UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

POSGRADO EN CIENCIAS BIOLÓGICAS
INSTITUTO DE INVESTIGACIONES EN ECOSISTEMAS Y SUSTENTABILIDAD
BIOLOGÍA EVOLUTIVA

PATRONES DE DISTRIBUCIÓN Y FILOGEOGRAFÍA DE LOS ENCINOS (*QUERCUS*: FAGACEAE) EN MESOAMÉRICA Y LOS ANDES

TESIS

QUE PARA OPTAR POR EL GRADO DE:
DOCTOR EN CIENCIAS

PRESENTA:
HERNANDO ALONSO RODRÍGUEZ CORREA

TUTOR PRINCIPAL DE TESIS: DOCTOR ANTONIO GONZÁLEZ RODRÍGUEZ
INSTITUTO DE INVESTIGACIONES EN ECOSISTEMAS Y SUSTENTABILIDAD, UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
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INSTITUTO DE ECOLOGÍA, UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO

MÉXICO, D.F. JULIO 2015

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UNAM
POSGRADO
Ciencias Biológicas

COORDINACIÓN

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

Por medio de la presente, me permito informar a usted, que en reunión ordinaria del Subcomité por Campo de Conocimiento (Ecología y Manejo Integral de Ecosistemas) del Posgrado en Ciencias Biológicas, se aprobó el siguiente jurado para el examen de grado de **Doctor en Ciencias** del alumno **Hernando Alonso Rodríguez Correa** con número de cuenta **510450957** con la tesis titulada **"Patrones de distribución y filogeografía de los encinos *(Quercus Fagaceae)* en Mesoamérica y los Andes"**, bajo la dirección del **Dr. Antonio González Rodríguez** Tutor Principal.-

Presidente:	Dr. Mauricio Ricardo Quesada Avendaño
Vocal:	Dr. Juan Servando Núñez Farfán
Secretario:	Dr. Alberto Ken Oyama Nakagawa
Suplente:	Dr. Eduardo Ruiz Sánchez
Suplente:	Dra. Ella Gloria Vázquez Domínguez

Sin otro particular, quedo de usted.

Atentamente
"POR MI RAZA HABLARÁ EL ESPÍRITU"
Cd. Universitaria, D.F., a 26 de junio de 2015

Dra. María del Coro Arizmendi Arriaga
Coordinadora del Programa

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que con sudor mestizo y pujanza de arrieros
nos abrieron el camino para llegar más lejos
sin olvidar nuestros pasos.

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que llenan de preguntas la mente de generaciones de biólogos,
preguntas que hoy y siempre inspirarán cuentos,
cuentos de historia natural.

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Table 1. *Quercus costaricensis* and *Q. bumelioides* populations geographic information summary, genetic diversity and genetic structure estimates. n: sample size, h_S: within population genetic diversity, D^2sh: mean pairwise genetic distance among individuals within a population under a stepwise mutation model. CG: Cordillera de Guanacaste, CC: Cordillera Central, CT: Cordillera de Talamanca, VC: Valle Central.

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Figure 1. Study area. Elevation of southern Central America is represented in a grey scale from lowlands (soft gray) to highlands (black). CG: Cordillera de Guanacaste, VC: Valle Central, CT: Cordillera de Talamanca, CCP: Panamanian Cordillera Central. White circles and triangles represent *Q. costaricensis* and *Q. bumelioides* populations, respectively. White-dashed line represents the Costa Rica-Panama border. White solid line corresponds to the 1000 meters line of the Costa Rican mountains.

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Table 1. Principal components analysis for climatic variation among the Andean forest belts.

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Figure 2. Principal components analysis for climatic variation among the Andean forest belts.

Figure 3. Ecological niche models for *Quercus humboldtii* during the Present-day and Last Glacial Maximum periods. Boxes a, b and c (Present-day, LGM according to MIROC and LGM according to CCSM models, respectively) contain the raw outputs suitability values increasing from cero (white) to one (black). Boxes d, e and f represent the same sequence (Present-day, LGM according to MIROC and LGM according to CCSM models respectively) of the models under the fixed cumulative value 1 logistic threshold. Boxes g, h and i represent the same sequence of models using the Balance training omission, predicted area and threshold value logistic threshold.

Capítulo 5

Table1. Population geographic information summary, genetic diversity and genetic structure estimates. n: sample size, h_S: within population genetic diversity, D^2SH, mean pairwise genetic distance among individuals within a population under a stepwise mutation model.

Table 2. Hierarchical analysis of molecular variance (AMOVA) using F_{ST} and R_{ST} for *Quercus humboldtii*. Groups correspond to the three Cordilleras in the Colombian Andes (see Fig. 1). * Significant values ($p<0.001$).

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Figure 2. Distribution of the 18 haplotypes identified from cpSSRs and haplotype network inferred using median-joining in *Quercus humboldtii*. Name of the haplotype is coded as H*n* followed by a number in parenthesis corresponding to the population for unique haplotypes. Purple circles represent unique haplotypes derived from H18, lima green circles represent unique haplotypes derived from H7 and orange circles represent unique haplotypes derived from H2. Elevation of the Colombian Andes is represented in a grey scale from lowlands (white) to highlands (black). COc: Cordillera Occidental,

CC: Cordillera Central, COr: cordillera oriental, CM: Macizo Colombiano (Colombian Massif).

Figure 3. Geographical location of the most important genetic discontinuities (red lines) using the Monmomier's maximum difference algorithm for the *Quercus humboldtii* populations (white triangles). Elevation of the Colombian Andes is represented in a grey scale from lowlands (white) to highlands (black). COc: Cordillera Occidental, CC: Cordillera Central, COr: cordillera oriental, CM: Macizo Colombiano (Colombian Massif).

Resumen

El género *Quercus* es un grupo con una distribución amplia desde la región Holártica hasta la Tropical. A través de su distribución las especies del género hacen parte fundamental de diferentes ecosistemas entre los que destacan los bosques deciduos templados, bosques perennes subtropicales y templados, bosque de pino-encino y bosques montanos. En la región Neotropical los bosques de encino se distribuyen desde la Zona de Transición Mexicana hasta los Andes Colombianos. A través de esta distribución las especies del género *Quercus* habitan ecosistemas desde el nivel del mar hasta los 3500 metros. Las especies de encino abarcan un espacio geográfico heterogéneo con una historia geológica y climática compleja como es el sur de México, Centroamérica y los Andes. En esta región se han estudiado una cantidad importante de procesos evolutivos como extinción, diferenciación intraespecífica, especiación y adaptación en diferentes taxa, relacionados estrechamente con la historia geológica compleja del Neotrópico. Las especies Neotropicales de *Quercus* no han sido la excepción pues exhiben procesos de diferenciación intraespecífica asociados a elementos geográficos como el Istmo de Tehuantepec (para *Quercus oleoides*) y un efecto moderado de las fluctuaciones climáticas del Cuaternario en su demografía histórica (para *Q. affinis, Q. laurina* y *Q. castanea*). La presente tesis en su Capítulo Uno ilustra los cambios en la distribución de las especies Neotropicales de *Quercus* tanto en relación con discontinuidades geográficas de las zonas montañosas (p. e. el Istmo de Tehuantepec, la Depresión de Nicaragua y el Istmo de Panamá) como para regiones con actividad tectónica reciente y constante (p. e. la zona de contacto entre las fallas Polochic y Motagua). A nivel genético, el Capítulo Dos describe niveles de diversidad genética más altos en *Q. insignis* y *Q. sapotiifolia* respecto a especies Holárticas del género. Las redes de haplotipos de ambas especies muestran procesos de colonización desde el Centro de Centroamérica (Guatemala y Honduras) hacia México y Costa Rica, e incluso procesos de re-colonización desde Costa Rica hacia Honduras. Está dinámica de dispersión, colonización y regresión es atribuida a procesos tectónicos y volcánicos característicos de estas regiones. El Capítulo Tres describe como *Q. costaricensis* a pesar de tener una distribución restringida al sur de Centro América, presenta una estructura filogeográfica fuerte a través de los bosques montanos altos, la cual responde a niveles moderados de flujo génico en sentido norte-sur influenciados por cambios en la distribución altitudinal de la especie durante la transición entre

periodos glaciales e integlaciales del Cuaternario. Por su parte *Q. bumelioides* exhibe un patrón opuesto, caracterizado por la ausencia de estructura genética y filogeográfica, evidencia de una colonización reciente, niveles altos de diversidad y flujo génico producto de distribución continua en los bosques montanos bajos durante el Cuaternario. A nivel regional y con base en estudios paleoecológicos para los Andes el Capítulo Cuatro describe la dinámica de la distribución de *Q. humboldtii* con respecto a los cambios climáticos durante el Último Máximo Glacial. A partir de estos análisis se identificó que las poblaciones del roble Andino presentan estructura altitudinal a través de los cinturones de vegetación de los Andes. Esta estructura es definida por cambios en variables climáticas asociadas principalmente a la temperatura. Igualmente se determinó que las fluctuaciones climáticas entre periodos glaciales e interglaciales no afectaron de forma significativa la extensión total del nicho climático disponible entre el presente y el Último Máximo Glacial ni generaron parches de bosque de encino aislados, por el contrario se presentó un desplazamiento de los cinturones de vegetación hacia las zonas bajas que presentaron niveles de conectividad importantes.Finalmente el Capítulo Cinco sugiere una expansión poblacional reciente para *Q. humboldtii*, lo cual es consistente con la aparición de polen fósil hace aproximadamente 480 *ka* AP. De igual forma, a partir de esta migración reciente se explican los valores bajos de diversidad genética, estructura genética y una red de haplotipos definida por dos haplotipos frecuentes de distribución amplia. Bajo este escenario se determinó que la llegada reciente de esta especie a los Andes Colombianos estuvo definida por eventos de cuello de botella seguidos por una expansión poblacional reciente (Holoceno temprano), lo que limitó el tiempo para acumular variación genética y promover procesos de diferenciación intraespecífica. Esta suma de patrones para las especies analizadas es consistente con procesos demográficos asociados a la estabilidad de la precipitación durante los periodos glaciales como se ha observado en otras especies con una distribución similar a la de las especies analizadas. Bajo estos modelos las condiciones climáticas favorecieron distribuciones continuas, lo que favoreció procesos de migración altitudinal. Debido al efecto moderado del cambio climático durante el Cuaternario, la estructura filogeográfica de las especies analizadas puede interpretarse a partir de procesos geológicos y características intraespecíficas de las especies (p.e. tamaño de las bellotas y distribución altitudinal) que pudieron determinar el éxito en los procesos de dispersión y colonización de ambientes nuevos durante la migración de las especies del género desde las montañas de México hasta los Andes Colombianos.

Abstract

Genus *Quercus* distributes from the Holarctic region through the Tropics. Along its distribution *Quercus* species represent fundamental elements of various ecosystems such as template deciduous forest, subtropical and template evergreen forest, pine-oak forest, cloud forest and montane forest. Neotropical oak forest distributes from the Mexican Transition Zone down to the Colombian Andes. Through its distribution *Quercus* species inhabit ecosystems from the sea-level up to the 3500 meters line. Moreover, oak species embrace a wide and heterogenic geographic space, characterized by a complex tectonic, volcanic and climatic history, as it could be observed in southern Mexico, Middle America and Colombian Andes. Along the mentioned area, there have been studied a wide variety of evolutionary processes such as species extinction, intra-specific differentiation, speciation and adaptation for different lineages, close related to the complex and dynamic geological history of the Neotropics. Neotropical oak species have not been the exception to this kind of studies as long as it has been described intra-specific divergence processes in several oak species related to geographic elements such as the Tehuantepec Isthmus (for *Q. oleoides*) and a moderate effect of the Quaternary climatic fluctuations in the oak historical demography (for *Q. affinis, Q. laurina* and *Q. castanea*). Chapter One of this thesis illustrates changes in the Neotropical *Quercus* species not only related to geographic discontinuities of the mountain ranges, particularly the Tehuantepec Isthmus, the Nicaraguan Depression and the Panamanian Isthmus, but also regions with recent tectonic and volcanic activity such as the contact zone between the Polochic and Motagua fault system. Genetically, Chapter Two described that both, *Q. insignis* and *Q. sapotiifolia,* exhibited higher genetic diversity values than other Holartic oak species. Haplotype networks from both species suggested colonization processes starting from Middle America to both, Mexico and Costa Rica. Interestingly, re-colonization processes from the Costa Rican mountains to the Middle America core distribution also were inferred from the haplotype network of *Q. sapotiifolia.* This dispersal, colonization and re-colonization processes were attributed to geological and volcanic events that characterize the Middle America region. Chapter Three describes a high phylogeographic structure in the upper montane forest oak *Q. costaricensis,* which suggested moderate levels og gene flow in a north-to-south direction influenced by altitudinal migrations during the Quaternary glacial to interglacial transitions. *Q. bumelioides* exhibited a contrasting pattern characterized by

the absence of phylogeographic structure, evidence of a recent colonization, high levels of genetic diversity and gene flow product of continuous lower montane extension through the lowlands during the Quaternary. Regionally and based on palaeoecological studies for the Colombian Andes, Chapter Four describes *Q, humboldtii* distribution dynamics respect to climatic changes during the Last Glacial Maximum. In that sense, results indicated the Andean oak populations have a well-defined altitudinal structure through the Andean forest belts. This altitudinal-based structure was defined mostly by climatic variables related to temperature values. Similarly it was defined that climatic fluctuations between the glacial and interglacial cycles did not importantly affected the total environmental climatic niche extension available between the present-day and Last Glacial Maximum periods, but it did configured a differential response between Andean forest belts oak communities. Generally, it was also determined that the effect of environmental changes did not promoted the a patchy and isolated distribution of the oak populations, on the contrary, vegetation belts elevation changes downwards favored important connectivity levels at the Andean lowlands. Finally, Chapter Five suggested a recent population expansion in *Q. humboldtii* consistently with the observed palynological records dated 48 *ka BP*. The recent migration evidenced in the pollen records also explains low gene diversity, genetic structure and a star-shaped haplotype network. Genetic results suggested a recent immigration of *Q. humboldtii* into the Colombian Andes defined by an initial bottleneck followed by a recent population demographic expansion (during the early Holocene), which limited the time available to observe genetic variation and did not allowed intra-specific differentiation. The patterns observed for the analyzed species were associated to stable climatic conditions during glacial stages as it has been reported for other species with similar distribution in the Neotropics. Under a model of few precipitation changes continuous forest were expected, which favored altitudinal migration of the species. The observed phylogeographical patterns could be attributed to geological processes and intra-specific traits of the studied species (e.g. acorn size and altitudinal distribution), which may have determined dispersal and colonization success into new available environments during the *Quercus* migration from Mexican mountains into the Colombian Andes.

Introducción

La distribución del género *Quercus* (Fagaceae) en la región Neotropical abarca áreas desde la zona de transición Mexicana, pasando por el domino Mesoamericano hasta el dominio del Pacífico y parte de la zona de transición Suramericana. A nivel biológico estas regiones son consideradas muy importantes debido a diferentes razones, entre las cuales se destacan que: i. Dentro de esta área se encuentra la zona de transición entre la biota Neotropical y la Neártica; ii. Dentro de su territorio se encuentran puntos calientes de diversidad como el Bosque de Pino-Encino, la región Tumbes-Chocó-Magdalena y Mesoamérica; iii. Una gran diversidad de especies de animales y plantas han sufrido procesos importantes de diversificación y extinción dentro de este territorio (Stehli & Webb 1985; Hooghiemstra & Van der Hammen 2004; Dacosta & Klicka 2008; Gutiérrez-García & Vázquez-Domínguez 2013). Los patrones de distribución actual para la biota del norte del Neotrópico y la heterogeneidad de eventos geológicos y climáticos que han moldeado el paisaje en este territorio a lo largo de la historia sugieren una relación causal entre ambos, que recientemente ha sido objeto de revisiones minuciosas (Bennett 2012; Gutiérrez-García & Vázquez-Domínguez 2013; Bagley & Johnson 2014; Ramírez-Barahona & Eguiarte 2014).

El género *Quercus* pertenece a la familia Fagaceae, y es uno de los grupos de árboles más estudiados en la región Holártica. Este género presenta niveles altos de diversidad en el sureste de los Estados Unidos de Norte América y el sur de México (Valencia-A 2004), además, juega un papel ecológico importante en ecosistemas tanto Neárticos como Neotropicales (Nixon 2006). La riqueza de especies del género *Quercus* dentro del territorio Americano ha sido un tema revisado de forma continua a través de los años, en particular para la región Mexicana (Lawrence 1951; Nixon et al. 1997;

Govaerts & Frodin 1998; Manos et al. 1999; Valencia-A 2004; Torres-Miranda et al. 2011, 2013; Rodríguez-Correa et al. 2015). Dentro de esta, la zona montañosa ubicada al sur de México es considerada como uno de los centros de diversidad para el grupo a nivel mundial (Nixon 2006). Las revisiones sobre el tema consideran que el número total de especies en América se encuentra alrededor de 220 especies. El género se distribuye desde Canadá y Estados Unidos, que cuentan con 4 y 90 especies respectivamente, pasando por México donde se encuentra un centro de diversidad representado con aproximadamente 160 especies (Valencia-A 2004), Centroamérica con alrededor de 35 especies, hasta llegar a Colombia donde únicamente se encuentra un representante del género, *Quercus humboldtii* (Pulido et al. 2006).

Nixon (2006) llama la atención sobre la reducción gradual en la diversidad de especies del género y los cambios en las características fenotípicas presentes a nivel latitudinal. Estos cambios ligados a la historia de la región Neotropical pueden ser una consecuencia de procesos no excluyentes entre los que es posible mencionar: i. La formación de una zona de transición entre las regiones Neártica y Neotropical a partir del levantamiento del puente centroamericano hace aproximadamente 25 *Ma* (Montes et al. 2012), la cual (en particular la región del Sur de Centroamérica) ha sido descrita por autores como Bagley & Johnson 2014 como una de las regiones más heterogéneas en términos físicos y biológicos en el mundo; y ii. Una historia geológica y climática compleja que incluye actividad volcánica reciente y elementos heterogéneos del paisaje como el Eje Neo-volcánico Mexicano, el Istmo de Tehuantepec y la Depresión de Nicaragua. Aunque en el norte de la región Neotropical se han estudiado diversas especies con el fin de reconstruir la historia evolutiva de los taxa que se distribuyen en la región (ver Gutiérrez-García & Vázquez-Domínguez 2013 y Bagley & Johnson 2014), existen zonas cuya importancia a nivel evolutivo han sido subestimadas. Este es el caso de el sur de Centroamérica, donde la mayoría de estudios filogeográficos se han basado en el papel que ha cumplido esta zona como un área de contacto y tránsito entre las biotas Neotropical y Neártica (Bagley & Johnson 2014), sin considerar los procesos regionales de diferenciación que pueden ser identificados a partir de especies con una distribución restringida a los sistemas montañosos Centroamericanos (p. e. Cordillera de Talamanca).

Para las especies del género *Quercus* distribuidas en el norte del Neotrópico que han estado sujetas a cambios de la actividad geológica y climática de esta región tan dinámica, no se ha estudiado en detalle el efecto de la heterogeneidad física sobre los patrones de diversidad y composición para el género. Gran parte de los estudios que analizan la distribución de la diversidad de las comunidades de roble se han enfocado en describir los efectos locales de gradientes altitudinales en la composición y estructura de las comunidades (Kapelle 1996; Kapelle & Uffelen 2006; Luna-Vega et al. 2006), o bien, en inferir la relación entre la diversidad de robles con respecto a la altitud y latitud a nivel regional, a partir de revisiones taxonómicas (p. e. Valencia-A 2004). Desde la perspectiva genética, la variación geográfica también es considerada como un tema fundamental pues los cambios en la variación genética a diferentes escalas espaciales son usados para explicar los principios y procesos que determinan la distribución de los linajes genealógicos tanto a nivel intra- como ínter-específico (Avise 1998; Manel 2003). Sin embargo, es importante tener en cuenta que los análisis moleculares requieren de un conocimiento muy completo sobre las especies evaluadas para lograr una interpretación apropiada de los resultados, y en particular para los estudios de la distribución geográfica de la variación genética intra-específica, es fundamental contar con un marco filogenético y biogeográfico que permita definir hipótesis evolutivas sólidas.

Los análisis biogeográficos son todavía escasos en el caso de los encinos Neotropicales, aun siendo especies importantes de los bosques montanos en Centroamérica (Nixon 2006; Pulido 2006). Recientemente diferentes estudios han iniciado una caracterización importante de los patrones biogeográficos y macroecológicos observados en las especies del género *Quercus* para la región del Neotrópico (Torres-Miranda 2011, 2013; Rodríguez-Correa et al. 2015). Debido a estos vacíos de información para el caso de las especies del género *Quercus,* no se contaba con la información suficiente para formular hipótesis filogenéticas y filogeográficas con bases biogeográficas claras. Sin embargo, avances recientes en el desarrollo de herramientas para análisis espacial, como los sistemas de información geográfica, el modelamiento de distribución de especies y la disponibilidad de información biológica a través de bases de datos tanto globales (p. e. Global Biological Information Facility) como regionales (p. e. Sistema de Información Biológica, SIB Colombia; Instituto Nacional de Biodiversidad, INBio-Costa Rica; Comisión Nacional para el Conocimiento y Uso de la Biodiversidad, CONABIO-México), han permitido elaborar bases de datos con una representación taxonómica amplia dentro del género *Quercus,* y por tanto han abierto la puerta para entender los patrones geográficos que exhiben las especies de encino en la región Neotropical como se describirá en el Capítulo Uno.

Diversidad del género *Quercus* en el Neotrópico

El género *Quercus* tiene un origen Holártico y se distribuye a través de diferentes tipos de hábitats, por ejemplo: bosques deciduos templados, bosques siempre-verdes templados y sub-tropicales, sabana subtropical y tropical, bosques subtropicales, bosques de pino-encino, varios tipos de bosque de niebla y bosques tropicales montanos (Kapelle et al. 1995; Nixon et al. 1997; Barbour & Billings 1999; Nixon 2006). Sin embargo, los anteriores son solo un ejemplo de los diversos ecosistemas donde los robles pueden establecerse y formar parte importante. En el continente Americano las especies del género se distribuyen en Canadá, Estados Unidos de Norte América, México, Belice, Guatemala, El Salvador, Honduras, Nicaragua, Costa Rica, Panamá y Colombia. Vale la pena resaltar que en el norte de la región Neotropical se encuentra uno de los centros de diversidad más importantes para el grupo (Valencia-A, 2004). Dicho centro se encuentra ubicado en la zona montañosa del sur de México, la cual se caracteriza por una alta actividad volcánica reciente (Ferrari et al. 1991; Ferrari et al. 2000; García-Palomo 2000; Belloti et al. 2006), y ha sido señalada por varios autores como una de las causas para los altos niveles de diversidad y endemismo reportados en varios grupos (Marshall & Liebherr 2000; Escalante et al. 2004; Corona et al. 2007).

El territorio correspondiente a la zona baja de Centroamérica (Costa Rica y Panamá) y Colombia alberga elementos menos diversos y diferentes a las demás especies del linaje Neártico, que siguen teniendo un papel importante a nivel ecosistémico (Nixon 2006). Este grupo del sur se caracteriza por una maduración anual de las bellotas y está formado principalmente por el complejo de *Quercus seemannii* (*Q. rapurahuensis* y *Q. gulielmitreleasei*), *Q. costaricensis* y *Q. eugenifolia* en la zona baja de Centroamérica y *Q. humboldtii* en Colombia (Nixon 2006). Uno de los patrones más interesantes de distribución de los robles en Mesoamérica es la disminución marcada en el número de especies a medida que la latitud disminuye a partir del centro de diversidad Mexicano hasta los Andes Colombianos. Dicho patrón se refleja en la presencia de al menos 160 especies de roble en México (Valencia-A 2004; Nixon 2006), 9 especies en Belice, 25-26 especies en Guatemala, 8-10 especies en El Salvador, 15-17 especies en Honduras, 14 especies en Nicaragua, 14 especies en Costa Rica, 12 especies en Panamá y tan solo una especie en Colombia (Nixon 2006).

A través de esta área de distribución es posible observar cambios importantes en algunos puntos claves de la distribución, como son: i. Eje Neovolcánico Mexicano, ii. Istmo de Tehuantepec y, iii. Istmo de Panamá (Torres-Miranda 2013; Rodríguez-Correa et al. 2015). Dicho comportamiento podría indicar una relación causa-efecto entre la historia geológica del norte del Neotrópico y la distribución de este grupo. Sin embargo, dentro de la literatura este fenómeno ha sido estudiado en su mayoría a través de revisiones taxonómicas (Valencia-A 2004) y análisis locales o regionales (Kappelle 1996).

Patrones biogeográficos del género *Quercus* en el norte del Neotrópico

Las áreas de endemismo juegan un papel fundamental ya que representan unidades básicas de análisis para la biogeografía evolutiva (Plantick 1991; Morrone 2008). Existen una gran variedad de trabajos biogeográficos para varias zonas del Neotrópico (Cole et al. 1994; Marshall & Liebherr 2000; Luna-Vega et al. 2001; Escalante et al. 2004; Méndez-Larios et al. 2005; Corona et al. 2007; Vargas et al. 2008; Escalante et al. 2009). Dichos trabajos mencionan diferencias entre algunos límites de las regiones Neotropical y Neártica. Sin embargo, en su mayoría coinciden en señalar la región del sur de México junto con el sur de Centroamérica como una región de gran importancia a nivel histórico para explicar los patrones de distribución de los organismos que se distribuyen en esta zona en el presente. Aún con una amplia variedad de estudios biogeográficos (concentrados en su mayoría en México), hace tan solo un par de años se han realizado estudios que describen los patrones biogeográficos en la región Neotropical para el género *Quercus* (Torres-Miranda et al. 2011, 2013; Rodríguez-Correa et al. 2015).

Estos patrones recién mencionados serán analizados en detalle en el Capítulo 1 de la presente tesis, sin embargo, es fundamental mencionar que existe una relación geográfica importante entre las áreas de endemismo observadas para el género *Quercus* en el Neotrópico y las zonas de mayor recambio de especies en esta misma región (Istmo de Tehuantepec, Falla Polochic-Motagua, Depresión de Nicaragua e Istmo de Panamá), donde las zonas de recambio parecen determinar la distribución y extensión de la mayoría de áreas de endemismo (Rodríguez-Correa et al. 2015). Bajo este escenario, es fundamental conocer cuál es el contexto geográfico que caracteriza el área donde se distribuyen las especies del género *Quercus* con el fin de incorporar elementos históricos (p.e. paleoclimáticos, tectónicos y volcánicos) que puedan explicar los patrones macroecológicos, biogeográficos y filogeográficos observados en el Neotrópico.

Diversidad, biogeografía y distribución del género *Quercus* en un contexto geológico

Las observaciones recopiladas en la presente tesis a partir de información ecológica, climática y genética exhiben una relación importante de la historia natural de las especies que forman el género *Quercus* con respecto a la historia geológica a través de su área de distribución. Considerando lo anterior, a continuación se hará referencia a las zonas geográficas más relevantes que serán discutidas en los capítulos siguientes. La importancia de estas zonas radica en su identificación como límites naturales de las especies que forman áreas de endemismo, áreas de recambio atípico de especies o bien, barreras importantes que determinan la estructura filogeográfica y patrones de flujo génico de las especies analizadas.

Sinopsis geológica del norte del Neotrópico

Dengo (1973) describe la estructura geológica del norte del Neotrópico (América Central incluyendo el centro y sur de México) en dos partes: la Septentrional y la Meridional. La primera forma parte del territorio Norteamericano y su límite sureño lo sitúa en la mitad del territorio de Nicaragua; está constituida por rocas metamórficas e ígneas originadas a principios o mediados de la era Paleozoica. La Meridional parte de este punto hasta llegar a las tierras bajas del Atrato en el nor-occidente Colombiano y está formada por una base de tipo oceánico emergente en la era Mesozoica. En el aspecto tectónico la región septentrional muestra durante las eras Paleozoica y Mesozoica actividad volcánica intensa, la cual se acentúa en el periodo Terciario Superior. En el territorio Nicaragüense dicha actividad aumenta desde el periodo Cretácico hasta el Cuaternario. En América Central Meridional, la historia tectónica no muestra las características de la anterior. En su lugar observamos levantamientos verticales en toda la región durante el Plioceno (López-Calleja 1980). Al final de la fase orogénica surge una intensa actividad volcánica semejante a la descrita anteriormente para la zona Septentrional. El puente terrestre entre Sur y Norte América se consolidó hace 15 Ma (Montes et al. 2012) y durante el Periodo Cuaternario la cadena volcánica de Centroamérica se transformó en una de las más activas del mundo (López-Calleja 1980; Marshall 2007).

Por otro lado, el territorio central y sureño de México está constituido por tipos diferentes de rocas con una estructura muy compleja en sedimentos, depósitos y afloramientos. La región correspondiente a la costa del Golfo es rica en sedimentos aluviales continentales y marinos. En la península de Yucatán abundan los depósitos de calizas, mientras que en las porciones centrales y del sur lo hacen rocas del Mesozoico (López-Calleja 1980). En la región Occidental y Central son comunes los depósitos de lava y ceniza volcánica del Cenozoico. En la zona Sur, en una parte del Istmo de

Tehuantepec, hay afloramientos de rocas del Precámbrico y en la porción más externa de ella se hayan intrusiones del Mesozoico y Cenozoico (López-Calleja 1980).

Eje Neovolcánico

El Eje Neovolcánico (ENV) es una provincia morfo-tectónica que se extiende en dirección oriente-occidente en el territorio mexicano y presenta una gran variedad de zonas climáticas (Ferrusquía-Villafranca 1993). Para esta región ha sido reportada una actividad volcánica extensa y reciente (Ferrari et al. 1991; García- Palomo 2000; Ferrari et al. 2000; Bellotti et al. 2006). Belloti et al. (2006) describe la evolución tectónica del sector centro-oriental del Eje Neovolcánico, de dicho estudio es fundamental resaltar la presencia de volcanes activos para periodos recientes como son el Nevado de Toluca, el Pico de Orizaba y el Volcán Popocátepetl. Estos volcanes hacen parte de un cinturón volcánico más amplio que se extiende a través de todo el eje y cuyo origen está relacionado con la interacción entre la Placa Cocos y la Norteaméricana (Ponce et al. 1992; Ferrusquía-Villafranca 1993; Pardo & Suárez 1993). Finalmente, esta actividad volcánica se puede considerar reciente tomando en cuenta que hasta hace aproximadamente 0.1 Ma dicha actividad parece haber disminuido en el caso del Nevado del Toluca (Belloti 2006). Una característica fundamental del Eje Neovolcánico que lo hace un sistema de desarrollo muy heterogéneo es su zonificación en tres regiones distintas: Oriental, Central y Occidental (Pasquarè et al. 1988; Ferrari et al. 2000) además de exhibir la Unión Triple de Colima-Chapala-Tepic en el límite occidental del Eje Neovolcánico. Dichas diferencias pueden ser un factor importante para procesos de diferenciación, extinción local y distribución en general para los organismos que se distribuyen a través de la zona.

Sierra Madre Oriental

Esta área en particular presentó actividad volcánica continua durante el Cuaternario y es considerada como un dominio tectónico único en comparación a las demás zonas colindantes como el ENV (Demant & Robin 1975; Ruiz-Martínez et al. 2000). La Sierra Madre Oriental (SMO) se caracteriza por tener elevaciones desde los 200 hasta >3000 metros. En la mayoría de su territorio se presentan elementos entre los 1000 y 2000 metros y un 20% se encuentra entre los 2000 y 3000 metros. Esta variación hace de la SMO una región de paisaje heterogéneo con elevaciones importantes agrupadas hacia la zona nor-oriental y sierras aisladas en el territorio restante (Ferrusquía-Villafranca 1993). Sumado a esta diversidad del paisaje, existen elementos climáticos importantes a considerar, pues durante el invierno corrientes de aire polar llamadas “nortes” se extienden por la costa oriental del territorio mexicano, a través de la Sierra Madre Oriental trayendo lluvias intensas en estas laderas orientales, al igual que en los estados de Chiapas y Oaxaca (Metcalfe et al. 2000). Históricamente, en términos climáticos la región de la SMO representa un nivel intermedio del efecto del último glacial máximo (UGM) en el territorio mexicano, pues los valores de ELA (del inglés Equilibrium Line Altitude) de los glaciares son menores a los presentados para el interior de México, pero mayores que en la vertiente del Pacífico (Lachnieta & Vazquez-Selem 2005).

Istmo de Tehuantepec

El Istmo de Tehuantepec (IT) es una de las barreras biogeográficas y filogeográficas esperadas en el planteamiento inicial de este proyecto para las especies del género *Quercus*; debido al cambio marcado en altitud que se presentan en esta zona, donde terminan los sistemas montañosos del norte convirtiéndose en una zona de dispersión difícil para los elementos montanos como son las especies de *Quercus*. Esta zona está representada en dos sub-provincias de la Provincia Tectónica de Chiapas denominadas la Depresión Central y la Planicie Costera del Pacífico. Ambas corresponden a zonas entre los 0 msnm hasta un máximo de 1000 metros (en la Depresión Central; Ferrusquía-Villafranca 1993). La zona correspondiente a la Planicie Costera del Pacífico consiste en playas y depósitos fluviales del Cuaternario con yacimientos dispersos de varias edades y composiciones, mientras que la Depresión Central presenta formaciones del Jurásico bien definidas hacia el nor-occidente, cuerpos de composición calcárea hacia el sur-oriente y pequeñas áreas de cuerpos Cenozoicos al sur de Tuxtla Gutiérrez (Ferrusquía-Villafranca 1993).

Depresión de Nicaragua

Nicaragua se ubica sobre el borde occidental de la Placa del Caribe. La interacción de esta placa con la Placa de Cocos ha producido una actividad volcánica y sísmica frecuente en la región. La Depresión de Nicaragua (DN) se caracteriza por presentar una cadena de volcanes Cuaternarios activos o de actividad reciente, sedimentos volcaniclasticos, albergar los lagos Managua y Nicaragua y estar rodeada por un grupo discontinuo de fallas prominentes (Arengi & Hodson 2000). Hacia el Sur de la Depresión de Nicaragua es importante resaltar la presencia de una zona de división en dos elementos estructurales del Istmo Centroamericano, los bloques Chorotega y Chortis (Arengi & Hodgson 2000).

Cordilleras de Costa Rica e Istmo de Panamá

El sur de América Central consiste en un cinturón volcánico del Neógeno-Cuaternario inmerso en el bloque Chorotega. La geomorfología de esta zona refleja una historia dinámica del vulcanismo del Cenozoico y una actividad tectónica compleja (Marshall 2007). Junto con el bloque del Chocó, el bloque Chorotega está ubicado en una zona de contacto entre cuatro placas: la placa del Caribe, la placa de Suramérica, la placa Cocos y la placa Nazca (Marshall 2007). Debido a su ubicación, los márgenes del bloque Chorotega se han deformado formando un sistema de micro placas que generan deformaciones regionales y una heterogeneidad topológica importante (Marshall 2007). Los elementos que distinguen el bloque Chorotega son el arco volcánico Chorotega, que a su vez agrupa los sistemas montañosos de Costa Rica (Cordillera de Guanacaste, Cordillera Central, Cordillera de Aguacate y la Cordillera de Talamanca), la zona del canal de Panamá y el Istmo del Darién (Marshall 2007). Tal vez el elemento histórico más importante relacionado con esta zona es la consolidación del puente terrestre entre Sur y Norte América hace 15 Ma (Montes et al. 2012), debido al impacto que esto implicó para las biotas tanto Neárticas como Neotropicales (Zonas de contacto, diversificación de linajes y migraciones entre Norte y Sur América; Coates et al. 1992).

Contexto filogeográfico del norte del Neotrópico

Recientemente se han desarrollado una cantidad importante de estudios sobre la distribución de la variación genética de diferentes taxa en el norte del Neotrópico. Este desarrollo conceptual fue revisado profundamente por Gutiérrez-García & Vázquez-Domínguez (2013) y Bagley & Johnson (2014) para el sur de Centroamérica. De forma complementaria, y enfocados en los bosques nubosos del Neotrópico Ramírez-Barahona & Eguiarte (2014), revisaron las diferentes propuestas planteadas a la fecha para explicar el efecto de las fluctuaciones climáticas durante el UGM en la demografía histórica de los organismos. En términos generales Gutiérrez-García & Vázquez-Domínguez (2013) describen tres grupos evolutivos a partir de patrones filogenéticos y filogeográficos comunes: el grupo Maya, el grupo Medio-Centroamericano y el grupo Panameño. Las características principales de estos grupos están definidas por la heterogeneidad geológica que determina una estructuración y divergencia fuerte además de presencia de refugios para el grupo Maya, diferenciación entre tierras altas y bajas asociada con actividad volcánica intensa para el grupo Medio-Centroamericano y migraciones latitudinales bidireccionales a través del Istmo de Tehuantepec que promovió divergencia de especies y procesos de especiación dentro del grupo Panameño.

Para el caso del sur de Centroamérica, ubicado entre el norte de Costa Rica y el sur de Panamá (incluyendo las islas cercanas a este territorio, p. e. Quepos, Boca del Toro, Coiba, Las Perlas y Cocos), Bagley & Johnson (2014) reportaron la existencia de dos patrones filogeográficos generales al comparar diferentes taxa. Según los autores mencionados anteriormente, diferentes grupos de organismos han mostrado una estructura genética definida por la presencia de la DN, el Frente Volcánico Chorotega y el istmo del Darién, al igual que diferentes patrones filogeográficos entre las vertientes del Pacífico y el Caribe en la zona montañosa de Panamá. Estos patrones comunes son atribuidos por Bagley & Johnson (2014) a respuestas históricas comunes entre los taxa del sur de Centro América ante procesos geológicos regionales. En contraste a este patrón otros organismos se caracterizan por no presentar estructura filogeográfica ni evidencia de cambios demográficos asociados a eventos físicos (geológicos o climáticos) de la región, hecho que resalta la importancia de procesos biológicos locales como el flujo génico y la dispersión a larga distancia como determinantes de la estructura genética de las especies (Bagley et al. 2014).

De forma complementaria, Ramírez-Barahona & Eguiarte (2014) al estudiar los patrones filogeográficos de las especies de los bosques de niebla del Neotrópico relacionados con la dinámica climática durante el UGM reportan inconsistencias entre los estudios filogeográficos y los modelos propuestos de refugios para dicho periodo.

En este aspecto, se ha propuesto la prevalencia de condiciones áridas que generaron compresión, fragmentación y aislamiento de los bosques tropicales en parches de bosques sin conexión durante el UGM (Haffer 1969; Toledo 1982; Carnaval et al. 2009). En el lado opuesto, autores como Colinvaux et al. (2000) y Baker (2003) proponen que no existieron estos cambios drásticos en precipitación durante el UGM, por lo tanto los bosques tropicales debieron tener distribuciones estables y continuas durante este periodo. La falta de evidencia que soporte alguno de los dos modelos de forma contundente según Ramírez-Barahona & Eguiarte (2014) se atribuye a respuestas diferenciales de las especies a los eventos de cambio climático o bien, a diferencias en el contexto analítico usado para el estudio de las especies.

Estudios posteriores a las revisiones recién mencionadas o bien no considerados en las mismas, suman un nivel de complejidad más al estudio de los patrones filogeográficos de especies Neotropicales. Ornelas et al. (2013) describen el papel de elementos geográficos como el IT en la configuración de la estructura filogeográfica de 15 especies distribuidas en los bosques Mesófilos de México. Dicho estudio plantea que aún cuando algunos elementos geológicos promueven eventos de diferenciación intraespecífica, cuando se comparan los mismos patrones entre especies existen periodos diferentes durante los cuales ocurrió el proceso de diferenciación. Debido a esta observación Ornelas et al. (2013) sugieren que la historia evolutiva de la biota que forma los bosques Mesófilos es específica para linajes particulares y está definida por diferencias en el nicho ecológico y capacidad de dispersión entre las especies. Este estudio es de suma importancia al identificar la variación temporal para procesos de diferenciación entre taxa co-distribuidos a partir de elementos geológicos comunes. Finalmente, Ornelas & González (2014) al caracterizar la historia demográfica de *Moussonia deppeana* continúan suministrando información que ejemplifica las diferentes respuestas de las especies ante eventos históricos de cambio climático. En este caso, *M. deppeana* exhibió una estructura filogeográfica y demográfica que siguieren que la especie persistió durante los periodos glaciales, mientras que durante los periodos interglaciales experimentó procesos de aislamiento y divergencia. Estos resultados según Ornelas & González (2014) resumen de forma consistente un modelo de refugios bajo condiciones secas durante los ciclos glaciales e interglaciales para los bosques Mesófilos.

Bajo este contexto para la región Neotropical y ante la cantidad creciente de información que relaciona la historia geológica del norte del Neotrópico con la historia demográfica de diferentes taxa distribuidos en esta región, y que describe respuestas diferentes entre taxa co-distribuidos ante la dinámica geológica y climática de la región (para una revisión detallada consultar Gutiérrez-García & Vázquez-Domínguez 2013 y Bagley et al. 2014), en esta tesis se pretende definir un contexto biogeográfico como marco de referencia para estudios filogeográficos posteriores en especies del género *Quercus* y otros taxa distribuidos en el norte del Neotrópico y, describir los procesos evolutivos que han determinado la migración de las especies de encino desde el centro de México hasta los Andes Colombianos a partir de análisis filogeográficos. Para cumplir con estos objetivos la estructura de la presente tesis fue definida de manera tal que a partir de análisis tanto biogeográficos como filogeográficos, se respondieran los objetivos siguientes: i. definir los patrones de distribución de las especies del género *Quercus* en el norte del Neotrópico (Capítulo Uno) utilizando análisis biogeográficos y macroecológicos, ii. describir la historia evolutiva de un grupo selecto de especies con patrones de distribución contrastantes, al caracterizar la estructura filogeográfica y los procesos demográficos históricos de dos especies de distribución amplia, *Quercus insignis* y *Q. sapotiifolia*, que se distribuyen a lo largo de la Zona de Transición

Mexicana y las Serranías Trans-Ístmicas (Capítulo Dos), iii. describir la historia evolutiva por medio de la caracterización filogeográfica de dos especies de distribución regional, *Q. bumelioides* y *Q. costaricensis*, distribuidas en las Cordilleras de Costa Rica (Capítulo Tres), iv. describir la relación entre procesos históricos como las fluctuaciones climáticas durante el Último Glacial Máximo (~21 ka BP) con los patrones de migración de las especies del género a partir de una especie modelo (*Q. humboldtii*), utilizando información paleobotánica y contemporánea para construir y comparar la distribución de la especie a partir de modelos de nicho ecológico desde el UGM (Capítulo Cuatro) y, v. caracterizar la historia demográfica y la estructura filogeográfica de la única especie del género *Quercus* distribuida en los Andes Colombianos, *Q. humboldtii* (Capítulo Cinco).

