b) One-way ANOVA tests between-subjects effects				
Source	**Response variables**	*F*	*df*	*p*
Sites	**Total phenols (mg g^{-1})**	2.7001	4	< 0.0403*
	Flavonoids (mg g^{-1})	5.1704	4	< 0.0001*
	PAS (mg g^{-1})	3.5051	4	< 0.0001*
	Gallotannins (mg g^{-1})	2.8487	4	< 0.0001*

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794   **Table 3.** Results of analyses of covariance (ANCOVA tests) that show the effects of site,

795   herbivory and secondary metabolites

Response variable	Explanatory variable	Covariate	df	F	P
Herbivory	Sites	------------	4	6.96	0.001*
		Total phenols	1	0.37	0.54
		Flavonoids	1	0.25	0.63
		PAS	1	4.87	0.01*
		Gallotannis	1	3.12	0.02*

* Significant values

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For Peer Review

Table 4. OLS regression testing on the effect community composition of oak species on the abundance and richness of canopy insect herbivores and foliar secondary metabolites along the oak diversity gradient. The parameters of oak community are: Oak species richness (OSR), Shannon diversity index (H'), total density of all oak species (TDOS), density of oaks Lobatae section (DOLS), density of oaks Quercus section (DOQS).

Variables response	Insect herbivore community						Leaf secondary metabolites											
	Abundance			Richness			Total phenols			Flavonoids			PAS			Gallotannins		
	F	$d.f.$	P	F	$d.f.$	P	F	$d.f.$	P	F	$d.f.$	P	F	$d.f.$	P	F	$d.f.$	P
OSR	3.20	4	**0.003(+)**	1.95	4	0.06	-2.66	4	**0.01(-)**	1.27	4	0.20	1.40	4	0.17	2.55	4	**0.01(+)**
H'	2.26	4	**0.03(+)**	1.17	4	0.17	-.143	4	0.16	0.84	4	0.40	1.64	4	0.11	2.35	4	**0.02(+)**
TDOS	5.37	4	**0.001(+)**	2.47	4	**0.02(+)**	-.294	4	**0.01(-)**	1.08	4	0.29	2.21	4	**0.03(+)**	2.72	4	**0.01(+)**
DOLS	5.48	4	**0.001(+)**	2.66	4	**0.01(+)**	-3.54	4	**0.01(-)**	1.08	4	0.29	1.49	4	0.15	2.15	4	**0.04(+)**
DOQS	1.33	4	0.19	0.92	4	0.36	0.32	4	0.75	0.29	4	0.77	1.04	4	0.30	0.73	4	0.47

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Legends to figures

Figure 1. Differences in the frequency of insect herbivore orders associated to the canopy of *Q. laurina* along oak diversity gradient.

Figure 2. Rarefaction curves of canopy arthropods of *Q. laurina* for each of the study sites along the oak diversity gradient. The data were rarefied considering the number of individuals observed (white circles) to allow a valid comparison of arthropod family richness between the study sites.

Figure 3. Diversity patterns of trophic guilds of insect herbivores associated to canopy of *Q. laurina*. A) Canopy insect herbivore abundance guilds. B) Species richness of insect herbivore guilds. Common letters identify means that were similar according to LSMeans test (P < 0.05).

Figure 4. Non-metric dimensional scaling (NMDS) ordinations illustrating similarity of phytophagous insects taxonomic composition between the study sites along oak diversity gradient. Each point is a two-dimensional (axis 1 and axis 2) representation of phytophagous insects species composition on an individual tree based on global, non-metric multidimensional scaling (NMDS).
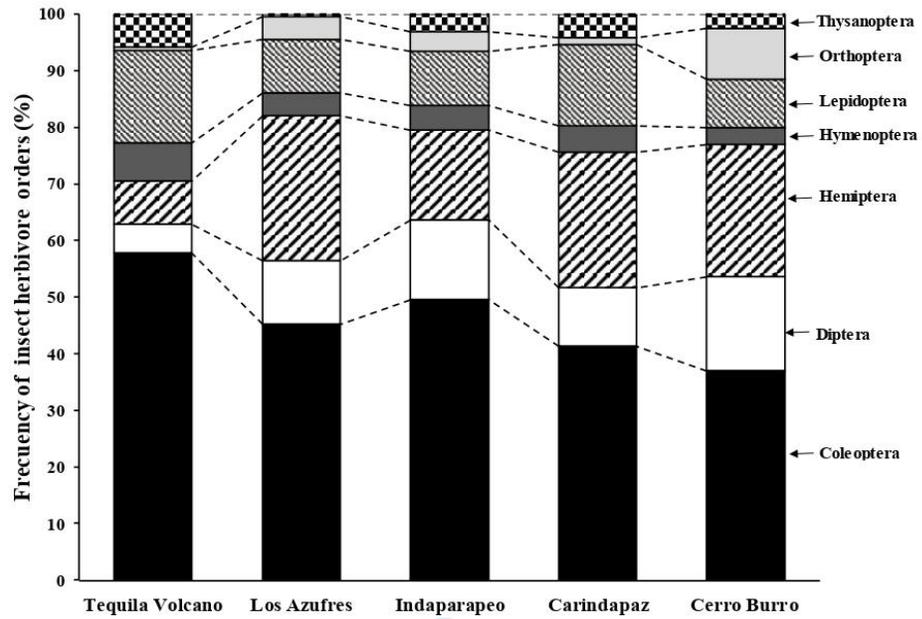
Figure 5. Herbivory levels in *Q. laurina* trees at each of the study sites along the oak diversity gradient. Common letters identify means that were similar according to LSMeans test (P < 0.05).

Figure 6. Canonical correspondence analyses (CCA) between climatic variables, foliar chemical defense (secondary metabolites) and the phytophagous insect community along the oak diversity gradient. A) Biplot showing the result of CCA foliar chemical defense and the phytophagous insect community. B) Biplot showing the result of CCA for climatic variables Mean Annual Temperature (MAT), Mean Annual Precipitation (MAP), mean

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temperature of the warmest quarter (TWQ) and mean temperature of the coldest quarter (TCQ) and phytophagous insect community. Each point is a two-dimensional (axis 1 and axis 2) representation of phytophagous insects composition on an individual tree

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Figure 1.

Figure 2.

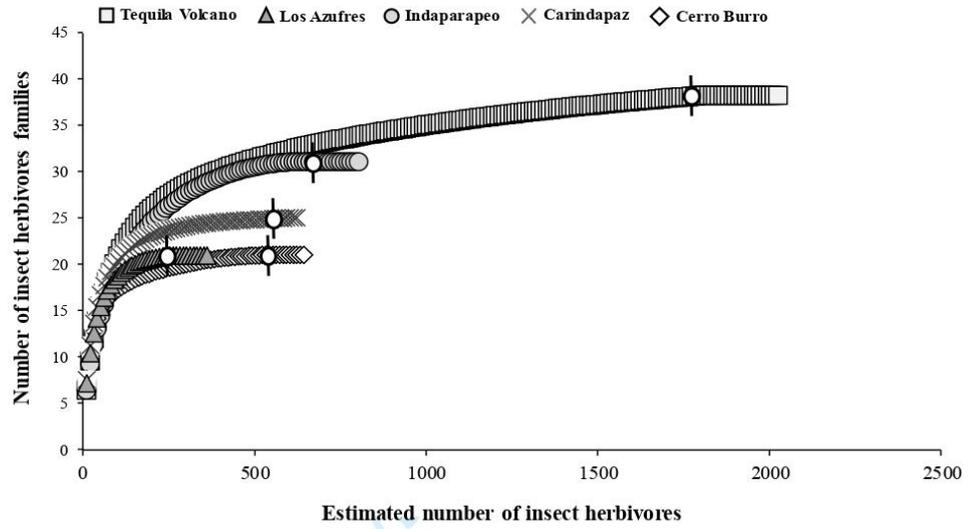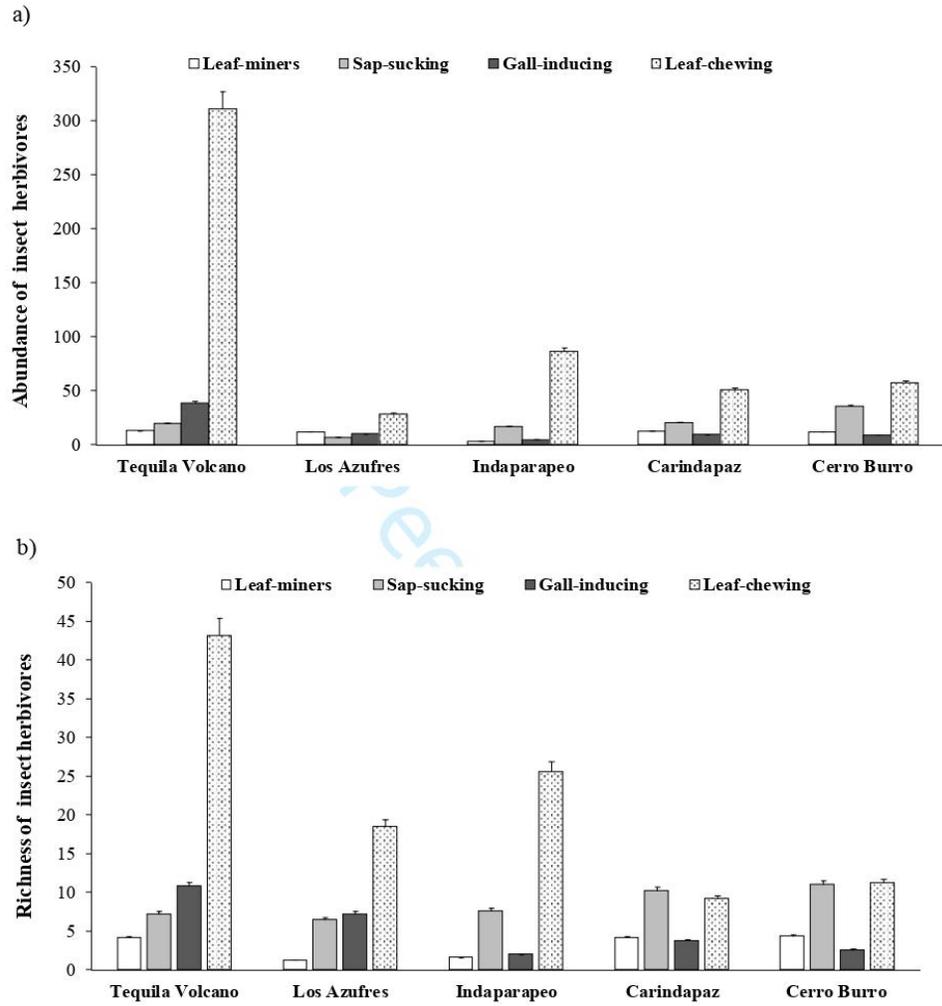Entomologia Experimentalis et Applicata

Figure 3.

a)

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Figure 4.