Capítulo Uno

How Are Oaks Distributed in the Neotropics? A Perspective from Species Turnover, Areas of Endemism, and Climatic Niches

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Abstract

Premise of the research: The most important diversity hotspot of genus *Quercus* (Fagaceae) in America is situated in southern Mexico. From this area down to the Colombian Andes, oak species diversity decreases considerably, but the pattern of species distribution and turnover has not been analyzed. This study aimed at determining geographical patterns of species turnover, species distribution and endemism for Neotropical *Quercus* species.

Methodology: Occurrence records for 58 oak species belonging to the *Quercus* and *Lobatae* sections were obtained. Patterns of species turnover were determined by comparing species composition among latitudinal/longitudinal units. areas of endemism were determined using weighted networks. The potential distribution of oak species was determined using ecological niche models. Finally, niche divergence tests were used to identify changes in the oak species ecological niche across areas.

Pivotal results: The species composition analysis indicated that the Tehuantepec Isthmus, the Nicaraguan Depression and the Panamanian Isthmus represent species turnover points. Nine areas of endemism were recovered, distributed through mountainous ranges from Mexico to Costa Rica. Most of these areas were delimited by the species turnover points detected. ENMs indicated that the turnover points represent areas with low climatic suitability for most oak species and represent discontinuities in the distribution of *Quercus*. Niche comparisons suggest niche divergence among species distributed in different areas of endemism or on opposite sides of turnover points.

Conclusions: The results indicate that the Tehuantepec Isthmus, the Nicaraguan Depression and the Panamanian Isthmus have acted as important barriers to the dispersal of oak species, influencing species diversity, biogeographic patterns and niche divergence.

Keywords: biogeography, distribution, diversity, Neotropical trees, *Quercus.*

Introduction

The area between the tropics of Mexico and the Colombian Andes is characterized by a high biological diversity and includes three of the most important global biodiversity hotspots (Mesoamerica, Chocó/Darién and Tropical Andes; Myers et al. 2000). This area is very important biogeographically and in terms of conservation of biodiversity due to various reasons, including: i. It represents a transition zone between the Neotropical and the Nearctic biota (Morrone 2010) ii. Within this area a great number of animal and plant taxa have experienced processes of diversification, extinction and migration (Gutiérrez-García and Vázquez-Domínguez 2013). iii. This area is undergoing exceptional loss of habitat (Myers et al. 2000). Oak species (*Quercus*: Fagaceae) are widely distributed within this region. This genus has high levels of diversity in the southeastern United States of America. However, the greatest oak species diversity occurs in the mountains of southern Mexico (Nixon 2006), although Valencia-A (2004) pointed out that also the central and northeastern regions of the country bear considerable numbers of species. In Central America, although oak species richness is lower than in Mexico, *Quercus* occupies the second place of importance in terms of richness in the montane forests.

The taxonomy and species richness of *Quercus* L. in the American territory has been a continuously revised topic over the years, particularly for the Mexican region (Nixon 1997; Valencia-A 2004). The reviews on the subject indicate that the total number of species in America is around 220, distributed from Canada and the United States with 4 and 90 species respectively, through Mexico with approximately 161 species (Valencia-A 2004), Central America with about 40 (Nixon 2006), and Colombia, where there is only a single species, *Quercus humboldtii* (Pulido et al. 2006).

Nixon (2006) described these changes in the richness of *Quercus* and the variation in phenotypic characteristics associated with the latitudinal range of Neotropical oak species. These patterns represent an interesting topic considering geological and biological processes in Mexico and Central America, including: i. the formation of a transition zone between the Nearctic and the Neotropical biotas as a result of the lifting of the Panamanian Isthmus (Panama, Costa Rica and southern Nicaragua), and ii. geological activity at morphotectonic provinces such as the Trans-Mexican Volcanic Belt and the Tehuantepec Isthmus in Mexico, the Nicaraguan Depression, and the mountains of Costa Rica. The first event caused a bidirectional biotic exchange that occurred from the Eocene to the present, reaching a peak during the Pliocene and the Quaternary (Webb 1991). During this period, specifically at 470 ka BP, nearctic elements such as *Quercus* appeared in the fossil record at the north of the Andes (Van't Veer and Hooghiemstra 2000). The second set of events promoted a new landscape configuration, product of a constant and recent volcanic and geologic activity that could have favored the expansion of various lineages in a latitudinal gradient, the formation of barriers to dispersal, and local diversification and extinction processes (Cavender-Bares et al. 2011, Ornelas et al. 2013).

Patterns of change in species diversity and species composition for Neotropical *Quercus* species have been considered obvious. Most of the studies examining the diversity of oak communities have focused on describing or commenting the effect of altitudinal gradients on community composition and structure (Kappelle 1996, Kapelle and Uffelen 2006, Luna-Vega et al. 2006), and on the relationship between the diversity of oak species and elevation and/or latitude at the regional level (e. g. Valencia-A 2004). Recently, Torres-Miranda et al. (2011, 2013) described the biogeography of the *Lobatae* section in Mexico and Central America. These studies identified as centers of species richness, with high levels of endemism, the north of the Sierra Madre Oriental and the southern foothills of Jalisco (both located in Mexico), and proposed to redesign the protected areas system to ensure the conservation of the *Lobatae* key elements. However, the distribution patterns for the whole *Quercus* genus within the Neotropics have not been analyzed, particularly in regard to how species composition changes across the region.

Recent advances in the development of spatial analysis tools, geographic information systems, species distribution modeling and the availability of information on the occurrence of species at both global (e. g. Global Biological Information Facility) and regional (e.g. Biological Information System, SIB-Colombia; Biodiversity National Institute, INBio-Costa Rica; National Commission for Knowledge and Use of Biodiversity, CONABIO-Mexico) databases, allow to apply various analysis of distribution patterns in terms of ecology and biogeography from local to regional scales. Fortunately, the genus *Quercus* is significantly represented in these databases and herbarium collections, a fact that allowed us to propose a broad scale analysis. Therefore, we have set as the main goal of this paper to analyze distribution patterns of Neotropical *Quercus* species using macroecological and biogeographic approaches including: i. The identification of areas of particularly high species turnover across the Neotropics; ii. The determination of areas of endemism for the genus and their possible relationship with the areas of species turnover, iii. The evaluation of climatic niche differences across areas of endemism, and iv. The modeling of the potential distribution and *Quercus* species co-ocurrence patterns.

Materials and methods

Study area

The study area (Fig. 1) was delimited to the north by the Mexican Transition Zone *sensu* Morrone and Márquez (2001) and Morrone (2001, 2006) and to the south by the Colombian Andes. These edges encompass the distribution area of most of the oaks species within the Neotropics.

Species studied and occurrence records

The oak species distributed in the study area (Table 1) were defined using taxonomical databases (Flora Mesoamericana Project, Missouri Botanical Garden available at http://www.tropicos.org/Project/FM) and reviews of the genus (Valencia-A 2004; Morales 2010). Geographical information was compiled using oak species presence records reported on the National Biodiversity Institute-INBio (Costa Rica; available at http://atta.inbio.ac.cr/ last accessed 2012-08-20) and The Missouri Botanical Garden Tropicos® (available at http://www.tropicos.org/Home.aspx last accessed 2012-08-20) datasets, and data published by: Herbario del Instituto de Ecología, A.C., MEXU/Tipos de plantas vasculares, Catálogo de autoridad taxonómica del género *Quercus*, Fagaceae en México, Herbarium of The New York Botanical Garden and Instituto Alexander von Humboldt through the GBIF Data Portal (available at http://www.data.gbif.org, last accessed 2012-08-20). This information was complemented with our own database, compiled from specimens deposited in several Mexican herbaria and field observations.

Sections *Quercus* and *Lobatae* differ in their distribution (*Lobatae* reaches the Colombian Andes while the southernmost distribution limit for *Quercus* is in Panama) and phenological traits (e. g. annual vs. biannual acorn maturation in white and red oaks, respectively). In view of such differences, turnover and niche distribution analyses were performed considering the genus *Quercus* as the study unit, but also for both sections separately.

Species turnover

The study area was divided separately in two ways: in rectangles of a latitudinal degree of height encompassing from 0° to 30 ° N, and in rectangles of a longitudinal degree of width from -117º to -75º W. Similar subdivisions have been used in studies of avifauna in humid montane forests along Mesoamerica (Sánchez-González and Navarro-Siguenza 2009). Species turnover was calculated using the dissimilarity index proposed by Lennon et al. (2001) (βsim hereinafter) for each latitudinal or longitudinal rectangle relative to its upper/lower or left/right neighbors for the whole genus and separately for the *Quercus* and *Lobatae* sections. This turnover index quantifies the relative magnitude of species gain and loss relative to the minimum value of species richness. Therefore, it allows identifying changes in the composition or species richness in relation to the unit with the lower richness value. The mean and standard deviation were estimated for 1-βsim (similarity) values across all study units. Units showing 1- βsim values beyond one standard deviation of the mean were considered as areas of atypically high species turnover. Several microendemic oak species in our dataset (*Quercus furfuracea, Q. grahami, Q. hirtifolia, Q. macdougalli, Q. mulleri, Q. pachucana, Q. paxtalensis* and *Q. rubramenta*) were not considered for the species turnover analysis.

Areas of endemism

In order to determine the geographical association of the neotropical oak species in terms of co-occurrence patterns, the Network Analysis Method (NAM; Dos Santos et al. 2008, 2012) was employed. NAM uses species punctual records directly and is different from the traditional procedures (such as the ones described by Szumik et al. 2004) in which sympatry is determined by overlapping species using grids of square cells of an arbitrary size delimited a priori. As with NAM no grids or polygons are needed, the uncertainty about the appropriate dimension of study units is not a major topic (Dos Santos et al. 2012). After creating sympatry networks, NAM identifies clusters of cohesively sympatric species that are simultaneously allopatric with others clusters. NAM was implemented using the software SyNet 2.0 (available at http://www.cran.r-project.org; Dos Santos et al. 2008), which is an add-on package for the statistical software R, using standard parameters. Once the cleavogram was obtained, branches were selected using the backward search as our interest was to recover the smallest sympatry areas.

Ecological niche modeling

Ecological niche modeling (ENM) was used to define environmental affinities among areas where *Quercus* species are present, and to identify the location of possible gaps in their distribution. For this goal we used the maximum entropy algorithm implemented in MAXENT version 3.3.3a (Phillips et al. 2006) using default parameters. In order to avoid over fitting due to autocorrelation between climatic variables, within the distribution range of each oak species, between 500 and 2500 random points were calculated, and the values corresponding to the 19 climatic variables reported by Hijmans et al. (2005) were extracted at a 30 arc-seconds (~1 Km) spatial resolution (available at http://www.worldclim.org). For the data of each species, correlation matrices were calculated among all 19 variables and from each pair of highly correlated variables (r>0.7) the more specific variable was discarded. Additionally, the soil type (FAO-UN Digital soil map of the world; available at http://www.fao.org) and elevation variables were also considered as scenopoetic data to construct the ENMs (Table A1).

Considering that not all oak species had a sufficient number of records, only 43 species were used for this analysis (species over 30 occurrences). Occurrence records of each species were filtered altitudinally and latitudinally by comparison to reported distribution information (following Valencia-A. 2004 and Morales 2010). In order to decrease possible effects of spatial autocorrelation due to proximity and aggregation of records, we only used points separated by more than 0.1 decimal degrees with respect to its nearest neighbors. Finally, ENMs were restricted to the biogeographic provinces where oak species are present; in order to avoid including possible climatically suitable areas that *Quercus* species have not occupied historically (e. g. the Yucatán peninsula).

ENMs were implemented using the bootstrap resampling method with 50 replicas. From the initial data set 30% of the presence records were used as a sub-run to calculate various estimates of quality and the remaining 70% was used to run the models. Models were initially evaluated with a threshold-independent method, the receiver operating characteristic (ROC) curve analysis, to determine model quality. As a threshold-dependent method we implemented the intrinsic omission rate, using the cumulative value of 10%. This threshold was selected considering that databases could include several erroneous occurrence records even after extensive depuration processes. Using the sum of the binomial outputs generated considering the threshold rule mentioned above, we identified the areas with a low number of potentially co-occurring species (low suitability areas) that separate regions with a high number of co-occurring species (high suitability areas). Additionally, the values of the climatic variables used to build the ENMs were compared among low and high suitability areas to identify the most important climatic factors that limit the distribution of oak species.

Results

Species turnover

Species turnover values exceeding the defined threshold of one standard deviation of the 1-βsim mean value were observed in both latitudinal and longitudinal gradients at the whole genus level, and also for the *Quercus* and *Lobatae* sections separately (fig. 2). Species composition changes (figs. 2a, 2b, 3a and 3b) at the genus level were observed in the Tehuantepec Isthmus (TI), the Motagua-Polochic fault (MPF), the Nicaraguan Depression (ND) and the Panamanian Isthmus (PI).

Red oak species (*Quercus* sect. *Lobatae;* Figs. 2c, 2d, 3c and 3d) showed a similar pattern of turnover areas. The TI appears as the first turnover area, followed by the ND and finally the Panamanian Isthmus. Species turnover values for *Quercus* sect. *Quercus* (figs. 2e, 2f, 3e and 3f) indicated that the limit between the Sierra Madre de Chiapas (SMC) and the TIM is the area where the first atypically high species turnover occurs. At the MPF region there is a second area of species turnover where several species with wide latitudinal distributions reach their southern distribution limit. Finally, the ND is the last turnover area for white oaks. The PI did not appear as a turnover area, as no white oak species reaches the Colombian Andes.

Areas of endemism

Nine network partitions or units of co-occurence (UCs) where recovered in the cleavogram derived through NAM analysis. These are shown in fig. 4: a. Supported by four species (*Q. martinezii, Q. nixoniana, Q. salicifolia* and *Q. uxoris*) distributed in the north of the Sierra Madre del Sur (SMS), Serranías de Guerrero (SG) and Serranías de Jalisco (SJ) (fig. 4a); b. Supported by three species (*Q. deserticola, Q. frutex* and *Q. rugosa*) distributed in the Sierra Madre Occidental (SMOc), Trans-Mexican Volcanic Belt (TMVB) and the Sierra Madre de Chiapas (SMC) (fig. 4b); c. Supported by four species (*Q. acutifolia, Q. crassipes, Q. glaucoides* and *Q. laurina*) distributed in the

TMVB, southern Sierra Madre Oriental (SMOr) and SMS (fig. 4c); d. Supported by three species (*Q. cortesii, Q. lancifolia* and *Q. xalapensis*) distributed in SMC, southern SMOr and north of the Trans-Isthmian Mountains (TIM) (fig. 4d); e. Supported by *Q. acherdophylla, Q. depressa* and *Q. germana*, distributed in the SMOr (fig. 4e); f. Supported by three species (*Q. segoviensis, Q. skinerii* and *purulhana*) distributed in the TIM (fig. 4f); g. Supported by two species (*Q. pachucana* and *Q. repanda*) distributed in the eastern TMVB (fig. 4g); h. Supported by two species (*Q. liebmanii* and *Q. rubramenta*) distributed in the SMS (fig. 4h) and, i. Supported by two species (*Q. bumelioides* and *Q. costaricensis*) distributed in the Costa Rican mountains (CRM) (fig. 4i). It can be observed that the distribution of all the units of co-occurrence were delimited at least at one edge by the turnover points described above, as follows: UCs a, b, c, e, h are delimited by the TI at the south; UC d is delimited by the MPF at the south; UC f is delimited by the TI at the north and the ND at the south; and UC i is delimited by the ND at the north and the PI at the south.

Ecological niche modeling

The models for all the species evaluated showed a good performance (AUC values over 0.89). For most oak species, the climatic variables with the highest influence on the ENMs were annual mean precipitation, temperature seasonality, temperature annual range, annual precipitation and precipitation seasonality (Table 1). The map with the sum of the models for all individual species shows the gaps in the *Quercus* species distribution (fig. 5a). In the northern part of the studied region, high levels of *Quercus* species co-occurrence is observed in mountainous areas of central and southern Mexico, particularly the TMVB and the SMS. The Balsas River Depression (BD) is an area with low presence of *Quercus* species that separates the TMVB and the SMS. The TI constitutes a clear gap in the distribution of *Quercus* species. From Guatemala to Nicaragua, the Trans-Isthmian Mountains stand out as the area with the highest *Quercus* species overlapping, while the lowlands of eastern Nicaragua and the ND configure an important gap of the genus distribution. In Costa Rica, the higher species concentration is observed in the Talamanca Mountains and part of the Pacific lowlands. Finally, species concentration falls down in the territory corresponding to the PI and the lowlands of northwestern Colombia. The map reflects a marked reduction in the number of species co-ocurring from southern Mexico to the Colombian Andes. The maximum values of predicted number of species co-ocurring for the different countries are distributed as follows: 18 species in Mexico, 10 species in Guatemala, 9 species in Honduras, 7 species in Nicaragua, 6 species in Costa Rica, and 3 species in Panama.

Section *Lobatae* (fig. 5b) showed a higher number of co-distributed species in central and southern Mexico than section *Quercus*. From southern Nicaragua down to the Colombian Andes *Lobatae* species appear considerably restricted to the mountainous regions of Costa Rica, but in the Colombian Andes a single species (*Q. humboldtii*) has a very broad distribution with a wide altitudinal range (between 800 and 3500 meters). For section *Quercus* (fig. 5c), species co-ocurrence values are higher in the central TMVB and the SMS, followed by the area from Guatemala down to northern Nicaragua. Costa Rica and Panama exhibit only three and two white oak species respectively. The observed distribution of sections *Quercus* and *Lobatae* species showed that areas such as TI, ND and PI are not suitable habitat for *Quercus* species. The comparisons between low suitability areas and high suitability areas indicated highly significant differences in annual mean temperature, temperature seasonality, temperature annual range, annual precipitation and precipitation seasonality (Table 2, Fig. 6).

Niche divergence across areas of endemism

The principal components analysis (Fig. 7) indicated that groups of species that defined most of the UCs have partially overlapping climatic niches. In particular, UCs b and c have wide and overlapping climatic envelopes that also contain the relatively narrower envelopes of UCs g and h. A second, recognizable group was formed by UCs a, e and f. Finally, UCs d and i seem to be the most distinct in terms of their climatic niches. Niche divergence tests among the nine UCs indicated that there is at least one divergent niche axis in every pairwise comparison (Tables 3 and 4). UCs f and i showed significant divergence in the second and third niche axes compared to most of the other UCs. The variables with the higher loadings in these two axes were annual mean precipitation, temperature seasonality and precipitation seasonality. The UCs a, b and c, conformed by species with wide distributions in Mexico also differed from each other in the three niche axes. Meanwhile, the UCs constituted by species with restricted geographical distributions in Mexico (UCs e, g and h) showed a conserved first niche axis with respect to the other UCs in almost all comparisons. Finally, UC i (restricted to the Talamanca Mountains) showed the strongest divergence in the three axes in comparison to all other UCs.

Discussion

Studies on the turnover patterns of *Quercus* species in the Neotropics have mainly focused on altitudinal patterns (Gentry 2001, Kappelle 2006, Kappelle and Van Uffelen 2006). The present study considered latitudinal and also longitudinal units in order to complement the current knowledge about the distribution patterns of Neotropical *Quercus*. Most of the previous studies on oak distribution have highlighted not only the change in oak species diversity from southern Mexico to Colombia, but also the fact that mountainous regions corresponding to southern Mexico are important diversity hot spots for the *Quercus* genus in America (Valencia-A 2004, Nixon 2006, Torres-Miranda et al. 2011, 2013).

Kapelle (2006) suggested that the distribution patterns of oaks can be explained by the geological and climatic history of the American continent, and the evolution of its flora. Recent phylogeographic evidence from *Quercus* species (Cavender-Bares et al. 2011) and other taxa (see Gutiérrez-García and Vázquez-Domínguez 2013 and Ornelas et al. 2013 for a detailed per species description) indicates that intra- and interspecific processes such as divergence, speciation and migration, coincide with historical geological and climatic features of Central America. Our results, based on biogeographical and macroecological approaches, suggest that most of the areas identified as barriers to gene flow for different taxa, are also important *Quercus* species turnover points, areas of climatic discontinuities and boundaries for areas of endemism. In the following, we provide a detailed discussion of the patterns found at each region.

Central and southern Mexico

This region is particularly interesting due to the high number of co-occurring oak species and the presence of several UCs limited at the south by the Tehuantepec isthmus. Both the Trans-Mexican Volcanic Belt and the southern Sierra Madre Oriental have been recovered as regions with high levels of endemism in several similar analysis performed for groups such as birds and mammals (Corona et al. 2007, Vargas et al. 2008). For the Trans-Mexican Volcanic Belt, Escalante et al. (2009) reported low levels of mammal endemism, proposing a review of the importance of the area as the limit between the Nearctic and the Neotropical regions. However, our analysis suggests an important role of the Trans-Mexican Volcanic Belt on the history of oak species distribution, reflected in the presence of three units of co-occurrence (UCs b, c and g) within this area. This morphotectonic province is located between latitudes 17º 30’ and 20º 25’ N and longitudes -96º 20’ and -105º 20’ W and spans from coast to coast presenting a wide variety of climatic zones (Ferrusquía-Villafranca 1993), which may have allowed the establishment of different oak species with different climatic requirements or niches. Recent studies have also highlighted the importance of the Trans-Mexican Volcanic Belt as an area of high haplotype diversity and endemism within particular oak species (González-Rodríguez et al. 2004).

The Sierra Madre Oriental and the Sierra Madre Occidental are also key areas for the distribution and endemism of oak species. There was evidence of niche divergence among the species groups that conformed UCs a, b, c, d and e (located through the Trans-Mexican Volcanic Belt, the Sierra Madre Oriental and the Sierra Madre Occidental) (Tables 3 and 4) which, added to the limited dispersal ability of the oaks, may have limited their migration to other regions. Both, the Sierra Madre Oriental and the Sierra Madre Occidental are characterized by elevations ranging from 200 to 3000 m.a.s.l. and a heterogeneous physiographic landscape (Ferrusquía-Villafranca 1993). Climatically, the Sierra Madre Occidental is more stable, and elevation seems to be the more important variable that defines the province (Ferrusquía-Villafranca, 1993). Meanwhile, the Sierra Madre Oriental exhibits important climatic factors that probably also influenced the distribution patterns observed. During the winter, polar air currents called "nortes” are spread over the eastern coast of Mexico through the Sierra Madre Oriental, bringing heavy rains in the eastern slope (Metcalfe et al. 2000). This precipitation regime could have allowed the colonization of habitats by different oak species considering that areas with high levels of precipitation usually have high oak diversity levels (as the humid montane oak forests; Luna-Vega et al. 2006).

Tehuantepec Isthmus

Species composition analysis identified the Tehuantepec isthmus as an area of species turnover, but only for red oaks (section *Lobatae*), and not for white oaks (section *Quercus*). This can be explained by the fact that there are several white oak species distributed through the isthmus lowlands (e. g. *Q. oleoides*) or on both sides of the Tehuantepec isthmus (e. g. *Q. corrugata*, *Q. insignis* and *Q. lancifolia*). On the contrary, several red oaks are only found to the west of the Tehuantepec isthmus (e. g. *Q. acutifolia*, *Q. crassipes*, *Q. laurina* and *Q. salicifolia*). These differences in distribution between red and white oak species may be due to the ecological differences between the two species groups. For example, apparently a higher proportion of white oak species are able to predominate in drier regions where normally red oaks seem not to develop well (Nixon 1993). Other differences between red and white oak species relate to seed dormancy (Struve 1998), which may be important determining the dispersal patterns of the seeds. It is possible that a combination of these and other traits has influenced to some extent the distribution of both sections.

ENMs showed that to the east of the Tehuantepec isthmus there are areas with a higher potential number of co-occurring species than are actually observed, suggesting that some species distributed to the west of the isthmus could have found climatically suitable areas but probably failed to disperse across this barrier. In fact, the NAM analysis identified several units of co-occurrence (a, b, c, e, g, f and h; Fig. 4) that have a geographical distribution delimited to the south by the presence of the isthmus. Interestingly, a principal components analysis based on climatic variables for the different UCs showed that several geographical units separated by the Tehuantepec isthmus, such as UCs b and c in comparison to UCs f and i, also differ from each other climatically. According to the niche divergence test, UC f (distributed to the east/south the Tehuantepec isthmus and over the ND) differentiates from UCs d, e, g and h (distributed to the west/north of the Tehuantepec isthmus, excepting UC d which distributes to the Nicaraguan Depression). The effect of the Tehuantepec isthmus as a barrier to oak species distribution was reported also by Torres-Miranda et al. (2013) considering only red oak (section *Lobatae*) species. However, it is also true that some role for local adaptation cannot be discarded, since the niche comparison tests indicated significant niche divergence among the species groups that constituted the UCs at both sides of the isthmus.

Geologically speaking, the Tehuantepec isthmus has several characteristics that could explain the above mentioned patterns. It is formed by two tectonic sub-provinces of Chiapas called the Central Depression and the Pacific Coastal Plain. Both correspond to areas from 0 up to 1000 m.a.s.l. (Ferrusquía-Villafranca 1993). The isthmus is characterized by a sudden change in elevation between central and southern Mexico that can represent a barrier for the dispersal of oak species, especially considering their seed dispersal largely mediated by gravity. Similarly, by separating tropical ecosystems from those with higher Nearctic influence, the isthmus represents an important turnover point for the distribution of species. Interestingly, this area has been reported in several phylogeographic studies as an area separating different haplotype lineages and/or impeding gene flow in different periods (Ornelas et al. 2013 and references therein).

Sierra Madre de Chiapas and north Trans-Isthmian Mountains

Although areas of co-occurrence of high number of species are not present to the east/south of the Tehuantepec isthmus, there are other two major species turnover points: Sierra Madre de Chiapas and the North of the Trans-Isthmian Mountains (located in Chiapas and Guatemala). These locations represent sites where species composition of the white oak section changes significantly and the limit to the distribution of UCs formed by species that cross through the Tehuantepec isthmus. Apparently, several species made it through the Tehuantepec isthmus but not all of them continued their migration southward, reaching their southern limit at the Motagua-Polochic system. The region between the Tehuantepec isthmus and the Motagua-Polochic system is a tremendously heterogeneous area, as the portion within the Mexican territory is characterized by discontinuous sierras and a series of transverse straight rivers, tributaries of the río Grande de Chiapas (Ferrusquía-Villafranca 1993), while the Guatemalan portion is defined by the tectonic boundary between the North American plate and the Caribbean plate in Guatemala, a region that consists of a complex system of large-scale faults that separate blocks with contrasting geological features (Ortega-Obregon et al. 2008).

Nicaraguan Depression

Volcanic activity in southern Nicaragua is largely a product of the interaction of the Caribbean Plate with the Cocos Plate. Particularly, the Nicaraguan Depression is characterized by a chain of Quaternary volcanoes, recent volcanic activity, volcaniclastic sediments, the presence of Lakes Managua and Nicaragua, and being surrounded by a discontinuous group of prominent faults (Arengi and Hodgson 2000). These events, along with the formation of the Cordillera de Guanacaste in Costa Rica (0.6 Ma) led to important climate changes (particularly in Costa Rica; van Wyk de Vries et al. 2007), which may have molded the distribution patterns of the oak species (Cavender-Bares et al. 2011). This is particularly true for species that constitute the UC i, which are distributed in the Costa Rican mountains, and exhibited evidence of strong niche divergence with respect to the UCs distributed to the north of the Nicaraguan Depression (Tables 3 and 4). .

The peaks of volcanic activity in the region also could have led to changes in the distribution of species and favored the isolation of populations. Likewise, volcanic activity may have determined a significant barrier to dispersal of species that are distributed through the mountain ranges. This case is particularly clear for the Nicaraguan Depression, which not only reports volcanic activity, but also a significant change in elevation that limits the distribution of the predominant species in the mountainous areas. Based on their analysis of red oaks distribution, Torres-Miranda et al. (2013) also suggested that the Nicaraguan Depression may have played a role as an important barrier. Our results indicate that the Nicaraguan Depression also has had an effect on the distribution of the white oaks and on the diversification of the genus as a whole. In the case of other biological groups, phylogenetic and phylogeographic analyses have reported this area as a major feature determining genetic and biogeographic patterns (Gutiérrez-García and Vázquez-Domínguez 2013).

Costa Rican mountains and Panamanian Isthmus

The southern border of Costa Rica represents the southernmost barrier to the migration of genus *Quercus* into the Colombian Andes. This region has low oak species diversity and also a low number of potentially co-occurring species as indicated by the ENMs. This area is known as Boca del Toro (boundary between Costa Rica and Panama) and defines the end of the mountainous region as well as the start of the Panamanian lowlands. Boca del Toro is also recognized as an important area that has influenced the current distribution patterns of several species of amphibians (Crawford et al. 2005, Wang et al. 2008). Finally, in the Darien region (border between Panama and

Colombia) the last important point of oak species turnover was identified. Even considering that Costa Rican and Panamanian mountains show a low number of oak species, the Darien region is crucial in order to understand the distribution of the genus *Quercus* in the neotropics. Important facts such as its recent geological origin, landscape heterogenity, climatic contrasts (particularly between the Caribbean and Pacific slopes), and proximity to the Andean region should have determined the arrival of the oaks into the Colombian Andes, where *Quercus humboldtii* is a key element of the montane ecosystems between 800 and 3500 m.a.s.l. (Pulido 2006, Fernández-M 2007).

Conclusions

This study is the first one to analyze the changes in the oak species composition throughout the Neotropics. We found that there are different regions that have acted as barriers to species dispersal, influencing the composition of forest communities by limiting the number of species that colonized southward areas, and probably impacting speciation processes as well. The ENMs also supported the role of these barriers by indicating that some areas in Central America could potentially harbor a higher number of species than is actually observed. These barriers are the Tehuantepec Isthmus, the Motagua-Polochic system, the Nicaraguan Depression, and the Panamanian Isthmus. According to the ENMs, these areas are regions with low climatic suitability for oak species that also define the borders of the endemism areas identified.

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Tables

Table 1. *Quercus* species studied, scenopoetic variables used to build the ecological niche model of each species, and the variables with the highest influence on the prediction of the distribution of each species. See Table S1 for the identity of each variable.

Species	**Section**	**ENMs Variables**	**Explanatory Variables**
Quercus acherdophylla Trel. 1924	Lobatae	NM	NM
Quercus acutifolia Née 1801	Lobatae	1,4,7,9,10,12,13,14,15,16,e,s	4,7
Quercus affinis Scheidw. 1837	Lobatae	1,4,7,12,13,15,16,e,s	4,15
Quercus benthamii A.DC. 1864	Lobatae	1,4,7,12,13,15,16,e,s	4,7
Quercus bumeloides Liebm. 1854	Quercus	1,4,7,12,13,e,s	4,7
Quercus candicans Née 1801	Lobatae	1,4,7,10,12,13,15,16,e,s	7,4
Quercus castanea Née 1801	Lobatae	1,4,6,7,10,11,12,13,15,16,e,s	7,15
Quercus conspersa Benth. 1842	Lobatae	1,4,7,10,11,12,13,15,16,e,s	7,12
Quercus cortesii Liebm. 1854	Lobatae	1,4,7,12,15,e,s	7,4
Quercus costaricensis Liebm. 1854	Lobatae	1,4,7,11,12,13,14,15,e,s	7,4
Quercus crassifolia Humb. et Bonpl. 1801	Lobatae	1,4,6,7,10,11,12,13,15,16,e,s	7,1
Quercus crassipes Humb. et Bonpl. 1809	Lobatae	1,4,6,7,10,11,12,13,14,15,e,s	7,4
Quercus crispifolia Trel. 1924	Lobatae	1,4,7,12,13,14,15,e,s	4,7
Quercus crispipilis Trel. 1924	Lobatae	NM	NM
Quercus depressa Humb. et Bonpl. 1809	Lobatae	NM	NM
Quercus deserticola Trel. 1924	Quercus	1,4,6,7,10,12,13,15,16,e,s	7,4
Quercus diversifolia Née 1801	Quercus	NM	NM
Quercus elliptica Née 1801	Lobatae	1,4,7,10,11,12,13,15,e,s	7,4
Quercus frutex Trel. 1924	Quercus	1,4,6,7,11,12,13,15,16,e,s	4,1
Quercus furfuraceae Liebm. 1854	Lobatae	NM	NM
Quercus germana Cham. et Schlecht 1830	Quercus	1,4,7,10,12,13,15,16,e,s	7,4
Quercus glabrescens Benth. 1840	Quercus	1,4,7,10,12,13,14,15,16,e,s	1,4
Quercus glaucescens Humb. et Bonpl. 1809	Quercus	1,4,7,10,12,13,15,e,s	7,15
Quercus glaucoides M.Martens et Galeotti 1843	Quercus	1,4,7,10,12,13,15,e,s	4,7,15
Quercus grahami Benth. 1840	Lobatae	NM	NM
Quercus hirtifolia Vázquez-Villagrán, Valencia y Nixon.	Lobatae	NM	NM
Quercus humboldtii Humb. et Bonpl. 1801	Lobatae	1,4,7,12,13,14,16,e,s	4,7,1
Quercus insignis M. Martens et Galeotti 1843	Quercus	1,4,7,10,12,13,15,e,s	4,7
Quercus lancifolia Cham. et Schltdl 1830	Quercus	1,4,7,12,13,15,17,e,s	4,12
Quercus laurina Humb. et Bonpl 1809	Lobatae	1,4,6,7,10,11,12,13,15,16,e,s	7,4,1
Quercus liebmanii Oerst 1869	Quercus	NM	NM
Quercus macdougalli M.Martínez 1964	Quercus	NM	NM
Quercus magnoliifolia Née 1801	Quercus	1,4,6,10,11,12,13,15,e,s	7,4,15
Quercus martinezii C.H.M?ll. 1953	Quercus	1,4,6,7,10,11,12,13,15,e,s	15,4
Quercus mexicana Humb. et Bonpl. 1809	Lobatae	1,4,6,7,11,12,13,15,e,s	7,4
Quercus mulleri Martínez 1953	Lobatae	NM	NM
Quercus nixoniana Valencia y Lozada 2003	Lobatae	NM	NM
Quercus ocoteifolia Liebm. 1854	Lobatae	1,4,7,10,12,13,15,e,s	4
Quercus oleoides Schltdl. et Cham. 1830	Quercus	1,4,7,10,12,13,15,16,e,s	4,7
Quercus pachucana Zavala-Chávez 2000	Lobatae	NM	NM
Quercus paxtalensis C.H.M?ll. 1942	Lobatae	NM	NM
Quercus peduncularis Née 1801	Quercus	1,4,7,10,12,13,15,e,s	7,4
Quercus polymorpha Schltdl. et Cham. 1830	Quercus	1,4,7,12,13,14,15,16,e,s	4,7
Quercus purulhana Trel. 1924	Quercus	1,4,7,10,12,13,15,e,s	4,7
Quercus repanda Humb. et Bonpl. 1809	Quercus	1,4,7,12,13,18,19,e,s	4,1
Quercus rubramenta Trel. 1934	Lobatae	NM	NM
Quercus rugosa Née 1801	Quercus	1,2,6,7,10,11,12,13,15,16,19,e,s	1,7
Quercus salicifolia Née 1801	Lobatae	NM	NM
Quercus sapotiifolia Liebm. 1854	Lobatae	1,4,7,12,14,15,e,s	7,4
Quercus sartorii Liebm. 1854	Lobatae	1,4,7,12,13,15,19,e,s	15,7
Quercus scytophylla Liebm. 1854	Lobatae	1,4,7,10,11,12,13,15,16,e,s	4,15
Quercus sebifera Trel. 1924	Quercus	1,4,7,12,13,15,e,s	4,15
Quercus segoviensis Liebm. 1854	Quercus	1,4,7,12,13,14,15,e,s	4,7
Quercus skineri Benth. 1841	Lobatae	1,4,7,10,12,13,14,15,16,e,s	4,7
Quercus splendens Née 1801	Quercus	1,4,7,10,11,12,13,15,17,e,s	15,4
Quercus uxoris McVaugh 1972	Lobatae	1,4,6,7,10,11,12,13,14,15,e,s	15,4
Quercus vicentensis Trel. 1924	Quercus	1,4,6,7,12,13,15,e,s	4,7
Quercus xalapensis Humb. et Bonpl. 1809	Lobatae	1,4,7,12,13,15,e,s	4,12

*NM: Not modeled species due to low occurences (<10)

Table 2. Principal components analysis for climatic variation between high and low suitability areas for oak species.

Variables	PC1	PC2	PC3
Annual mean temperature	-0.2001937	0.90051569	0.34128628
Temperature seasonality	0.45700872	-0.0876238	0.70774579
Temperature annual range	0.55232916	-0.0135147	0.19327178
Annual precipitation	-0.5081348	-0.0942476	0.26481402
Precipitation seasonality	0.43335559	0.41512444	-0.5245371
% explained	58.97	19.58	13.72
Cummulative percentage	58.97	78.56	92.28
Top variable loadings	Temp. ann. range	Ann. mean Temp.	Temp. seas.

Table 3. Principal components analysis for climatic niche variation among the groups of species constituting the nine units of co-occurrence.

Variables	PC1	PC2	PC3
Annual mean temperature	0.08422716	0.91373876	0.34104264
Temperature seasonality	0.48880015	-0.1665056	0.57461648
Temperature annual range	0.56415078	-0.1074303	0.15361553
Annual precipitation	-0.5130006	0.1378603	0.2074451
Precipitation seasonality	0.41538477	0.32681897	-0.697764
% explained	57.79	21.34	13.27
Cummulative percentage	57.96	79.11	92.38
Top variable loadings	Temp. ann. range	Ann. mean temp.	(Prec. seas.)

Tabla A1. Climatic and topographic variables considered to build the ENMs.

ID	Variables
1	Annual mean temperature. (WorldClim, Bio 1)
2	Mean diurnal range (WorldClim, Bio 2)
3	Isothermality (WorldClim, Bio 3)
4	Temperature seasonality (WorldClim, Bio 4)
5	Max. temp. of warmest month (WorldClim, Bio 5)
6	Min. temp. of coldest month (WorldClim, Bio 6)
7	Temperature annual range (WorldClim, Bio7)
8	Mean temp. of wettest quarter (WorldClim, Bio 8)
9	Mean temp. of driest quarter (WorldClim, Bio 9)
10	Mean temp. of warmest quarter (WorldClim, Bio 10)
11	Mean temp. of coldest quarter (WorldClim, Bio 11)
12	Annual precipitation (WorldClim, Bio 12)
13	Precipitation of wettest month (WolrdClim, Bio13)
14	Precipitation of driest month (WorldClim, Bio 14)
15	Precipitation seasonality (WorldClim, Bio 15)
16	Precipitation of wettest quarter (WorldClim, Bio 16)
17	Precipitation of driest quarter (WorldClim, Bio 17)
18	Precipitation of warmest quarter (WorldClim, Bio 18)
19	Precipitation of coldest quarter (WorldClim, Bio 19)
e	Elevation
s	Soil (FAO-UN)

Table A2. One-way ANOVA comparing the values of the five climatic variables that explained most of the oaks ecological niche models between high and low suitability areas.

	Mean	**ee**	**F**	**p**
	Annual mean temperature			
Optimal suitability areas	16.2322208	0.05	3728.344	<0.001
Low suitability areas	25.86728232	0.14		
	Temperature seasonality			
Optimal suitability areas	14.72288404	0.1	39.4458	<0.001
Low suitability areas	12.99846966	0.25		
	Temperature annual range			
Optimal suitability areas	19.58810441	0.08	191.0421	<0.001
Low suitability areas	16.50290237	0.2		
	Annual precipitation			
Optimal suitability areas	1324.25246	13.12	305.3459	<0.001
Low suitability areas	1938.168865	32.58		
	Precipitation seasonality			
Optimal suitability areas	86.83311938	0.32	47.9836	<0.001
Low suitability areas	80.83641161	0.8		

Figures

Figure 1 Study area and its principal geological elements. TMVB: Trans-Mexican volcanic Belt, SMOc: Sierra Madre Occidental, SMOr: Sierra Madre Oriental, SJ: Serranías de Jalisco, SG: Serranías de Guerrero, SMS: Sierra Madre del Sur, SMC: Sierra Madre de Chiapas, TI: Tehuantepec Isthmus, TIM: Trans-Isthmian Mountains, PMF: Polochic-Motagua Fault, ND: Nicaraguan Depression, CRM: Costa Rica Mountains, PI-DAR: Panamanian Isthmus and Darien region, CA: Colombian Andes. Black areas represent mountainous systems (> 1000 m.a.s.l.).