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Figure 5

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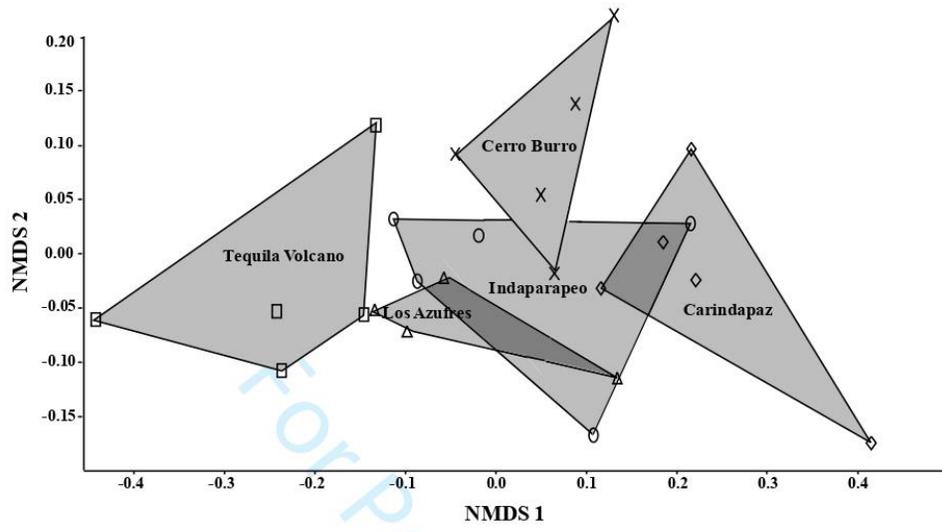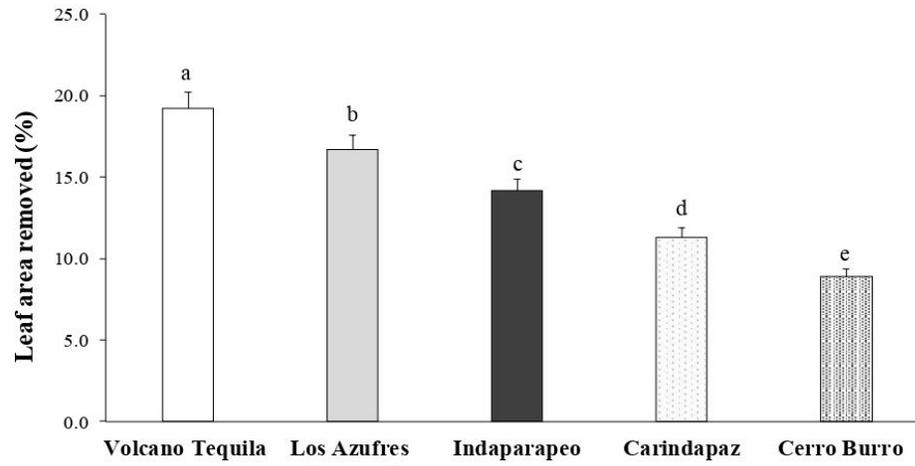
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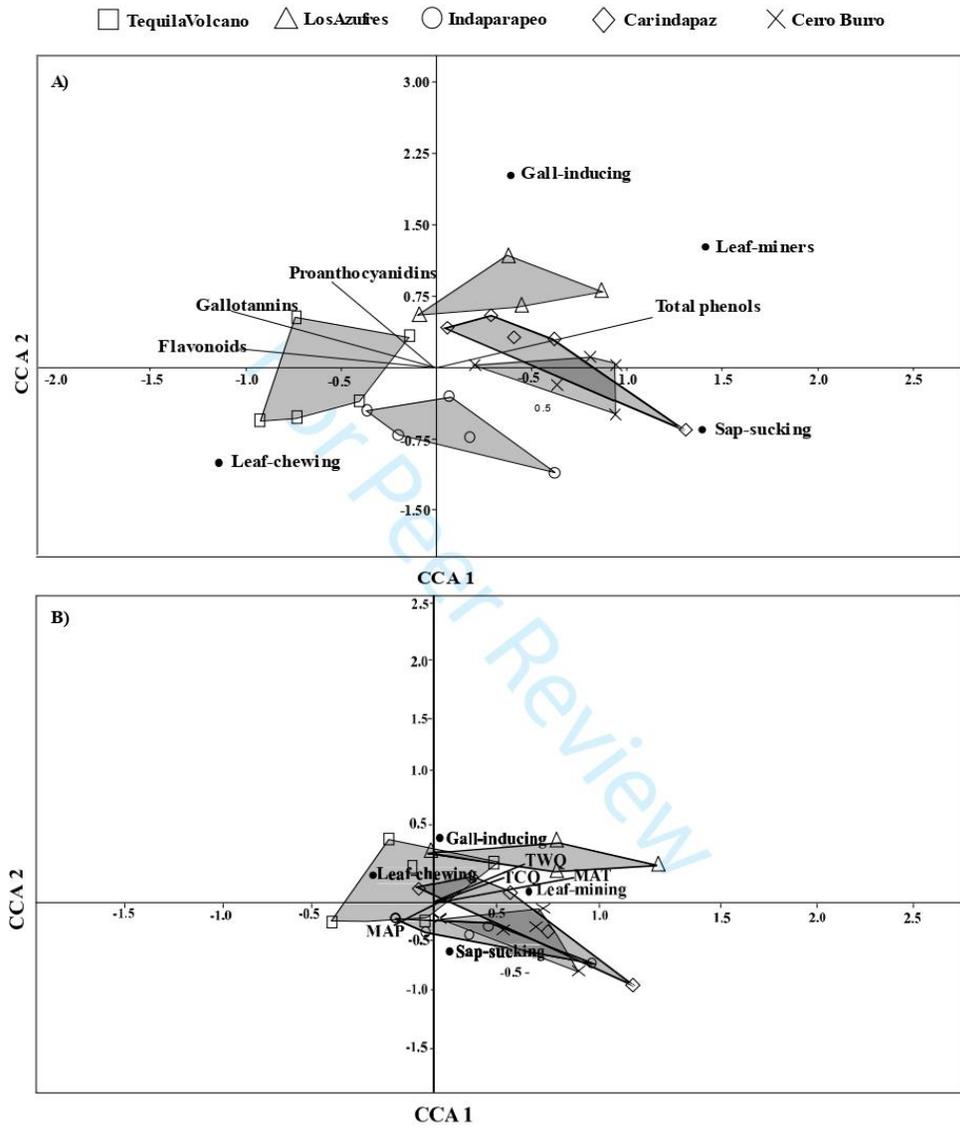
DISCUSION GENERAL

Los encinos representan uno de los grupos de plantas leñosas de mayor diversidad en el hemisferio norte (Cavender-Bares, 2016). En este trabajo de tesis se detectaron comunidades de encinos con una riqueza de especies que varío entre dos y cuatro especies de encinos coexistiendo. En cuanto al número de especies en la sección Lobatae, observamos la coexistencia de *Q. laurina* con uno o dos especies de encinos rojos (*Q. crassifolia* y *Q. calophylla*). El sitio de mayor riqueza y abundancia de encinos fue el Volcán de Tequila. Además, en todos los sitios de estudio, los individuos pertenecientes a la sección Lobatae fueron dominantes en relación a los individuos de encinos de la sección *Quercus*. De forma interesante, *Q. laurina* fue siempre la especie dominante en todos los sitios de estudio; representando en promedio el 88% de los individuos encontrados en todos los sitios de estudio. Se detectaron altos niveles de diversidad genética para *Q. laurina* en todos los sitios de estudio; oscilando el número de alelos por locus (N_a) y el número de alelos efectivos por locus (N_e) entre 10.42 y 12.42 y entre 5.87 y 8.07, respectivamente entre los sitios de estudio. Estos valores son similares y superiores a los reportados por Ramos-Ortiz et al. (2016) para una zona de hibridación entre *Q. laurina x Q. affinis*. En el caso de los niveles de heterocigosidad, encontramos valores altos de H_O (rango de 0.51 a 0.60) y H_E (rango de 0.81 a 0.86). El sitio de Carindapaz, el cual presentó una baja riqueza y abundancia de especies de encinos tuvo los niveles más altos de diversidad genética. En contraste, el sitio de mayor riqueza y abundancia de especies de encino (i.e. Volcán de Tequila) fue el sitio que presentó los niveles más bajos de diversidad genética. En el caso del cpDNA, se presentó el mismo patrón de diversidad que con los marcadores de núcleo, identificando en total 15 haplotipos diferentes y 12 haplotipos privados. En el sitio Los Azufres, se encontraron dos especies de encino rojo, además de *Q. laurina*, siendo el sitio