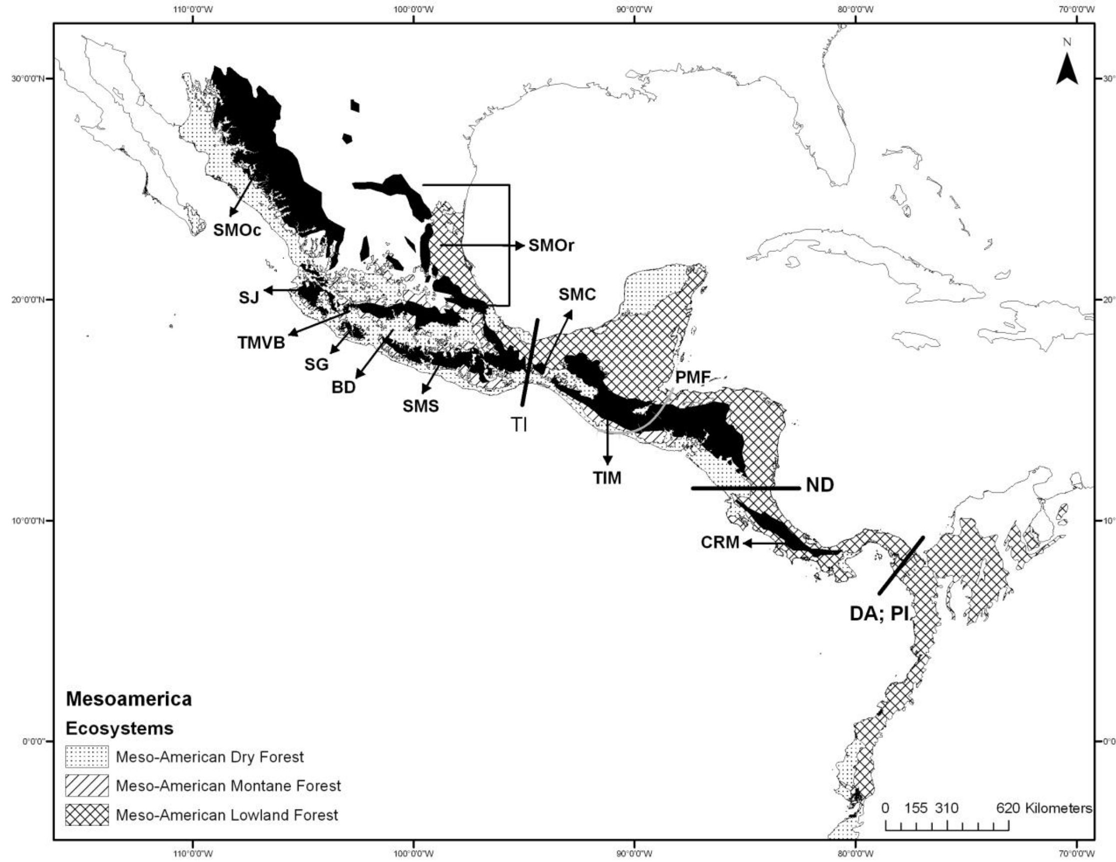


Figure 2 Neotropical *Quercus* species turnover patterns. The horizontal dotted line represents the threshold of similitude values beyond the expected variation. a. Longitudinal turnover pattern for the whole genus. b. Latitudinal turnover pattern for the whole genus. c. Longitudinal turnover pattern for red oaks (Sect. *Lobatae*). d. Latitudinal turnover pattern for red oaks (Sect. *Lobatae*). e. Longitudinal turnover pattern for white oaks (Sect. *Quercus*). f. Latitudinal turnover pattern for white oaks (Sect. *Quercus*). Numbers indicate the turnover points (for geographical location see Figures 1 and 3) as follows: 1-2 TI, 3 PMF, 4 ND, 5 PI, 6 ND, 7 CRM, 8 PI, 9-10 ND, 11 PI, 12 ND, 13 PI, 14-15 SMC, 16 PMF, 17 ND, 18 ND.

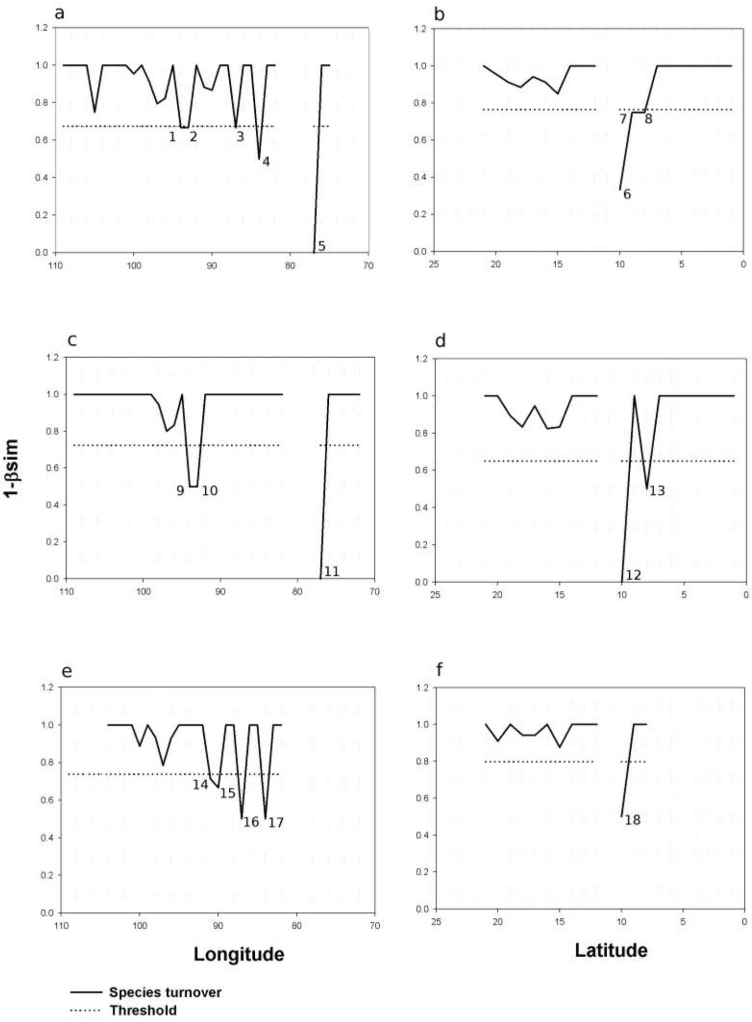


Figure 3 Geographical location of the main turnover points for *Quercus* species. Black bars represent the longitudinal and latitudinal units where marked species turnover occurs (see also Fig. 2). a. Longitudinal turnover pattern for the whole genus. b. Latitudinal turnover pattern for the whole genus. c. Longitudinal turnover pattern for red oaks (Sect. *Lobatae*). d. Latitudinal turnover pattern for red oaks (Sect. *Lobatae*). e. Longitudinal turnover pattern for white oaks (Sect. *Quercus*). f. Latitudinal turnover pattern for white oaks (Sect. *Quercus*). Numbers correspond to those shown in Fig. 2 for values of 1-βsim and correspond to the following areas: 1-2 TI, 3 PMF, 4 ND, 5 PI, 6 ND, 7 CRM, 8 PI, 9-10 ND, 11 PI, 12 ND, 13 PI, 14-15 SMC, 16 PMF, 17 ND, 18 ND.

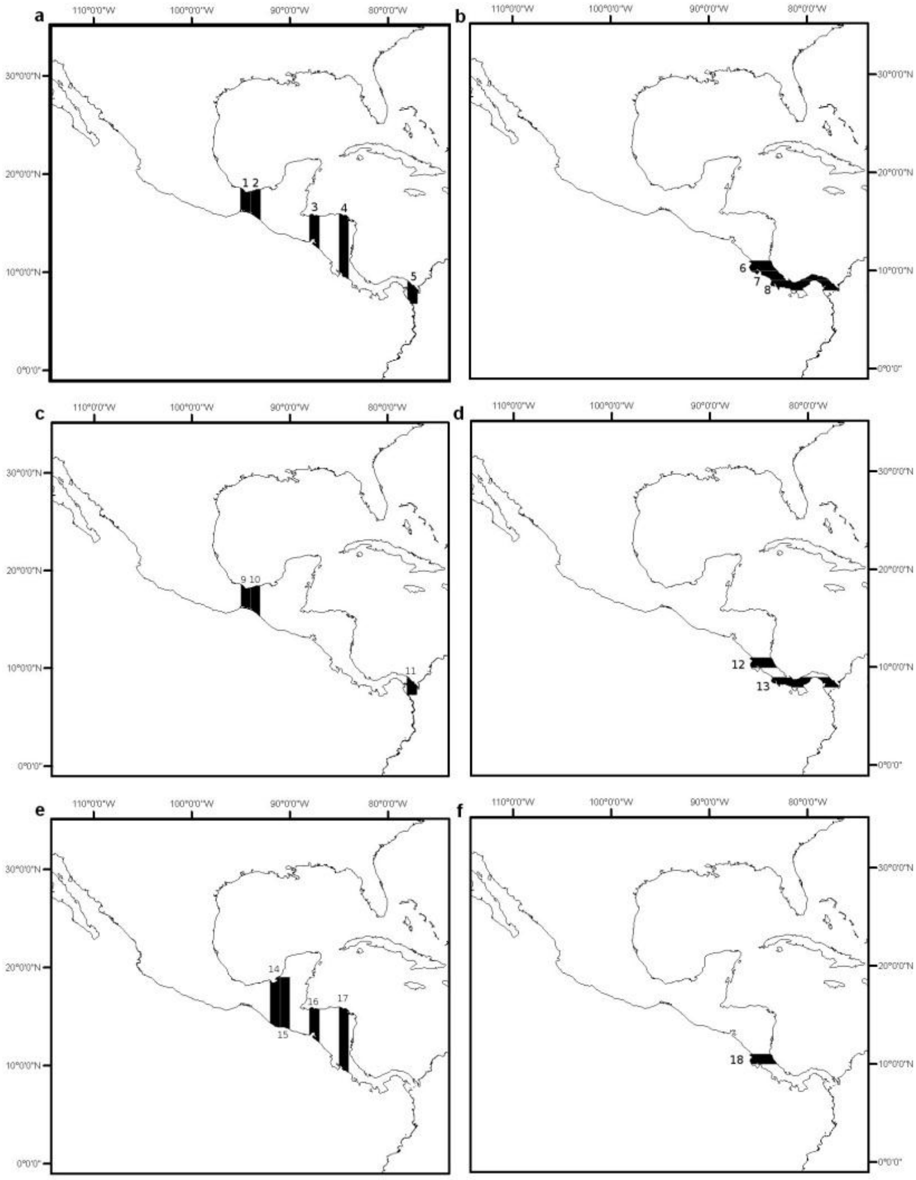


Figure 4 Cleavogram representing units of co-occurrence (or areas of endemism) for Neotropical oak species estimated using NAM analysis. Letters from a to i indicate each of the identified units of co-occurrence and the maps on the right side indicate the geographical distribution of the species groups that constitute each unit of co-occurrence.

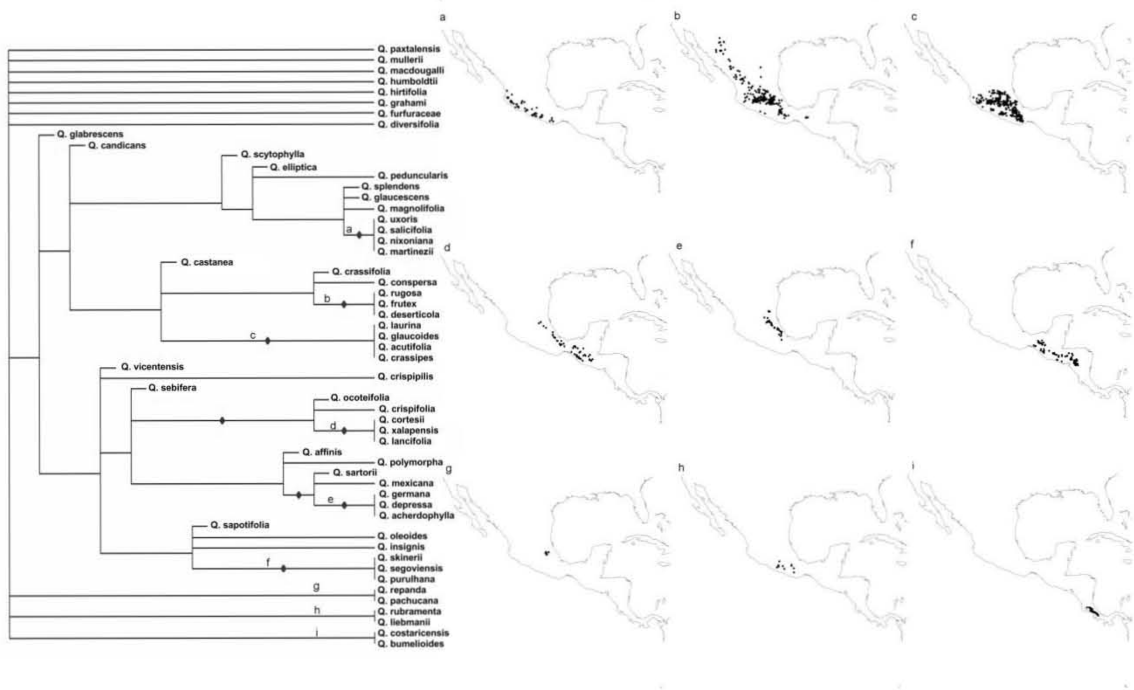


Figure 5 Potential co-occurrence patterns for neotropical *Quercus* species determined using ecological niche modeling. a. Distribution of the potential number of co-occurring species for the whole *Quercus* genus. b. Distribution of the potential number of co-occurring species for red oaks (Sect. *Lobatae)*. c. Distribution of the potential number of co-occurring species for white oaks (Sect. *Quercus*).

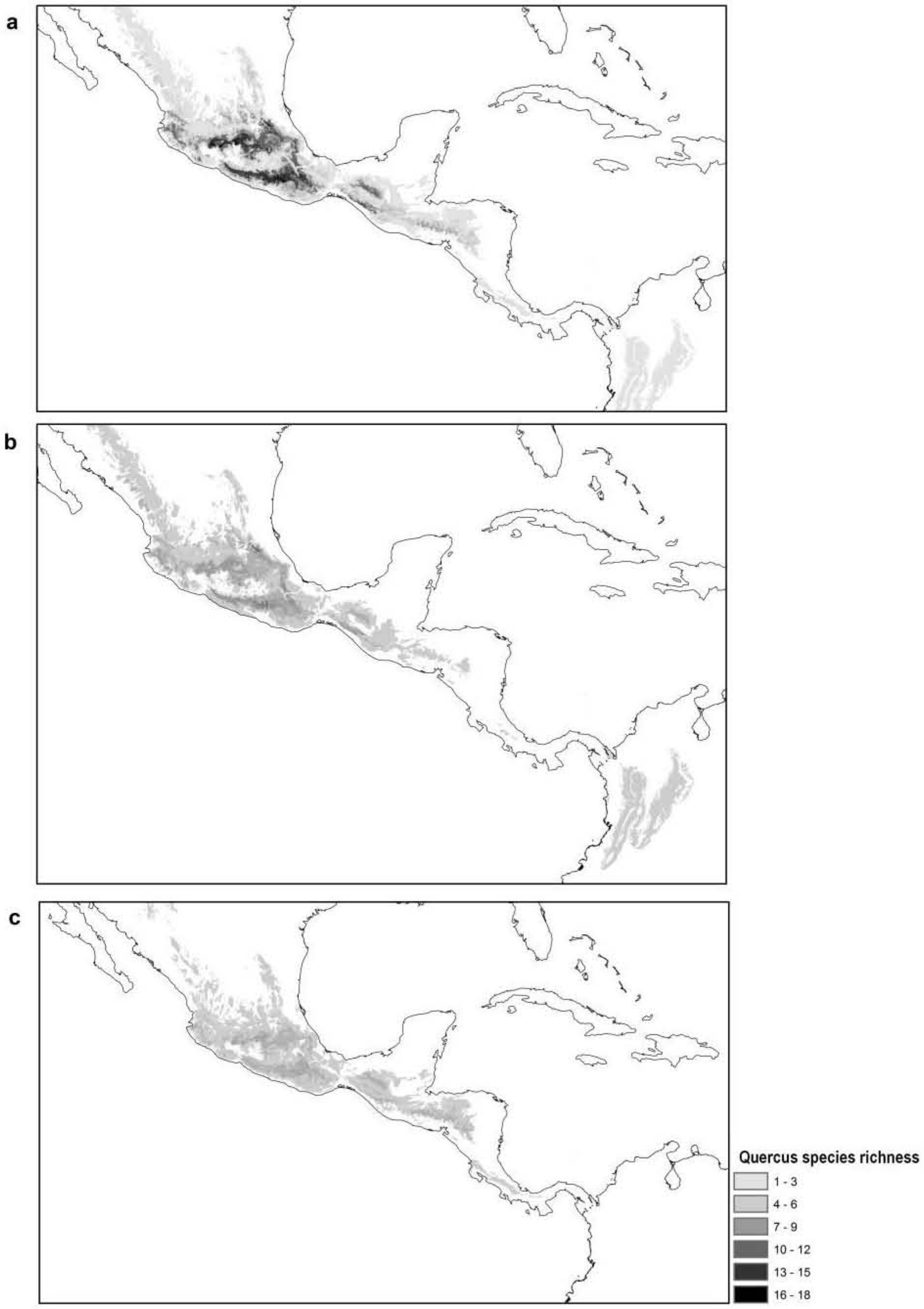


Figure 6 Principal components analysis showing climatic differences between areas with low suitability (barriers) and high suitability (highlands) for *Quercus* species detected using ENMs.

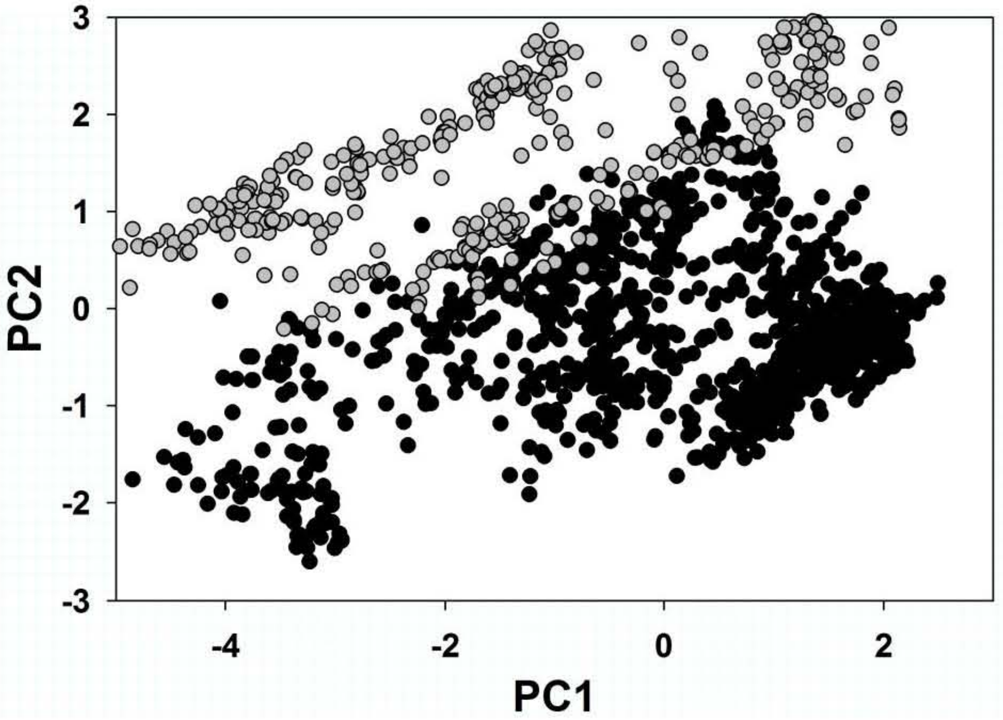
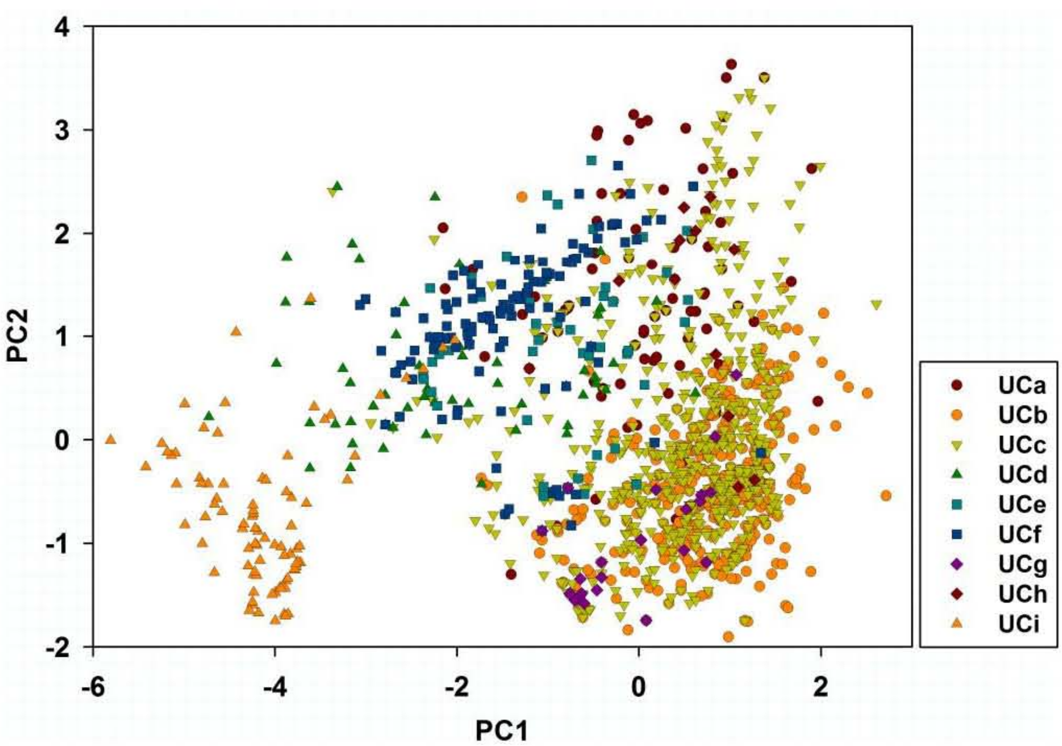


Figure 7 Principal components analysis showing ecological niche model envelopes for groups of species constituting the nine units of co-occurrence.



Capítulo Dos

Complex phylogeographic patterns and possible Central American origin in two widespread Mesoamerican *Quercus* (Fagaceae) species

Hernando Rodríguez-Correa, Ken Oyama, Mauricio Quesada, Erick Fucs, Maura Quezada, Lilian Ferrufino, Susana Valencia-Ávalos, Antonio González-Rodríguez

Abstract

The northern Neotropical region is characterized by a heterogeneous geological and climatic history. Recent studies have shown contrasting patterns regarding the role of geographic elements as barriers that could have determined phylogeographic structure in various species. Recently, the phylogeography and biogeography of *Quercus* species have been studied intensively, and the patterns observed so far suggest contrasting evolutionary histories for Neotropical species in comparison with their Holarctic relatives. The goal of this study was to describe the phylogeographic structure of two Neotropical oak species (*Q. insignis* and *Q. sapotiifolia*) in the context of the geological and palaeoclimatic history of the northern Neotropics. Populations through the distribution range of both species were characterized using nine chloroplast DNA microsatellite loci. Both oak species showed high levels of genetic diversity and strong phylogeographic structure. The distribution of genetic variation in *Q. insignis* suggested an influence of two major barriers, the Tehuantepec Isthmus and the Nicaraguan Depression, while *Q. sapotiifolia* exhibited a genetic structure defined by the heterogeneity of the Chortis highlands. The haplotype networks of both species indicated complex histories, suggesting that colonization from the Sierra Madre de Chiapas to central Mexico and from the north of the Nicaraguan Depression to the Costa Rican mountains may have occurred during different stages, and apparently more than one time. In conclusion, the phylogeographic structure of Neotropical oak species seems to be defined by a combination of geological and climatic events.

Keywords: Neotropical trees, Middle America, Mesoamerica, palaeodistribution, phylogeography, historical demography.

Introduction

The area of the Neotropics comprising southern Mexico and Central America is important for the study of evolutionary processes such as migration, extinction and diversification of different organismal groups, in the context of the geological and climatic heterogeneity that characterizes this region (Gutiérrez-García and Vázquez-Domínguez 2014). Even though consensual patterns have not emerged yet, several studies coincide on the importance of geological elements such as the Tehuantepec Isthmus (TI), the Polochic- Motagua fault system (PMF), the Nicaraguan Depression (ND) and the Panama Isthmus (PI) as promoters of phylogeographic structure in different organisms (see table 1 in Gutíerrez-García and Vázquez-Domínguez 2014), and also in the geographical organization of entire lineages such as *Quercus* (Rodríguez-Correa et al. 2015) and ferns (Ramírez-Barahona and Luna-Vega 2015) in the Neotropics. On the other hand, a lack of phylogeographic structure related to these geographic elements also has been observed mainly (but not exclusively) in lowland species (see table 1 in Gutíerrez-García and Vázquez-Domínguez 2014). More interesting, is the fact that even when the phylogeographic structure of the species appears to be determined by these geographic barriers, it seems like processes of intraspecific divergence took place at different time periods, suggesting differential responses to the geological dynamics of the region, and therefore, linage-specific evolutionary histories (Ornelas et al. 2013).

Historical environmental fluctuations have also been considered important in structuring genetic diversity of Neotropical species. However, available evidence (reviewed in Ramírez-Barahona and Eguiarte 2013) indicates differential responses to climate change according to the distribution, ecology and phylogenetic relationships of species. Therefore, the identification of common patterns and how these can be associated to the species' characteristics requires further studies dealing with co-distributed species and the integration of paleoclimatic, ecological and molecular data (Ramírez-Barahona and Eguiarte 2013). In northern latitudes, several *Quercus* species have been studied using phylogeographic approaches (Dumolin-Lepègue et al. 1997; Fineschi et al. 2002; Cottrell et al. 2002; Csaikl et al. 2002; Olalde et al. 2002; Petit et al. 2002a; Petit et al. 2002b; Grivet et al. 2006; Lopéz de Heredia et al. 2007; Chen et al. 2012; Liu et al. 2013; Alexander & Woeste 2014) and the results obtained have been a fundamental basis to understand historical colonization processes related to climatic fluctuations during the last glacial cycle particularly at the European continent. Interestingly, oak species situated in the Neotropics exhibit contrasting patterns with respect to their Nearctic and Palearctic relatives. For example, Mexican oak species show higher levels of within population variation and lower among population differentiation than northern latitude species (Tovar-Sánchez et al. 2008, Ramos-Ortiz et al. *unpublished data* and Peñaloza-Ramírez et al. *unpublished data*), probably because at lower latitudes the effects of glaciations were less severe and oak populations did not isolate into small refugia but remained large and maintained relatively stable geographic ranges (González-Rodríguez et al. 2004; Cavender-Bares et al. 2011). In contrast, it seems like the most important effect of the Pleistocene climatic changes on oak species in the Neotropics were related to changes in their altitudinal distribution (Hooghiemstra and van der Hammen 2004; Rodríguez-Correa et al. *unpublished data*).

Recently, it has been suggested that geographical areas such as the Tehuantepec Isthmus (TI), the Polochic-Motagua fault system (PMF), the Nicaraguan Depression (ND) and the Panamanian Isthmus (PI) have acted as important barriers to the dispersal of oak species influencing species diversity, biogeographic patterns and niche divergence processes (Torres-Miranda et al. 2013; Rodríguez-Correa et al. 2015). However, a few oak species (*Q. corrugata*, *Q. insignis*, *Q. oleoides* and *Q. sapotiifolia*, and perhaps also *Q. benthamii*, *Q. cortesii* and *Q. salicifolia*) (Valencia 2004; Morales 2010) have a distribution range that extends across all these barriers (except the PI), eliciting questions about the timing and process of dispersal and the influence of species' traits and ecological niche on dispersal success. Also, an evident hypothesis is that the mentioned barriers probably correspond to phylogeographic breaks within these species. However, the lowland *Q. oleoides* (distributed from northeastern Mexico to northern Costa Rica) is the only species so far analyzed (Cavender-Bares et al. 2011; Cavender-Bares et al. 2015), and showed no genetic discontinuities across the TI and the PMF, but strong differentiation across the ND, estimated at about 1.9 my BP using next-generation sequence data, implicating the formation of the Nicaraguan depression and associated volcanic activity as the cause (Cavender-Bares et al. 2015).

In this study, we used chloroplast DNA microsatellite data to reconstruct phylogeographic patterns in two Neotropical oak species (*Quercus insignis* and *Quercus sapotiifolia*) distributed from southern Mexico to northern Panama. Both species are cataloged with different levels of thread for the Mexican cloud forest tree species (critically endangered for *Q. insignis* and vulnerable for *Q. sapotiifolia*; González-Espinosa et al. 2011). *Quercus insignis* is a white oak (section *Quercus*) and *Q. sapotiifolia* is a red oak (section *Lobatae*), but both have roughly similar geographical distributions and are mainly montane species. The specific goals of the study were: i. to describe the phylogeographic structure of two widespread oak species (*Quercus insignis* and *Quercus sapotiifolia*) distributed from southern Mexico to Costa Rica; ii. to evaluate the importance of geological and climatic variables as barriers determining the geographic distribution of the genetic variation for both species by comparing phylogeographic patterns with present-day and palaeoclimatic modeled distributions; and iii. to compare historical demographic patterns observed in the oak species studied with previously characterized oak species distributed in other biogeographical realms.

Methods

Studied species and sampled populations

The two oak species were sampled across their whole distribution range (Figure 1). *Quercus insignis* M. Martens et Galeotti (1843) is found in the Mexican states of Jalisco, Guerrero, Oaxaca, Chiapas, Veracruz and in Belize, Guatemala, Honduras, Nicaragua Costa Rica and Panama in Central America. The altitudinal range of *Q. insignis* varies from approximately 1500 to 2000 m (Valencia-A 2004). The species is emblematic for producing very large acorns (7-8 cm in diameter; Montes-Hernández and López-Barrera 2013), probably the largest of all oaks species. *Quercus sapotiifolia* Liebm. (1854) in Mexico is present in the states of Hidalgo, Oaxaca, Chiapas and Veracruz and in Central America in Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica and Panama, with an altitudinal range between 250 and 2000 m (Valencia-A 2004). This species is characterized by considerable morphological variation across its distribution. Despite their wide ranges, both species are rare and populations are difficult to locate because of their low density. In total, 13 populations of *Q. insignis* and 15 populations of *Q. sapotiifolia* were sampled (Table 1).

DNA isolation and microsatellite amplification

Fresh leaf tissue was collected in the field and stored in silica gel until processing in the laboratory. Dried tissue was disrupted using liquid nitrogen and DNA isolation was carried out using a commercial DNA isolation kit (QIAGEN DNeasy plant mini kit). A set of nine chloroplast DNA (cpDNA) microsatellite loci (cmcs2, cmcs3, cmcs4, cmcs5, cmcs6, cmcs7, cmcs10, cmcs12 and cmcs14) previously characterized for Fagaceae species by Sebastiani et al. (2004) were tested for polymorphism in both *Q. insignis* and *Q. sapotiifolia*. Two groups of primers (cmcs3, cmcs4, cmcs5, cmcs6 in the first group and cmcs2, cmcs7, cmcs10, cmcs12, cmcs14 in the second group) with different combinations of fluorescent dyes and expected size were employed in multiplexed polymerase chain reactions (PCR). These were performed using the QIAGEN multiplex PCR kit with a final volume of 5 µL containing 1 X multiplex PCR master mix, 0.25 mM of each primer, dH_2O and 20 ng of template DNA. Amplification was performed using an initial denaturation step for 15 min at 95°C, followed by 35 cycles of 30 seg at 95°C, 1.5 min at 55°C, 1 min at 72°C, and a final extension step for 30 min at 60°C. PCR products were run in a ABI-PRISM 3300 Avant sequencer (Applied Biosystem) and allele size was determined using a standard (GeneScan-600 LIZ) with the Peak Scanner program version 2.0 (Applied Biosystem).

Genetic analysis

Genetic diversity and genetic structure

Each unique combination of size variants for the nine loci was defined as a different haplotype. Genetic diversity was described in terms of haplotype richness (*AR*), haplotype diversity with unordered alleles (*h sensu* Pons and Petit 1996), non-standardized haplotype diversity with ordered alleles (*v sensu* Pons and Petit 1996) and the pairwise genetic distance among individuals within a population under a stepwise mutation model (D^2_{SH}; Goldstein et al. 1995) using SPAGeDi version 1.1 (Hardy & Vekemans 2002). These estimators were calculated both at the population and region levels. The regions were defined as follows: 1. populations located to the north/east of the Tehuantepec Isthmus (TI) belonged to the Upper Mesoamerica (UM) region; 2. populations found between the TI and the Nicaraguan Depression (ND) belonged to the Middle Mesoamerica (MM) region and, 3. populations to the south of the ND formed the lower Mesoamerica (LM) group. Population i14 of *Q. insignis* (from Chiapas, Mexico) only had one individual and was considered for graphical purposes only, but it was excluded from population structure and historical demography analysis.

In order to infer haplotype relationships, a minimum spanning network was computed using Network version 4.6 (available at www.fluxus-engineering.com) with the median-joining method (Bandelt et al. 1999) and a maximum parsimony search (Polzin and Daneschmand 2003). Genetic differentiation and phylogeographic structure were analyzed by calculating G_{ST} and N_{ST} (a G_{ST} analogue which takes into account the genetic distances among haplotypes) with SPAGeDi version 1.1 (Hardy & Vekemans 2002). A permutation test implemented in this program was used to test whether the value of N_{ST} was significantly greater than the value of G_{ST}, what indicates that there is phylogeographic structure in the populations (Pons & Petit 1996). The F_S statistic (Fu, 1997) was estimated for each population and region in order to test for events of demographic expansion. Calculations were carried out using Arlequin version 3.5 (Excoffier et al. 2005) and codifying cpSSR data in binary form as suggested by Navascues et al. (2006).

The partitioning of the genetic variation among regions, among populations within regions and within populations was described using a hierarchical analysis of molecular variance (AMOVA) implemented in Arlequin version 3.5 (Excoffier et al. 2005). The grouping of populations in regions was the same explained above. AMOVA was calculated using both F_{ST} (based on the infinite alleles mutation model, IAM) and

R_{ST} (based on the stepwise mutation model, SMM) with 10,000 permutations to determine significance of the estimates (Excoffier et al. 2005). To further understand the geographic distribution of genetic variation, a spatial analysis of molecular variance was implemented using SAMOVA version 1.0 (Dupanloup et al. 2002). The number of groups was evaluated using values of K from two to ten so that the genetic differentiation among the groups (Φ_{CT}) was maximized. Consistence between different runs was determined by repeating twice every K-value evaluated.

Also, the geographical location of the most important genetic discontinuities among populations was determined using the Monmonier's maximum difference algorithm implemented in BARRIER ver. 2.2 (Manni et al. 2004). To provide bootstrap support to the observed genetic barriers, a set of 100 pairwise matrices of average square genetic distance (ASD; Goldstein *et al.,* 1995; Slatkin, 1995) among populations was generated by manual resampling of individuals in the original matrix. Finally, genetic diversity indexes (AR, h, v and D^2_{SH}) were correlated with the altitude and latitude of the sampled populations and also compared among regions using a Kruskal-Wallis rank sum test in order to determine the existence of geographical patterns in the distribution of genetic diversity. These tests were implemented in R ver. 3.0.2.

Ecological Niche Modelling

Ecological niche models (ENMs) were estimated using a maximum entropy approach implemented in MAXENT version 3.3.3a (Phillips et al. 2006) in order to determine the climatically suitable areas for both *Quercus insignis* and *Q. sapotiifolia* during the Last Interglacial (LIG; ~120 *ka* BP), the Last Glacial Maximum (LGM; ~21 *ka* BP) and the present-day (PD) periods. In order to build the models, occurrence data were downloaded from a public repository (Global Biodiversity Information Facility). The data were filtered using as reference the reported distribution and altitudinal range of the species (Valencia-A 2004).

Climatic information used to run the models was based on the 19 bioclimatic variables proposed by Hijmans et al. (2005) at a spatial resolution of 30 arc-seconds (~1 Km). However, in order to avoid correlation among variables, a correlation matrix was calculated, and from each pair of highly correlated variables (r>0.7) the more specific variable was discarded. To decrease possible effects of spatial autocorrelation due to the aggregation of records, we only used points separated by more than 0.1 decimal degrees from the nearest neighbour. ENMs were estimated after 100 replicas using the bootstrap resampling method in MAXENT. Thirty percent of the presence records were used to calculate estimators of quality and the remaining 70% was used to run the models. In the absence of palaeodistribution data to build the LGM and LIG models, the ‘projection’ option in MAXENT was used.

Model quality was determined with a threshold-independent method, the area under the receiver operating characteristic (ROC) curve analysis (AUC; Fielding & Bell 1997); and the fixed cumulative value 1 logistic threshold (FCV) was applied in order to obtain binomial outputs. FCV was used as it has been suggested as an appropriated threshold value in other Neotropical species analysed with palaeoecological methods involving ENM (Rodríguez-Correa et al. *unpublished data*). Finally, in the models we determined the areas where the distribution of both species remained stable during the LIG, LGM and PD periods. The location of the main phylogeographic breaks obtained with the molecular data was compared with the discontinuities in climatically suitable areas obtained in the Maxent models to evaluate the possible role of present-day and historical climatic conditions in structuring the genetic diversity of the two species.

Results

Quercus insignis

Genetic diversity, genetic structure and historical demography

A total of 76 individuals from 14 populations were characterized (Table 1, Figure 2). The total number of haplotypes was 28, 14 of them corresponding to singletons. The number of haplotypes per population ranged from one to six. At the region level the number of haplotypes was as follows: two haplotypes were observed in UM, 16 haplotypes (nine singletons) in MM and 12 haplotypes (five singletons) for LM. Within-population haplotype diversity (h_S) ranged between 0.4 and 1. Mean (s. e.) h_S and total diversity (h_T) were 0.65 (0.05) and 0.95 (0.03), respectively. Genetic differentiation among populations was 0.315 (0.05) for the unordered alleles (G_{ST}) and 0.716 (0.07) for ordered alleles (N_{ST}). G_{ST} and N_{ST} values were significantly different ($P<0.001$) indicating the presence of phylogeographic structure. Fu's F_S values for all populations and geographic regions were non-significant ($p>0.02$; Table 1).

The partitioning of the genetic variation according to results of the AMOVA (Table 2) showed that for both F_{ST} and R_{ST} the differences among regions explained a considerable amount of variation (20 and 30% respectively; $p<0.001$). Meanwhile, most of the genetic variation was distributed among populations within regions under both mutation models, 42.22% ($p<0.001$) for IAM and 45.18% ($p<0.001$) for SMM. The haplotype network (Figure 2) indicated a complex scenario. Despite the presence of haplotypes with high frequencies such as H27 (found in 15 individuals), there is not a widely distributed haplotype through the whole *Q. insignis* distribution. The distribution of haplotypes in MM and SM suggested that several haplotypes are more closely related between regions that within regions (e. g. H10 and H5, H6 and H7; Figure 2).

Geographic distribution of the genetic variation

The results of the SAMOVA analysis indicated that the maximum value of genetic differentiation among groups of populations corresponded to four groups ($\Phi_{CT} = 0.72$; $p<0.001$). According to this result one group was formed by populations from Veracruz, México (i13, i12, i11) and three populations from Honduras (i10, i8 and i6), thus including populations from both UM and MM. A second group was constituted by two populations from Honduras (MM; i9 and i7) and two populations from Costa Rica (LM; i4, i2); a third group included two populations from Costa Rica (i5 and i3) and the fourth group included only the southernmost population (i1).

The analysis of genetic discontinuities suggests that the TI and the ND are important barriers for *Q. insignis* populations (94 and 95% bootstrap support). In Honduras the two southernmost populations i7 and i10 were separated from the northern populations (95% bootstrap support). In Costa Rica, the heterogeneous composition of populations was indicated by the presence of barriers separating populations (95% bootstrap support) into three groups corresponding to northern, central and southern Costa Rica. Finally, all the correlations of genetic diversity statistics with elevation and latitude were not significant ($p>0.05$), as well as the comparisons among regions ($p>0.05$).

Ecological niche modelling

A total of 61 records representing *Quercus insignis* distribution were used to run the ENM. The ENMs of *Quercus insignis* for the present-day period showed a good performance according to the AUC value (0.991± 0.005). This model showed two main distribution areas, the first one located from the Sierra Madre de Chiapas (SMC) through the trans-isthmian mountains (TIM) in Guatemala, Honduras and northern

Nicaragua. The second distribution area is located in the Costa Rica Mountains (CRM) (Figure 3). The northernmost part of the species' range (i.e. southern Mexico) is characterized by a fragmented distribution. ENMs also suggested an increase in the area available for *Q. insignis* during the LGM and an important reduction in the available climatic niche during the LIG (Figure 3). The increase in available area during the LGM could have favored the contact or the proximity of populations across barriers such as the TI and ND, suggesting the possibility of intermittent gene flow across these barriers at some time periods. Finally, it can be observed that distribution areas that have remained stable are located in SMS, SMC, TIM (including an important portion of the Nicaraguan southern region) and CRM (Figure 6).

Quercus sapotiifolia

Genetic diversity and structure

A total of 139 individuals from 15 populations were characterized (Table 1, Figure 4). Total number of haplotypes was 34, with 17 singletons. The number of haplotypes per population ranged from one to seven. At the regional level haplotype richness was seven (four singletons) in UM, 22 (11 singletons) in MM and five (two singletons) in LM. Within-population genetic diversity (h_S) ranged between 0.4 and 0.95. Mean (s. e.) h_S and total haplotype diversity (h_T) were 0.51 (0.06) and 0.96 (0.01), respectively. Genetic differentiation among populations was 0.471 (0.06) for unordered alleles (G_{ST}) and 0.752 (0.05) for ordered alleles (N_{ST}). A significant difference between G_{ST} and N_{ST} ($p<0.001$) was observed, indicating the presence of phylogeographic structure. Fu's F_{S} values for all populations and geographic regions were not significant ($p>0.02$; Table 1).

The partitioning of the genetic variation according to the AMOVA results (Table 2) showed that for F_{ST} differences among regions explained 21% ($P<0.001$) of the variation. However, for the case of R_{ST} the differentiation value was very small and non-significant (2%; $P>0.05$). On the other hand, most of the genetic variation was distributed among populations within regions under both mutation models (68%; $P<0.001$, for IAM and 64%; $P<0.001$ for SMM). The haplotype network (Figure 4) showed that the majority of haplotypes are found in the MM region. Interestingly, the haplotypes found within the UM region seem to belong to two lineages independently derived from H34 and H3, and a similar pattern seems to be true for the haplotypes present in the LM region (Figure 4).

Geographic distribution of the genetic variation

SAMOVA results showed that genetic differentiation among groups is maximum when K=5 ($\Phi_{CT} = 0.72$; $p<0.001$). According to this result the grouping of populations was as follows: one group formed by UM and northern MM populations (s13, s14 and s15), groups two and three each formed by one population from MM (s10 and s12, respectively), group four was constituted by eight MM and one LM populations (s2, s3, s4, s5, s6, s7, s8, s9 and s11) and group five formed by population s1 (LM). The analysis of genetic discontinuities showed several significant barriers that define the geographic configuration of the genetic differentiation. From north to south the first barrier separates the Mexican populations from the remaining populations (100% bootstrap support), and it is located at the SMC close to the PMF. Adjacent to this barrier and to population s10 a second barrier separates this population (s10) from the remaining groups (98% bootstrap support). At the middle portion of MM a third barrier separates Guatemalan from Honduran oaks (90% bootstrap support). In the Honduras territory population s7 differentiates from the rest of the oak populations. LM populations also were separated in two groups (100% bootstrap support) similarly as for *Q. insignis*: northern and central Costa Rica. Correlations of genetic diversity measures with elevation and latitude as well as comparisons among geographic regions did not show any significant result (p>0.05).

Ecological niche modelling

A total of 120 records representing *Quercus sapotiifolia* distribution were used to run the ENM. *Quercus sapotiifolia* ENM for the present-day period exhibited a good performance according to the AUC value (0.090±0.001). This model predicted two main distribution areas as was also observed for *Q. insignis.* These areas are formed by: i. the MM region including the Sierra Madre de Chiapas (SMC) and the trans-isthmian mountains (TIM) in Guatemala, Honduras and northern Honduras, and ii: the Costa Rica Mountains (CRM) in LM. The first area showed a more continuous distribution than the observed in *Q. insignis*, particularly at Honduras and northern Nicaragua (Figure 5). The *Q. sapotiifolia* distribution at UM is almost restricted to the southern SMS and SMO. For the LGM, both models (CCSM and MIROC) suggested an increase in the connectivity among areas across the TI region, but a gap between the SMS and TIM at least in the CCSM model. At the ND both models showed an important gap with a shallow connection between Nicaragua and north-western Costa Rica according to the MIROC model (Figure 5). During the LIG the climatic niche distribution is restricted to the SMS and northern TIM, southern Honduras and northern Nicaragua portions of the TIM and the CRM. When all models were considered together it was observed that, as in *Q. insignis,* most stable areas were located between the SMS and the TIM, as well as at the CRM. In contrast to the climatic niche extension in *Q. insignis*, *Q. sapotiifolia* has more restricted suitable and stable areas at southern Nicaragua (Figure 6).

Discussion

Recent studies regarding the Central American biota have focused on the description of evolutionary processes such as speciation, extinction, and diversification of flora and fauna considering the intricate geologic history of this region, its habitat diversity, palaeoclimatic dynamics and tectonic history (Gutíerrez-García and Vázquez-Domínguez 2013). Gutiérrez-García and Vázquez-Domínguez (2013) proposed the presence of at least three evolutionary groups in the northern Neotropics based on the coincidence of genetic differentiation patterns: The Mayan group (located between the TI and the PMF), the Mid-Central group (between the PMF and the Hess escarpment) and the Panamanian group (from the HE to the Andes). Within this area other oak species studied have shown a strong differentiation between Costa Rica and Honduras associated to the formation of the ND (*Q. oleoides*; Cavender-Bares et al. 2011; Cavender-Bares et al. 2015). However, little is known so far about the effects of past geological and climatic events on the population history of other oak species in the same area, but that differ from *Q. oleoides* in climatic niche and habitat.

Quercus insignis and *Q. sapotiifolia,* exhibited higher haplotype richness (28 and 34 haplotypes, respectively) in comparison to higher latitude oak species that have been characterized with comparable chloroplast microsatellite molecular markers, such as the European white oaks complex (from France and the Iberian Peninsula; *Quercus robur*, *Q. petraea*, *Q. canariensis*, *Q. faginea* and *Q. pyrenaica*) which showed 11 haplotypes (Grivet et al. 2006); *Q. suber* in the western Mediterranean with five haplotypes (Magri et al. 2007); and *Q. garryana* in the Pacific northwestern region of

North America with six haplotypes (Marsico et al. 2009). However, haplotype richness was lower than in Mexican oak species such as *Q. castanea* (Peñaloza-Ramirez et al. *unpublished data*) which had 90 haplotypes; and similar to haplotype richness in the *Q. crassifolia x Q. crassipes* hybrid complex that showed 26 haplotypes (Tovar-Sánchez et al. 2008) and the *Q. affinis x Q. laurina* hybrid complex that had 35 haplotypes (Ramos-Ortiz et al. *unpublished data*). Also, the values were similar to haplotype richness in *Q. lobata* in California, with 39 haplotypes (Grivet et al. 2006). Similarly, within population diversity (h_S) and total diversity (h_T) observed in *Q. insignis* (0.65 and 0.95, respectively) and *Q. sapotiifolia* (0.51 and 0.96) were higher than in most of the above-mentioned mid- and high-latitude oak species (0.28 and 0.97 for *Q. lobata*; 0.11 and 0.75 for the European white oak complex; and 0.08 and 0.67 for *Q. garryana*) and comparable to the Mexican species (0.73 and 0.98 for *Q. castanea*; 0.9 and 0.99 for the *Q. affinis x Q. laurina* hybrid complex).

Despite that diversity values seem to be clearly associated with the different geographic regions (higher values within the Neotropics and lower in the temperate zone), these comparisons should be treated with caution considering that not only the set but also the number of cpSSR loci used only partially coincide among studies. In terms of the total number of haplotypes, the genetic diversity was higher at the regional level for both *Q. insignis* and *Q. sapotiifolia* in the MM region than in the UM and LM regions (Table 1). However, the mean values of within population diversity were not significantly different according to the Kruskal-Wallis test ($p>0.05$). Several authors have attributed high levels of chloroplast DNA diversity to processes such as introgression and the persistence of large populations through different periods (Lumaret et al. 2002; Jiménez et al. 2004). It is probable for both Neotropical oak species (particularly *Q. sapotiifolia*) that not only processes such as hybridization may have been important drivers of the high observed genetic diversity, but also the persistence of large areas with suitable climatic niches at least since the LGM (Figure 6), may have promoted large effective population sizes through time. This affirmation is also supported by the absence of historical population demographic expansion signal and therefore population equilibrium evidenced by the calculated Fu's F_S values (Table 1) for both species, not only at the population level, but also regionally.

Both oak species exhibited considerable values of genetic structure. Moreover, significant differences between G_{ST} and N_{ST} ($p<0.001$) indicated a well-defined phylogeographical structure. The AMOVA analysis grouping the populations into three regions (UM, MM and LM) defined by the main geographic barriers (TI and ND) that were identified through a biogeographic analysis of oak species distribution indicated that 20.57-30.41% of the genetic variation in *Q. insignis* (depending on the mutation model, IAM and SMM, respectively) and 21.21-2.79% in *Q. sapotiifolia* is found among the three regions. However, a SAMOVA analysis maximizing the genetic variance among population groups indicates that in the case of *Q. insignis* the TI and ND have not been effective barriers, meanwhile the Barriers analysis suggested that the same areas (TI and ND) represent zones where genetic distance differ more that it would be expected by chance. Simultaneously considering the haplotype network of *Q. insignis*, it is plausible to infer a complex historical dynamics. The relation between UM and MM suggests a regional differentiation of the oak populations, with only one shared haplotype (H27; Figure 2) and the presence of H24 in one individual at eastern TI.

Differences between SAMOVA and Barriers results reflect methodological differences. SAMOVA search for groups of populations geographically homogeneous and maximally differentiated from each other, and Barriers identifies boundaries between points or study sites based on differences of an attribute distance matrix. Therefore, SAMOVA results suggested that regional groups of *Q. insignis* populations were not defined by major geographic barriers (TI and ND), however, Barriers identify punctual differences (between population) in genetic variation at both the TI and ND. These results reflect that although significant genetic differentiation is observed between populations at the TI and ND according to Barriers, such differentiation have not been strong enough to define major genetic linages from the mentioned barriers. The lack of a well-defined SAMOVA geographic structure concordant with Barriers results may also reflect the complex evolutionary history of Neotropical oaks characterized by events of multiple colonization and re-colonization of upper and lower Mesoamerica, events that could have diluted the geographic structure of the oaks species.

The relation between MM and LM suggests an initial event of colonization of LM from Honduras, probably followed by at least two events of migration on the opposite direction (LM to MM) and finally another MM to LM colonization. The Barriers analysis also showed important differentiation of populations within MM and LM. Particularly, in the LM region there was a sub-structure dividing the populations into northern, central and southern groups. Both regions are characterized by high levels of haplotype diversity and a considerable number of singletons. Complementarily, ENMs suggested that precisely these areas (MM and LM) have exhibited stable climatic niche suitability values since the LIG (Figure 6) and, despite that geographic barriers (particularly TI and ND) seem to interrupt the suitable climatic areas for the species, through time there have been episodes when connectivity may have been possible. Other important fact that may have influenced processes of colonization and re-colonization between MM and LM is the unstable geological dynamics of the region, characterized by the persistence of intense volcanic activity (as most of southern Central America consists of a Neogene-Quaternary volcanic belt) and tectonic activity related to the Nicaraguan volcanic front, Los Gatusos and San Carlos lowlands and the Chorotega volcanic front described by Marshall (2007), which together configure a highly dynamic and heterogeneous geographic area.

Considering the observed differentiation of the LM populations it is also important to mention the diversity of geological histories that characterize the CRM. *Quercus insignis* populations in Costa Rica distribute in the Cordillera de Guanacaste (CG; i3; Figures 1 and 4), Cordillera Central (CC; i2 and i4; Figures 1 and 4) and Cordillera de Talamanca (CT; i1; Figures 1 and 4). This distribution includes a Quarternary chain of shield-like stratovolcanos at CG, composite shield volcanoes and strong climate gradients at CC; the Central Valley (between CG, CC and CT) consisting of a low-relief upland surface with deeply incised river canyons, active faulting and a thick accumulation of andesitic to dacitic lavas, pyroclastic rocks, and lacustrine sediments throughout the Quarternary. Meanwhile, the CT is characterized by a suite of Neogene-Quaternary intrusive and extrusive rocks and a rapid Quaternary uplift and glaciated peaks during the Pleistocene (Marshall, 2007). Under such a geological diverse scenario mainly characterized by high volcanic activity and heterogeneous mountain systems, the differentiation of oak populations would be expected, even more considering the limited capability of the oak species to disperse their seeds. *Quercus insignis* probably has the largest acorns of any oak in the world (between 7-8 cm in diameter; Montes-Hernández and López-Barrera 2013), which could have implied some restrictions in seed movement.

For *Q. sapotiifolia* both AMOVA and SAMOVA results suggested a pattern of genetic differentiation poorly congruent with our a priori regionalization based on the presence of the TI and ND. SAMOVA suggested that Mexican populations (s13, s14 and s15; Figure 4) formed a unique group, and results from Barriers also showed a lack of differentiation associated to the TI (Figure 7). Three populations (s1, s10 and s12) did not group with other populations, and all the remaining sites formed a unique group including MM and LM samples. Again the Barriers analysis did not support significant differences across the ND. Most of the differentiation observed in the Barriers analysis concentrated at the MM region, particularly near the PMF. The first main barrier was located at the eastern portion of the TI in the SMC followed by a second barrier separating Guatemala from Honduras populations. The MM is an area of convergence among the Maya highlands, the Motagua fault zone and the Chortis highlands. The potential distribution area of *Q. sapotiifolia* in Guatemala and Honduras is interrupted by the Chuacus range and Las Minas range (in the Motagua fault zone) and the western rifted Chortis highlands. Differentiation among populations indicated by the Barriers analysis could be attributed to prominent river valleys (such as the Motagua and Polochic valleys) and major structural depressions associated to the valleys as is it described by Marshall (2007).

The haplotype network of *Q. sapotiifolia* (Figure 4) indicates processes of multiple colonization from MM to UM and LM. Interestingly, the data suggested that the major barriers (TI and ND) were not an important factor defining the genetic structure of the species, meanwhile barriers that were not expected as strong drivers of differentiation as the CRM showed an important effect on the genetic variation distribution. This unexpected pattern may reflect for the CRM case the importance of the recent geological, volcanic and climatic heterogenity of the Chortis block on the distribution of the genetic variation in montane oak species. It is necessary to consider that *Q. sapotiifolia* has a wide altitudinal distribution (from 250 up to 2000 m) which suggests that this species could have been more successful during processes of dispersion through the lowlands than other oak species such as *Q. insignis* that distributes above the 1500 m. Second, seed dispersal in *Q. sapotiifolia* should be easier due to the smaller size of their acorns (~1.5 cm in diameter) (Morales 2010). Therefore, even under a scenario of few climatic suitable areas connecting MM with UM and LM, it is possible to suggest long-distance dispersal (e.g. via birds such as passenger pigeons as it was hypothesized by Nixon, 1985) as a mechanism of dispersal through the main barriers. Finally, similarly to what was observed for *Q. insignis* in LM, *Q. sapotiifolia* populations located in the CC are genetically differentiated from the population of the CT, suggesting that Central Valley and the characteristic geology of the CRM described above may have played an important role in the genetic differentiation of several *Quercus* species including *Q. sapotiifolia* and *Q. insignis*.

Conclusions

Understanding the effect of historical processes such as tectonics, volcanism and climate fluctuations on the evolutionary history of the biotas distributed in the northern Neotropics still requires considerable effort. In the present study, two *Quercus* species exemplify the diversity of possible evolutionary histories that can be observed and reconstructed from a common region. In the case of *Q. insignis* we observed coincidences with previous proposed barriers that may have determined the species phylogeographic structure, the TI and the ND. The influence of both barriers have been observed in different biological groups such as mammals (Arellano et al. 2005), reptiles (Castoe et al. 2003; Hasbún et al. 2005; Daza et al. 2010), birds (DaCosta and Klicka 2008; Vázquez-Miranda et al. 2009), amphibious (Mulcahy et al. 2006) and plants (Cavender-Bares et al. 2013). All the mentioned studies attributed a role as barriers to the TI and ND based on different geological attributes (such as volcanism, tectonics, and physiography) and/or palaeoclimatic events.

On the other hand, the lack of influence of geographic elements such as the TI and ND, but significant levels of population differentiation due to the geologic heterogeneity of areas such as the PMF and the Central American volcanic arc also have been reported for different organismal lineages such as plants (Novick et al. 2003; Jardón-Barbolla et al. 2011) and mammals (Eizirik et al. 1998, 2001; Ordóñez-Garza et al. 2010). Once again the mentioned studies not only considered different geological features, but also palaeoclimatic information in order to explain the patterns observed in their studies. The findings of the present study suggest a complex evolutionary history of the oaks populations in the northern Neotropics. The most striking indirect finding is the possibility of a MM origin of the species analyzed; this is particularly clear for *Q. sapotiifolia*. This affirmation is based on the important diversity exhibited in its populations and the evidence of processes of multiple colonization from MM to both UM and LM and even re-colonizations from LM to MM. The evaluation of possible Central American origin of this species should be considered in future oak species (or even other taxa) phylogeographic and phylogenetic studies considering that northern Neotropics characterized by being the encounter point between Nearctic and Neotropical biotas under an extremely complex geologic and climatic scenario, the perfect combination for novel evolutionary processes as we observe in this study.

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Tables

Table 1. *Quercus insignis* and *Q. sapotiifolia* population geographic information summary, genetic diversity and genetic structure estimates. n: sample size, h_S: within population genetic diversity. UM: upper Mesoamerica, MM: middle Mesoamerica, LM: lower Mesoamerica, CR: Costa Rica, HON: Honduras, GUA: Guatemala, MEX: Mexico.

	ID	Longitud	Latitude	Country/ Region	Elevation (m.a.s.l)	n	Total haplotypes	Allelic richness (rarefacted)	h_S	Fu´s F_S	p-value
Quercus insignis	i1	-82.93	9.4	CR	725	5	2	1.60	0.40	0.2	0.94
	i2	-83.98	9.88	CR	1715	7	4	2.46	0.80	0.5	0.15
	i3	-84.8	10.3	CR	1401	3	3	3.00	1.00	0.66	0.62
	i4	-84.01	9.95	CR	1372	5	2	1.60	0.40	0.68	0.94
	i5	-83.81	9.54	CR	2451	10	5	2.50	0.82	0.23	0.07
			LM			30	12	8.56	0.91	0.22	0.58
	i6	-87.95	14.53	HON	1638	7	3	1.86	0.52	0.18	0.76
	i7	-87.81	14.76	HON	1505	7	6	2.86	0.95	0.13	0.65
	i8	-87.95	14.76	HON	846	6	3	2.00	0.60	0.25	0.51
	i9	-87.12	14.24	HON	1754	5	2	1.90	0.60	0.37	0.4
	i10	-87.07	14.04	HON	1507	6	4	2.45	0.80	0.27	0.21
			MM			31	16	10.07	0.93	-1.89	0.25
	i11	-97.04	19.18	MEX	1408	7	2	1.71	0.47	0.72	0.93
	i12	-96.94	17.43	MEX	802	5	2	1.60	0.40	0.68	0.49
	i13	-96.96	19.37	MEX	1059	3	2	2.00	0.66	1	0.56
	i14*	-92.9	16.8	MEX	1108	1	1	NA	NA	NA	NA
			UM			15	2	2	0.42	4.14	0.96
Quercus sapotiifolia	*s1*	-83.96	9.7	CR	1671	5	2	1.60	0.40	1.68	0.76
	s2	-84.25	10.14	CR	2276	9	3	2.05	0.63	1.85	0.48
			LM			14	5	5.00	0.79	1.35	0.77
	s3	-86.79	13.39	HON	1938	11	3	1.76	0.47	-0.65	0.1
	s4	-87.13	13.9	HON	1832	9	3	1.67	0.41	-1.08	0.07
	s5	-87.09	13.98	HON	1317	6	3	2.00	0.60	-0.85	0.08
	s6	-87.07	14.04	HON	1122	8	7	2.89	0.96	-2.62	0.05
	s7	-87.06	14.22	HON	2004	8	5	2.59	0.85	-1.74	0.06
	s8	-88.21	14.27	GUA	1298	10	3	1.60	0.37	0.286	1.45
	s9	-96.46	14.69	GUA	1468	9	4	1.99	0.58	2.21	0.86
	s10	-91.88	14.83	GUA	1524	14	2	1.54	0.36	0.65	0.45
	s11	-90.98	15.49	GUA	1507	18	2	1.44	0.29	0.46	0.38
	s12	-90.98	15.49	GUA	1914	3	1	1.00	0.00	NA	NA
	s13	-91.7	16.11	MEX	1501	6	2	1.50	0.33	1.6	0.72
	s14	-94.18	16.89	MEX	1908	3	2	2.00	0.66	0.38	0.2
			MM			105	22	7.82	0.88	-0.65	0.47
	s15	-97.04	19.18	MEX	1178	20	7	5.64	0.64	3.02	0.91
			UM			20	7	5.64	0.64	3.02	0.91

Table 2. Hierarchical analysis of molecular variance (AMOVA) using F_{ST} and R_{ST} for *Quercus insignis* and *Q. sapotiifolia*. Groups category corresponds to UM, MM and LM.

	Source of variation	d.f.	s.s.	Variance components	Percentage of variation	
Quercus insignis			**F_{ST}**			
	Among groups	2	42.33	0.6	20.57	Φ_{CT}=0.20*
	Among populations within groups	10	83.24	1.24	42.22	Φ_{SC}=0.53*
	Within populations	63	69.19	1.09	37.2	Φ_{ST}=0.62*
	Total	75	194.77	2.95		
			R_{ST}			
	Among groups	2	550.86	9.01	30.41	Φ_{CT}=0.30*
	Among populations within groups	10	848.43	13.38	45.18	Φ_{SC}=0.64*
	Within populations	63	455.6	7.23	24.41	Φ_{ST}=0.75*
	Total	75	1854.67	29.62		
Quercus sapotiifolia			**F_{ST}**			
	Among groups	2	65.96	0.52	21.21	Φ_{CT}=0.21**
	Among populations within groups	12	142.36	1.33	53.93	Φ_{SC}=0.68*
	Within populations	124	76.58	0.61	24.87	Φ_{ST}=0.75*
	Total	138	284.91	2.48		
			R_{ST}			
	Among groups	2	588.97	0.87	2.79	Φ_{CT}=0.02
	Among populations within groups	12	2099.73	19.54	62.39	Φ_{SC}=0.64*
	Within populations	124	1352.21	10.9	34.82	Φ_{ST}=0.65*
	Total	138	4040.92	31.32		

Figures

Figure 1. *Quercus insignis* and *Q. sapotiifolia* distribution represented by white and black triangles respectively. Elevation of the northern Neotropics is represented in a grey scale from lowlands (white) to highlands (black). SMO: Sierra Madre Oriental, SMS: Sierra Madre del Sur, TI: Tehuantepec Isthmus, SMC: Sierra Madre de Chiapas, PMF: Polochic-Motagua fault system, TIM: Trans-Isthmian mountains, ND: Nicaraguan Depression, CRM: Costa Rica mountains. Inside dotted and solid rectangles were represented details of MM and LM as follows: MH: Maya highlands, CRa: Chuacus range, LM: Las Minas range, MFZ: Motagua fault system, wCH: western rifted Chortis highlands, CH: central Chortis highlands, CG: Cordillera de Guanacaste, VC: Valle Central, CC: Cordillera Central, CG: Cordillera de Talamanca, CVF (solid line): Chorotega volcanic front.

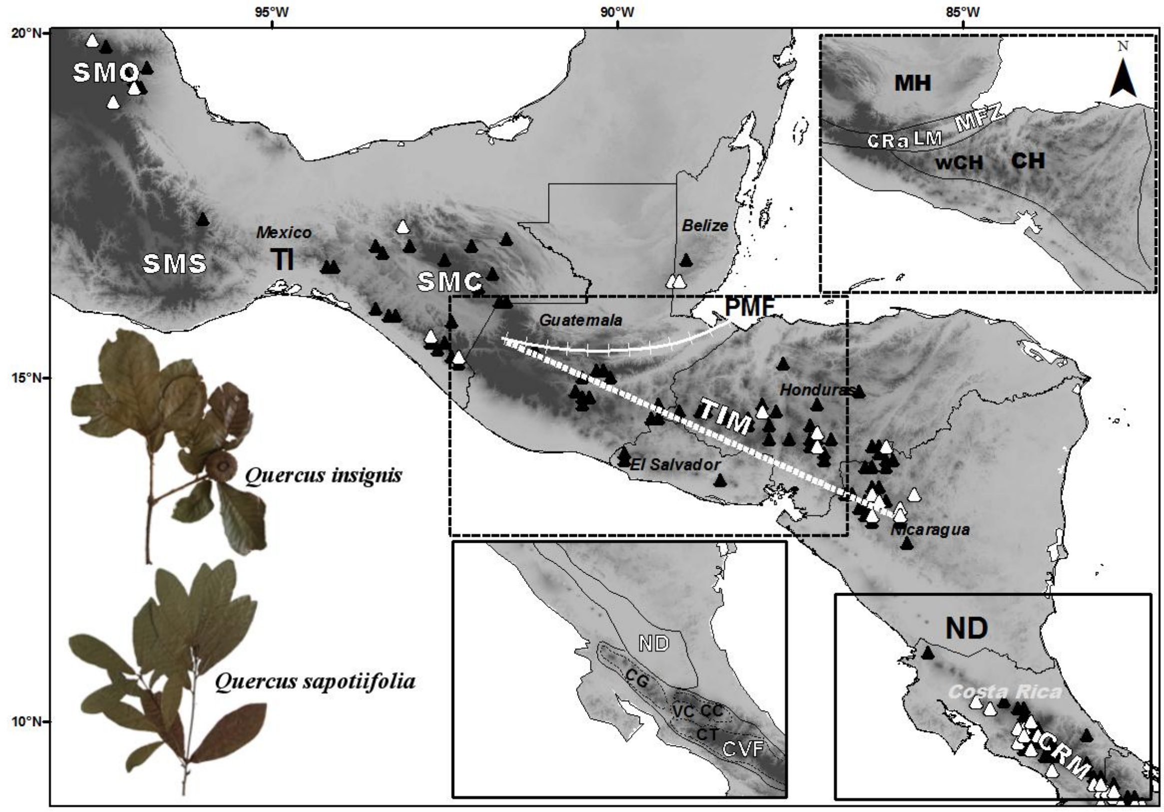


Figure 2. Haplotype distribution for *Quercus insignis*, circles diameter is proportional to populations number and haplotype frequency. Northern Neotropics is divided in upper Mesoamerica (red line), middle Mesoamerica (green line) and lower Mesoamerica (yellow line). Haplotype network is also represented by the presence of the haplotypes per region. Grey and White haplotypes correspond to unique haplotypes in MM and LM respectively. Numbers inside parenthesis correspond to populations where the haplotype was observed. Bold numbers correspond to the number of observed differences between haplotypes. Orange circles represent expected haplotypes (not observed).

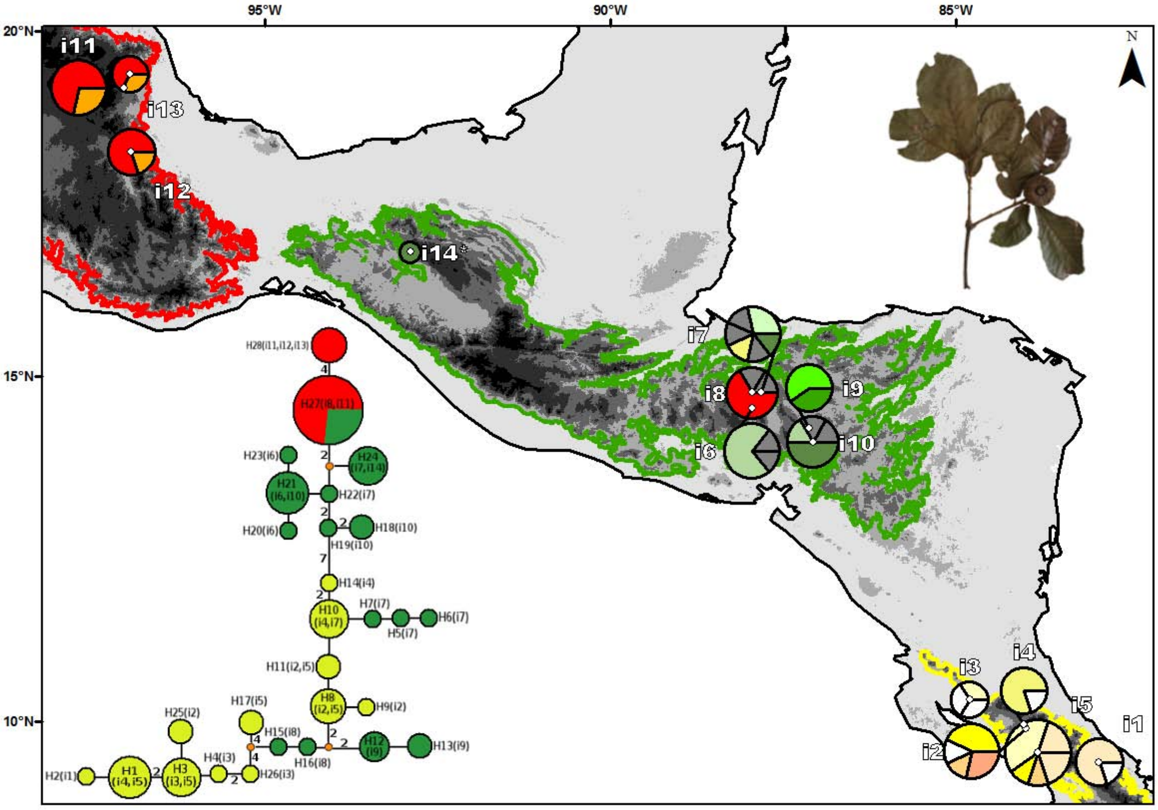


Figure 3. Present-day (1950-2000), Last Glacial Maximum (~21 ka BP) and Last Interglacial (~120 ka BP) Ecological Niche Models for *Quercus insignis* in northern Neotropics.

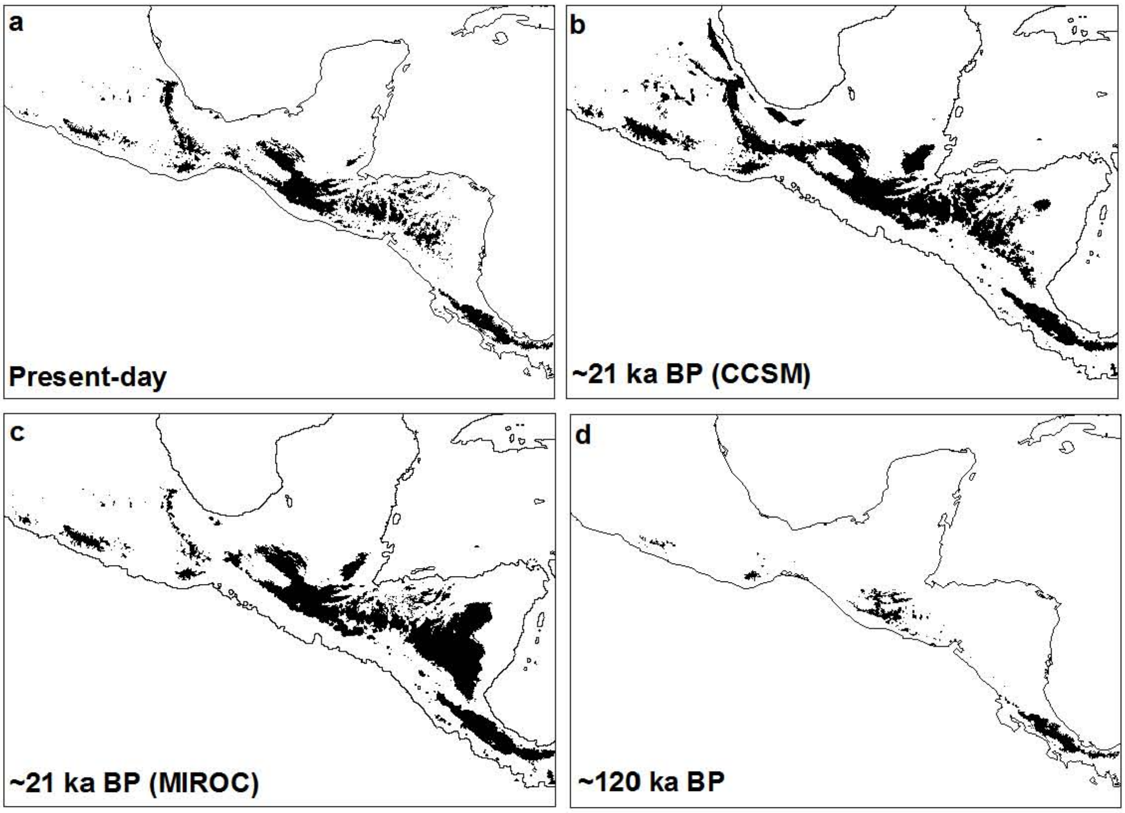


Figure 4. Haplotype distribution for *Quercus sapotiifolia*, circles diameter is proportional to populations number and haplotype frequency. Northern Neotropics is divided in upper Mesoamerica (red line), middle Mesoamerica (green line) and lower Mesoamerica (yellow line). Haplotype network is also represented by the presence of the haplotypes per region. Dark grey, light grey and white haplotypes correspond to unique haplotypes in UM, MM and LM respectively; green and blue dotted haplotypes correspond to the most widely distributed haplotypes in MM. Numbers inside parenthesis correspond to populations where the haplotype was observed. Bold numbers correspond to the number of observed differences between haplotypes. Orange circles represent expected haplotypes (not observed).

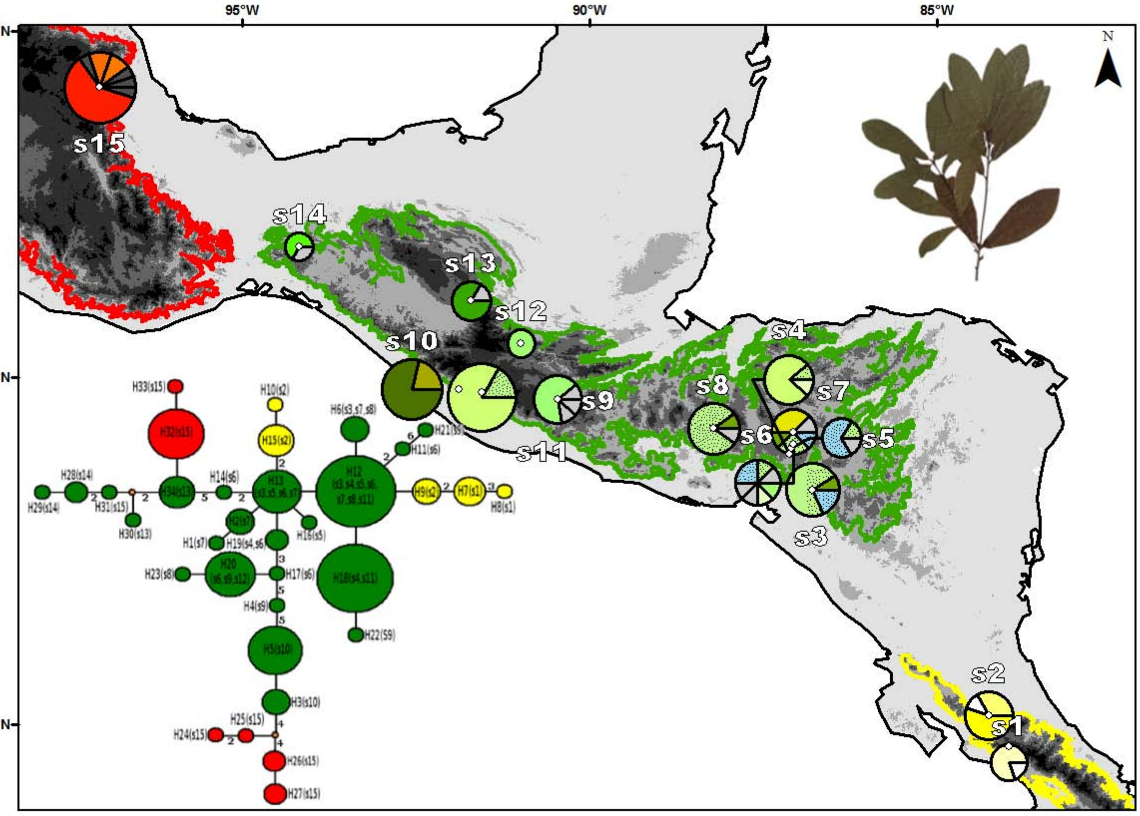


Figure 5. Present-day (1950-2000), Last Glacial Maximum (~21 ka BP) and Last Interglacial (~120 ka BP) Ecological Niche Models for *Quercus sapotiifolia* in northern Neotropics.

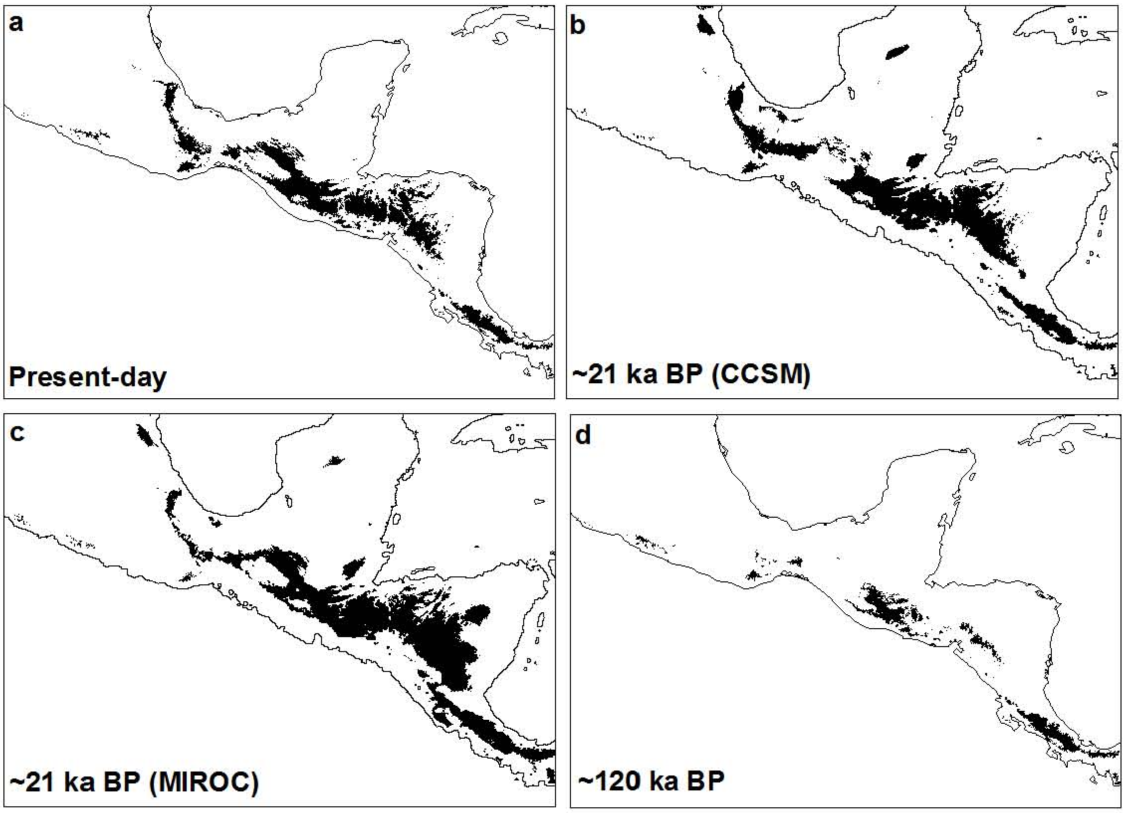


Figure 6. *Quercus insignis* and *Quercus sapotiifolia* stable areas of distribution since the Last Interglacial (~120 ka BP). Black category corresponds to the areas where the distribution converge in the four models used. Dark grey corresponds to the areas where models for the present-day and LGM models converge.