de mayor diversidad de haplotipos (i.e. nueve haplotipos en total y seis haplotipos privados). En cambio, la población con menos haplotipos fue el Volcán Tequila con solo uno, el cual fue compartido con otras poblaciones. Lo anterior no apoya nuestra hipótesis planteada que predice mayores niveles de diversidad genética de *Q. laurina* en sitios con mayor presencia de especies de encinos de la misma sección. Esto sugiere que para este sitio no están ocurriendo procesos de hibridación e introgresión. Esto podría atribuirse a factores climáticos, los cuales pueden ser las causas de la baja diversidad genética detectada en el Volcán Tequila; debido a que los factores climáticos potencialmente pueden incidir sobre los patrones fenológicos reproductivos (Zeng et al. 2011; Kim et al. 2018). Una explicación alternativa a este patrón podría deberse a la baja abundancia relativa de cada especie de encino en comparación con la abundancia de *Q. laurina*. Algunos estudios han demostrado que las diferencias en la abundancia local de cada especie de encino y las condiciones ambientales locales pueden determinar las tasas de hibridación entre diferentes taxones y reducir la hibridación (Dumolin-Lapegue et al. 1999; Lepais et al. 2009; Ramos-Ortiz et al. 2016).

Por otra parte, se encontraron diferencias significativas en los atributos funcionales foliares de *Q. laurina* entre los sitios de estudio. Los atributos funcionales foliares que mostraron diferencias significativas entre los sitios fueron el área foliar, el peso seco foliar, el área foliar específica, la densidad foliar y el contenido de clorofila. Además de presentar mayor variación en los sitios con mayor riqueza de especies de encinos. En particular, el área foliar específica, el área foliar y la densidad foliar están asociados con la adquisición y uso de recursos (Reich, 2014). Se ha documentado que el área foliar específica, el grosor de la hoja, la densidad estomática y el contenido de clorofila, pueden variar en el espacio y a lo largo del tiempo (Filella y Peñuelas 1999; Wagner et al., 2004); resultado de cambios en

las condiciones ambientales (e.g. temperatura, precipitación, incidencia de luz) y por factores genéticos (e.g. flujo de genes, hibridación e introgresión) (Violle et al. 2007). Esto concuerda con algunos estudios que han demostrado que las comunidades de plantas más diversas tienen una mayor diversidad funcional o mayores coeficientes de variación en algunos atributos funcionales; favoreciendo el acceso a más recursos como nutrientes, agua y luz (Maherali y Klironomos 2007; Fornara y Tilman 2008). Nuestros hallazgos concuerdan con esta idea y sugieren que un aumento en la diversidad funcional de *Q. laurina* (e.g. área foliar específica, área foliar y densidad foliar) está asociado con la riqueza de especies de encinos. Para el caso de las variables climáticas, se encontró una asociación significativa de los atributos funcionales foliares con variables climáticas; pero no con la diversidad genética de *Q. laurina* a lo largo del gradiente de diversidad de encinos. Este resultado apoya la idea de que la variación de los atributos funcionales foliares de *Q. laurina* podría estar más relacionada con factores ambientales (temperatura y precipitación) y ecológicos (estructura de la comunidad de encinos) y no a factores genéticos.

En cuanto a la comunidad de artrópodos asociados al dosel de *Q. laurina* se colectaron un total de 7,479 de artrópodos agrupados en 15 órdenes. Se encontró que los sitios con mayor diversidad de encinos presentaron la mayor riqueza, abundancia, así como diferencias en la composición de la comunidad de artrópodos, en comparación con los sitios de menor diversidad de encinos. Estos resultados apoyan "La hipótesis de la riqueza de especies de plantas" (Fernandes y Price 1988), la cual propone que comunidades de plantas más diversas representan sitios potenciales para ser colonizados por más especies de insectos (efectos botton-up) (Hertzog, 2017). En este estudio, las comunidades de encinos más diversas representan para los artrópodos sitios de una mayor disponibilidad de recursos

y nichos para ser colonizados por un mayor número de especies donde la riqueza de especies de artrópodos en el dosel de *Q. laurina* aumenta a medida que aumenta el número de especies y el número de especies disponibles de encinos. De forma similar, para el Volcán Tequila se encontró una mayor abundancia y riqueza de especies de fitófagos, depredadores y parasitoides. Esta mayor diversidad de depredadores y parasitoides encontrados en los sitios con mayor diversidad de encinos sugiere que los enemigos naturales podrían estar regulando la diversidad de la comunidad de artrópodos del dosel. Este resultado concuerda con lo predicho por "La hipótesis del enemigo natural" (Root 1973), que sugiere que la diversidad de enemigos naturales (e.g. depredadores y parasitoides) aumenta en comunidades con una gran diversidad de plantas debido a la presencia de más presas potenciales y disponibilidad de microambientes (efectos top-down) (Sobek et al. 2009). Por ejemplo, la diversidad de plantas puede incrementar la abundancia de herbívoros a través de una mayor disponibilidad y/o calidad de alimentos, nichos, microambientes y refugio, lo que a su vez determinará la diversidad de depredadores (Forkner y Hunter 2000). Particularmente, en las comunidades de encinos; algunos estudios han sugerido a los efectos bottom-up como el principal mecanismo regulador de la distribución y abundancia de insectos herbívoros, debido a cambios en la calidad nutricional de las hojas y en los patrones fenológicos (Feeny 1970, Faeth y Bultman 1986, Rossiter et al. 1988). Adicionalmente, la presencia de barreras geográficas como cadenas montañosas que son característicos del Eje Neovolcánico Transversal, también podría estar afectando a la comunidad de artrópodos del dosel, limitando su distribución y reduciendo la migración de artrópodos entre localidades (Novotny & Weiblen 2005; Arriaga-Jiménez et al.2018).

Los resultados de este estudio son consistentes con la premisa de que, para los

bosques templados, las comunidades de árboles representan un elemento clave para dar forma a la estructura física ambiental; razón por la que se ha sugerido que la diversidad de especies de árboles podría influir en un aumento en la riqueza y abundancia de artrópodos del dosel (Affeld 2008; Moreira et al. 2016). Este hecho es consistente con la ausencia de relaciones significativas entre las variables ambientales (e.g. temperatura, precipitación) con la comunidad de artrópodos del dosel en todos los sitios de estudio, lo que sugiere que las diferencias en la estructura de la comunidad de encinos entre localidades son el factor principal que está modulando los cambios en la comunidad de artrópodos asociados al dosel de *Q. laurina* a lo largo del gradiente de diversidad de encinos.