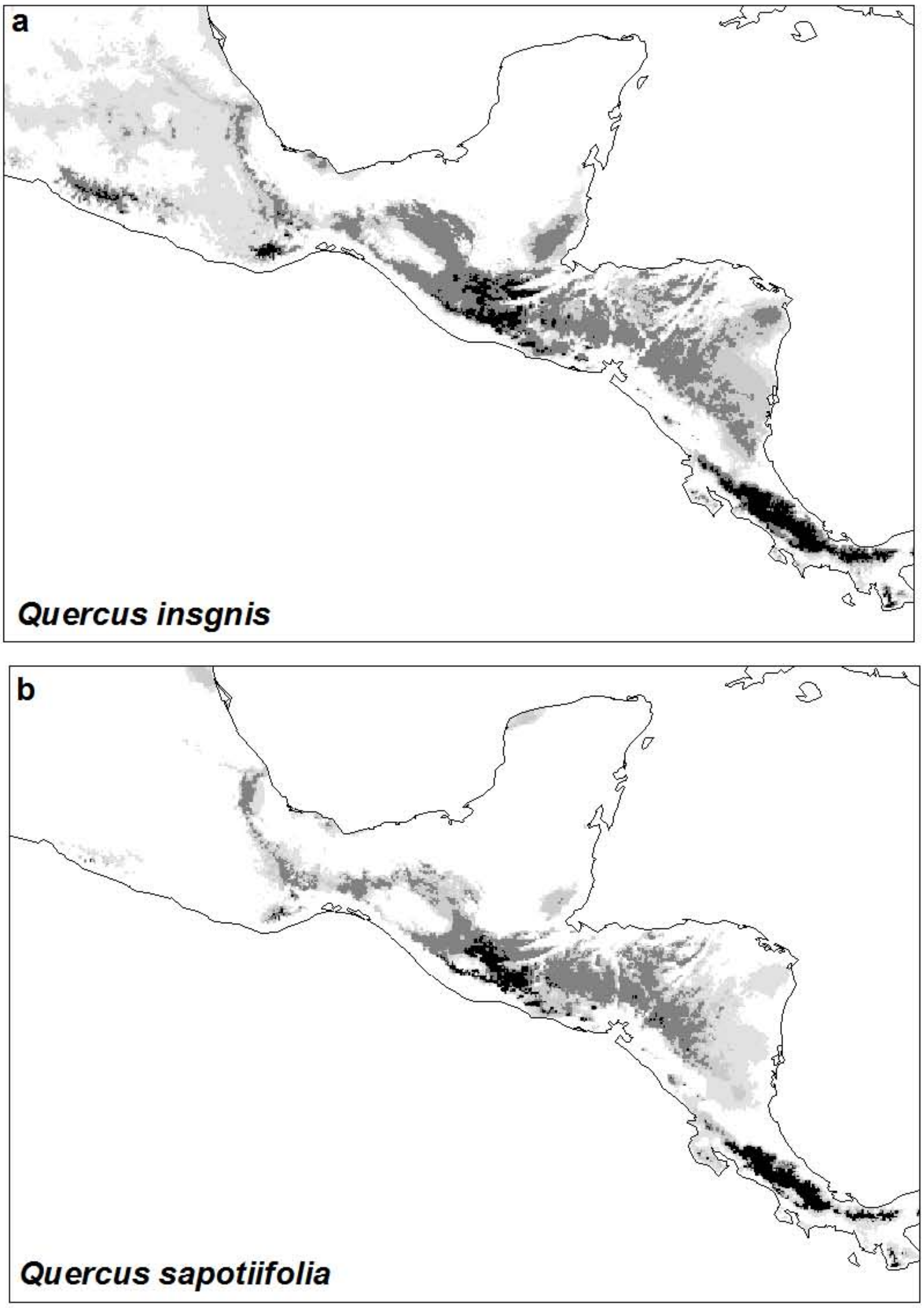
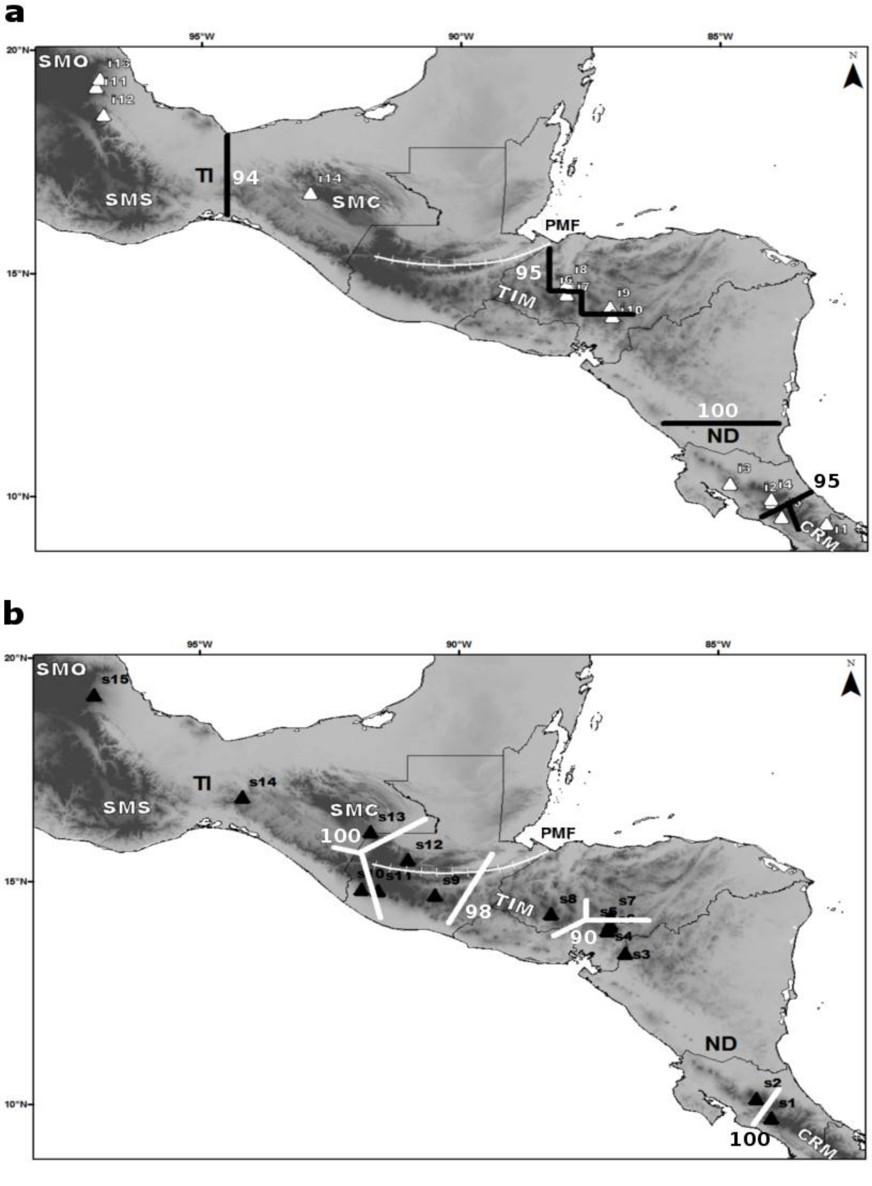


Figure 7. Geographical location of the most important genetic discontinuities (black lines for *Quercus insignis* and white lines for *Q. sapotiifolia*) using the Monmomier´s maximum difference algorithm for both *Quercus* species populations (white triangles for *Q. insignis* and black triangles for *Q. sapotiifolia*). Elevation of the northern Neotropics is represented in a grey scale from lowlands (light gray) to highlands (black). SMO: Sierra Madre Oriental, SMS: Sierra Madre del Sur, TI: Tehuantepec Isthmus, SMC: Sierra Madre de Chiapas, PMF: Polochic-Motagua fault system, TIM: Trans-Isthmian mountains, ND: Nicaraguan Depression, CRM: Costa Rica mountains.



Capítulo Tres

Contrasting patterns of population history and seed-mediated gene flow in two endemic Costa Rican oak species

Hernando Rodríguez-Correa, Ken Oyama, Mauricio Quesada, Erick Fuchs, Antonio González-Rodríguez

Abstract

Lower Central America has been proposed as a fundamental area to study recent historical assembly and diversification of Neotropical species due to its complex and dynamic geological, climatic and biological history. Studies regarding phylogeography of Neotropical species are few in comparison with other regions and even less for tree species. We set as the aim of the present study to characterize the phylogeographic structure in two co-distributed oak species (*Quercus costaricensis* and *Q. bumelioides*) of the Costa Rican mountains and to describe its historical demography by characterizing populations of both species using chloroplast short sequence repeats. Genetic diversity, genetic structure, minimum-spanning haplotype networks, patterns of gene-flow and historical demography were described for both species and discussed based on the geologic and climatic history of Costa Rica. Results suggested contrasting phylogeographic patterns between both species. *Q. costaricensis* exhibited high values of genetic diversity, a marked phylogeographic structure defined by mountain ranges, a north-to-south genetic diversity gradient and evidence of a demographic expansion during the Quaternary. *Quercus bumelioides* did not show any genetic structure and both, the haplotype network and historical demography estimates suggested a recent colonization and population expansion, probably during the Upper Pleistocene-Holocene transition. Phylogeographic structure of *Q. costaricensis* seems to be related to Pleistocene altitudinal migration due to its (mostly) upper montane forest distribution. Meanwhile, the high levels of gene-flow and lower montane forest distribution of *Q. bumelioides* may have promoted the homogenization of genetic variation. Population expansion and stable suitable climatic areas in both species probably indicate that historical climatic change did not promote the species isolation in higher lands. On the contrary, downwards altitudinal migration allowed both species to expand their distribution into the Panamanian mountains during interglacial stages.

Keywords: *Quercus*, Fagaceae, Middle America, phylogeography, gene-flow, chloroplast microsatellites.

Introduction

Neotropical oaks distribute from the Mexican transition zone down to the northern Andes. Along this distribution, there is a marked reduction in species richness from north to south (Nixon 2006). There are only nine *Quercus* species in Costa Rica (Morales 2010), but oaks still have a great ecological importance in this area, in terms of abundance and biomass. Even more, some individuals in oak stands in Costa Rica are among the tallest for the genus in America (Nixon 2006). In terms of *Quercus* biogeography, the Costa Rican mountains are the only area of endemism in the Central American region, while the limit between the Nicaraguan depression and the Costa Rican mountains are an important turnover point for *Quercus* species composition (Rodríguez-Correa et al. 2015) as different species reach its natural boundary in this region (*Quercus elliptica*, *Q. skinerii, Q. peduncularis, Q. segoviensis, Q. purulhana*). The importance of the Costa Rican mountains as drivers of evolutionary processes has been recognized not only in biogeographic studies. Genetic studies also have suggested a close relation between the tectonic and climatic history of the area with the colonization history of lower Central America by plant species such as oaks (Cavender-Bares et al. 2011, 2015) and orchids (Kartzinel et al. 2013). The congruence between genetic structure and geological history has also been reviewed by Gutiérrez-García and Vázquez-Domínguez (2013) for a wide variety of taxa. The authors suggested that Costa Rica, Panama and a portion of the Colombian Darien together configure an evolutionary group defined by common patterns of genetic arrangement, the Panamian group.

The Panamian group proposed by Gutiérrez-García and Vázquez-Domínguez (2013) is characterized mainly by: i. the presence of species that are genetically differentiated from those present in other geological regions such as the Mayan block and Mid-Central America, ii. marked genetic structure between the north and south, and also between the Pacific and the Caribbean limits of the region and iii. the presence of barriers to gene flow in areas such as Boca del Toro (between Costa Rica and Panama) and the Darien Isthmus. All of the mentioned patterns are related to geological and climatic processes characteristic of the region, which biogeographically is included in the Pacific dominion of the Neotropics. This dominion is in turn divided into two biogeographic units, the Guatuso-Talamanca and the Puntarenas-Chiriquí provinces (Morrone 2014). Both provinces are characterized by a dynamic and heterogeneous tectonic and climatic history as it is highlighted by Gutiérrez-García and Vázquez-Domínguez (2013). Geologically, both the Guatuso-Talamanca and the Puntarenas-Chiriquí provinces are included in the Chorotega block that is characterized by the presence of a Neogene-Quaternary volcanic belt. The Chorotega block is limited by a major fault lineament from Costa Rica's Elena peninsula eastward to the Hess escarpment at the north, and a basement suture at the Panama Canal Zone at the east that separates the Chorotega block from the Choco block (Marshall 2007).

Despite the climatic, geologic and biogeography heterogeneity exhibited by the Chorotega block, until recent times, the evolution of the biota in this area has been discussed focusing on the idea that the region represents a mere biogeographic crossroad between Nearctic and Neotropical taxa (Bagley and Johnson 2014). However, in the last few years, different authors have remarked the fact that areas such as the Nicaraguan depression, the Boca del Toro region, the Talamanca range and the Darien isthmus have played an important role in shaping the distribution and genetic structure of several Neotropical species (including oak species) (Torres-Miranda et al. 2011; Gutiérrez-García and Vázquez-Domínguez 2013; Torres-Miranda et al. 2013; Bagley and Johnson 2014; Rodríguez-Correa et al. 2015). Bagley and Johnson (2014) emphasized that the apparent lack of phylogeographic structure in plant species of Lower Central America can be explain by the effect of using low resolution genetic markers and a bias towards studying species with high relative dispersal potential. They also remarked an important taxonomic sampling bias between animals and plants, favoring animal studies with a 9:1 ratio. Bagley and Johnson (2014) also mentioned the need of more phylogeographic studies dealing with co-distributed plant species inhabiting premontane and montane forest belts. So far, few studies have attempted to describe seed-mediated gene flow and species historical demography in plat species in this region (e.g. Kartzinel et al. 2013).

In Costa Rica, a wide altitudinal distribution has been described for *Quercus* species (from zero up to 3500 meters), as well as an important diversity of plant taxa associated to oak forests (more than 253 vascular plant genera; Kappelle 2006). There are also differences in oak species composition between the Costa Rican Pacific and Atlantic slopes (Kappelle and van Uffelen 2006). Within the Costa Rican oak forests is common to observe co-occurring species; this is particularly true for *Quercus costaricensis* and *Q. bumelioides* in the upper montane forests and *Q. seemanni* and *Q. bumelioides* in the lower montane forests. It is interesting that both *Quercus costaricensis* (a red oak belonging to section *Lobatae*) and *Q. bumelioides* (a white oak in section *Quercus*) exhibit strikingly similar foliage characterized by elliptic leaves with impressed venation and usually conspicuous tomentum on the lower surface leaves (Nixon 2006). Therefore, these similarities have been attributed to parallelism in response to similar environments (Nixon, 2006). The main differences between the species lies on the acorn production as *Q. costaricensis* seems to produce large crops of acorns and *Q. bumelioides* produce acorns sporadically (Nixon 2006), and their altitudinal distribution, that although overlaps, suggests that *Q. costarricensis* is more dominant in higher elevations ((1800-)2300-3600 meters) than *Q. bumelioides* ((600-)1100-2900 meters) (Morales 2010).

This scenario of two congeneric species with similar seed-mediated dispersal, an important degree of simpatry and therefore similar environmental niches, configures an important opportunity to test if historical processes associated to geology and climate have shaped equally the species population dynamics in a heterogeneous landscape as the Costa Rican mountains. Therefore, we set as aim of this study: i. to describe the phylogeographic structure in two partially co-distributed oak species (*Quercus costaricensis* and *Q. bumelioides*) in the Costa Rican mountains, ii. to characterize historical demographic patterns in these two species, iii. to describe patterns of seed-mediated gene flow among populations within the two oak species, and iv. to compare phylogeographic and gene-flow patterns observed in the Costa Rican montane oak species with other *Quercus* species.

Methods

Studied species

Quercus costaricensis and *Q. bumelioides* belong to the *Lobatae* and *Quercus* sections of *Quercus* (Fagaceae), respectively. *Quercus costaricensis* is a black bark tree that reaches a height between 3 and 50 meters, with obovate and tomentose leaves. The species is distributed in montane forests and paramo ecosystems in both slopes of the Costa Rica Central Cordillera and Cordillera de Talamanca. *Quercus bumelioides* has a gray to white-gray bark with obovate to elliptic leaves. It is distributed in montane forests (including primary and secondary forests) of the Cordillera de Guanacaste, Cordillera de Tirilán, Cordillera Central and Cordillera de Talamanca. Overall, we sampled between five and nine individuals per species from seven populations of *Q. costaricensis*, and between four and 15 individuals in six populations of *Q. bumelioides* across their distribution range in Costa Rica.

DNA isolation and microsatellite amplification

Total DNA was extracted using the QIAGEN DNeasy plant mini kit. Nine chloroplast DNA (cpDNA) microsatellite loci designed for Fagaceae species (cmcs2, cmcs3, cmcs4, cmcs5, cmcs6, cmcs7, cmcs10, cmcs12 and cmcs14; Sebastiani et al. 2004) were screened and tested for polymorphism, using two primer groups (cmcs3, cmcs4, cmcs5, cmcs6 formed the first group and cmcs2, cmcs7, cmcs10, cmcs12, cmcs14 the second group) for multiplexing reactions grouped by expected allels size and flourecense. Polymerase chain reactions (PCR) were performed using a QIAGEN multiplex PCR kit with a final volume of 5 µL containing 1X multiplex PCR master mix, 0.25 mM of each primer, 10 ng of DNA and dH_2O. Amplification was performed using an initial denaturation step for 15 min at 95°C, followed by 35 cycles, each of 30 s at 95°C, 1.5 min at 55°C and 1 min at 72°C, and a final extension step for 30 min at 60°C. PCR products were analyzed in a ABI-PRISM 3300 Avant sequencer (Applied Biosystems) and the resulting electropherograms were processed using the Peak Scanner program version 2.0 (Applied Biosystems).

Genetic diversity and structure

Haplotypes were defined as unique combinations of size variants for the evaluated chloroplast microsatellite loci. Rarefacted haplotype richness (*AR*), haplotype diversity with unordered alleles (*h*; Pons and Petit 1996), non-standardised gene diversity with ordered alleles (*v*; Pons and Petit 1996) and the mean pairwise genetic distance among individuals within a population under a stepwise mutation model (D^2_{SH}; Goldstein et al.

1995) were calculated for the populations of both species using SPAGeDi version 1.1 (Hardy and Vekemans 2002). Genetic differentiation and phylogeographic structuring were assessed by calculating G_{ST} (the coefficient of genetic differentiation with unordered alleles) and N_{ST} (the coefficient of genetic differentiation with ordered alleles) with SPAGeDi version 1.1 (Hardy and Vekemans 2002). This program also implements a permutation test to evaluate if the values of G_{ST} and N_{ST} are significantly different. A higher value of N_{ST} than G_{ST} indicates phylogeographic structure in the populations, resulting from the presence of closely related haplotypes within the same populations (Pons and Petit 1996).

The partitioning of the genetic variation between and within populations was estimated using a hierarchical analysis of molecular variance (AMOVA). AMOVA was calculated considering both F_{ST}-like (based on the infinite alleles mutation model, IAM) and R_{ST}-like (based on the stepwise mutation model, SMM) measures and using 10,000 permutations in Arlequin version 3.5 (Excoffier et al. 2005). Haplotype relationships were inferred using a minimum spanning network with the median-joining method (Bandelt et al. 1999) and a maximum parsimony search (Polzin and Daneschmand 2003) in Network version 4.6 (available at www.fluxus-engineering.com). To assess possible geographical patterns in genetic diversity levels, the correlation of *AR*, h_S and D^2_{SH} and the latitude of the populations was determined using R version 3.1.2. However, this analysis was only performed for *Q. costaricensis* because of the small latitudinal range of *Q. bumelioides* samples.

Genetic barriers and gene flow estimation

The location of the most important genetic discontinuities for *Q. costaricensis* was determined using the Monmomier's maximum difference algorithm implemented in

BARRIER version 2.2 (Manni et al. 2004). For this purpose, a pairwise matrix of average square genetic distance (ASD; Goldstein *et al.* 1995; Slatkin, 1995) was calculated. To provide bootstrap support to the observed genetic barriers the matrix was randomly resampled 100 times. Only barriers with a bootstrap support value over 80 were reported. *Quercus bumelioides* was excluded from this analysis considering the lack of significant genetic structure (see results).

In order to estimate gene flow patterns, mutation scaled immigration rate (*M*) between populations was estimated for *Q. costaricensis* and *Q. bumelioides*, using a maximum likelihood coalescent approach implemented in MIGRATE version 3.2.19 (Beerli and Felsenstein 2001). In this analysis an initial genealogy started from a random tree and initial migration rate and theta parameters were estimated from F_{ST}. Ten short chains (5×10^5 genealogies sampled) and three long chains (1×10^7 genealogies sampled) after discarding 1×10^4 genealogies as a burn-in were run under a Brownian motion approximation as the mutation model. Analyses were run three times using different starting seeds in order to check for parameters estimates consistency.

Historical demography

Events of demographic expansion were assessed using the F_S statistic (Fu, 1997) and the mismatch distribution test (Rogers and Harpending 1992) for *Q. costaricensis* and *Q. bumelioides.* F_S estimations were calculated using Arlequin version 3.5 (Excoffier et al. 2005) using cpSSR data coded in binary form as suggested by Navascués et al. (2006). This analysis was performed at the population level and also considering all populations of each species as a single group due to the low genetic structure observed. For those populations or groups of populations that exhibited negative and significant F_S values and non-significant raggedness values, the estimation of the time to the population expansion in mutational units (τ) was calculated using the maximum-pseudolikelihood method implemented in the LMSE software (Navascués et al. 2009), which accounts for homoplasy. The time to expansion in years was calculated using the expression $\tau = 2l\mu t$ (l: number of microsatellite loci, μ: microsatellite mutation rate and t: generation time; Rogers and Harpending 1992) considering a per-locus mutation rate between 1×10^{-5} and 1×10^{-4}, as well as a generation time between 50 and 100 years (Navascués et al. 2009; Heuertz et al. 2010).

Ecological niche modelling

Climatically suitable areas for *Q. costaricensis* and *Q. bumelioides* during the Last Interglacial (LIG; ~120 *ka* BP), the Last Glacial Maximum (LGM; ~21 *ka* BP) and the present-day (PD) periods were estimated using ecological niche models (ENMs) in Maxent version 3.3.3a (Phillips et al. 2006). Occurrence data were downloaded for both species from a public repository (Global Biodiversity Information Facility; www.gbif.org). Geographic information was filtered according to the reported distribution and altitudinal range of each species. Environmental data used to run the models were obtained from a set of 19 bioclimatic variables described by Hijmans et al. (2005; www.worldclim.org) at a spatial resolution of 30 arc-seconds (~1 Km). From this 19 variables, a sub-set was defined by eliminating one variable from each pair of highly correlated bioclimatic variables (r>0.7) and prioritizing more general versus more specific variables. Only data points separated by more than 0.1 decimal degrees with respect to their nearest neighbour were used in order to reduce aggregation of geographic records.

ENMs were estimated after 100 replicas using the bootstrap resampling method using the 30% of the presence records to calculate quality estimators and 70% to run the models. In the absence of palaeoecological data to build the LGM and LIG models, the 'projection' option in Maxent was used to obtain palaeodistribution maps for both species. Projections into past periods were estimated using three general circulation model layers, the Community Climate System Model (CCSM) and the Model for Interdisciplinary Research on Climate (MIROC) for the LGM, and a model proposed by Otto-Bliesner et al. (2008) for the LIG. The area under the receiver operating characteristic (ROC) curve analysis (AUC; Fielding & Bell, 1997) was used to determine the model quality, and the fixed cumulative value 1 logistic threshold (FCV) was used to calculate binomial outputs. FCV was used as it has been suggested as an appropriated threshold criteria in other Neotropical species analysed with palaeoecological methods involving ENM (Rodríguez-Correa et al. *unpublished data*). Finally the stability of climatically suitable areas through the different time periods was determined by overlaying the obtained distribution maps.

Results

Genetic diversity and genetic structure

A total of 42 individuals distributed in six populations and 75 individual distributed in seven populations were characterized for *Q. costaricensis* and *Q. bumelioides*, respectively (Figure 1, Table 1). All the nine cpSSR were polymorphic and the number of alleles for each locus varied from two to four in both species. The number of haplotypes per species was 18 including eight singletons and six haplotypes private to a single population for *Q. costaricensis* and 26, with 15 singletons and two private haplotypes for *Q. bumelioides*. Mean (s.e.) h_S and total gene diversity (h_T) were 0.68 (0.021) and 0.95 (0.028), respectively for *Q. costaricensis*, and 0.825 (0.038) and 0.84 (0.037) for *Q. bumelioides*. Genetic differentiation (s. e.) among populations in *Q.*

costaricensis was 0.275 (0.029; $p<0.05$) for unordered alleles (G_{ST}) and 0.485 (0.045; $p<0.05$) for ordered alleles (N_{ST}). Meanwhile, *Q. bumelioides* exhibited non-significant ($p>0.05$) values for both G_{ST} (0.003 ± 0.029) and N_{ST} (0.005 ± 0.028). The presence of phylogeographic structure in *Q. costaricensis* was supported by significant $N_{ST} > G_{ST}$ ($p<0.001$), but in *Q. bumelioides* the difference between G_{ST} and N_{ST} was not significant. AMOVA (Table 2) showed for that both F_{ST} and G_{ST}-like measures in *Q. costaricensis* the partitioning of the genetic variation was similar among populations (49.52% for IAM and 52.81% for SMM). In *Q. bumelioides* both differentiation values were non-significant. Correlation tests of latitude versus *AR* ($r=-0.91$, $p<0.01$), h_S ($r=-0.88$, $p<0.01$) and latitude of the *Q. costaricensis* populations showed a significant increment of genetic diversity as population latitude decrease and no relation between D^2_{SH} and latitude for the sampled populations ($r=0.072$, $p>0.05$).

The haplotype network in *Q. costaricensis* (Figure 2a) showed that haplotype H14 was present in four populations across the range of the species. Other three haplotypes (H2, H6 and H7) were present in two populations each, and the rest of the haplotypes were private to a single population or were singletons. All of the haplotypes were separated by one mutational step with the exception of haplotype H16 that was separated by three mutations from haplotype H10. The haplotype network of *Q. bumelioides* (Figure 2b) was characterized by having a star-shaped pattern, with two frequent and widely distributed haplotypes (H12 and H13), and a large number of singletons (16) and two private haplotypes (H6 and H19). This haplotype network indicated the presence of four non-sampled haplotypes and suggested that haplotypes H6, H17 and H26 were separated from the main network by two mutational steps.

Genetic barriers and gene flow estimation

Significant discontinuities in the *Quercus costaricensis* genetic distances were identified separating populations c2 located at northern CG, c1 located at southern CG and c3 located at the CC. Populations restricted to the CT (c4, c5 and c6) were grouped together without any significant differentiation between them (Figure 3). Mutation scaled immigration rate (*M*) between populations of *Q. costaricensis* ranged from cero to 6.58 and 11 comparisons (from a total of 30 comparisons) showed *M* values significantly greater than 1 (Table 3). Paired comparisons between populations c1/c5 and c5/c6 the only ones that exhibited gene flow in both directions. Population c4 was identified as a source population and the southern populations (c4, c5 and c6) maintained differential levels of gene flow between them (c5→c4, c5↔c6, c6→c4). Meanwhile population c1 (northernmost population) appears to be a sink population from the southern (CT) populations. *Quercus bumelioides* mutation scaled immigration rate (*M*) between populations (42 comparisons) exhibited *M* values significant greater than 1 ranging from 1.2 to 2.9 (Table 3). Paired comparisons between populations b4/b2 and b4/b7 exhibited gene flow in both directions. Two populations located at the CC acted as important source populations, b1 (b1→b2, b1→b5 and b1→b7) and b3 (b3→b2, b3→b4 and b3→b7); meanwhile populations b4 and b7 were identified as the most common sink populations (b2→b4, b3→b4, b5→b4, b3↔b7 and b1→b7, b2→b7, b3→b7, b4↔b7).

Historical demography

Four *Q. costaricensis* populations (c1, c2, c3 and c4) as well as all populations as a whole exhibited significant negative F_S values and non-significant raggedness values (Table 4). The estimation of the time to the population expansion (low mutation rate and long generation time/high mutation rate and short generation time) ranged between

826/41 and 1210/60 *ka* BP, meanwhile considering all populations as a unit the demographic expansion was dated 2355/117 *ka* BP. *Q. bumelioides* populations b1, b3, b4 and b7 also exhibited significant negative F_S values and non-significant raggedness values (Table 4), however the estimation of the time to the population expansion showed a different pattern from *Q. costaricensis*. Demographic expansion ranged between 588/29 and 1110/55 *ka* BP and considering all populations as a unit event more recently, 316/15 *ka* BP.

Ecological niche modeling

Ecological niche modeling for *Q. costaricensis* and *Q. bumelioides* presented a good performance as indicated by the AUC values (0.997 and 0.998 respectively). *Q. costaricensis* climatic suitable areas distributed over the 1000 meters line through the highlands in the present-day and both 21 *ka* BP scenarios (Figure 4a, b and c). In the 120 *ka* BP scenario (Figure 4d) even though the climatic suitability values did not decrease, the potential distribution of the species showed a shift southwards into the Panamanian mountains. In contrast, *Q. bumelioides* climatic suitable areas extended above the 1000 meters line all over the mountainous region of Costa Rica and Panama during the present-day and 21 *ka* BP scenarios (Figure 5a, b and c). However, in the 120 *ka* BP scenario (Figure 5d) the distribution of the species importantly decreased and got restricted to the Panamanian mountains. When temporal scenarios were considered together for *Q. costaricensis* (Figure 6a) it was noticed that the areas that have remained stable since the 21 *ka* BP (grey areas) and the 120 *ka* BP (black areas) periods are found mainly in the CT. Meanwhile, for *Q. bumelioides* stable areas covered most of the Costa Rican mountains under both 21 *ka* BP (gray areas) scenarios but are restricted to the

Panamanian mountains for the period between 120 *ka* BP (black areas) and the present (Figure 6b).

Discussion

Southern Central America is a complex and dynamic region not only from the geological perspective, but also from the point of view of the evolutionary history of the biotas that distribute therein. The phylogeographic structure of different taxa has been characterized within the area comprising from northern Costa Rica to the Darien Isthmus. However most of these studies have been focused on animal species such as amphibians (Johnson et al. 1999; García-Paris et al. 2000; Crawford et al. 2007; Streicher et al. 2009; Hauswaldt et al. 2011; Brusa et al. 2013), mammals (Ruíz-García et al. 2012) and fish (Larson et al. 2012; Bagley and Johnson 2014b). Meanwhile, studies of plants have been almost restricted to lowland species (in many cases with wide distribution range) from genus such as *Bursera*, *Brosimum*, *Ficus* (Poelchau and Hamrick 2011), *Jacaranda*, *Luehea*, *Simarouba* (Jones et al. 2013), *Symphonia* (Dick and Heuertz 2008; Jones et al. 2013), *Quercus* (Cavender-Bares et al. 2011 and 2015), *Cedrela* (Cavers et al. 2003) and *Cordia* (Rymer et al 2013). Studies of the phylogeographic structure of plants in southern Central America dealing with montane species are practically absent. Apparently, only the patterns of contemporary and historical gene-flow in orchid species across southern Central America mountain ranges have been analyzed so far (Kartzinel et al. 2013).

In comparison to other oak species, *Q. costaricensis* and *Q. bumelioides* exhibited lower haplotype richness (18 and 26 haplotypes, respectively) and similar values of h_S and h_T (0.68 and 0.97 respectively for *Q. costaricensis,* 0.825 and 0.84 for *Q. bumelioides*) than other Neotropical oak species (characterized using similar cpSSR loci) such as: i. the *Quercus affinis x Quercus laurina* hybrid complex with 35 haplotypes, $h_S = 0.9$ and $h_T = 0.99$ (Ramos-Ortiz et al. *unpublished data*), ii. *Quercus castanea* with 90 haplotypes, $h_S = 0.73$ and $h_T = 0.98$ (Peñaloza-Ramírez *unpublished data*), iii. *Q. castanea* studied at a regional scale with 21 haplotypes, $h_S = 0.71$ and $h_T = 0.54$ (Valencia-Cuevas et al. 2014), iv. *Q. insignis* and *Q. sapotiifolia* with 28 and 34 haplotypes respectively, $h_S = 0.65$ and 0.95 and $h_T = 0.51$ and 0.96 (Rodríguez-Correa et al. *unpublished data*). In contrast, both, *Q. costaricensis* and *Q. bumelioides* exhibited higher genetic diversity values than the Andean oak (*Q. humboldtii;* 18 haplotypes, $h_S = 0.418$ and $h_T = 0.453$; Rodríguez-Correa et al. *unpublished results*), as well as other *Quercus* species such as the European white oaks complex (11 haplotypes; Grivet et al. 2006), *Q. suber* in the western Mediterranean (five haplotypes , Magri et al. 2007) and *Q. garryana* in the Pacific northwestern region of North America (six haplotypes; Marsico et al. 2009). Although diversity values seem to be consistently higher within Neotropical oak species compared to Nearctic *Quercus* species, this comparisons should be treated carefully considering that not only the set but also the number of cpSSR loci used are not exactly the same between studies.

In terms of genetic structure, *Q. costaricensis* exhibited a significant $N_{ST} > G_S$ ($P<0.001$) difference, which indicated phylogeographic structure. AMOVA also suggested that a significant proportion of the genetic variation is distributed among populations under both F_{ST} and R_{ST}-based measures (Table 2). The haplotype network showed a considerable number of unique and private haplotypes and only one haplotype distributed through almost all the populations (H14; Figure 2). Correlation tests between latitude and genetic diversity suggested that number of haplotypes, haplotype richness and h_S tended to increase in a north to south direction. Together, the phylogeographic structure, the partitioning of the genetic variation between populations and within populations and the haplotypes distribution suggested that the spatial configuration of the genetic variation may have been shaped by historical (e.g. geologic and climatic) events. This idea is supported by the fact that the most important genetic discontinuities for *Q. costaricensis* were observed separating the most important mountain systems in Costa Rica, the CG, CC and CT (Figure 1 and Figure 3). Besides to the geographic genetic discontinuities, the gene flow analysis suggested that the c2 population (north, CG) acted as a sink population and population c4 (south, CT) acted as source population, at the time that maintained gene flow with its CT neighbours (c5 and c6). *Q. costaricensis* is dominant at higher elevations (1800-2300 and up to 3600 meters; Morales 2010), therefore the geologic heterogeneity of the Costa Rican mountains may have affected historical patterns of seed-mediated gene flow, considering that most of the acorn dispersal in oaks is determined by gravity and in a lesser extent by animals. Even though *Q. costaricensis* populations are distributed within a single physiographic province, the Chorotega block, the observed phylogeographic structure makes sense when considering the geological heterogeneity of the Costa Rican mountains.

The Chorotega block limits at the east (in the Panama canal zone) with the Chocó block, and both are situated within a region of complex tectonics between the Caribbean, South American, Cocos and Nazca plates. The Chorotega block is characterized by Cenozoic volcanism and upper-plate deformation mediated by complex tectonics along the southern Middle America trench (Marshall 2007). Inside the Chorotega block, the Chorotega volcanic front is characterized by the presence of a complex volcanic belt that includes the CG, Cordillera de Tirilán, Cordillera de Aguacate, CC and CT, all generated by Cenozoic tectonics (Marshall 2007). CG is formed by distinct mountains configured by a Quaternary chain of shield–like stratovolcanos distributed above a surrounding low-relief matrix (Marshall 2007) which may explain the presence of geographical barriers between north and south of CG. The low-relief matrix separating mountains represents a climatically unsuitable area for *Q. costaricensis* (Figures 4 and 6) determining a low level of gene flow from this region (Table 3) and therefore low genetic diversity in CG populations c1 and c2 (Table 1).

Southwards from the CT, two important genetic discontinuities for *Q. costaricensis* were observed separating CC population c3 from CG populations and a second one separating CC from CT populations c4, c5 and c6. CC is composed by a NW-trending mountain range with peak elevations from 2000 to 3400 meters, and a strong climatic gradient across the range (Marshall 2007). Once again, the limits between CC with CG and CT are determined by a lower elevations matrix such as the CV that may have limited gene flow between cordilleras. Despite that authors such as Islebe and Hooghiemstra (2006) have described that during glacial to interglacial conditions there have been important downward and upward migrations reflected in the Costa Rican oak palinological records, continuous changes of the altitudinal distribution due to historical climate change may have promoted weak intermittent connections at the lowlands due to the higher elevation distribution of species such as *Q. costaricensis,* therefore this intermittent connection may have not been strong enough to promote important levels of historical gene flow favouring the observed population genetic discontinuities.

Southeast of the CV there is the CT, with a volcanic gap that extends into Panama, where the major southern Central American elevations are found (over 4000 meters). Interestingly, according to the ENMs, it is in this non-volcanic region where the climatic niche for *Q. costaricensis* has remained more stable and where the higher levels of genetic diversity and gene flow were observed (Table 3; Figure 2). According to the results of the historical demography analysis important demographic expansion may have occurred for *Q. costaricensis* during the 2.3 My-117 *ka* BP period. This signal of demographic expansion agrees with the idea that the Quaternary ice ages have been a time of redistribution of populations (Bennet 2015). In contrast, *Q. bumelioides* exhibited a very different pattern. AMOVA suggested that most of the genetic variation is found within populations (Table 2) and there was no detectable phylogeographic structure. For that reason geographic discontinuities in the genetic structure were not calculated. Gene flow analysis (Table 3) suggested that all populations have significant gene flow levels either as source or sink populations. The haplotype network exhibited a strong star-shaped structure that suggests a recent population expansion with the presence of 15 unique haplotypes and two widespread haplotypes (H12 and H13; Figure 3).

Such patterns could be explained considering that *Q. bumelioides* is mainly distributed in the lower montane forest belt (between (600-)1100 to 2900 meters; Morales 2010), therefore connectivity between mountains is more likely to occur in comparison to upper montane oak species. Interestingly, the lack of genetic structure implies important levels of gene-flow that have homogenized genetic variation thought the species population or very recent spread throughout the current geographic range. The observed sporadic acorn production of *Q. bumeloioides* during the rainy season (Nixon 2006) and the observed gene flow estimates suggest a moderate but extremely effective dispersal and hence seed-mediated gene flow. ENM results (Figures 5 and 6) suggested that during glacial stages climatic suitability values were similar to the present-day period, characterized by a wide distribution, meanwhile during the 120 *ka* BP period, distribution of the climatic suitable values was concentrated southwards (CCP). The observed *Q. bumelioides* ENM and stable climatic conditions (Figures 5 and 6) support a scenario of recent population expansion from the Costa Rica-Panama border (more stable area; Figure 6) northwards up to the CC, evidenced by the negative F_S and non-significant raggedness values (Table 4) dated between 316 and 15.8*ka* BP (Middle to Upper Pleistocene).

Similar patterns of connectivity during glacial stages, high levels of genetic diversity, absence of phylogeographic structure and population expansion only have been observed in one Neotropical species, *Quercus humboldtii.* However, the Andean oak population expansion dated the Pleistocene-Holocene transition; probably due to a recent Andean colonization (Rodríguez-Correa et al *unpublished results*). Both species, *Q. bumelioides* and *Q. humboldtii,* partially fit genetic patterns expected under the Neotropical moist forest refugia model, which state that unchanging humidity conditions as the ones inferred by van der Hammen (1961), promoted Neotropical forests continuous and stable distributions (Farrera et al. 1999; Caballero 2010). Genetic consequences of this model include homogenization of genetic variation due to gene flow, high levels of genetic variation, diffuse genetic structure, common widely distribute alleles (as observed in *Q. bumelioides*) and little to no demographic growth (Ramírez-Barahona and Eguiarte 2013).

Conclusions

The present study showed that even in a smaller and geographically restricted area, with less conspicuous geographic barriers than other well-defined phylogeographic breaks such as the Tehuantepec Isthmus and the Nicaraguan Depression, differential patterns of historical demography, phylogeographic structure and gene flow could be observed in co-distributed and strikingly in congener species. Both *Q. costaricensis* and *Q. bumelioides,* representing upper montane and lower montane ecosystems respectively, showed differential responses to historical changes at the geological and climatic levels. Unfortunately the phylogeographic patterns observed in this study are hard to compare with other montane tree species in southern Central America, and even with montane tree species of regions with similar floristic composition, climatic and palaeoclimatic dynamics (such as the Colombian Andes *sensu* Isbele and Hooghiemstra 2006, Kappelle 2006, Kappelle and van Uffelen 2006) as little (probably none) phylogeographic studies have been published yet.

From the contrasting patterns observed in this study between congener species, we strongly suggest to consider the phylogenetic relations between species as criteria for selecting species to develop phylogeographic studies. A clear phylogenetic framework could lead to better historical interpretations of the phylogeographical patterns as historical demographic processes could be successfully related and dated depending on temporal diversification and speciation processes observed within a determined linage. Therefore, it could be important to include not only co-distributed species to test phylogeographic hypothesis, but also congener species for which there is an initial phylogenetic framework defined. Hopefully, with the development of a framework phylogeny of the American oaks by Hipp et al. (2014), soon it will be possible to test more robust comparative phylogeographic hypothesis in the northern Neotropics as it has been initially done for a mostly nearctic oak group, the *Quercus* subsection *Virentes* (Cavender-Bares et al. 2015).

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Tables

Table 1. *Quercus costaricensis* and *Q. bumelioides* populations geographic information summary, genetic diversity and genetic structure estimates. n: sample size, h_S: within population genetic diversity, D^2sh: mean pairwise genetic distance among individuals within a population under a stepwise mutation model. CG: Cordillera de Guanacaste, CC: Cordillera Central, CT: Cordillera de Talamanca, VC: Valle Central.

	ID	Longitud	Latitude	Region	Elevation (m.a.s.l)	n	Total haplotypes	Allelic richness (rarefacted)	h_S	D^2sh
Quercus costaricensis	c1	-84.8	10.37	CG	1642	7	4	3.38	0.81	0.01
	c2	-85.33	10.82	CG	1664	8	1	1.00	0.00	0.00
	c3	-84.2	10.2	CC	2132	7	2	1.95	0.48	0.66
	c4	-83.81	9.54	CT	2451	9	7	4.44	0.94	0.03
	c5	-83.67	9.56	CT	2639	6	6	5.00	1.00	0.14
	c6	-83.88	9.67	CT	2720	5	4	4.00	0.90	0.07
			Total			42	18	4.18	0.94	0.02
Quercus bumelioides	b1	-84.12	10.08	CC	1750	15	7	3.15	0.84	0.02
	b2	-83.85	9.97	CC	2500	13	10	3.71	0.95	0.04
	b3	-83.98	9.88	VC	1515	6	6	4.00	1.00	0.04
	b4	-83.95	9.73	CT	2298	9	5	2.77	0.72	0.17
	b5	-83.8	9.56	CT	2463	14	6	2.83	0.75	0.10
	b6	-83.69	9.59	CT	2542	14	7	3.09	0.82	0.05
	b7	-84.13	9.85	CT	1933	4	3	3.00	0.83	0.04
			Total			75	26	3.23	0.84	0.01

Table 2. Hierarchical analysis of molecular variance (AMOVA) using F_{ST} and R_{ST} for *Quercus costaricensis* and *Q. bumelioides*.

	Source of variation	**d.f.**	**s.s.**	**Variance components**	**Percentage of variation**
Quercus costaricensis			F_{ST}		
	Among populations	5	33.69	0.84	49.52
	Within populations	36	31.01	0.86	50.48
	Total	41	64.71	1.7	
			R_{ST}		
	Among populations	5	89.21	2.27	52.81
	Within populations	36	73.16	2.03	47.19
	Total	41	162.38	4.3	
Quercus bumelioides			F_{ST}		
	Among populations	6	4.98	-0.0003	-0.04
	Within populations	68	56.75	0.83	100.04
	Total	74	61.74	0.834	
			R_{ST}		
	Among populations	6	19.92	-0.054	-1.41
	Within populations	68	264.21	3.885	101.41
	Total	74	284.13	3.831	

Table 3. Estimates of mutation scaled immigration rate (*M*) between populations of *Q. costaricensis* (a) and *Q. bumelioides* (b) based on cpDNA haplotypes. Recipient populations (sink) are shown above the diagonal, and the source (donor) populations are below the diagonal. 95% confidence intervals are located below each population pair *M* value in parenthesis. Bold numbers correspond to significant higher than 1 *M* values. For populations codes refer to Table 1.