Finalmente, en este estudio analizamos los efectos de la diversidad de especies encinos sobre los patrones de herbivoría, defensa química y la comunidad de insectos fitófagos asociados al dosel de *Q. laurina*. Se ha propuesto que la diversidad de plantas es un factor clave que puede aumentar la herbivoría por insectos; donde comunidades de plantas más diversas (i.e. mayor presencia de plantas hetero-específicas) pueden disminuir la resistencia de una planta (i.e. resistencia asociativa) o aumentar la susceptibilidad a la herbivoría (i.e. susceptibilidad asociativa), aumentando la probabilidad de que las plantas sean atacadas por insectos herbívoros. (Root, 1973; White y Whitham, 2000).

Nuestros resultados muestran que el porcentaje de herbivoría por insectos fitófagos fue mayor en los sitios con mayor diversidad de especies de encinos. A lo largo del gradiente de diversidad, el sitio del Volcán Tequila presentó los mayores niveles de herbivoría. Esto es consistente con la idea de que la diversidad de plantas es un factor clave que puede aumentar o disminuir los porcentajes de herbivoría por insectos en las plantas (i.e. susceptibilidad asociativa). En nuestro estudio, los niveles de herbivoría podrían ser el resultado de la mayor proporción de insectos masticadores de hojas, los cuales en gran

parte pertenecen a familias de insectos generalistas; donde los sitios con mayor diversidad de encinos, representan zonas con más recursos y disponibilidad de nichos para ser ubicados y colonizados. Estos resultados pueden ser resultado del recambio de insectos masticadores entre diferentes especies de encinos, debido a la alimentación por insecto fitófagos generalistas de una mezcla de diferentes especies de plantas (Unsicker et al. 2008; Karban et al. 2010).

Se ha propuesto que la modificación de la composición química foliar y sus efectos sobre la comunidad de insectos fitófagos, es el vínculo indirecto entre las interacciones planta-herbívoro (Zunjarrao et al. 2019). En nuestro estudio, la composición química foliar asociada a defensa química fue diferente entre los sitios de estudio; donde las concentraciones más altas de metabolitos asociados a defensa vegetal fueron mayores en individuos de sitios con mayor diversidad de encinos. Encontramos tres relaciones significativas entre la comunidad de encinos y tres metabolitos de defensa, donde las proantocianidinas (PAS) y galotaninos mostraron una relación positiva con la diversidad de encinos y los fenoles totales mostraron una relación negativa con la diversidad de encinos. Este efecto de la comunidad del encinos se podría explicar por " La Hipótesis de compensación entre crecimiento y defensa" (Eisenhauer et al. 2009), donde establece que las plantas asignarán más recursos para la defensa química en comunidades de plantas más diversas, debido al incremento de la competencia por nutrientes, agua y luz, reduciendo el crecimiento de plantas. Esto podría explicar la relación encontrada entre la de diversidad de encinos y las proantocianidinas (PAS) y los galotaninos asociados a defensa química. De forma contraria, los fenoles pueden estar más asociados a otras funciones fisiológicas, como la fotoprotección y no actuar como defensa contra los fitófagos generalistas (Barbehenn et al. 2011; Castagneyrol et al. 2014). De forma similar, se detectó una fuerte

relación entre la concentración de fenoles totales, flavonoides, PAS y galotaninos con la composición de insectos fitófagos; particularmente con el gremio de los masticadores de hojas. Esta relación podría explicarse por "La hipótesis del dilema especialista-generalista" (Van der Meijden 1996), la cual propone que la concentración de las defensas químicas en una planta depende de la proporción de herbívoros generalistas y especialistas.

En total encontramos 3,693 de insectos fitófagos agrupados en 7 órdenes asociados al dosel de *Q.laurina*, presentando mayor abundancia y riqueza de insectos fitófagos los sitios de mayor diversidad de encinos. Este resultado es similar a lo encontrado con la comunidad de artrópodos en general; reforzando para este caso de estudio "La hipótesis de riqueza de especies de plantas" (Fernades y Price 1988). En el caso de los gremios de insectos fitófagos, el gremio masticador de hojas presentó la mayor proporción dentro de la comunidad de insectos fitófagos en el Volcán Tequila (81,50%) y una menor proporción en Cerro Burro (47,63%). En contraste, los insectos chupadores de savia fueron más abundantes en Cerro Burro (33,45%) y menos abundantes en el Volcán Tequila (5,08%). En nuestro caso, las comunidades de encino representan para los insectos fitófagos una mayor disponibilidad de recursos y nichos para ser localizados y colonizados por un mayor número de insectos fitófagos (Gómez, Verdú & Perfectti 2010). El hecho de que los masticadores de hojas sean el gremio de mayor abundancia y riqueza en los sitios con mayor diversidad de encinos, podría explicar los mayores niveles de herbivoría en estos sitios. Castagneyrol et al. (2014) encontró que la distancia filogenética entre las especies hospederas es un factor que determina los porcentajes de daño foliar causado por insectos fitófagos; donde las plantas más relacionadas filogenéticamente tienen una mayor probabilidad de compartir atributos funcionales involucrados en el reconocimiento y explotación de recursos por parte de los insectos fitófagos hacia la planta hospedera ,

siendo propensos a compartir especies de insectos fitófagos (Ødegaard, Diserud & Østbye 2005; Wiens et al.2010).

Nosotros concluimos que los cambios en la diversidad de especies de encinos son un factor que influye sobre la variación de los atributos funcionales foliares, estando más asociados a factores tanto ambientales (e.g. temperatura, precipitación) como ecológicos (estructura de la comunidad de encinos). Además, nuestros resultados no apoyan la hipótesis que predice mayores niveles de diversidad genética en comunidades con mayor ocurrencia de especies de encino de la misma sección. Este hecho podría estar relacionado con las diferencias detectadas en las variables climáticas a lo largo del gradiente de diversidad de encino. En el mismo sentido, la diversidad de artrópodos del dosel y de insectos fitófagos a lo largo de un gradiente de diversidad de especies de encinos fue en el Volcán Tequila, el cual representó el sitio con mayor diversidad de encinos. Nuestros hallazgos confirman que, a escala local, la riqueza de especies de encinos es el principal factor que determina la diversidad de artrópodos asociada al dosel de los encinos, afectando los niveles tróficos y la estructura y composición de los gremios de artrópodos asociados a *Quercus laurina*. México es el principal centro de riqueza, diversificación y endemismo de encinos en el hemisferio norte. Por lo cual, destacamos la importancia de la conservación de las especies de encino ya que estas representan elementos clave de los bosques templados, albergando una alta diversidad de especies y siendo zonas de alta actividad ecológica.

REFERENCIAS GENERALES

Abbas M, Ebeling A, Oelmann Y, Ptacnik R, Roscher C, Weigelt A, Weisser WW, Wilcke
W, Hillebrand H. 2013. Biodiversity effects on plant stoichiometry. PLoS ONE 8:
e58179. doi:10.1371/journal.pone.0058179

Affeld K (2008) Spatial complexity and microclimatic responses of epiphyte communities
and their invertebrate fauna in the canopy of northern rata (*Metrosideros robusta* A.
Cunn.: Myrtaceae) on the West Coast of the South Island, New Zealand.
Dissertation, Lincoln University

Arnold ML (1997) Natural hybridization and evolution. Oxford University Press, New
York, USA

Arnold ML (2006) Evolution through genetic exchange. Oxford University Press,New
York, USA

Arriaga-Jiménez A, Rös M, Halffter G (2018) High variability of dung beetle diversity
patterns at four mountains of the Trans-Mexican Volcanic Belt. PeerJ 6:e4468
https://doi.org/10.7717/peerj.4468

Barbehenn, R. V., & Constabel, C. P. (2011). Tannins in plant–herbivore interactions.

Bravo-Oviedo, Andres et al. European Mixed Forests: definition and research perspectives.
Forest Systems, [S.l.], v. 23, n. 3, p. 518-533, dec. 2014. ISSN 2171-9845.
https://revistas.inia.es/index.php/fs/article/view/6256>

Caseys C, Stritt C, Glauser G, Blanchard T, Lexer C (2015) Effects of hybridization and
evolutionary constraints on secondary metabolites: the genetic architecture of
phenylpropanoids in European *Populus* species. PloS one 10:128-200. https:// doi:
10.1371/journal.pone.0128200

Castagneyrol B, Giffard B, Pere C, Jactel H. 2013. Plant apparency, an overlooked driver of

associational resistance to insect herbivory. Journal of Ecology 101: 418–429.
https://doi:doi: 10.1111/1365-2745.12055

Castagneyrol, B., Jactel, H., Vacher, C., Brockerhoff, E. G., & Koricheva, J. (2014). Effects of plant phylogenetic diversity on herbivory depend on herbivore specialization. Journal of Applied Ecology, 51(1), 134-141.

Cavender-Bares J (2016) Diversity, distribution and ecosystem services of the North American oaks. Int. Oaks 27:37–49.

Cavender-Bares J (2019) Diversification, adaptation, and community assembly of the American oaks (Quercus), a model clade for integrating ecology and evolution. New Phytol. 221(2):669-692. https://doi.org/10.1111/nph.15450

Chamagne, J., Paine, C. T., Schoolmaster Jr, D. R., Stejskal, R., Volarřík, D., Šebesta, J., ... & Matula, R. (2016). Do the rich get richer? Varying effects of tree species identity and diversity on the richness of understory taxa. Ecology, 97(9), 2364-2373.

Cuevas-Reyes P, Quesada M, Hanson P, Dirzo R, Oyama, K (2004) Diversity of gall-inducing insects in a Mexican tropical dry forest: the importance of plant species richness, life-forms, host plant age and plant density. J Ecol 707-716. https://doi.org/10.1111/j.0022-0477.2004.00896.x

Curtu AL, Gailing O & Finkeldey R (2007a) Evidence for hybridization and introgression within a species-rich oak (*Quercus* spp.) community. Evol Biol 7:1-15. https://doi: 10.1186/1471-2148-7-218

Dumolin-Lapegue S, Kremer A, Petit RJ (1999) Are chloroplast and mitochondrial DNA variation species independent in oaks? Evolution 53:1406–1413. https://doi:10.2307/2640887

Eisenhauer, N., Milcu, A., Nitschke, N., Sabais, A. C. W., Scherber, C., & Scheu, S.

(2009). Earthworm and belowground competition effects on plant productivity in a

plant diversity gradient. Oecologia, 161(2), 291–301. doi:10.1007/s00442-009-

1374-1

Faeth SH, Bultman TL (1986) Interacting effects of increased tannin levels on leaf-mining

insects. Entomol 40(3):297-301. https://doi.org/10.1111/j.1570-

7458.1986.tb00515.x

Feeny P (1970) Seasonal changes in oak leaf tannins and nutrients as a cause of spring

feeding by winter moth caterpillars. Ecology 51(4):565-

581.https://doi.org/10.2307/1934037

Fernandes GW, Price PW (1988) Biogeographical gradients in galling species richness.

Oecologia 76(2):161-167.https://doi.org/10.1007/BF00379948

Filella I, Peñuelas J (1999) Altitudinal differences in UV absorbance, UV reflectance and

related morphological traits of *Quercus ilex* and *Rhododendron ferrugineum* in the

Mediterranean region. Plant Ecol 145:157–65.

Forkner RE, Hunter MD (2000) What goes up must come down? Nutrient addition and

predation pressure on oak herbivores. Ecology 81(6):1588-1600.

https://doi.org/10.1890/0012-9658(2000)081

Fornara DA, Tilman D (2008) Plant functional composition influences rates of soil carbon

and nitrogen accumulation. J Ecol 96:314–322. https://doi: 10.1111/j.1365-

2745.2007.01345.x

García-Guzmán OM, Garibay-Orijel R, Hernández E, Arellano-Torres E, Oyama K (2017)

Word-wide meta-analysis of *Quercus forests* ectomycorrhizal fungal diversity

reveals southwestern Mexico as a hotspot. Mycorrhiza, 27(8):811-822.

https://doi.org/10.1007/s00572-017-0793-9

Gómez, J. M., Verdú, M., & Perfectti, F. (2010). Ecological interactions are evolutionarily conserved across the entire tree of life. Nature, 465(7300), 918-921.

González-Rodríguez A, Oyama Ken (2005) Leaf morphometric variation in *Quercus affinis* and *Q. laurina* (Fagaceae), two hybridizing Mexican red oaks. Bot J Linn Soc 147(4):427-435.https://doi: 10.1111/j.1095-8339.2004.00394.x

Haddad NM, Crutsinger GM, Gross K, Haarstad J, Tilman D (2011) Plant diversity and the stability of foodwebs. Ecol Lett 14:42-46. https://doi.org/10.1111/j.1461-0248.2010.01548.x

Harrison RG, & Larson EL (2014) Hybridization, introgression, and the nature of species boundaries. J Hered 105:795-809. https://doi.org/10.1093/jhered/esu033

Himrane H, Camarero JJ, Gil-Pelegrín E (2004) Morphological and ecophysiological variation of the hybrid oak *Quercus subpyrenaica* (*Q. faginea x Q. pubescens*). Trees 18:566–575.https://doi: 10.1007/s00468-004-0340-0

Hipp AL, & Weber, J.A (2008) Taxonomy of Hill's oak (*Quercus ellipsoidalis*: Fagaceae): Evidence from AFLP data. Syst Bot 33:148-158.