(a)

	c1	**c2**	**c3**	**c4**	**c5**	**c6**
c1		*0.161*	*0.000*	*0.565*	***2.176***	*0.000*
	-	(0.009-0.294)	(2.207e-013-0.242)	(0.175-1.315)	***(1.082-4.233)***	(7.268e-014-1.050)
c2	*9.358E-14*		*8.829E-01*	*5.655E-01*	**4.022**	*9.358E-14*
	(7.268E-14-0.049)	-	(0.378-1.714)	(0.175-1.318)	**(2.132-6.704)**	(7.268e-014-1.853)
c3	*6.030E-13*	*7.093E-01*		**2.017**	**2.176**	*1.990E-01*
	(4.547E-13-0.048)	(0.205-0.984)	-	**(1.097-3.323)**	**(1.929-4.236)**	(0.149-1.514)
c4	**2.316**	*0.161*	*0.126*		**1.658**	**1.746**
	(1.333-2.831)	(0.009-0.710)	(0.007-0.557)	-	**(1.185-3.557)**	**(01.370-3.232)**
c5	***2.092***	*0.321*	1.076	*0.435*		***2.420***
	(1.175-2.577)	(0.053-0.993)	(0.480-2.022)	(0.106-1.140)	-	***(1.307-4.084)***
c6	**1.241**	*0.400*	*0.253*	*0.000*	***6.588***	
	(1.057-1.625)	(0.349-1.173)	(0.041-0.785)	(8.992e-014-0.271)	***(3.951-9.553)***	-

(b)

	b1	**b2**	**b3**	**b4**	**b5**	**b6**	**b7**
b1		**2.966**	0.336	1.093	**1.531**	0.678	**2.286**
	-	**(1.841-4.468)**	(0.104-0.781)	(0.691-2.038)	**(1.171-2.479)**	(0.309-1.264)	**(1.313-3.649)**
b2	0.320		1.007	***1.239***	0.000	0.927	**1.225**
	(0.000-0.744)	-	(0.538-1.690)	***(1.079-2.639)***	(8.910E-0.12-0.485)	(0.695-1.590)	**(1.128-2.286)**
b3	0.562	**1.635**		**2.186**	0.547	0.760	**1.521**
	(0.241-1.087)	**(1.121-2.802)**	-	**(1.071-3.443)**	(0.196-1.177)	(0.365-1.371)	**(1.219-2.672)**
b4	0.216	***1.494***	1.346		0.109	0.422	***1.983***
	(0.239-1.083)	***(1.096-2.992)***	(0.878-2.117)	-	(0.006-0.485)	(0.151-0.907)	***(1.088-3.265)***
b5	0.900	0.900	0.168	**1.371**		0.935	0.458
	(0.356-1.834)	(0.356-1.834)	(0.027-1.187)	**(1.058-2.468)**	-	(0.483-1.912)	(0.343-1.191)
b6	0.799	0.146	**2.183**	0.827	**2.508**		0.610
	(0.399-1.40)	(0.109-0.651)	**(1.445-3.135)**	(0.328-1.683)	**(1.615-3.679)**	-	(0.457-1.418)
b7	0.400	0.900	0.588	***1.642***	0.547	**1.354**	
	(0.143-0.860)	(0.356-2.217)	(0.252-1.137)	***(1.242-2.754)***	(0.195-1.178)	**(1.107-2.129)**	-

Table 4. Estimates for historical population expansion and demographic parameters for *Quercus costaricensis* and *Q. bumelioides*. Θ_0: ancestral population sizes scaled by mutation rate, Θ_1: current population sizes scaled by mutation rate, τ: number of generations since the expansion occurred scaled by mutation rate, *-log [CL]*: computed pseudo-likelihood values of the model with homoplasy. Populations without historical demographic information were not considered as Fu´s F_S values were not significant or positive.

Population	F_S	**p-value**	**Raggedness index**	**p-value**	Θ_0	Θ_1	τ	***-log[CL]***
Quercus costaricensis								
c1	-4.68	0.001	0.14	0.43	0.00	4.E+10	2.18	9.13
c2	-0.91	0.021	0.37	0.94	0.27	4.E+05	1.86	17.82
c3	-3.23	0.01	0.29	0.21	3.41	1.E+12	1.49	7.32
c4	-1.94	0.02	0.05	1.00	0.00	2.E+13	1.99	6.58
c5	0.00	0.00	0.00	0.00	-	-	-	-
c6	3.75	0.95	0.73	0.94	-	-	-	-
all-populations	-8.40	0.01	0.06	0.14	1.30	10.48	4.24	55.40
Quercus bumelioides								
b1	-2.30	0.02	0.05	0.69	0.00	6.054E+12	1.94	21.64
b2	-0.35	0.37	0.65	0.04	-	-	-	-
b3	-4.98	0.003	0.07	0.46	2.88	6.857E+12	1.20	13.38
b4	-4.66	0.00	0.24	0.28	0.00	7.149E+13	1.99	5.76
b5	-0.34	0.42	0.07	0.79	-	-	-	-
b6	-1.83	0.10	0.08	0.49	-	-	-	-
b7	-0.89	0.01	0.53	0.42	0.00	1.816E+13	1.06	4.75
all-populations	-20.11	0.00	0.06	0.33	2.23	1.032E+12	0.57	80.62

Figures

Figure 1. Study area. Elevation of southern Central America is represented in a grey scale from lowlands (soft gray) to highlands (black). CG: Cordillera de Guanacaste, VC: Valle Central, CT: Cordillera de Talamanca, CCP: Panamanian Cordillera Central. White circles and triangles represent *Q. costaricensis* and *Q. bumelioides* populations, respectively. White-dashed line represents the Costa Rica-Panama border. White solid line corresponds to the 1000 meters line of the Costa Rican mountains.

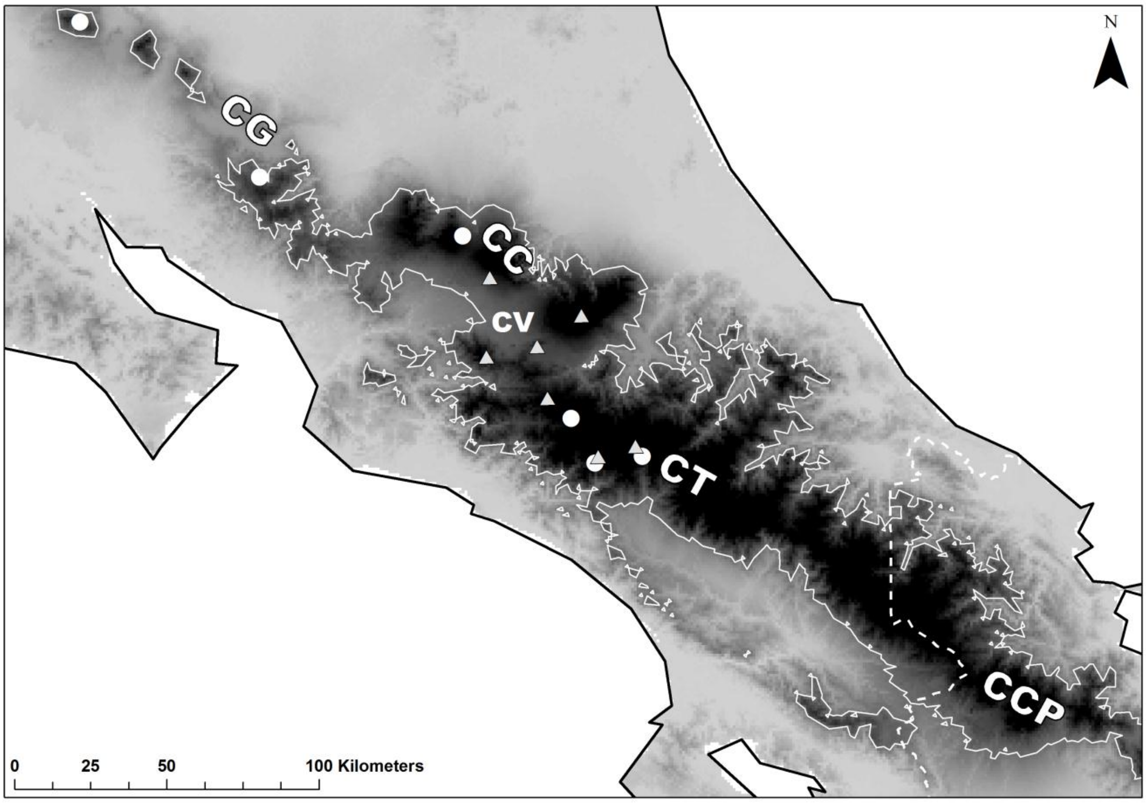


Figure 2. Haplotype distribution for *Quercus insignis* (a) and *Q. bumelioides* (b) in Costa Rica. Size of haplotype circles and populations are proportional to haplotype frequency and number of individuals per population, respectively. White and gray haplotypes represent unique and private haplotypes respectively, with their corresponding populations in parenthesis. Bold numbers correspond to the number of mutations between haplotypes if the observed steps were higher than one.

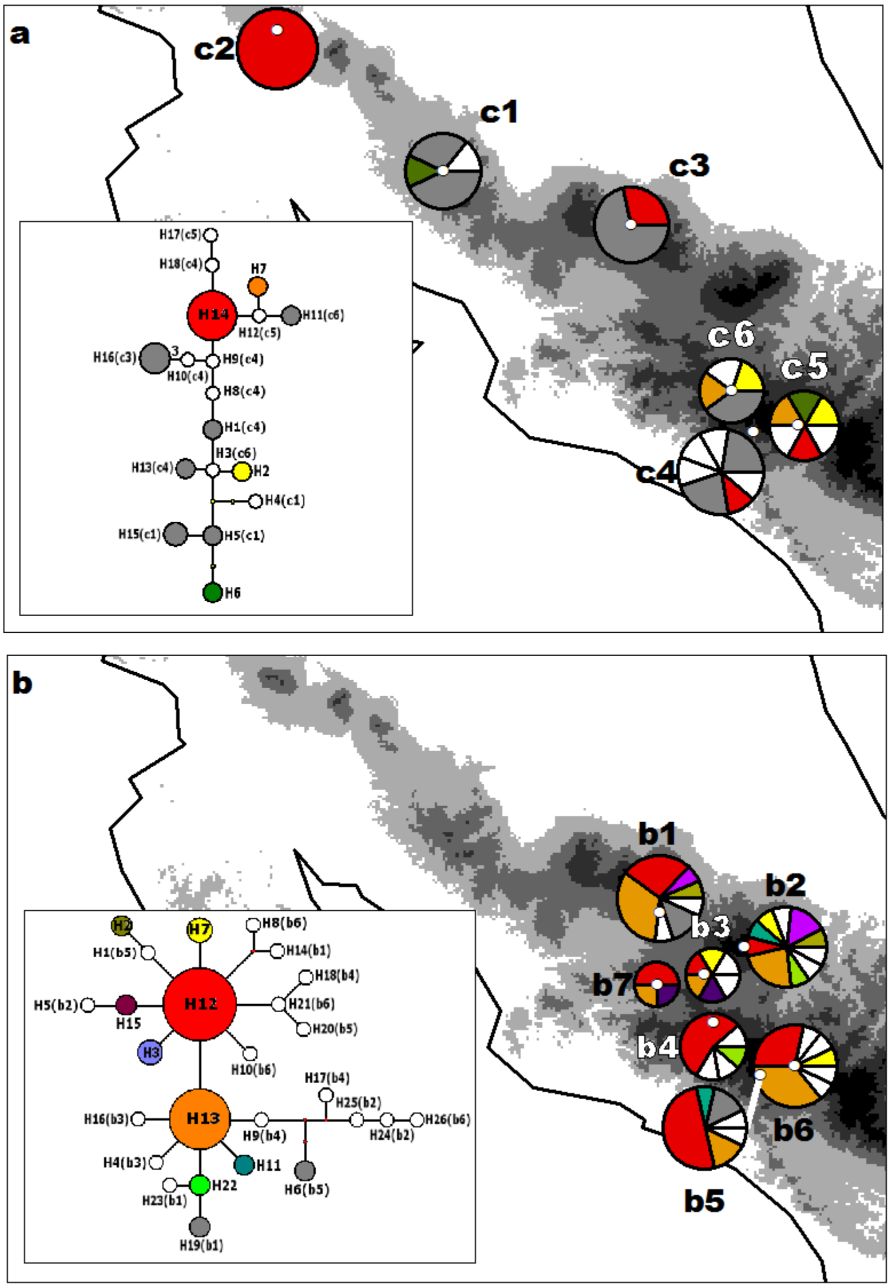


Figure 3. Geographical location of the most important genetic discontinuities for *Quercus costaricensis* (black lines) using the Monmomier´s maximum difference algorithm. Elevation is represented in a grey scale from lowlands (light gray) to highlands (black). Populations show their respective haplotypes with size proportional to the number of individuals (see Figure 2 for complementary information).

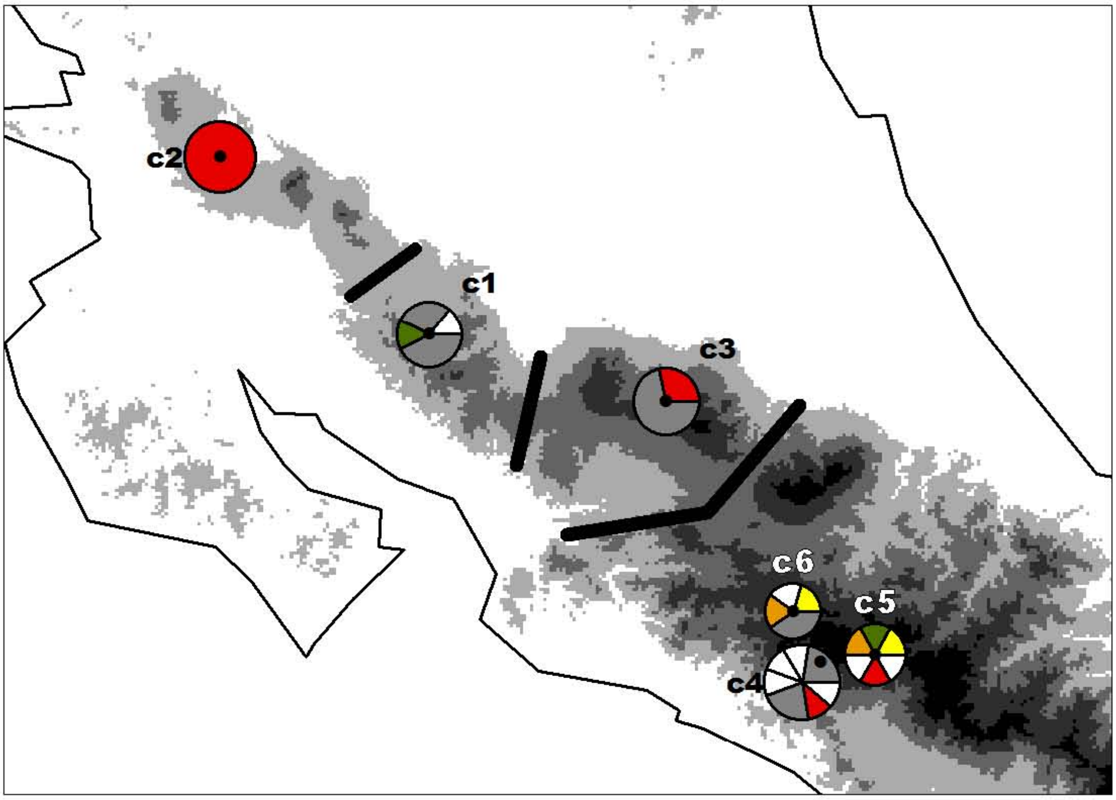


Figure 4. Present-day (4a; 0 *ka*; 1950-2000), Last Glacial Maximum (~21 *ka* BP; according to CCSM (4b) and MIROC (4c) global circulation models) and Last Interglacial (4d; ~120 *ka* BP) Ecological Niche Models for *Quercus costaricensis* in Costa Rica. Black-dotted polygon corresponds to the mountainous region over 1000 meters.

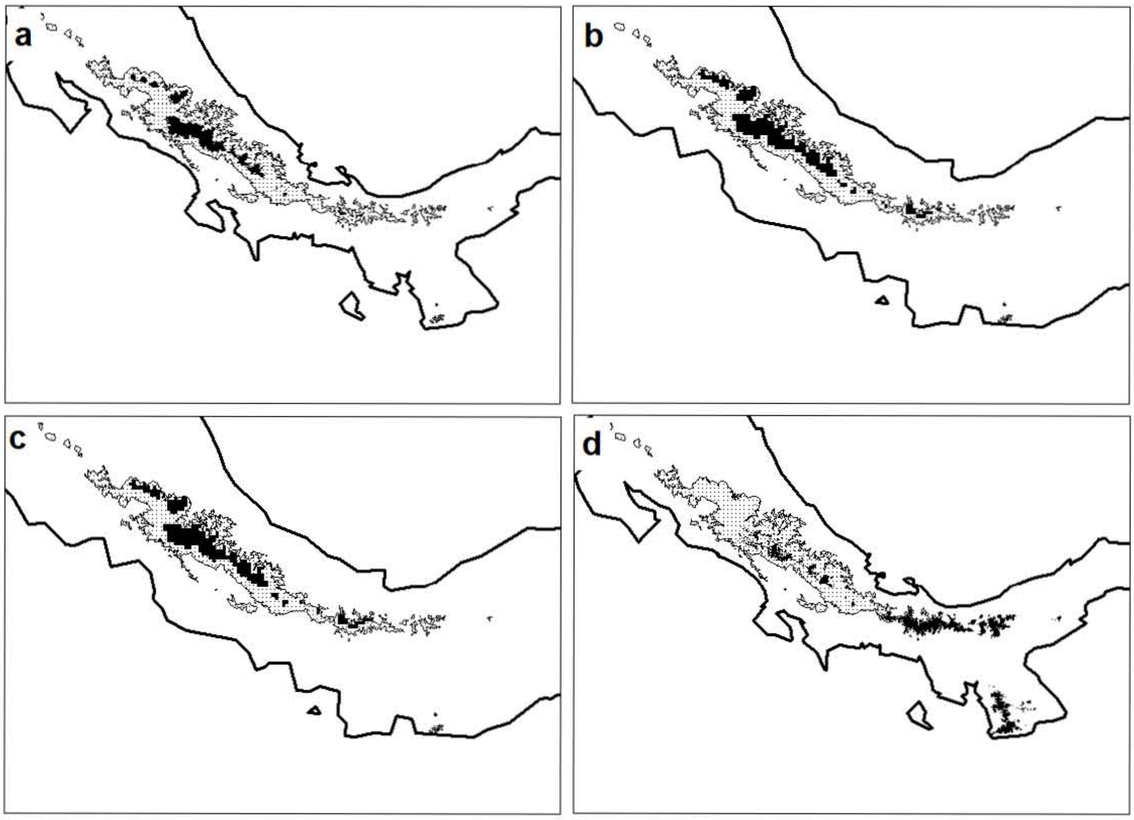


Figure 5. Present-day (5a; 0 *ka*; 1950-2000), Last Glacial Maximum (~21 *ka* BP; according to CCSM (5b) and MIROC (5c) global circulation models) and Last Interglacial (5d; ~120 *ka* BP) Ecological Niche Models for *Quercus bumelioides* in Costa Rica. Black-dotted polygon corresponds to the mountainous region over 1000 meters.

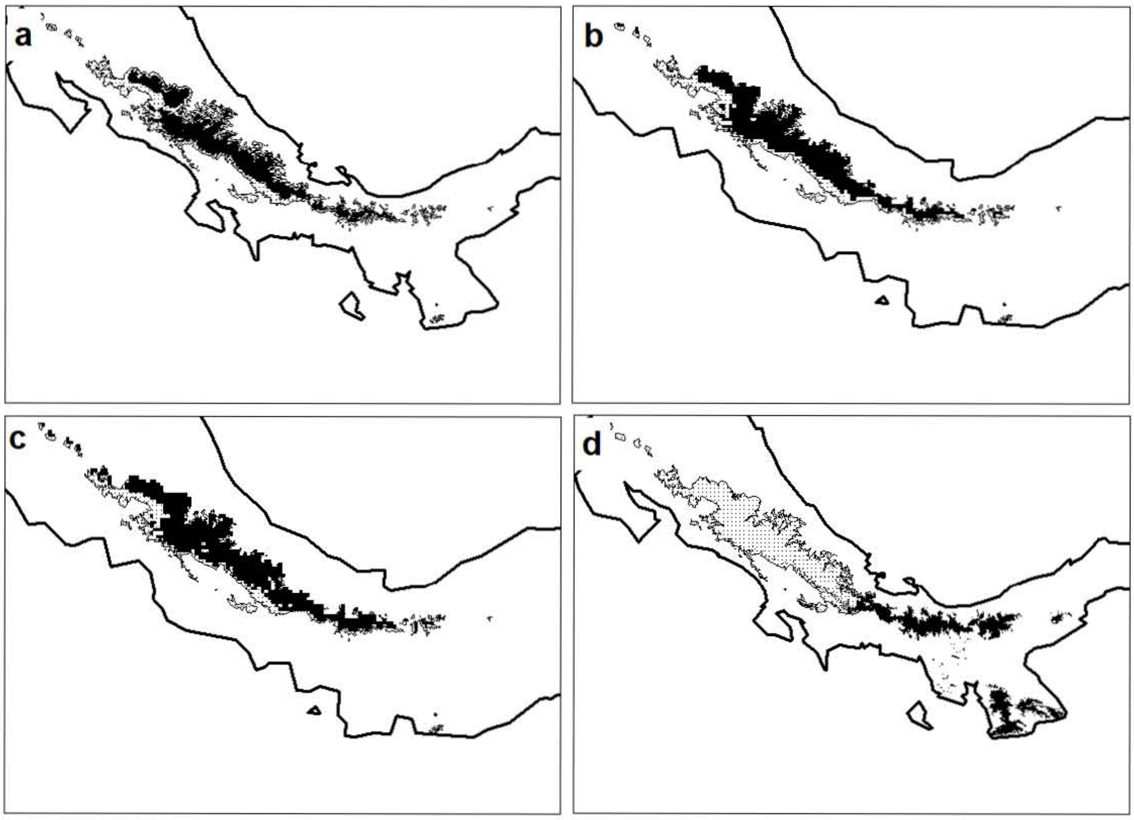
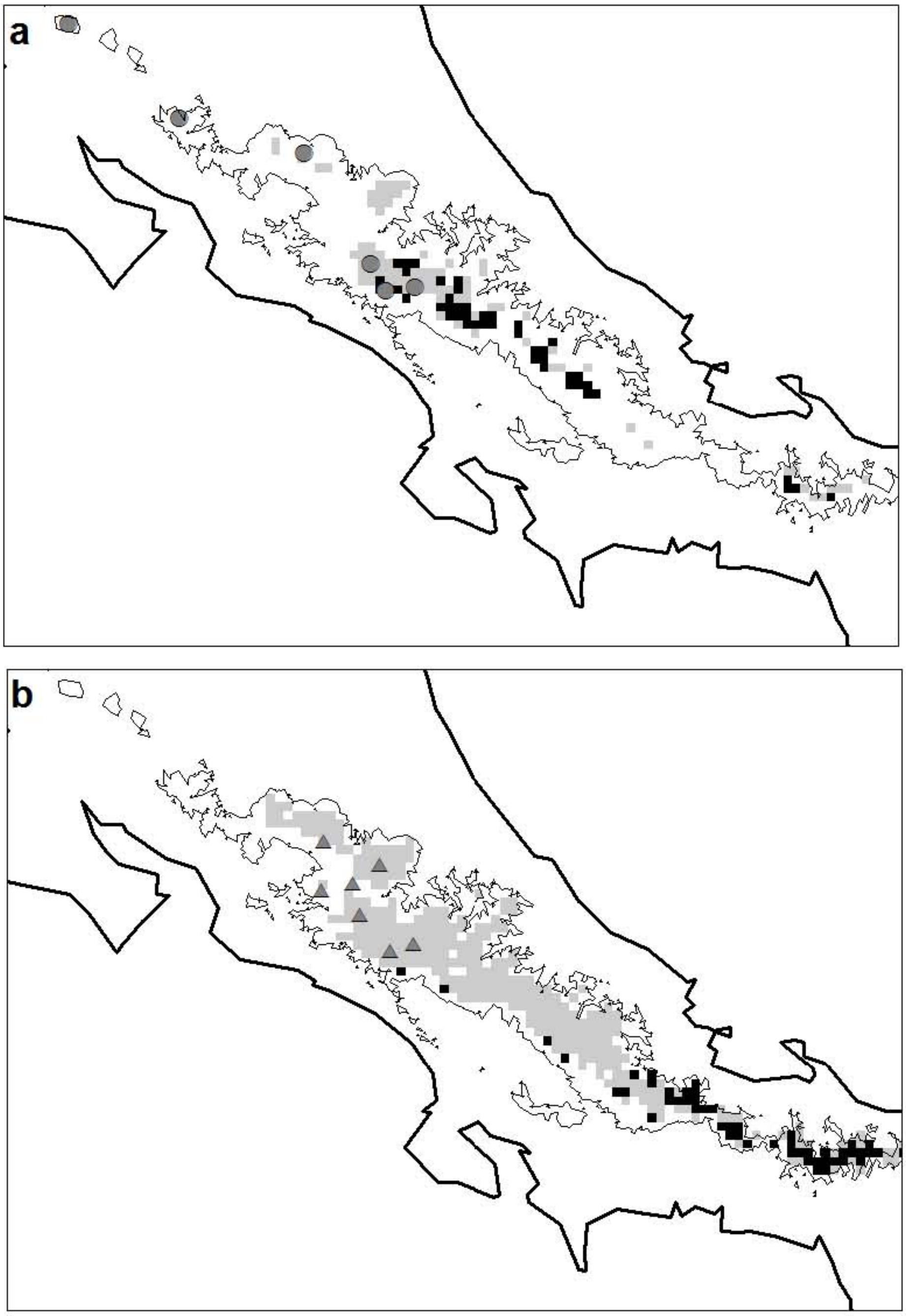


Figure 6. *Quercus costaricensis* (6a) and *Q. bumelioides* (6b) climatically suitable stable areas since the Last Interglacial (~120 *ka* BP). Black category corresponds to the areas where the distribution is predicted in the four models used. Grey category corresponds to the areas where models for the present-day and LGM (~21 *ka* BP) predict presence of the species. Black-lined polygon corresponds to the 1000 meters line.



Capítulo Cuatro

An oak climbing the Andes: analyzing the distribution dynamics of *Quercus humboldtii* during the Last Glacial Maximum

Hernando Rodríguez-Correa, Ken Oyama, Antonio González-Rodríguez

Abstract

Aim: To analyze altitudinal changes in the climatic niche of *Quercus humboldtii* between the Last Glacial Maximum (LGM) and the present-day.

Location: Colombian Andes.

Methods: The climatic niche of *Q. humboldtii* was characterized using a principal components analysis considering four elevation belts. The species geographic distribution was determined using ecological niche models through an entropy approach. Present-day distributions were projected from present to the LGM period using the current distribution occurrences and results were validated using available *Quercus* pollen fossil records. Finally, changes in the oak distribution extent were compared with the montane forest compression patterns observed in palaeoecological studies of the Andean flora.

Results: *Quercus humboldtii* showed a well-defined altitudinal distribution from the tropical lowland forest up to the upper montane forest/sub-paramo border. Ecological niche models described properly the known oak distribution in the Colombian Andes. LGM projections showed evidence of an altitudinal displacement towards the Andean lowlands. Comparisons of available niche among forest belts through time showed differential responses depending on forest type, suggesting an increase in the available habitat in the upper montane forest, and a decrease in available habitat in the lower montane and tropical lowland forests.

Main conclusions: Migration patterns in *Q. humboldtii* showed an altitudinal displacement towards the lowlands during the LGM compared to present-day distribution, with higher values of climatic suitability in the upper montane forest, where the species currently reaches its altitudinal distribution limit in the present. The observed data showed that *Q. humboldtii* fits a scenario of “moist forests” during the LGM. However, even if this model is congruent with the observed changes in distribution, variables as temperature in elevation gradients were fundamental to define the population structure of the specues during the Quaternary in the Andean montane forest.

Keywords: Neotropical trees, Fagaceae, climatic niche, ecological niche modeling, biogeography, Quaternary.

Introduction

The effect of the Pleistocene glacial periods on the distribution of species has been widely studied, particularly for European and North American taxa. There is a consensus view that reductions in species distribution and the formation of refugial areas at mid- to high latitude regions of the Northern Hemisphere resulted from the advance of the ice sheets (Bennet et al. 1991, Hewitt 1996, Taberlet et al. 1998, Brewer et al. 2002). However, the effect of the Pleistocene glaciations on the distribution of species in the Neotropics is still under discussion, although it is recognized that climate change during this period constituted a major event that shaped the distribution and genetic structure of many species in the region (Gutiérrez-García and Vázquez-Domínguez 2013).

Two models have been proposed to describe the population responses of tropical species during the last glacial cycle. The first is a scenario of widespread aridity that caused tropical forests to be compressed, fragmented and isolated into forest patches during the Last Glacial Maximum (LGM; ~21 ka BP) (Haffer 1969; Toledo 1982; Carnaval and Moritz 2008; Carnaval et al. 2009; de Mello-Martins et al. 2011, Ramírez-Barahona and Eguiarte 2014), while the second model proposes a scenario of switching conditions between wet-cold and dry-warmer phases without significant reduction of precipitation during the LGM (van der Hammen 1961; Colinvaux et al. 2000; Colinvaux and de Oliveira 2001; Baker et al. 2003; Ramírez-Barahona and Eguiarte 2014), promoting stable and continuous distribution of the tropical forests (Farrera et al. 1999 Hoestler and Mix 1999; Caballero et al. 2010). Ramírez-Barahona and Eguiarte (2014) found that available phylogeographic data for Neotropical montane forest species do not clearly support any of the two models and called for the inclusion of palaeoclimatic and ecological information when conducting phylogeographic studies in the Neotropics.

Recent studies such as Ornelas and González (2014), employing molecular and palaeoclimatic data, described a phylogeographic pattern in *Moussonia deppeana* (Gesneriaceae) congruent with cloud forest fragmentation during the last interglacial (Colinvaux et al. 2000).

The Colombian Andes have been a fundamental region to understand species population dynamics during the LGM on the basis of paleobotanical reconstructions (Helmens and Kuhry 1986; Bakker and Solomons 1989; Hooghiemstra and van der Hammen 1993; Hooghiemstra and Ran 1994; Helmens et al. 1996; Wille et al. 2000; Wilee et al. 2001; Marchant et al. 2002; van der Hammen and Hooghiemstra 2003; Hooghiestra and Van der Hammen 2004), which indicate complex and dynamic changes in altitudinal distribution driven mostly by variables as temperature, precipitation and carbon dioxide concentration (Hooghiemstra and van der Hammen 2004). The study of species population history in a region well characterized in paleoecological terms such as the Colombian Andes therefore represents an important opportunity to apply multidisciplinary approaches combining ecological niche modeling, biogeography and phylogeography, that could allow us to test paleoecological scenarios based on information from available fossil records, and eventually help us to understand the complex species dynamics in response to the climatic changes during the Pleistocene, and particularly the LGM.

Quercus humboldtii Bonpl. (Fagaceae) is the only oak species that inhabits the Colombian Andes region. Consequently, it is possible to associate the abundant oak fossil pollen records to this species. Also, the current distribution of this oak is well characterized. Therefore, this species represents an exceptional study system to test palaeoecological models of species dynamics through phylogeographic and biogeographical methods. The distribution of *Q. humboldtii* extends from the Darien region at the northwestern boundary between Colombia and Panama, to the Nariño region at southern Colombia (Fig. 1), with an altitudinal range between 774 and 3200 meters (Pulido *et al.* 2006; Rangel and Avella 2011). The species forms stands of an almost monospecific canopy in the inner slopes of the three Andean mountain systems. Those monospecific stands also known as "rodales" are highly diverse in understory and epiphytic vascular plant species. Like other oaks species, *Q. humboldtii* is wind pollinated, monoecious, and produces acorns dispersed by gravity, large frugivorous birds, and rodents (Fernandez-M and Sork 2005). The *Quercus* first appearance date (FAD) in the Colombian Andes has been established between 423 Ka before present (BP; Hooghiestra and van der Hammen 2004) and 478 Ka BP in the Funza-I pollen record (Van´t Veer and Hooghiemstra 2000). This relatively recent migration from North and Central America into South America correlates with the uplifting of the Andes during the Pliocene (Pulido *et al.* 2006) and its posterior to the closing of the Central American seaway (15 My BP; Montes et al. 2012).

Because the Isthmus of Panama is low lying, Hooghiemstra and van der Hammen (2004) suggested that *Quercus humboldtii* entered Colombia as an element of the lower montane forest belt. During the following 200,000 years *Q. humboldtii* must have competed with elements of the existing montane forest and probably gradually reached higher elevations in the upper montane forest (Hooghiestra and van der Hammen 2004). Whereas the oak forest was first dominant during cool interglacial conditions, it gradually extended its ecological range probably due to adaptation to new niches during the glacial cold conditions. Thus, the ecological range of *Quercus* in Colombia may have double since its FAD (Hooghiemstra and van der Hammen 2004). In the course of the last 40 *ka* the northern Andes region has experienced complex and important vegetation changes. Those changes consisted mainly in altitudinal displacements of the different vegetation types that exhibited compression and expansion processes at different times, as well as the immigration of Nearctic taxa. In order to increase the understanding of the effect of the Last Glacial Maximum on the historical dynamics and distribution of montane tree species, particularly those distributed through the Colombian Andes, in this study we evaluated if: i. the distribution of *Q. humboldtii*, reconstructed using ecological niche modeling and projections of the present-day distribution into LGM climatic layers, is consistent with the palynological fossil records of the Colombian Andes, ii. the modeled distribution of *Q. humboldtii* fits the proposed altitudinal distribution changes described for montane forest belts in the Colombian Andes by Hooghiemstra and van der Hammen (2004), and therefore supports a scenario of wet and cold conditions during the LGM in the Colombian Andes and, iii. the historical distribution dynamics of *Q. humboldtii* provides new information to support previously hypothesized possible routes of colonization among the Colombian Andes Cordilleras sub-systems (Western, Central and Eastern mountains systems; van der Hammen et al. 2008).

Methods

Study area and occurrence data

Present-day presence records of *Q. humboldtii* (Fig. 1) were compiled from public databases (Global Biological Information Facility), field observations and bibliographic references. Additionally, fossil pollen records corresponding to the LGM period were compiled from palaeoecological studies developed for the Andean region (Kelmens and Kuhry 1986; Bakker and Solomons 1989; Hooghiemstra and van der Hammen 1993; Hooghiemstra and Ran 1994; Helmens et al. 1996; Wille et al. 2000; Wilee et al. 2001;

van der Hammen and Hooghiemstra 2002; Marchant et al. 2002; Hooghiemstra and Van der Hammen 2004; Table S1). Occurrence records were mapped and depurated by altitude, latitude and longitude based on the species natural history information. To diminish the possible spatial autocorrelation effects of the aggregation of occurrence records on the niche modeling, all records except one within the same one decimal degree unit were deleted from the database. As the number of records varies for different regions of the Andes due to unequal sampling efforts, which have been particularly high for the north of the Cordillera Central (CC; Fig. 1), a random 50% sub sample of observed points was used for this region, in order to avoid over prediction.

Climatic and topographic variables

For the compiled occurrence records, a total of 19 climatic variables values were extracted for the 1950-2000 and ~21 *Ka* BP periods. Climatic layers used were developed by Hijmans et al. (2005) for the contemporary period and two LGM general circulation models: The Community Climate System Model (CCSM) and the Model for Interdisciplinary Research on Climate (MIROC), both available in the Paleoclimate Modelling Intercomparison Project Phase II (PMIP2) website (http://www.pmip2.cnrs-gif.fr). All climate layers were used with a spatial resolution of 30 arc-seconds (~1 Km^2). Topographic information was based on the ETOPO1 global relief model of the Earth's surface developed by the National Geophysical Data Center, National Oceanic and Atmospheric Administration (NOAA; Amante and Eakins 2009) with an original spatial resolution of 1 arc-minute. In order to use the topographic information in the LGM projections, and considering that elevation shifts are fundamental to explain the Andean biota migration (Hooghiemstra and Van der Hammen 2004) an LGM elevation layer was calculated considering the changes in the sea level as described by Peltier (2004).

Climatic niche and topographic characterization

In order to avoid redundancy among climatic variables a sub-set of variables was defined using a pairwise correlation test between variables. When values of the correlation were higher than 0.7 the more specific variable of each pair was discarded (e.g. maximum temperature of the warmest month versus annual mean temperature). The dataset was divided into four categories following Hooghiemstra and van der Hammen (2004) depending on the altitude of the occurrence records as follows: tropical lowland forest (TLF; 0-1000 m), lower montane forest (LMF; 1000-2300 m), upper montane forest (UMF; 2300-3200 m) and sub-paramo/grass paramo (SP; 3200-4200 m). Observed records classified as belonging to the SP were slightly above the 3200 m upper limit of the UMF. A Principal Components Analysis was performed with R ver. 3.0.2 using the climatic information mentioned above, in order to visualize the variation in environmental conditions among the four forest belt categories.

Ecological niche modeling

Ecological niche modeling was used to characterize the climate at areas where *Q. humboldtii* is present and to build a climatic suitability based distribution model for the species through the Colombian Andes. For this goal we used the maximum entropy algorithm implemented in MAXENT version 3.3.3a (Phillips 2006). MAXENT was implemented using presence records along the Colombian Andes, seven independent climatic variables remaining after the pairwise correlation tests (annual mean temperature, mean diurnal range, temperature seasonality, temperature annual range, annual precipitation and precipitation seasonality) and elevation data. The palaeodistribution model for *Q. humboldtii* in the Colombian Andes during the LGM was obtained projecting the present-day model with both LGM scenarios (CCSM and MIROC) and topographic layers. Models were evaluated using the average AUC (area under the curve) as an independent threshold method after a 100 replication bootstrap. A sub-set formed by 30% of the total records was used for training AUC and the 70% remaining data were used to test AUC. In order to quantify and compare changes in the modeled distribution area among vegetation belts, binomial (presence/absence) distribution models were obtained using the 11 threshold criterias available in Maxent ver. 3.0.2. The presence/absence present-day maps were validated using binomial and chi-square tests implemented in Maxent ver. 3.0.2, while the LGM projections were validated using the pollen records for *Quercus* in the Andes region with a binomial test using R ver. 3.0.2 considering that Maxent validation does not include projected outputs. In this sense, if the number of observed pollen records falling within a predicted presence area was significantly higher than expected by chance, the threshold criteria was considered to be adequate to describe the species distribution.

Changes in oak forest altitudinal distribution between periods

Only binomial maps that accurately described the current distribution (in the case of the present-day models) and the fossil pollen records of *Q. humboldtii* (in the case of the LGM projections) were used to compare changes in the distribution area of the species for each vegetation belt between during the present-day and the LGM. In the present-day models, the climatic suitability based distribution area of *Q. humboldtii* corresponding to each vegetation belt (TLF, LMF, UPF and SP) was calculated considering the previously explained altitudinal ranges. For the LGM models, the climatic suitability based distribution area of the species was calculated considering the altitudinal range of the same vegetation belts during this period according to Hooghiemstra and van der Hammen (2004): TLF (0-800 m), LMF (800-1400 m), UMF (1400-2000 m) and SP (2000-3000 m). For each model, the calculations were performed for each of the 100 replicas generated by Maxent, and the mean estimated presence area (km^2) for each vegetation belt category were compared between LGM and present-day periods using a t-test in R ver. 3.0.2.

Results

Occurrence data

A total of 281 occurrences were recorded for *Q. humboldtii* in the Andes region. After depuration of the dataset considering over-represented areas, altitudinal distribution and aggregation of occurrences in small areas, a dataset with 82 records was used for ENM (two corresponding to TLF, 38 to LMF, 22 to UMF and three to SP). A second dataset of 10 fossil pollen localities was compiled for the validation of the projections for the LGM (two corresponding to LMF and eight to UMF; Table S1).

Climatic niche and topographic characterization

The PCA indicated that 89.25% of the variation in environmental conditions was explained by the first three main niche axes. Those three principal components were mainly associated to annual mean temperature, temperature seasonality and elevation (PC1), temperature annual range and precipitation seasonality (PC2) and annual precipitation (PC3) (Table 1). The plot of the PC1 versus the PC2 and PC3 (Fig. 2a, 2b) indicates that the four forest belt categories are clearly different climatically. However, the separation of the four groups was less clear in the plot of the PC2 versus the PC3 (Fig. 2C; Table 1).

Ecological niche modeling

Training and Test AUC indicated a good performance of the models (0.97±0.0043 and 0.95±0.01, respectively). The present-day presence/absence model accurately predicted the distribution of the species according to the binomial and/or chi-square test ($p<0.001$) results for the different 11 threshold criteria implemented by Maxent. However, for both LGM projections (with the CCSM and MIROC scenarios) only the presence/absence maps resulting from two of the threshold criteria adequately predicted the location of the fossil pollen records: the fixed cumulative value 1 logistic threshold (fcv1; binomial test $p<0.01$ for both LGM scenarios) and the balance training omission predicted area value logistic threshold (bto; binomial test $p<0.01$ for both LGM scenarios). Therefore, only the models resulting from these two threshold criteria were used for comparison between the LGM and the present-day distributions.