Hipp Andrew L., et al (2020) Sympatric parallel diversification of major oak clades in the Americas and the origins of Mexican species diversity. New Phytol 217:439-452. https://doi:10.1111/nph.14773

Jactel, H., Bauhus, J., Boberg, J., Bonal, D., Castagneyrol, B., Gardiner, B., ... & Brockerhoff, E. G. (2017). Tree diversity drives forest stand resistance to natural disturbances. Current Forestry Reports, 3(3), 223-243.

Karban, R., Karban, C., Huntzinger, M., Pearse, I., & Crutsinger, G. (2010). Diet mixing enhances the performance of a generalist caterpillar,Platyprepia virginalis. Ecological Entomology, 35(1), 92–99. doi:10.1111/j.1365-2311.2009.01162.x

Kim BY, Wei X, Fitz-Gibbon S, Lohmueller KE, Ortego J, Gugger PF, & Sork VL (2018)
RADseq data reveal ancient, but not pervasive, introgression between Californian
tree and scrub oak species (*Quercus* sect. Quercus: Fagaceae). Mol Ecol 22:4556-
4571.https://doi: 10.1111/mec.14869

Kos, M., Bukovinszky, T., Mulder, P. P. J., & Bezemer, T. M. (2015). Disentangling
above- and belowground neighbor effects on the growth, chemistry, and arthropod
community on a focal plant. Ecology, 96(1), 164–175. doi:10.1890/14-0563.1

Kostenko, O., Mulder, P. P. J., Courbois, M., & Bezemer, T. M. (2016). Effects of plant
diversity on the concentration of secondary plant metabolites and the density of
arthropods on focal plants in the field. Journal of Ecology, 105(3), 647–660.
doi:10.1111/1365-2745.12700

Lagache L, Klein EK, Guichoux E, Petit R (2013) Fine-scale environmental control of
hybridization in oaks. Mol Ecol 22:423–436. https://doi: 10.1111/mec.12121

Lavorel S, Díaz S, Cornelissen JHC, Garnier E, Harrison SP, McIntyre S, Urcelay C (2007)
Plant functional types: are we getting any closer to the Holy Grail? In: Springer.
Terrestrial ecosystems in a changing world. Berlin, Heidelberg. pp149-164

Lepais O, Petit RJ, Guichoux E, Lavabre JE, Alberto F, Kremer A, Gerber S (2009) Species
relative abundance and direction of introgression in oaks. Mol Ecol 18:2228–2242.
https://doi: 10.1111/j.1365-294X.2009.04137.x

Leroux SJ, Loreau M (2015) Theoretical perspectives on bottom-up and top-down
interactions across ecosystems. Trophic Ecology. 3:28.
https://doi.org/10.1017/CBO9781139924856.002.

Maherali H, Klironomos JN (2007) Influence of phylogeny on fungal community assembly

    and ecosystem functioning. Science 316: 1746–1748.

    https://doi:10.1126/science.1143082

Maldonado-López Y, Vaca-Sánchez MS, González-Rodríguez A, Oyama K, López-

    Barbosa., Fagundes M, Cuevas-Reyes P (2018) Hybridization increases canopy

    arthropod diversity in the *Quercus affinis*× *Quercus laurina* complex. J Insect

    Conserv 22(5-6):781-793. https://doi.org/10.1007/s10841-018-0103-7

McCauley RA, Cortés-Palomec AC, Oyama K (2019) Species diversification in a lineage

    of Mexican red oak (Quercus section Lobatae subsection Racemiflorae) the

    interplay between distance, habitat, and hybridization. Tree Genet 15:27.

    https://doi.org/10.1007/s11295-019-1333-x

McIntyre S, Lavorel S, Landsberg J, Forbes TDA (1999) Disturbance response in

    vegetation–towards a global perspective on functional traits. J Veg Sci 10(5): 621-

    630. https://doi:10.2307/3237077

Moreira X, Abdala-Roberts L, Rasmann S, Castagneyrol B, Mooney KA (2016) Plant

    diversity effects on insect herbivores and their natural enemies: current thinking,

    recent findings, and future directions. Curr Opin Insect 14, 1-7.

    https://doi.org/10.1016/j.cois.2015.10.003

Moreira, X., Abdala-Roberts, L., Parra-Tabla, V., & Mooney, K. A. (2014). Positive

    Effects of Plant Genotypic and Species Diversity on Anti-Herbivore Defenses in a

    Tropical Tree Species. PLoS ONE,9(8)e105438. doi:10.1371/journal.pone.0105438

Morin, X., Fahse, L., de Mazancourt, C., Scherer-Lorenzen, M., & Bugmann, H. (2014).

    Temporal stability in forest productivity increases with tree diversity due to

    asynchrony in species dynamics. Ecology letters, 17(12), 1526-1535. http//:

Novotny V, & Weiblen, GD (2005). From communities to continents: beta diversity of

    herbivorous insects. In: Annales Zoologici Fennici. Finnish Zoological and

    Botanical Publishing Board. pp 463-475

Novotny, V., Miller, S. E., Baje, L., Balagawi, S., Basset, Y., Cizek, L., ... & Weiblen, G.

    D. (2010). Guild-specific patterns of species richness and host specialization in

    plant–herbivore food webs from a tropical forest. Journal of Animal Ecology, 79(6),

    1193-1203. https://doi.org/10.1111/j.1365-2656.2010.01728.x

Ødegaard, F., Diserud, O. H., & Østbye, K. (2005). The importance of plant relatedness for

    host utilization among phytophagous insects. Ecology Letters, 8(6), 612-617.

    https://doi: 10.1111/j.1461-0248.2005.00758.x

    Phytochemistry, *72*(13), 1551-1565.

Ramos-Ortiz S, Oyama K, Rodríguez-Correa H, González-Rodríguez A (2015) Geographic

    structure of genetic and phenotypic variation in the hybrid zone between *Quercus*

    affinis and *Q. laurina* in Mexico. Plant Species Biol 31:219–232.

    https://doi:10.1111/1442-1984.12109

Reich PB (2014) The world-wide 'fast-slow' plant economics spectrum: A traits manifesto.