The climatic suitability based distribution model for the present-day period (Fig. 3a, d and g) describes well the known oak forest distribution, including the few lowland known records in the Caribbean region, Paramillo (PAR) and south of the Serranía del Perijá (SP), and excluding areas where oak populations have not been reported, such as the Cauca and Magdalena valleys and most of the Andean highlands (>3.500 m). Other areas where *Q. humboldtii* is known to occur, but due to the difficult access have not been studied or well-sampled, such as The Serranía del Darién (SD), also were predicted by both threshold criteria of the present-day model as isolated patches.

LGM projections (MIROC and CCSM under fcv1 and bto thresholds; Fig 3b, c, e, f, h and i) showed an increase in the area available in the lowlands, particularly at the north of the Cauca River Valley, and a reduction in area in the highlands, particularly at the north-eastern portion of the eastern cordillera (COr). The Magdalena Valley remains as a barrier between the Central (CC) and the Eastern cordilleras, but there is an increase in suitable habitat at the lowlands between both mountain systems (COc and CC). Current areas with *Quercus* presence in the Caribbean region are represented in the ENM outputs, but with a reduced distribution, particularly at PAR and north of SP. ENM also showed an important distribution area at the southern proportion of the Colombian Andes, particularly from the south of the Macizo Colombiano (MC) down to the Nudo de los Pastos (NP), which is the present-day southernmost *Quercus* distribution limit in America.

Changes in oak forest altitudinal distribution between periods

The comparison of the climatic suitability based distribution areas of *Q. humboldtii* between the two time periods (present-day and both LGM scenarios under fcv1 and bto thresholds) indicated differences in the oak forest extension through the different Andean forest belts (Table 2, Fig. 4). Both the fcv1 and bto threshold criteria showed an increase in the available area in the UMF and SP for both LGM models (CCSM and MIROC). On the contrary, LMF and TLF show larger climatically suitable areas for the present-day period. Generally, the climatic suitability based distribution extent of the oak forest in the TLF is 22 to 24% greater during the present-day period than during the LGM under the CCSM and MIROC scenarios, respectively. Meanwhile, the extension of the climatic suitability based distribution in the LMF is 29 to 39% greater during the present-day compared to the LGM CCSM and MIROC scenarios, respectively. The UMF showed the lowest proportion of change, as the models suggested that the available niche was between 6 to 10% greater during the LGM CCSM and MIROC scenarios respectively. All the observed differences between periods and forest belts were statistically significant (Table 2).

Discussion

Quercus humboldtii is a representative element of the present-day LMF and UMF vegetation (Hooghiemstra et al. 2006). It can also be found either at lower (770 m; Rangel and Avella 2011) and higher (>3000m; Rangel and Avella 2011) altitudinal levels. This wide altitudinal distribution and the associated morphological variation have led to controversy about the potential number of oak species in the Colombian Andes. However, Muller (1942) recognized only one species, and more recent molecular (Cavelier et al. 1993) and morphological studies found low levels of differentiation, which reflects the presence of only a single species of oak (Pulido et al. 2006). Rangel and Avello (2011) suggested that the wide climatic variation of the areas where the oak trees are present indicate high adaptability to different temperature regimes. Our results also showed that there are clear climatic differences among *Q. humboldtii* populations along the four altitudinal vegetation belts in which the species is present.

Most of the climatic variation related to the altitude of *Q. humboldtii* populations occurred for temperature variables (annual mean temperature, temperature seasonality and temperature annual range; components 1 and 2 in the PCA) rather than for precipitation variables. The importance of temperature variables have been highlighted by authors such as Wille et al. (2001) who suggested that changes in temperature explain better the migration dynamics in the Andean forest belts during the LGM based on paleoecological data, while precipitation explains better the vegetation dynamics for the subparamo and paramo forest belts. Kapelle and van Uffelen (2006) reported that temperature seems to be the principal factor controlling the montane oak forest altitudinal distribution in Costa Rican montane forests.

Also, effects of temperature on different traits of oak species have been widely studied. For example, variation in temperature have been associated to morphological variation (Aranda et al 2005, Ramírez-Valiente et al 2009, Mediavilla et al. 2012), period of leaf expansion (Gratany and Bonito 2009), phenology (Hernández-Calderón et al. 2013), adaptative genetic diversity (Ramírez-Valiente et al 2010) and forest structure (Kapelle and van Uffelen 2006 and references therein) in different oak species. The observed lack of altitudinal differences in precipitation are not unexpected considering that present-day precipitation regimens at least in Central and Western Colombia (important oak distribution areas) are characterized by a bimodal annual cycle with high-rain and low-rain seasons (Poveda et al. 2011), high levels of precipitation (1500 and 3500 mm) between the 1000 and 3500 m, pluviometric optimum between 1500 and 1800 m, with secondary pluviometric optimum in the highlands (over 3600 m), and the capacity of the montane forest to intercept water mist (Cavelier et al. 2001)

Together all of these features set up a scenario that does not imply a limitation by precipitation, as it would be expected in temperate zones where precipitation clearly increases with altitude (Hastenrath 1991). Even though there is little information regarding historical precipitation regimes, available palaeoecological reconstructions indicate a wet cold climate in the Colombian Andes during the LGM (Van der Hammen 1961) suggesting that precipitation was not significantly reduced during this period (Ramírez-Barahona and Eguiarte 2013). Therefore, it is likely that variation in temperature-related variables is more important in determining the current distribution of *Q. humboldtii*, and that changes in these variables during the Pleistocene have had an important impact on the population dynamics of the species. Interestingly, this conclusion agrees with the results of Moles et al. (2014), who recently concluded through a meta-analysis that traits of plant species are more strongly correlated with mean annual temperature than with mean annual precipitation.

As it is observed in the present-day and LGM ENMs built mainly from temperature variables, *Q. humboldtii* climatically suitable areas occur mostly in the LMF and UMF, with low representation in the lowlands and highlands (Fig. 3). The observed differences between models particularly those at the highlands result from the dryer and colder conditions in the MIROC scenario in comparison to the CCSM scenario. However, both scenarios showed similar results where historically the main barriers in the distribution of *Q. humboldtii* have been the Magdalena River Valley, the Cauca River Valley and the contact zone between COr and SP. Both scenarios suggested that the SP region is not a stable distribution area, not only at the present-day but also since the LGM. This idea contradicts the Rangel and Avella (2010) proposal that oak migration during interglacial/glacial cycles should have followed a route from the PAR region to Serranía de San Lucas (SSL) and finally the SP. We consider more reliable a colonization of the COr through bridges as proposed by Van der Hammen et al. (2008) during stronger glacial cycles, as connectivity between the CM and the COr also appears in both models. However, molecular studies and an intensive sampling in the PAR and SSL areas should be developed to appropriately test this hypothesis.

Quercus humboldtii **altitudinal distribution dynamics trough time**

When comparing the *Quercus humboldtii* climatic niche available in the present-day period to both LGM scenarios (Fig. 4, Table 2) it is observed that the oaks distribution shifted downwards. This is particularly notable in the inter-Andean valleys (Cauca and Magdalena), PAR and SSL, and at the highlands during the LGM. When considering the altitudinal range of the forest belts during the LGM proposed by Hooghiemstra and

Van der Hammen (2004) that we used to quantify the oaks suitable niche, it was observed that despite the observed downwards shift, the climatic niche available for the TLF and LMF was reduced during the LGM. This reduction suggests that even if *Quercus* entered to the Colombian Andes as an element of the LMF (Cleef and Hooghiemstra 1984) it seems that its optimal environment corresponds to the UMF, which during the LGM probably increased in climatically suitable area in about 6 to 10% according to the models. The low performance of *Quercus* in the lower forest belts is support by the consideration that the LMF received taxa from northern and southern migrations, which imply a stronger competition than in the UMF, which is floristically poorest than LMF. Besides, the Neartic origin of the *Quercus* genus may have influenced that *Quercus humboldtii* got over physical constrains as the night frost that define the limit between the LMF and UMF and may have played an important role in their floral composition (Hooghiemstra and Van der Hammen 2004).

Dry refugia or moist forests?

Recently, Ramírez-Barahona and Eguiarte (2014) reviewed the effects of the climatic history on the genetic diversity of Neotropical cloud forest species and the authors outlined two scenarios: the dry refugia or the moist forests. As it was mentioned before, comparisons between the distribution of climatically suitable areas during the LGM and present-day periods showed greater connectivity between the COc and the CC during the LGM, a shift of the oaks distribution from higher elevations to low-lying areas and finally, a differential change between the available climatic niche in the TLF, LMF and UPF.

The present case of study fits better the moist forest model that is characterized by a minor effect of precipitation on the continuity of forest cover, favoring down-slope range expansion and connectivity during the cold conditions (Ramírez-Barahona and Eguiarte 2014 and references therein). However, as the climatic characterization and ENMs showed (Fig. 3), this observed dynamic fits better with connectivity through the LMF, as both MIROC and CCSM models showed divergent patterns regarding distribution areas in the TLF. Therefore, considering that our study showed a differential response of each forest belt we found fundamental to consider in the scenarios proposed by Ramírez-Barahona and Eguiarte (2014) an explicit differentiation of the species altitudinal distribution, since the proposed model (Fig. 2B in Barahona and Eguiarte 2014) seems to propose a constant block displacement of the montane species range downwards.

Apparently the most important factor that may have defined the glacial/interglacial changes in the distributions of the oaks in the Neotropical Andes, and probably in oaks of other regions of the Neotropics is related to changes in the species elevation. Previous palaeoecological studies already mention the importance of altitudinal migration in the Colombian Andes (Hooghiemstra and van der Hammen 2004); however, this variable has received little to no attention particularly in phylogeographic studies. Altitudinal migration has been proposed as the consequence of different events such as: decreasing temperatures at higher elevations that forced taxa to move downwards (Bennett 2012) and significant reduction of CO_2 during the LGM and therefore even lower concentration at higher altitudes that leads to CO_2 stress (Bennett and Willis 2000). Together, these climatic factors configure a scenario of altitudinal taxa migrations (as the one that we reported in this study), that eventually may have promoted TLFs with a composition that includes montane and dry forest taxa (Bennett 2012).

Conclusions

Despite the important compression in the Andean vegetation belts that occurred during the LGM described by Hooghiemstra and van der Hammen (2004), ENMs did not show variation regarding the total available suitable climatic niche area between the present-day and LGM periods when considered the entire *Quercus* distribution as a whole. However a differential response was observed in the available suitable climatic niche area among forest belts. Our data suggested that the main effect of the transition between glacial and interglacial stages was to modify the *Q. humboldtii* altitudinal distribution, but not the total available climatic niche. Therefore, at least in terms of the species climatic niche, the Andean oak species should not have been affected by the LGM as intensely as it has been observed in Holartic oak species. This pattern coincide with several observations of downwards migration of higher-altitude taxa towards the lowlands during the cold-stages (Colinvaux et al. 2000, Ledru et al. 2007, Bennet et al. 2012) probably due to important modification of the atmospheric CO_2 concentrations (Bennet et al. 2012).

Apparently, the periods of climatic oscillation in the Neotropics did not have the strength and duration necessary to promote evolutionary processes as allopatric speciation (Bennet et al. 2012). In this sense Bennet et al. (2012) also mentioned that the available molecular evidence indicate processes of linage splits in different taxa that occurred before the Quaternary and little fossil evidence that suggest the emergence of new species during the Quaternary ice ages. Therefore the most important effect of the Quaternary ice ages is the redistribution of populations that resulted in extinction processes rather than speciation (Bennet 1990; Bennet 2004; Willis and Niklas 2004; Bennet 2012). However, a fine-scale reconstruction of the historical demography of the oaks in the Andes would require the use of molecular techniques in order to test the validity of these results. Meanwhile it is possible to hypothesize that according to palaeoecological data and the results obtained in this study, *Quercus humboldtii* historical demography was not importantly affected by the climatic fluctuation during the Quaternary in terms of area reduction, but rather the species experienced a differential redistribution through the different Andean montane forests.

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Tables

Table 1. Principal components analysis for climatic variation among *Q. humboldtii* localities trough the Andean forest belts.

Variables	PC1	PC2	PC3	PC4	PC5
Annual mean precipitation	0.51	-0.18	-0.22	-0.06	0.43
Mean diurnal range	0.35	0.44	0.41	-0.16	-0.02
Temperature seasonality	0.54	-0.08	-0.15	-0.07	0.38
Temperature annual range	0.28	0.57	0.30	-0.02	-0.16
Annual precipitation	0.17	-0.39	0.58	0.70	0.02
Precipitation seasonality	-0.03	0.50	-0.49	0.69	0.11
Elevation	-0.47	0.20	0.32	-0.05	0.79
Cumulative Percentage	46.98	74.61	89.25	97.14	98.99

Table 2. Mean *Quercus humboldtii* area extension of suitable climatic niche between periods for the Andean forest belts. TLF: tropical lowland forest; LMF: lower montane forest; UMF: upper montane forest; SP: sub-paramo. * Significant mean area differences (p<0.01) between present-day and each LGM scenarios according to the t-test.

	Fixed cumulative value 1 logistic threshold					***Balance training omission, predicted area and threshold value logistic threshold***				
	TLF	**LMF**	**UPF**	**SP**	**Total**	**TLF**	**LMF**	**UPF**	**SP**	**Total**
Present-day (0ka)	160346	148618	63977	14866	311318	83479	144647	63785	14714	393262
MIROC (21ka)	124908*	104549*	71055*	96278*	313217*	64218*	90372*	68142*	93674*	396792*
CCSM (21ka)	120708*	100256*	70846*	96201*	302782*	63436*	87261*	68808*	95182*	379684*

Table S1. *Quercus* localities used for the LGM validation test using palinological data.

Locality ID	Period (ka BP)	Longitude	Latitude
Pitalito	23-18	-76.03	1.87
Lusitania	23-18	-76.57	3.82
Fuquene	36-25	-73.77	5.45
Funza	350-24	-74.22	4.72
CIEGA	18	-72.33	6.50
CUX	18	-74.19	4.67
La Cachucha	24	-75.36	4.59
Timbio	21-16	-76.68	2.35
Agua Blanca	87-7,5	-74.17	5.00
La Depresion	21	-74.33	2.50

Figures

Figure 1. *Quercus humboldtii* geographical records (white triangles) distribution in the Colombian Andes. SD: Serranía del Darién; PAR: Paramillo; COc: Cordillera Occidental; CC: Cordillera Central; CM: Macizo Colombiano; NP: Nudo de los Pastos; COr: Cordillera Oriental; SSL: Serranía de San Lucas; COr-SP: Cordillera Oriental-Serranía del Perijá border; SP: Serranía del Perijá. Elevation is represented from lower (white) to higher (black) values

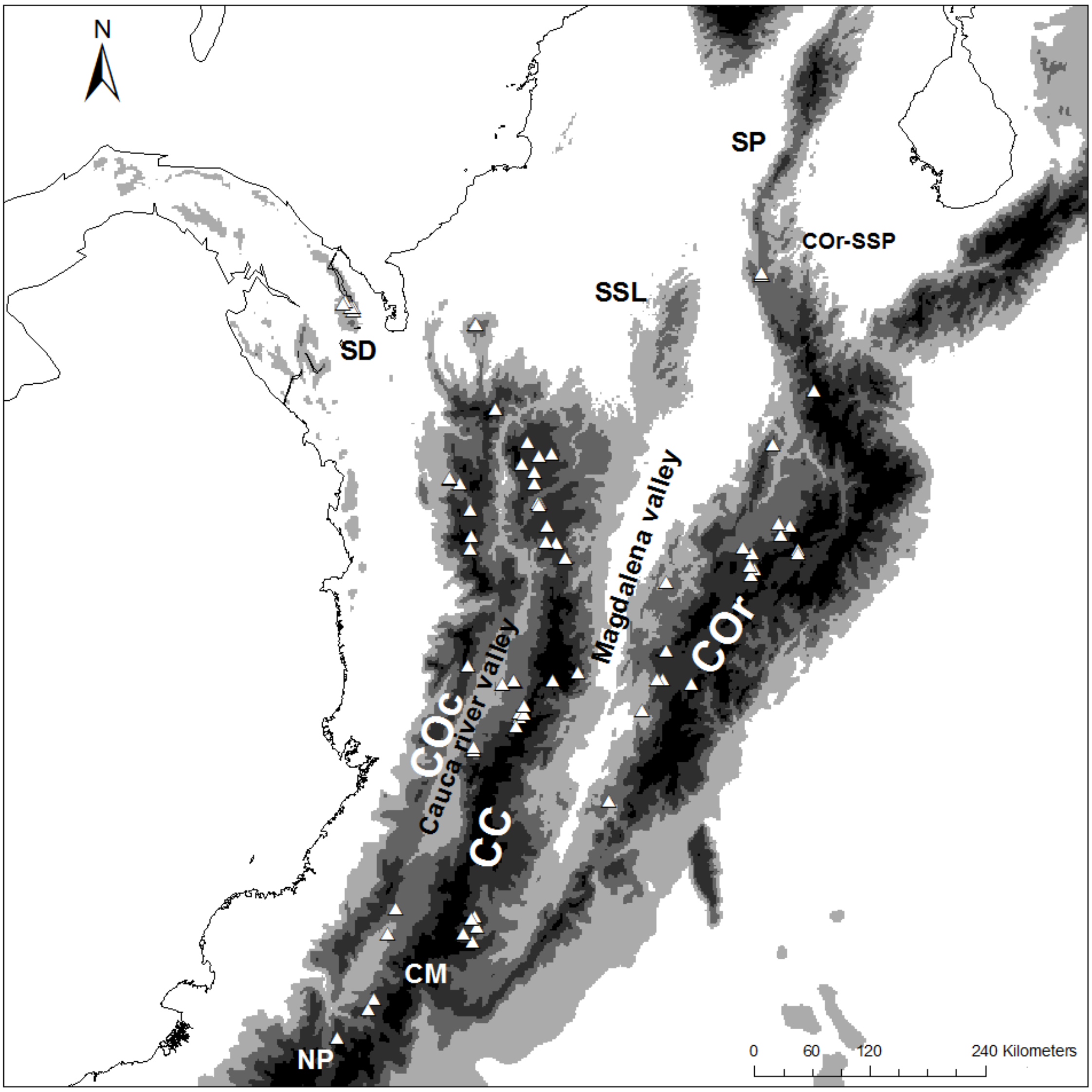


Figure 2. *Quercus humboldtii* principal components analysis for climatic variation among the Andean forest belts.

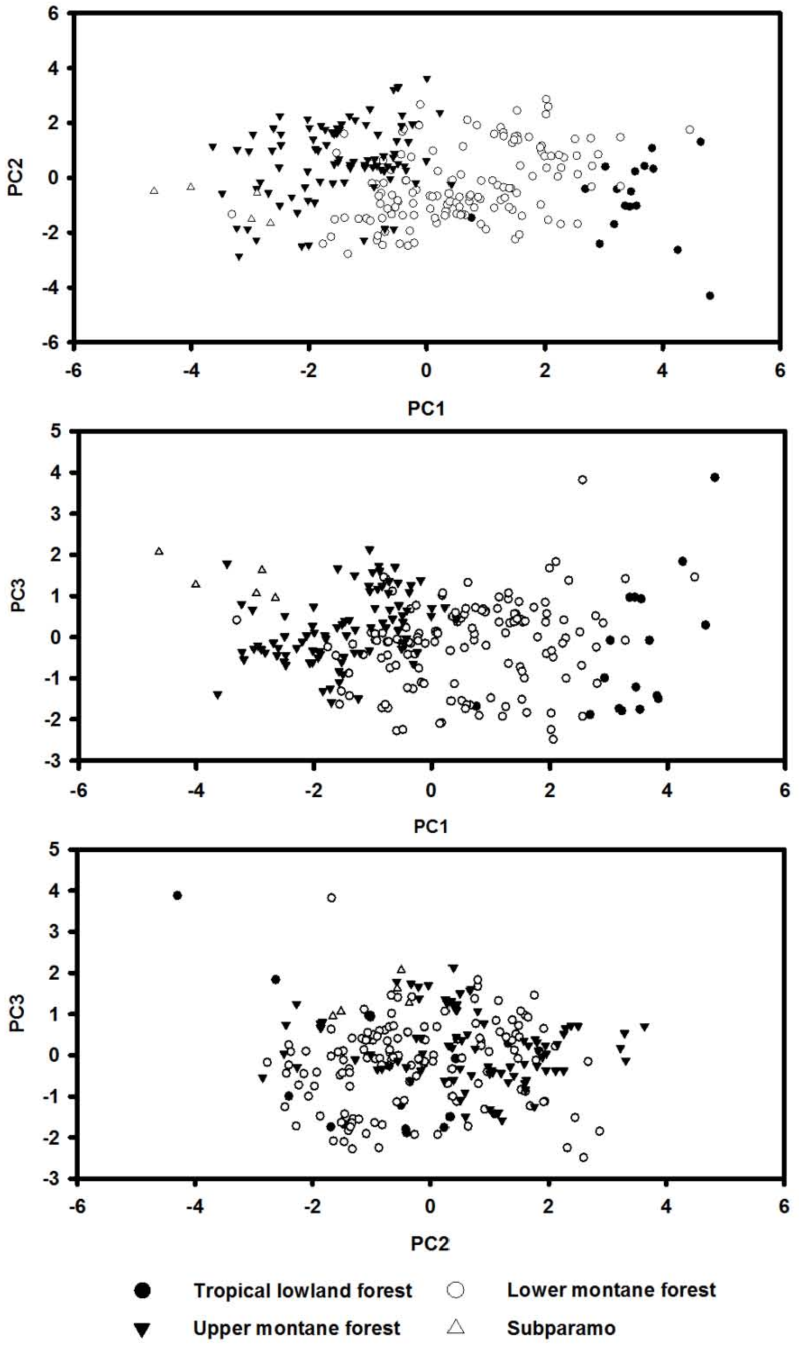
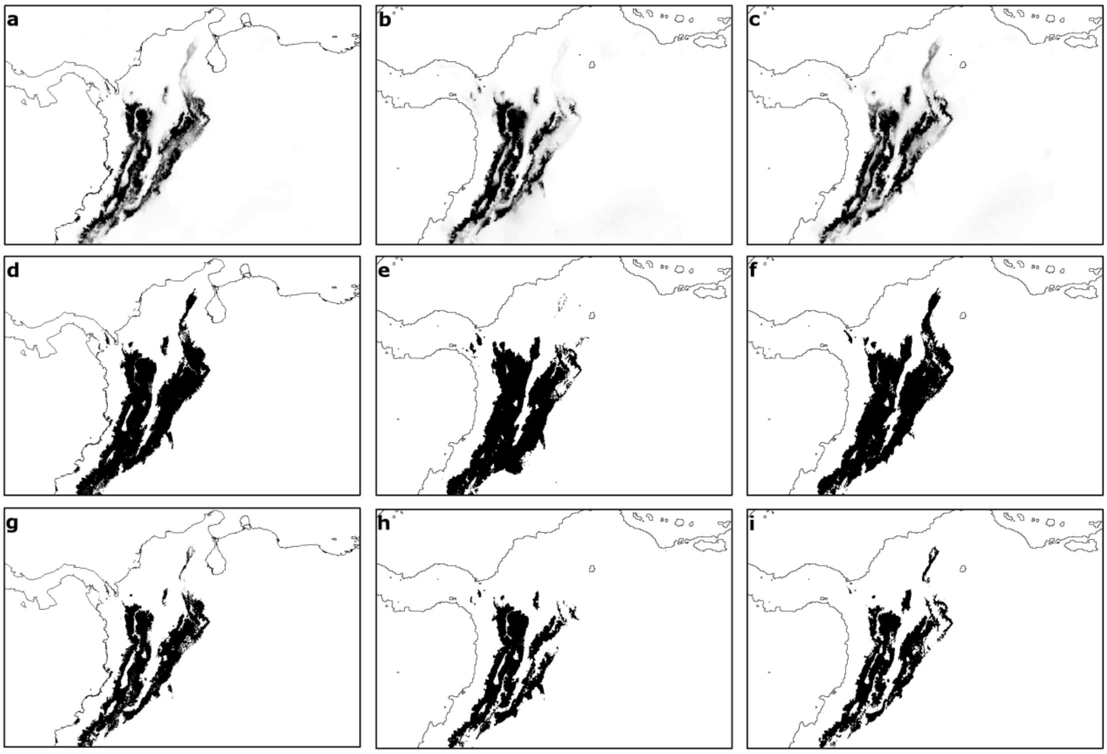


Figure 3. Ecological niche models for *Quercus humboldtii* during the Present-day and Last Glacial Maximum periods. Boxes a, b and c (Present-day, LGM according to MIROC and LGM according to CCSM models, respectively) contain the raw outputs suitability values increasing from cero (white) to one (black). Boxes d, e and f represent the same sequence (Present-day, LGM according to MIROC and LGM according to CCSM models respectively) of the models under the fixed cumulative value 1 logistic threshold. Boxes g, h and i represent the same sequence of models using the Balance training omission, predicted area and threshold value logistic threshold.



Capítulo Cinco

A lonely oak history: lack of phylogeographic structure and recent population expansion in *Quercus humboldtii* in the Colombian Andes.

Hernando Rodríguez-Correa, Ken Oyama, Mailyn González, Antonio González-Rodríguez

Abstract

Phylogeographic studies of *Quercus* species at northern latitudes have been the base to understand the response of the species to historical changes such as the glacial-integlacial cycles during the Pleistocene. Recently, oak species have been studied in other regions such as the Mexican Transition Zone. These studies have shown a differential response to historical environmental changes in comparison to their temperate congeners. In the present study, we analyzed the chloroplast DNA variation of the Andean oak, *Quercus humboldtii*, to describe the effect of historical environmental and geological changes in the Colombian Andes on the demographic history of oak populations, and to contrast these findings with patterns observed in other regions. Genetic data was obtained from chloroplast DNA microsatellite loci and analyzed using phylogeographic methods. We estimated values of genetic diversity, genetic and phylogeographical structure, computed minimum spanning haplotype networks, tested for historical demographic expansion and, finally, described patterns of geographic variation in the genetic structure and genetic diversity of oak populations. Our results indicated that *Q. humboldtii* does not exhibit phylogeographical structure and shows genetic diversity values lower than the observed for European, North American and Mexican oak species. Minimum spanning haplotype networks and demographic expansion tests also suggested a recent colonization of the Colombian Andes and a recent demographic expansion dated after the Late Glaciation period during the early Holocene, probably preceded by either a bottleneck during the *Quercus* immigration or a *Q. humboldtii* Andean origin. Such patterns also suggest a milder effect of the Pleistocene glaciations on the Andean oak populations in contrast to the patterns observed in northern latitude oaks.

Keywords: Historical demography, Neotropical trees, Neotropics, Pleistocene, Holocene.

Introduction

The genus *Quercus* (Fagaceae) is characterized by a wide distribution in the northern hemisphere, where oak species have a considerable ecological and economic importance (Nixon 2006). The oaks species diversity and ecological dominance in a wide variety of ecosystems, such as temperate deciduous and evergreen forests, sub-tropical evergreen forests, oak-pine forests and cloud forests, have caught the attention of many researchers that have studied oak species evolutionary history along its distribution. An important number of studies have focused on the paleoecology and biogeography (Crepet 1989a; Barbero et al. 1992; Manos et al 1999; Carrión et al. 2000; Fernández et al. 2007; Fletcher et al. 2007; Torres-Miranda et al. 2011; Voelker et al. 2012; Torres-Miranda et al. 2013; Kozharinov and Borisov 2014; Rodríguez-Correa et al 2015) and systematics (Burger 1975; Crepet 1989a; Crepet 1989b; Nixon 1989; Nixon 1997a; Nixon 1997b; Manos et al. 1999; Nixon 2002) of the genus. However, since the emergence of phylogeographic analyses, this discipline has provided an overwhelming amount of evolutionary information for oak species, particularly within the Palearctic and Nearctic regions (Dumolin-Lepègue et al. 1997; Fineschi et al. 2002; Cottrell et al. 2002; Csaikl et al. 2002; Petit et al. 2002a; Petit et al. 2002b; Olalde et al. 2002; Grivet et al. 2006; Lopéz de Heredia et al. 2007; Chen et al. 2012; Liu et al. 2013; Gugger et al. 2013; Gugger and Cavender-Bares 2013; Alexander and Woeste 2014; Cavender-Bares et al. 2015).

Recently, there have been some studies of oak species distributed from the Mexican transition zone between the Nearctic and the Neotropical regions (including species in the Caribbean islands) down to the Neotropical montane forest in the Colombian Andes. The review of Kappelle (2006) covers several topics such as the biogeography, paleoecology, biodiversity, community ecology, population genetics and conservation biology of Neotropical montane oak forests. Phylogeography and population genetics of oaks in this area have been studied mostly on Mexican species (González-Rodríguez et al. 2004; Tovar-Sánchez et al. 2008; Peñaloza-Ramírez et al. 2010; Cavender-Bares et al. 2011; Gugger et al. 2013; Gugger and Cavender-Bares 2013; Valencia-Cuevas et al. 2014; Cavender-Bares et al. 2015). From Mexico down to the Colombian Andes (the southernmost *Quercus* distribution limit) oaks species have not been studied except for *Quercus oleoides* (also distributed in Mexico; Cavender-Bares et al. 2011, Gugger et al. 2013, Gugger and Cavender-Bares 2013; Cavender-Bares et al. 2015) and *Quercus humboldtii* (Fernández-M et al. 2000; Fernández-M and Sork 2005; Fernández-M and Sork 2007).

Phylogegoraphic studies in oak species from the Neotropical regions have shown contrasting demographic patterns when compared to Nearctic and Palearctic species. Neotropical oak species exhibit higher levels of within population variation and lower among-population differentiation, probably as a result of the fact that *Quercus* populations did not isolate into refugia during the periods of glacial maxima as the higher latitude oak species did (González-Rodríguez et al. 2004; Cavender-Bares et al. 2011). Apparently, the most important effect of the Pleistocene climatic changes on Neotropical oak species was related to changes in their altitudinal distribution (Hooghiemstra and van der Hammen 2004, Rodríguez-Correa et al. *unpublished data*). However, there is still a considerable gap on the knowledge of historical demographic processes of oaks at the southernmost part of the distribution of the genus, and particularly at the Colombian Andes, where the single oak species present (*Quercus humboldtii*) has been studied only with traditional population genetics approaches, describing nuclear microsatellites cross-amplification, genetic diversity and matting patterns related to habitat fragmentation (Fernández-M et al. 2000; Fernández-M and Sork 2005; Fernández-M and Sork 2007).

The extensive paleoecological information about montane forest dynamics including clear oak palynological records (Hooghiemstra and van der Hammen 2004 and references therein), and the fact that *Quercus humboldtii* is the only oak species in the Colombian montane forest, configure an optimal scenario to test historical demography hypotheses about the responses of *Quercus* (a typical northern hemisphere taxon) species within neotropical environments and to provide more information about the effect of the historical climatic changes on the elements of the montane forests in the Neotropics. Therefore, we set as aims of this study: i. to describe the phylogeographic structure of a Neotropical oak (*Quercus humboldtii*) in the Colombian Andes, ii. to test the effect of topographic variables on the species genetic diversity and structure, and iii. to compare historical demography patterns observed for *Quercus humboldtii* with the previously reported studies for other neotropical, nearctic and palearctic oak species.

Methods

Study species and population sampling

Quercus humboldtii (Bonpl.) is distributed in northern South America from the Darién region at the Colombia/Panama border down to the Nariño region at southern Colombia through the Andean mountains (Fig. 1). Its altitudinal distribution ranges from 774 to 3200 m (Pulido et al. 2006; Rangel and Avella 2011). A total of 21 populations of *Q. humboldtii* were studied along this distribution (Fig. 1). Samples were obtained from the tissue collection of the *Instituto de Investigación de Recursos Biológicos Alexander von Humboldt* (IAvH) (http://www.humboldt.org.co/servicios/colecciones-biologicas/tejidos).

DNA isolation and microsatellite amplification

Total DNA was extracted using a commercial DNA isolation kit following the instructions provided by the manufacturer (QIAGEN DNeasy plant mini kit). A set of nine chloroplast DNA (cpDNA) microsatellite loci (cmcs2, cmcs3, cmcs4, cmcs5, cmcs6, cmcs7, cmcs10, cmcs12 and cmcs14) designed for Fagaceae species by Sebastiani et al. (2004) were screened and tested for polymorphism. Polymerase chain reactions (PCR) were performed using a QIAGEN multiplex PCR kit with a final volume of 5 μL, containing 1X multiplex PCR master mix, 0.25 mM of each primer, 20 ng of DNA and dH_2O. Two groups of cpDNA microsatellite loci with common annealing temperatures were used to amplify four (cmcs3, cmcs4, cmcs5 and cmcs6) and five (cmcs2, cmcs7, cmcs10, cmcs12 and cmcs14) loci at the same time for each individual. cpDNA microsatellite multiplex groups were defined so that different fluorescence and loci expected size differ importantly within the same reaction mix. Amplification was performed using an initial denaturation step for 15 min at 95°C, followed by 35 cycles, each of 30 s at 95°C, 1.5 min at 55°C, 1 min at 72°C, and a final extension step for 30 min at 60°C. PCR products were sized using an ABI-PRISM 3300 Avant sequencer (Applied Biosystem) and fitted with respect to a size standard (GeneScan-600 LIZ) using the Peak Scanner program version 2.0 (Applied Biosystem).

Genetic analysis

Genetic diversity and Genetic Structure

Each unique combination of size variants for the evaluated chloroplast microsatellite loci was defined as a different haplotype. Allelic richness corrected for sample size (*AR*), gene diversity with unordered alleles (h_S sensu Pons and Petit 1996) and non-standardized gene diversity with ordered alleles (v_S sensu Pons and Petit 1996) and the mean pairwise genetic distance among individuals within a population under a stepwise mutation model (D^2_{SH}; Goldstein et al. 1995) were calculated at the population and regional (Cordillera Occidental, COc; Cordillera Central, CC and Cordillera Oriental, COr; Fig. 1) levels, using SPAGeDi version 1.1 (Hardy and Vekemans 2002). To evaluate genetic differentiation both G_{ST} and N_{ST} (a G_{ST} analogue which takes into account the genetic distances between haplotypes) and their significances were calculated with SPAGeDi version 1.1 (Hardy and Vekemans 2002). Additionally, the significance of the difference between the values of G_{ST} and N_{ST} was determined through the allele permutation test with 200000 repetitions. A significantly higher value of N_{ST} than G_{ST} indicates phylogeographic structure in the populations, according to which more closely related haplotypes tend to occur in the same populations (Pons and Petit 1996).

A hierarchical analysis of molecular variance (AMOVA) was implemented in order to describe the partitioning of the genetic variation among population groups, among populations within groups, and within populations. The grouping of populations was defined by the three mountainous regions of the Colombian Andes (as described above). AMOVA was calculated considering both F_{ST} (based on the infinite alleles mutation model, IAM) and R_{ST} (based on the stepwise mutation model, SMM) and using 10,000 permutations in Arlequin version 3.5 (Excoffier et al. 2005). The relationships among haplotypes were depicted using a minimum spanning network computed with Network version 4.6 (available at www.fluxus-engineering.com) using the median-joining method (Bandelt et al. 1999) and a maximum parsimony search (Polzin and Daneschmand 2003).

Historical demography

Possible events of past demographic expansions were assessed using the F_S statistic (Fu, 1997) and the mismatch distribution test (Rogers and Harpending 1992) considering the entire *Quercus humboldtii* populations as a single population (considering the absence of genetic structure; see results) using Arlequin version 3.5 (Excoffier et al. 2005) and coding the cpSSR alleles as binary data as suggested by Navascues et al. (2006). The estimation of the time to the population expansion in mutational units (τ) was calculated using a maximum-pseudolikelihood method implemented in the LMSE software (Navascués et al. 2009) considering the homoplasy correction model. The time to expansion was calculated using the formula $\tau = 2l\mu t$ (where l corresponds to the number of loci; Rogers and Harpending 1992) under a per-locus average mutation rate of 1×10^{-5} as well as generation time for trees between 50 and 100 years (Navascués et al. 2009; Heuertz et al. 2010) .

Geographic patterns of genetic diversity and structure

The computed genetic diversity indexes were compared against the altitude of the sampled populations in order to determine the existence of geographical gradients of genetic diversity using correlation tests implemented in R ver. 3.0.2. Comparisons with latitude and longitude were not considered because the distribution of *Q. humboldti* was mainly determined by altitudinal migrations during the glacial cycles (Hooghiemstra and van der Hammen 2004). The geographical location of the most important genetic discontinuities was defined using the Monmomier´s maximum difference algorithm implemented in Barrier ver. 2.2 (Manni et al. 2004). For this analysis, a set of pairwise matrices of average square genetic distance (ASD; Goldstein *et al.,* 1995; Slatkin, 1995) resampled manually from random subsets of individuals within populations was used to define the bootstrap support values of the observed genetic breaks. Complementarily, the observed matrix of ASD was compared to a distance matrix inferred from the population geographical location in order to estimate if the observed genetic structure fitted an isolation by distance (IBD) model using a Mantel test (10000 permutations) implemented in R ver. 3.0.2.

Results

Genetic diversity and genetic structure

The nine cpDNA loci were polymorphic, with the number of alleles per locus ranging from two to five, as follows: csmc6 had five alleles, cmcs12 and cmcs14 had four alleles each, cmcs2, cmcs5 and cmcs7 had three alleles each, and cmcs3, cmcs4 and cmcs10 had three alleles each. Eighteen different haplotypes (Table S1) were identified in the 22 populations sampled, with 13 unique haplotypes (haplotypes with a frequency equal to one), one private haplotype (haplotype with frequency>1 restricted to a single population), two haplotypes present in 2−4 populations and two widely distributed haplotypes (Table 1; Fig. 2). The number of haplotypes per population ranged from one to seven. Considering the regions defined by the three Cordilleras, 10 haplotypes (five unique) were observed for the COr, 11 haplotypes (seven unique) for the CC and four haplotypes (one unique) for the COc. Within-population genetic diversity (h_S) varied between zero (populations 14 and 15; Fig. 2) and 0.846 (population 3; Fig. 2). Mean (s. e.) h_S and total gene diversity (h_T) were 0.418 (0.055) and 0.453 (0.059), respectively.

The genetic differentiation among populations was 0.077 (0.033) for the unordered alleles (G_{ST}) and 0.048 (0.021) for the ordered alleles (N_{ST}). Both, G_{ST} and N_{ST} values were significant ($P<0.001$), but the comparison between G_{ST} and N_{ST} does not support the presence of phylogeographic structure. The hierarchical analysis of molecular variance (AMOVA; Table 2) indicated that most of the genetic variation is found within populations (89.17% under the IAM and 87.41% under the SMM) followed by variation among populations within groups (10.01% under the IAM and 14.63% under the SMM). The percentage of genetic variation among the three regions was almost zero and not significant. The haplotype network showed that the 18 haplotypes are separated from each other by one mutational step in most cases, except haplotypes H15 and H17 that are separated by 5 steps (Fig. 2). Most haplotypes have a low frequency, except two haplotypes (H7 and H2), that have a wide distribution through the entire *Q. humboldtii* range (Fig. 2). The geographic distribution of the haplotypes did not show any clear structure; further supporting the absence of phylogeographic patterns.

Historical population demography

The calculation of the Fs statistic (Fu, 1997) resulted in a highly significant large negative value (-16.64, $p<0.001$), indicating a population expansion. The mismatch distribution analysis also suggested a population expansion scenario since the Harpending's raggedness index exhibited a non-significant value (0.129, $p=0.418$), which indicates that the observed distribution did not deviate from a unimodal shape. The time to the population expansion (τ) observed value (using a model under homoplasy) was 0.021 (θ_0= 0.76, θ_1=3.62E10, *–log[CL]*=528.31), which considering mutation rates ranging between 1×10^{-5} and generational times ranging between 50 and 100 years, suggested that a population expansion dated between 5833 and 11666 years BP could have occurred in the Colombian Andes oak population.

Effect of geography on the genetic diversity and genetic structure

Correlation tests showed significant negative relationships between genetic diversity values and elevation. Particularly, haplotype richness (corrected by rarefaction) showed the strongest negative relation with elevation (r= -0.46, p=0.023) followed by genetic diversity with unordered alleles (h_S; r= -0.39, p=0.047) and non-standardized gene diversity with ordered alleles (v_S; r= -0.37, p=0.047). However, the genetic diversity measured as the mean pairwise genetic distance among individuals within a population under a stepwise mutation model (D^2_{SH}; Goldstein et al., 1995) did not show any relation with elevation (r= -0.05, p= 0.46). The inter-population analysis of genetic discontinuities separated the populations in the Northern Andes (populations 2, 4, 7, 18 and 21) from populations in the southern and eastern Andes due to the prevalence of unique haplotypes in populations two (H9), seven (H10 and H14) and 18 (H16), and showed that populations 3 (situated in the central COc) and 9 (in the southern CC) are somewhat differentiated from other populations (Fig. 3) due to the presence of unique haplotypes (H1, H11, H13 and H17 in population 3 and H3 in population 9). All the estimated barriers exhibited a well-supported bootstrap value (over 90%) and, two populations (9 and 3) at southern CC and central COc respectively were observed isolated from the other sampled populations (Fig. 3). Finally, the Mantel test results did not support a pattern of isolation by distance in the sampled populations (r=-0.13 p>0.05).

Discussion

Comparing the genetic diversity values of *Quercus humboldtii* (Table 1) with the diversity observed in other *Quercus* species, a higher haplotype richness is observed in the Andean species than in European white oaks and some North American species, but lower diversity values than in Californian (USA) and Mexican species. For example, also using cpDNA microsatellites, Grivet et al. (2006) found a total of 11 haploptypes in the European white oak complex (*Quercus robur*, *Q. petraea*, *Q. canariensis*, *Q.*

faginea and *Q. pyrenaica*) in France and the Iberian Peninsula; Magri et al. (2007) reported five haplotypes for *Quercus suber* in the western Mediterranean basin and Marsico et al. (2009) observed six haplotypes for *Quercus garryana* in the northwest region of North America.