    J Ecol 102:275– 301. https://doi.org/10.1111/1365-2745.12211

Rieseberg LH, Archer MA, Wayne RK (1999) Transgressive segregation, adaptation and

    speciation. Heredity, 83(4):363-372. https://doi.org/10.1038/sj.hdy.6886170

Riordan EC, Gugger PF, Ortego J, Smith C, Gaddis K, Thompson P, & Sork, VL (2016)

    Association of genetic and phenotypic variability with geography and climate in

    three southern California oaks. Am J Bot 103(1):73-85.

    https://doi:10.3732/ajb.1500135

Rodríguez-Correa H, Oyama K, MacGregor-Fors I, González-Rodríguez A (2015) How are

oaks distributed in the neotropics? A perspective from species turnover, areas of
endemism, and climatic niches. Int J Plant Sci 176:222–231.
https://doi.org/10.1086/679904

Root RB (1973) Organization of a plant–arthropod association in simple and diverse
habitats: the fauna of collards (Brassica oleracea). Ecol Monogr 43:95-124.
https://doi.org/10.2307/1942161

Rosado‑Sánchez, S., Parra‑Tabla, V., Betancur‑Ancona, D., Moreira, X., & Abdala‑
Roberts, L. (2018). Effects of tree species diversity on insect herbivory and leaf
defences in *Cordia dodecandra*. Ecological Entomology, 43(6), 703-711.
http//:doi.org/10.1111/een.12648

Rossiter M, Schultz JC, Baldwin IT (1988) Relationships among defoliation, red oak
phenolics, and gypsy moth growth and reproduction. Ecology 69(1).267-277.
https://doi.org/10.2307/1943182

Salvini D, Bruschi P, Fineschi S, Grossoni P, Kjaer ED, Vendramin GG (2009) Natural
hybridization between *Quercus petraea* (Matt.) Liebl. and *Quercus pubescens*
(Willd.) within an Italian stand as revealed by microsatellite fingerprinting. Plant
Biol 11(5):758-765. https://doi: 10.1111/j.1438-8677.2008.00158.x

Schuldt, A., Baruffol, M., Böhnke, M., Bruelheide, H., Härdtle, W., Lang, A. C., ... &
Assmann, T. (2010). Tree diversity promotes insect herbivory in subtropical forests
of south-east China. Journal of Ecology, 98(4), 917-926.
https://doi.org/10.1111/j.1365-2745.2010.01659.x

Schwarzbach AE, Donovan LA, & Rieseberg LH (2001) Transgressive character
expression in a hybrid sunflower species. Am J Bot, 88(2):270-277.

https://doi:10.2307/2657018

Sobek S, Scherber C, Steffan-Dewenter I, Tscharntke T (2009) Sapling herbivory, invertebrate herbivores and predators across a natural tree diversity gradient in Germany's largest connected deciduous forest. Oecologia 160:279-288. https://doi.org/10.1007/s00442-009-1304-2

Soltis, PS, & Soltis, DE (2009) The role of hybridization in plant speciation. Annu Rev Plant Biol. 60:561-588.https://doi:10.1146/annurev.arplant.043008.092039

Torres-Miranda A, Luna-Vega I, Oyama K (2013) New approaches to the biogeography and areas of endemism of red oaks (Quercus L., Section Lobatae). Syst Biol 62(4), 555-573. https://doi.org/10.1093/sysbio/syt021

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

Tovar-Sánchez E, Oyama K (2006a) Community structure of canopy arthropods associated to *Quercus crassifolia* x *Quercus crassipes* complex. Oikos 112:370–381. https://doi.org/10.1111/j.0030-1299.2006.14029.x

Tovar-Sánchez, E., Valencia-Cuevas, L., Castillo-Mendoza, E., Mussali-Galante, P., Pérez-Ruiz, R. V., & Mendoza, A. (2013). Association between individual genetic diversity of two oak host species and canopy arthropod community structure. European Journal of Forest Research, 132(1), 165-179.

Ulyshen MD (2011) Arthropod vertical stratification in temperate deciduous forests: Implications for conservation-oriented management. For Ecol Manag 261:1479–1489. https://doi.org/10.1016/j.foreco.2011.01.033

Unsicker, S. B., Oswald, A., Köhler, G., & Weisser, W. W. (2008). Complementary

effects through dietary mixing enhance the performance of a generalist insect

herbivore. Oecologia, 156(2), 313-324. https://doi:10.1007/s00442-008-0973-6

Valencia S (2004) Diversidad del género Quercus (Fagaceae) en México. Bol Soc Bot

México 75:33–53. https://doi.org/10.17129/botsci.1692

Valencia-Cuevas L, Mussali-Galante P, Piñero D, Castillo-Mendoza E, Rangel-Altamirano

G, Tovar-Sánchez E (2015) Hybridization of *Quercus castanea* (Fagaceae) across a

red oak species gradient in Mexico. Plant Syst Evol 301:1085–1097.

https://doi.org/10.1007/s00606-014-1151-4

Van der Meijden, E. (1996). Plant defence, an evolutionary dilemma: contrasting effects of

(specialist and generalist) herbivores and natural enemies. Proceedings of the 9th

International Symposium on Insect-Plant Relationships, 307–310. doi:10.1007/978-

94-009-1720-0_69

Van der Plas, F., Manning, P., Soliveres, S., Allan, E., Scherer-Lorenzen, M., Verheyen,

K., ... & Fischer, M. (2016). Biotic homogenization can decrease landscape-scale

forest multifunctionality. Proceedings of the National Academy of Sciences,

113(13), 3557-3562.

Violle C, Navas ML, Vile D, Kazakou E, Fortunel C, Hummel I, Garnier E (2007) Let the

concept of trait be functional. Oikos 116:882–892. https://doi: 10.1111/j.2007.0030-

1299.15559.x

Wagner et al. (2004) The genetic basis of singlet oxygen-induced stress responses of

Arabidopsis thaliana. Science 306, 1183–1185.

White JA, Whitham TG (2000) Associational susceptibility of cottonwood to a box elder

herbivore. Ecology, 81(7), 1795-1803. https://doi.org/10.1890/0012-9658(2000)081

Wiens, J.J., Ackerly, D.D., Allen, A.P., Anacker, B.L., Buckley, L.B., Cornell, H.V.,

Damschen, E.I., Jonathan Davies, T., Grytnes, J.-A., Harrison, S.P., Hawkins, B.A., Holt, R.D., McCain, C.M. & Stephens, P.R. (2010) Niche conservatism as an emerging principle in ecology and conservation biology. Ecology Letters, 13, 1310–1324. doi: 10.1111/j.1461-0248.2010.01515.x

Zeng YF, Liao WJ, Petit RJ, Zhang DY (2011) Geographic variation in the structure of oak hybrid zones provides insights into the dynamics of speciation. Mol Ecol 20:4995–5011. https://doi.org/10.1111/j.1365-294X.2011.053x

Zunjarrao, S. S., Tellis, M. B., Joshi, S. N., & Joshi, R. S. (2020). Plant-insect interaction: the saga of molecular coevolution. Co-evolution of secondary metabolites, 19-45. https://doi/10.1007/978-3-319-96397-6_42