In contrast, Peñaloza-Ramírez et al. (*submitted*) found a total of 90 haplotypes for *Quercus castanea* along its whole distribution in Mexico, while Valencia-Cuevas (2014) reported 21 haplotypes for the same species in a more restricted geographic area in central Mexico. Other Mexican oak species also show high haplotype richness such as the *Quercus crassifolia x Quercus crassipes* hybrid complex with 26 haplotypes (Tovar-Sánchez et al. 2008) and the *Quercus affinis x Quercus laurina* hybrid complex with 35 haplotypes (Ramos-Ortiz et al. *submitted*). Finally, a Californian oak species (*Quercus lobata*) also exhibited considerable haplotype richness with 39 haplotypes (Grivet et al. 2006).

Overall, *Quercus humboldtii* showed a higher haplotype richness (18) than Nearctic oak species (except for *Quercus lobata*), but lower diversity than species distributed in the Neotropics within the Mexican Transition Zone (MTZ) (*sensu* Morrone 2010). A similar conclusion arises when comparing the values of average within population diversity (h_S) of *Q. humboldtii* with those of the above-mentioned oak species: diversity values are higher than in *Q. lobata*, the European white oak complex and *Q. suber,* but lower than the h_S values in *Q. castanea* and the *Q. affinis x laurina* complex. Regarding total gene diversity (h_T), *Q. humboldtii* exhibits lower values than the entire set of oak species mentioned above (including European and North American oaks).

However, these diversity comparisons should be taken with caution since, even though all the cited studies are based on chloroplast microsatellites, the set and number of cpSSR loci partially differs among studies. In terms of among-population variation, we observed low but significant values of genetic differentiation as indicated by both N_{ST} and G_{ST} and a lack of phylogeographic structure given the non-significant difference between the estimates of both statistics. This lack of phylogeographic structure indicates that haplotypes are randomly distributed in the populations with respect to their genealogical relationships (Pons and Petit 1996), probably due to a recent colonization (or origin) of the *Q. humboldtii* in the Colombian Andes as will be discussed later.

Interestingly, high levels of genetic diversity and genetic differentiation have been associated to hybridization between *Quercus* species in the MTZ (González-Rodríguez et al. 2005; Továr-Sánchez et al. 2008; Valencia-Cuevas et al. 2014). The limited interaction between *Q. humboldtii* and other oak species (assuming that no other oak species colonized the northern Andes or that the other colonizing lineages became extinct) may in part explain the comparatively lower genetic diversity and genetic structure of the Andean populations. However, historical demographic processes probably played the most important role. The AMOVA results (Table 2) indicated a lack of genetic differentiation among populations situated along the three main Andean mountains ranges in Colombia and indicated that genetic variation distributes mostly within populations.

cpSSR data considering all populations as a single group showed strong signals of a past demographic expansion according to Fu's F_S and Harpending's raggedness index (Table 3). The estimated time to the demographic expansion according to LMSE calculations date this event between 5.5 and 11.1 *ka* BP, configuring a scenario of recent (post-glacial) population expansion probably preceded by a recent bottleneck caused either by a recent immigration of the species into the Andes or a recent speciation process. Such patterns fit previous observations of forest fluctuations in the Colombian Andes during the last 650 *ka* BP that include higher values of *Quercus* pollen abundance during warmer climatic conditions than during cold climatic conditions (Van´t veer and Hooghiemstra 2000).

Different authors coincide on the idea that after the appearance of *Quercus* in the Colombian Andes (dated about 480 *ka* BP) a gradual colonization occurred, as a result of this colonization important changes in the composition of the previous Andean forest occurred as it has been observed in the palinological record (Van´t veer and Hooghiemstra 2000; Hooghimestra and van der Hammen 2004). *Quercus* has been considered as an important element of the Andean forest during interglacial and transitional climatic conditions (van´t veer and Hooghiemstra 2000). Despite that *Q. humboldtii* palinological records have suggested an effective colonization of the Colombian Andes during Pleistocene interglacial conditions (van´t veer and Hooghiemstra 2000), molecular analysis in this study, particularly the time to the last population demographic expansion, did not match Pleistocene interglacial periods, instead the observed demographic expansion signal to the Andean oaks was dated at the beginning of the Holocene as it could be expected considering the *Q. humboldtii* recent immigration.

It has also been hypothesized that colonization of *Quercus* in the Colombian Andes was accompanied by the evolution of the oak ecological niche. In this sense, although Van´t Veer and Hooghiemstra (2000) remarked the importance of *Quercus* in the pollen spectra corresponding to interglacial climatic conditions, they also mentioned that during the last 135 *ka* BP the maximal abundances of *Quercus* pollen did not coincide strictly with warm climatic conditions, which leads to the idea that following a period of competition between oaks and other Andean forest taxa, *Quercus* reached an equilibrium evidenced by the locally and permanent presence of *Quercetum*. Under this scenario and according to the genetic data obtained, we propose that following the *Quercus* equilibrium stage since its arrival, an important oak demographic expansion may have occurred after the Late Glaciation (between>40-20 *ka BP sensu* Coltrinari 1993) of the Quaternary period, and particularly at the initial stages of the Holocene, for which observations of very high percentages of *Quercus* pollen have been reported that pointed out the oak forest as a dominant element in (at least) the eastern Colombian Andes (van der Hammen and Hooghiemstra 2003).

Together, the recent immigration of the species into the Colombian Andes and a very recent population expansion could explain the lack of phylogeographic structure, as a recent migration leaves a short time for the appearance of new haplotypes. This lack of structure is evidenced by the haplotype network that exhibited two frequent haplotypes distributed across the entire species geographic range and randomly distributed rare haplotypes (Fig. 2). Despite the lack of geographic genetic structure, it was possible to identify a negative relation between the genetic diversity (expressed as haplotype richness, genetic diversity with unordered alleles and non-standardized gene diversity with ordered allels) and elevation. Previous studies of the Andean forest belts altitudinal migration described by Hooghiemstra and van der Hammen (2004), and also for the distribution dynamics of *Q. humboldtii* (Rodríguez-Correa et al. *unpublished results*) suggested that during the Last Glacial Maximum the lower elevation oak populations experienced an increase in their available climatic niche area, while higher elevation populations may have suffered a compression effect due to the reduction of their environmental optimal conditions and the expansion of the sub-paramo and grass-paramo vegetation. An increase in the lower forest belt area implies higher connectivity due to larger population sizes and could explain the current higher genetic diversity values in the low elevation oak populations and a reduced genetic variation at higher elevations due to upwards migration from the lowlands and isolation at the highlands.

Interestingly, even in the absence of phylogeographic structure, low but significant genetic differentiation was observed among populations (G_{ST} = 0.077) and the BARRIERS analysis showed that the most important genetic discontinuities separate the oak populations in the north of the CC and COc and the oak populations in central CC and COc. Similarly, oak populations south of the Colombian Massif (CM) also seem to differentiate from the populations located north of the CM. Two populations (3 and 9; Fig. 2) stand out due to their apparent isolation in relation to their nearest neighbors. However, both populations show different geographical conditions. Population 9 is located at the south of the CM, a tremendous geologically heterogeneous area formed by valleys and important mountains which may explain processes of isolation and differential gene flow with its neighbors. On the other hand, the Belen population is located near the Cauca River Valley (a mountain-lowland transition) and considering that oaks are mainly dispersed by gravity and pollinated by wind it is also expected a limited gene flow with other oak populations located mountain-top in the COc.

Conclusions

The historical demography of *Q. humboldtii* exhibited a complex dynamics. The demographic expansion analysis suggested an important signal of expansion during the early Holocene. Interestingly, palynological data for the COr indicates an important dominance of *Quercus* pollen during the Holocene over other taxa such as *Weinmannia* (dominant during interglacial periods), providing support for our results. The idea of a recent population expansion, added to an assumed recent Andean colonization of *Quercus*, could explain the low genetic structure and the lack of phylogeographic structure, with two common haplotypes widely distributed with high frequency and few rare haplotypes randomly distributed through the species geographical range. It is also interesting that the Andean oak exhibited different geographical structure patterns compared to their Palearctic, Nearctic and even MTZ congeners. Finally, this study also represents an important effort to complement the current understanding of the montane forest evolution with a genetic approach, as most of the hypothesis postulated about this topic came from palaeoecological studies meanwhile there are little or no phylogeographic studies of any Andean montane tree species.

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Tables

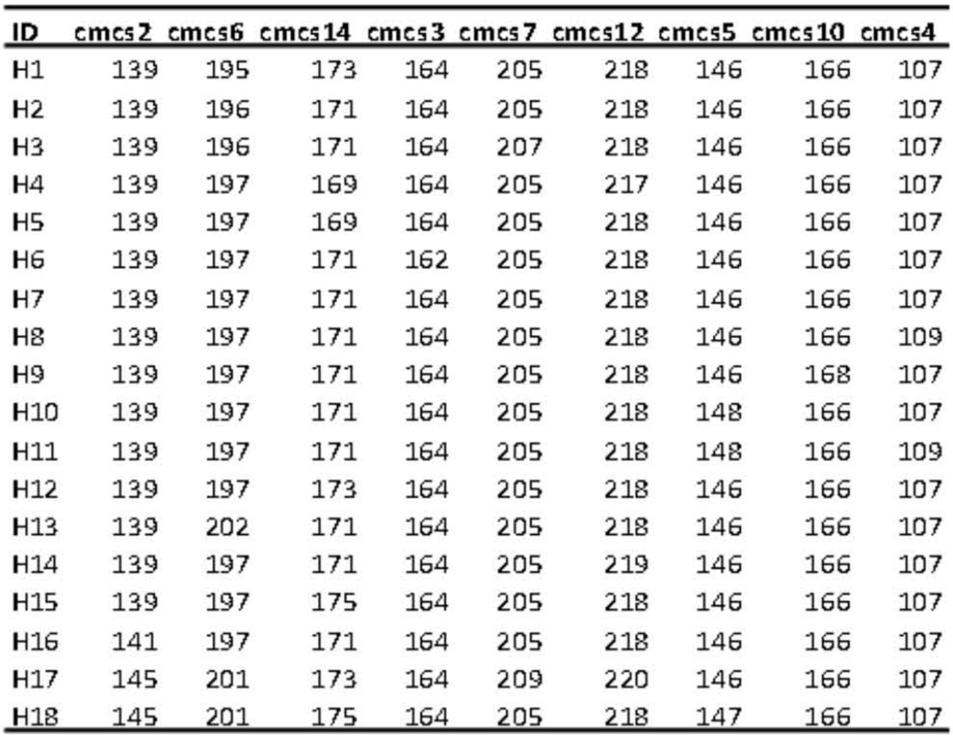
Table1. Population geographic information summary and genetic diversity and genetic structure estimates. n: sample size, h_S: within population genetic diversity, D^2SH, mean pairwise genetic distance among individuals within a population under a stepwise mutation model.

Id	Longitude	Latitude	Elevation (m.a.s.l)	n	Total haplotypes	Allelic richness (rarefacted)	h_S	D^2sh
1	-76.83	1.93	2591	8	2	1.55	0.18	0.01
2	-75.05	6.55	973	10	4	3.17	0.71	0.02
3	-75.86	5.2	1499	10	7	4.29	0.84	0.12
4	-75.65	6.61	2923	8	2	1.87	0.35	0.02
5	-75.62	4.55	1900	8	2	1.96	0.42	0.02
6	-75.64	5.56	2242	10	4	3.03	0.67	0.03
7	-75.32	6.78	2017	10	5	3.67	0.75	0.04
8	-77.26	1.33	2314	10	3	2.48	0.56	0.02
9	-77.15	1.38	1790	6	3	3	0.6	0.04
10	-76.74	3.32	1801	10	2	1.5	0.16	0.01
11	-73.65	5.45	2988	10	2	1.6	0.2	0.01
12	-75.86	5.55	1994	9	2	1.99	0.5	0.03
13	-76.79	2.01	2446	10	2	1.87	0.35	0.02
14	-76.1	1.63	1716	7	1	1	0	0
15	-75.49	6.21	2475	7	1	1	0	0
16	-75.7	4.33	2052	9	3	2.09	0.34	0.01
17	-75.56	4.65	1965	9	2	1.46	0.16	0.01
18	-76.08	6.42	2889	9	3	2.53	0.61	0.02
19	-73.46	5.71	2781	10	4	3.07	0.7	0.13
20	-73.15	5.95	3031	9	2	1.87	0.35	0.02
21	-75.51	6.93	2701	8	2	1.75	0.25	0.06

Table 2. Hierarchical analysis of molecular variance (AMOVA) using FST and RST for *Quercus humboldtii*. Groups correspond to the three Cordilleras in the Colombian Andes (see Fig. 1). * Significant values (p<0.001).

Source of variation	**d.f.**	**ss**	**Variance components**	**Percentage of variation**	**Fixation index**
			F_{ST}		
Among groups	2	8.59	0.01	0.73	ΦCT=0.007
Among populations within groups	10	61.19	0.17	10.1	ΦSC=0.101*
Within populations	184	315.48	1.53	89.17	ΦST=0.108*
Total	196	385.27	1.72		
			R_{ST}		
Among groups	2	0.87	0	0	ΦCT=0.001
Among populations within groups	10	14.51	0.05	14.36	ΦSC=0.141*
Within populations	184	60.02	0.29	87.41	ΦST=0.125*
Total	196	75.42	0.33		

Table S1. Haplotype list defined from each unique combination of size variants for the evaluated chloroplast microsatellite loci.



ID	cmcs2	cmcs6	cmcs14	cmcs3	cmcs7	cmcs12	cmcs5	cmcs10	cmcs4
H1	139	195	173	164	205	218	146	166	107
H2	139	196	171	164	205	218	146	166	107
H3	139	196	171	164	207	218	146	166	107
H4	139	197	169	164	205	217	146	166	107
H5	139	197	169	164	205	218	146	166	107
H6	139	197	171	162	205	218	146	166	107
H7	139	197	171	164	205	218	146	166	107
H8	139	197	171	164	205	218	146	166	109
H9	139	197	171	164	205	218	146	168	107
H10	139	197	171	164	205	218	148	166	107
H11	139	197	171	164	205	218	148	166	109
H12	139	197	173	164	205	218	146	166	107
H13	139	202	171	164	205	218	146	166	107
H14	139	197	171	164	205	219	146	166	107
H15	139	197	175	164	205	218	146	166	107
H16	141	197	171	164	205	218	146	166	107
H17	145	201	173	164	209	220	146	166	107
H18	145	201	175	164	205	218	147	166	107

Figure legends

Figure 1. *Quercus humboldtii* distribution represented by localities available with georeferenced information (white triangles) and populations included in this study (yellow triangles). Elevation of the Colombian Andes is represented in a grey scale from lowlands (white) to highlands (black). COc: Cordillera Occidental, CC: Cordillera Central, COr: cordillera oriental, CM: Macizo Colombiano (Colombian Massif).

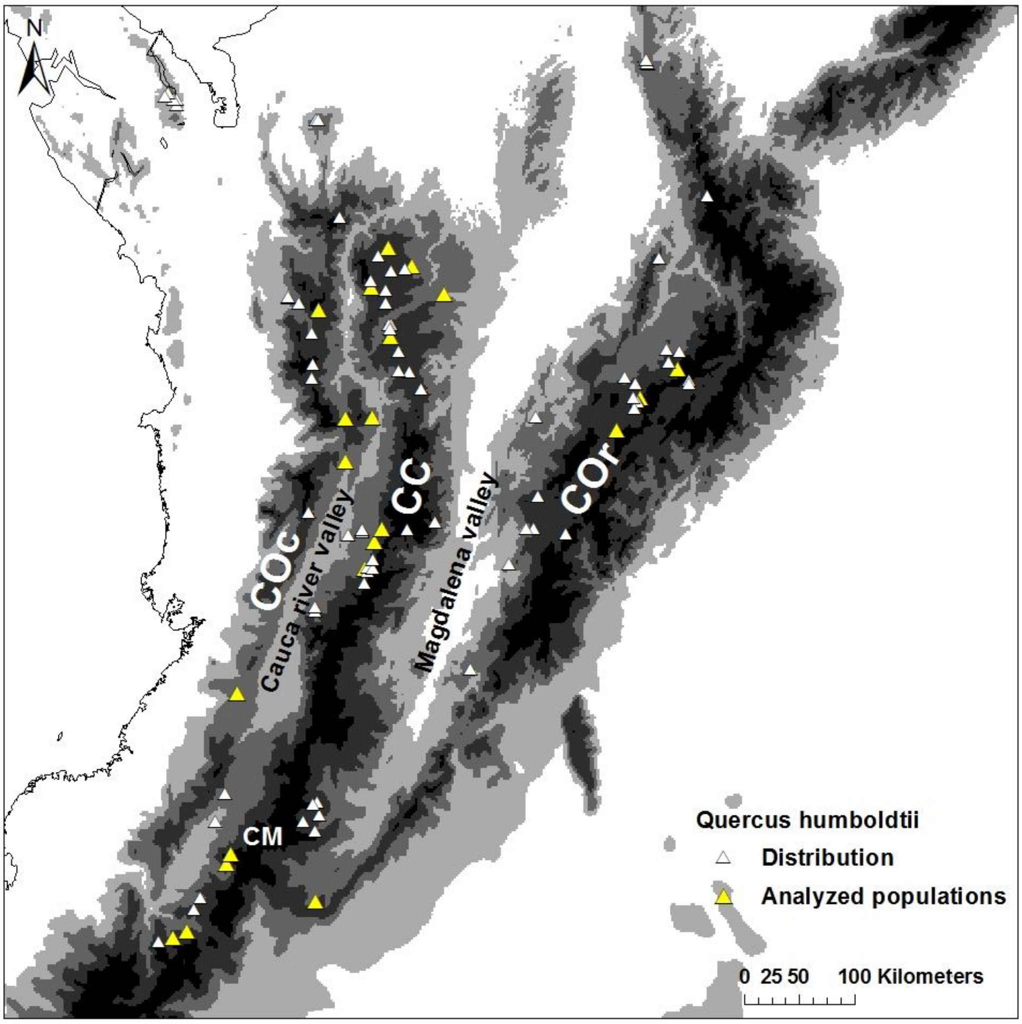


Figure 2. Distribution of the 18 haplotypes identified from cpSSRs and haplotype network inferred using median-joining in *Quercus humboldtii*. Name of the haplotype is coded as Hn followed by a number in parenthesis corresponding to the population for unique haplotypes. Purple circles represent unique haplotypes derived from H18, lima green circles represent unique haplotypes derived from H7 and orange circles represent unique haplotypes derived from H2. Elevation of the Colombian Andes is represented in a grey scale from lowlands (white) to highlands (black). COc: Cordillera Occidental, CC: Cordillera Central, COr: cordillera oriental, CM: Macizo Colombiano (Colombian Massif).

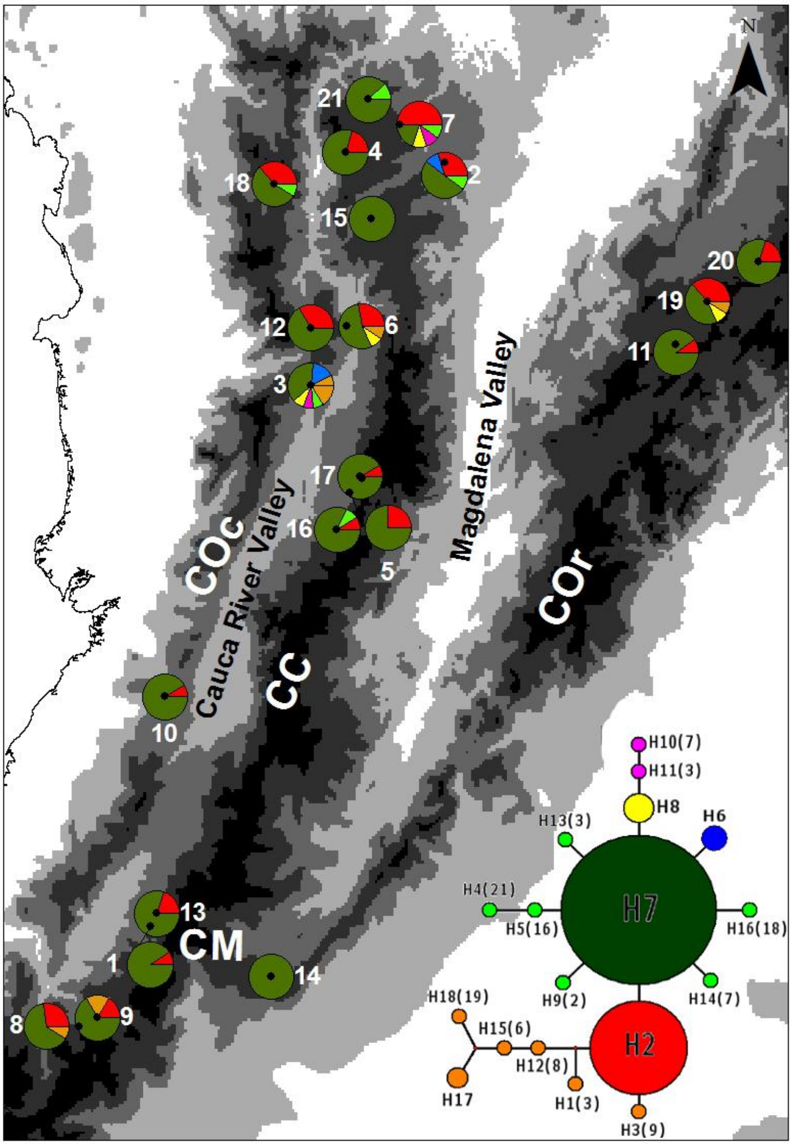
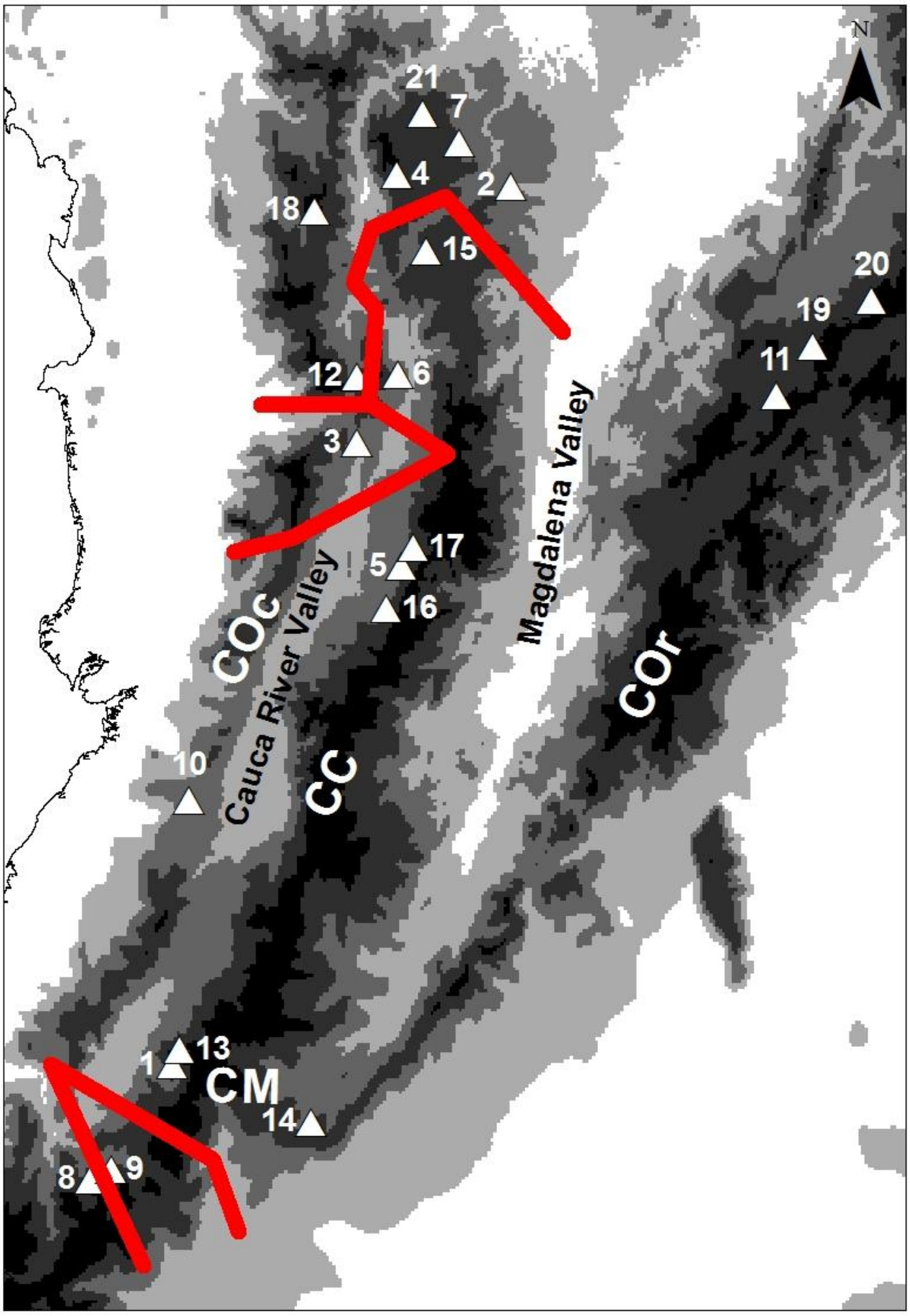


Figure 3. Geographical location of the most important genetic discontinuities (red lines) using the Monmomier's maximum difference algorithm for the *Quercus humboldtii* populations (white triangles). Elevation of the Colombian Andes is represented in a grey scale from lowlands (white) to highlands (black). COc: Cordillera Occidental, CC: Cordillera Central, COr: cordillera oriental, CM: Macizo Colombiano (Colombian Massif).



Discusión general y conclusiones

Los bosques de roble (*Quercus*: Fagaceae) son elementos clave de los ecosistemas Holárticos y Neotropicales, por sus niveles de diversidad biológica, dominancia ecológica y valor económico (Nixon 2006). Sumado a esta importancia, las diferentes especies que forman el género *Quercus* (y otros géneros de la familia Fagaceae como ha sido planteado por Petit et al. 2013) deben ser consideradas como elementos claves para reconstruir la historia evolutiva a nivel global, considerando que dichas especies han sido empleadas como grupos modelo para evaluar o bien, describir el efecto de cambios históricos asociados con fluctuaciones climáticas, dinámica tectónica o la heterogeneidad del paisaje con respecto a la historia de los linajes que habitan ecosistemas o regiones biogeográficas particulares, como es el caso de los sistemas montanos de los Andes Colombianos durante el cuaternario (Hooghiemstra & van der Hammen 2004) o las rutas de migración y formación de refugios durante el Pleistoceno en Europa (Dumolin-Lapègue et al. 1997).

Cuando se considera la región Neotropical (definida desde el extremo norte de la Zona de Transición Mexicana incluyendo el norte de la Sierra Madre Oriental y el norte de la Sierra Madre Occidental en México hasta el sur de la Zona de Transición Suramericana en la Provincia Monte), el género *Quercus* aparece como un grupo que potencialmente puede aportar información para describir y sustentar los patrones de distribución observados en diferentes taxa distribuidos en el extremo norte del Neotrópico, región que se caracteriza por tener niveles de biodiversidad abrumadores, incluyendo diversos puntos calientes de biodiversidad como Mesoamérica, parte importante del Chocó/Darién/Ecuador Occidental y parte de los Andes Tropicales (Myers et al. 2000).

A través de esta distribución las especies del género *Quercus* abarcan una gran variedad de ecosistemas pues cuenta con especies que se distribuyen desde México hasta Costa Rica a través de las zonas bajas como es el caso de *Q. oleoides* (desde los 150 hasta los 960 m.s.n.m; Valencia-A 2004), hasta especies con la misma distribución geográfica pero que se distribuyen en cinturones de vegetación montanos (entre los 1500 y los 2000 m.s.n.m como *Q. sapotiifolia* y *Q. Insignis;* Valencia-A 2004). Esta distribución altitudinal es aún más representativa si se consideran especies con una distribución más restringida como aquellas especies endémicas de México o bien aquellas especies que alcanzan el norte de Centroamérica. Allí la distribución de las especies de roble puede variar desde el nivel del mar hasta los 3500 m.s.n.m (Valencia-A 2004).

Los estudios de especies del género *Quercus* en esta región adquieren una importancia mayor cuando se consideran las investigaciones recientes que se han desarrollado en el norte del Neotrópico. En este sentido las revisiones de Bagley et al. (2014), Ramírez-Barahona & Eguiarte (2014) y Gutiérrez-García & Vázquez-Domínguez (2013), junto con los estudios recientes de Ornelas & Rodríguez-Gómez (2015), Rodríguez-Gómez & Ornelas (2015), González & Ornelas (2014), Ornelas & González (2014) y Ornelas et al. (2013), han ilustrado la gran variedad de patrones geográficos que explican la distribución de la variación genética y la demografía histórica de las especies que se distribuyen en la región norte del Neotrópico. Dentro de estos estudios es fundamental mencionar que las diferentes especies evaluadas sugieren la presencia de patrones comunes que explican la estructura filogeográfica de las especies Neotropicales asociados con elementos geológicos importantes como el IT, la zona de contacto entre las fallas Polochic-Motagua, el arco volcánico de Centroamérica, la DN y las cordilleras de Costa Rica y Panamá.

Dichos elementos geológicos como se pudo observar en el Capítulo Uno de la presente tesis representan zonas de gran importancia dentro de la distribución del género *Quercus* en el Neotrópico. El IT, la zona de contacto entre las fallas Polochic-Motagua y la DN son zonas donde la composición de especies de *Quercus* presenta valores de recambio mayores a los que se esperarían por la variación natural de los valores de diversidad. Sumado a lo anterior, zonas como las cordilleras de Costa Rica y Panamá representan sitios importantes para la distribución de áreas de endemismo del grupo. Aunque estos resultados representan la distribución actual de las especies de *Quercus* es necesario resaltar que esta distribución es el reflejo de los procesos históricos que han determinado los procesos de dispersión, flujo génico contemporáneo e histórico, diversificación y extinción de las especies de roble desde el centro y sur de México hasta los Andes Colombianos.

La idea de que la distribución actual de *Quercus* tiene una explicación histórica en el contexto de las barreras observadas que determinan cambios en la composición de especies, toma más fuerza cuando se consideran diferentes estudios filogeográficos de taxa Neotropicales. En este sentido, Ornelas et al. (2013) describen la convergencia de puntos geográficos (como el IT) donde especies diferentes (tanto de plantas, aves y mamíferos) con distribuciones en común presentan procesos de divergencia, pero con la característica de que dichos eventos tienen marcos temporales diferentes. Este comportamiento sugiere procesos múltiples de diversificación explicados por la dinámica tectónica y su efecto en la reducción de zonas altas a través del IT (Ornelas et al. 2013). Otros ejemplos de divergencia asociados a la presencia del IT en México también son reportados en diferentes grupos por Gutiérrez-García & Vázquez-Domínguez (2013). La importancia del IT en los procesos de diferenciación reportados en la revisión de Gutiérrez-García & Vázquez-Domínguez (2013) es tal, que dentro de los ejemplos que caracterizan el grupo Maya propuesto por las autoras, esta barrera es un elemento recurrente.

Una vez definido tanto el contexto biogeográfico y macroecólogico del género *Quercus* en el norte del Neotrópico como el marco conceptual para generar hipótesis y explicar los patrones filogeográficos de especies particulares del grupo, es posible complementar la historia de los encinos Neotropicales. De esta forma, el Capítulo Dos de la presente tesis ilustra como dos especies del género presentan respuestas complejas y en cierta medida contrastantes entre si cuando se describe su dinámica demográfica histórica y la distribución geográfica de la variabilidad genética. Las dos especies caracterizadas, *Quercus sapotiifolia* y *Q. insignis*, en primer lugar exhiben niveles de diversidad y estructura genética altos y similares a otras especies del género distribuidas en el Neotrópico (p. e. *Quercus castanea*, Peñaloza-Ramirez et al. e*nviado*; *Quercus crassifolia* x *Quercus crassipes*, Tovar-Sánchez et al. 2008; *Quercus affinis* x *Quercus laurina,* Ramos-Ortiz et al. *enviado*) y de igual forma, valores superiores a los reportados para la mayoría de especies de roble con una distribución Holártica (*Quercus robur*, *Q. petraea*, *Q. canariensis*, *Q. faginea* y *Q. pirenaica*, Grivet et al. 2006; *Quercus suber,* Magri et al. 2007; *Quercus garryana,* Marsico et al. 2009).

Referente a la distribución de la variación genética y la historia demográfica ambas especies muestran una afinidad Neotropical marcada, hecho particularmente claro para *Q. sapotiifolia,* donde la red de haplotipos sugiere un posible origen Centroamericano debido a la prevalencia de haplotipos frecuentes y distribuidos de forma amplia entre Guatemala y Honduras. Aún más interesante es el hecho de que las redes de haplotipos para las dos especies sugieren procesos de dispersión y re-colonización desde el centro de Centroamérica (Guatemala y Honduras) tanto hacia el sur de México, como hacia la zona montañosa de Costa Rica. Análisis complementarios también muestran como las barreras identificadas a nivel biogeográfico y macroecológico determinan la distribución de la variación genética (Istmo de Tehuantepec y Depresión de Nicaragua en el caso de *Q. insignis;* zona de contacto entre la falla Polochic-Motagua y arco volcánico de Centroamérica en *Q. sapotiifolia*).

Como se explica en el Capítulo Dos estos patrones de distribución de la variación genética son consistentes con la historia geológica compleja y heterogénea de Centroamérica, donde se destacan eventos tectónicos constantes (Marshall 2007), actividad volcánica reciente (Marshall 2007), fluctuaciones climáticas históricas importantes y heterogéneas (Islebe & Hooghiemstra 2006). Sin embargo, aunque ambas especies tienen cierto grado de co-distribución (habitan áreas cercanas pero rara vez se encuentran en simpatría) existe una diferencia en la distribución de la variación genética entre ambas. Dicha diferencia se refleja en las barreras al flujo génico observadas. En el Capítulo Dos se propone que dicha variación se puede atribuir a características intra-específicas relacionadas con la capacidad de dispersión, como el tamaño de las bellotas y la distribución altitudinal entre las especies. Esta variación entre especies apoya observaciones previas donde se atribuyen patrones contrastantes de distribución de la variación genética a procesos específicos para cada taxa (Ornelas et al. 2013; Ramírez-Barahona & Eguiarte 2013).

Hasta este momento los datos obtenidos y discutidos en los capítulos Uno y Dos reflejan dos patrones de distribución geográfica de las especies y su variación genética. En primer lugar está el cambio latitudinal tanto de la composición de las especies como de la estructura genética intra-específica y la influencia de los componente geológicos como el IT en ambos aspectos; en segundo lugar los patrones de variación genética observados en las redes de haplotipos en ambas especies (*Q. insignis* y *Q. sapotiifolia*), siguieren procesos de dispersión y colonización tanto hacia México como hacia Costa

Rica desde el centro de Centroamérica (Honduras y Guatemala). Estos procesos de dispersión y colonización se repiten de forma consistente en ambas especies e incluso sugieren la posibilidad de procesos de re-colonización desde Costa Rica hacia Honduras. De este patrón observado es posible plantear la hipótesis de que el cambio geográfico en la estructura genética está asociado o bien se expresa durante periodos diferentes como ha sido observado por Ornelas et al. (2013) al comparar diferentes taxa co-distribuidos. Sin embargo, a diferencia de los casos reportados por el autor, en nuestro sistema de estudio los cambios demográficos históricos asociados a puntos geográficos específicos podrían variar incluso dentro de una misma especie en periodos distintos.

La caracterización filogeográfica de las dos especies de encino con una distribución regional (cordilleras de Costa Rica; Capítulo Tres) refuerza patrones observados previamente y arroja resultados novedosos que incrementan el grado de complejidad de la historia evolutiva de las biotas Neotropicales. En este sentido, *Q. Costaricensis* a pesar de tener una distribución restringida al sur de Centro América, presentó una estructura filogeográfica bien definida a través de los bosques montanos altos. Esta estructuración responde a niveles moderados de flujo génico en sentido norte-sur influenciados por cambios en la distribución altitudinal de la especie durante la transición entre periodos glaciales e integlaciales del Cuaternario descrita por Islebe & Hooghiemstra (2006) para Costa Rica. Lo anterior evidencia que aún a escalas regionales los procesos físicos históricos pueden configurar cambios en la historia demográfica de las especies del género *Quercus*. Este patrón resalta la importancia de estudios regionales y contradice la tendencia de restringir la importancia del sur de Centro América a un punto de tránsito entre las migraciones bidirecionales entre el Neartíco y el Neotrópico. Por su parte, *Q. bumelioides* exhibe un patrón opuesto caracterizado por la ausencia de estructura genética y filogeográfica, evidencia de expansión poblacional reciente, niveles altos de diversidad genética y flujo génico asociados a la distribución en los bosques montanos bajos que caracteriza a *Q. bumeliodes*. Estos patrones (al menos a nivel demográfico y filogeográfico) observados son más afines a *Q. humboldtii* que a las demás especies del género estudiadas en el Neotrópico.

Sumado a estos dos ejes de variación, la latitud y el tiempo, en la presente tesis se describe una variable más que influye de forma importante en la distribución de la variación genética, la elevación. En este sentido en el Capítulo Cuatro se evaluaron los cambios en la distribución de *Quercus humboldtii* en los Andes Colombianos durante el UGM considerando estudios paleoecológicos para los bosques montanos desarrollados por Hooghiemstra & van der Hammen (2004). Los resultados obtenidos sugieren que las poblaciones de *Q. humboldtii* en los Andes Colombianos presentan una estructura altitudinal bien definida. En términos generales las poblaciones de roble no muestran diferencias importantes en la extensión del nicho climático disponible, pero al considerar las diferencias entre cinturones de vegetación fue posible identificar un incremento en el nicho disponible para los bosques montanos altos y reducciones en el nicho disponible para el bosque montano bajo y los bosques tropicales de zonas bajas. Aparentemente, los cambios en la distribución de los bosques de roble en los Andes Colombianos reflejan una migración altitudinal importante, donde las condiciones climáticas se desplazaron hacia las zonas bajas sin generar cambios importantes en la distribución.

A partir de estos resultados es posible establecer que los bosques de *Q. humboldtii* no siguen un modelo de reducción y aislamiento en parches durante el Pleistoceno como se ha propuesto por diferentes autores (Haffer 1969; Toledo 1982;

Carnaval & Moritz 2008; Carnaval et al. 2009; de Mello-Martins et al. 2011). En cambio, los patrones observados de distribución coinciden con un escenario de bosques continuos a través de los ciclos glaciales e interglaciales con eventos de migración altitudinal como el descrito por Farrera et al. (1999), Hoestler & Mix (1999) y Caballero et al. (2010). Ambos escenarios de respuesta ante las fluctuaciones climáticas durante el Pleistoceno han sido revisadas recientemente por Ramírez-Barahona & Eguiarte (2013) haciendo énfasis en los bosques Mésofilos y la información filogeográfica disponible para especies de estos bosques. Los autores sugieren que existe falta de evidencia contundente para definir la validez de los escenarios recién mencionados, por lo que sugieren la combinación de información ecológica, genética y paleoecológica para generar información más contundente.

Una vez definido el escenario biogeográfico de *Q. humboldtii* durante los periodos de cambio climático durante el Pleistoceno (al menos para el UGM), la misma especie fue caracterizada a nivel filogeográfico con el fin de describir los procesos demográficos históricos que determinaron la distribución actual de la especie (Capítulo Cinco). En este sentido los resultados obtenidos para *Q. humboldtii* sugieren la ausencia de estructura filogeográfica, mientras que la red de haplotipos muestra un patrón en forma de estrella que refleja una expansión poblacional reciente producto de un cuello de botella anterior, debido bien a un origen reciente de la especie o a la colonización reciente de *Q. humboldtii* de los Andes Colombianos. Estos resultados son consistentes con la aparición de esta especie en los registros palinológicos de los Andes hace aproximadamente 480 ka BP, fecha a partir de la cual se generaron cambios importantes en la composición de los cinturones de vegetación montanos (Van´t veer & Hooghiemstra 2000; Hooghimestra & van der Hammen 2004).

De igual forma los análisis demográficos sugieren que las poblaciones de *Q. humboldtii* presentaron una expansión poblacional reciente datada hace aproximadamente 11 *ka BP* a finales del Pleistoceno e inicio del Holoceno. A partir de esta información es posible establecer que los cambios climáticos asociados a las transiciones entre periodos glaciales e interglaciales tuvieron un efecto importante para la dinámica demográfica histórica de esta especie. Al analizar los cambios en la diversidad genética, fue posible identificar que las poblaciones de la especie presentan valores más altos de diversidad genética hacia las zonas bajas, la cual se reduce de forma importante y significativa cuando se consideran las poblaciones de los cinturones de vegetación más altos. El efecto moderado o nulo en términos de conectividad y asilamiento debido a fluctuaciones climáticas y los niveles de diversidad altos hacia las zonas bajas es consistente con la idea de condiciones húmedas durante el último glacial máximo las cuales favorecieron distribuciones estables y continuas (Farrera et al. 1999, Hoestler & Mix 1999 y Caballero et al. 2010).

Los resultados obtenidos en el Capítulo Cuatro complementan los resultados obtenidos en la caracterización filogeográfica de *Q. humboldtii* en la medida que no existe evidencia de un incremento general del nicho climático disponible durante el UGM. De igual forma, además de la estabilidad del área disponible, los modelos de nicho ecológico muestran un desplazamiento de los cinturones de vegetación hacia las zonas bajas lo que favoreció un paisaje conectado que pudo fomentar el flujo génico y los bajos de niveles de diferenciación genética observados en los robles Andinos. De forma complementaria, los análisis genéticos y biogeográficos de los Capítulos Dos y Cuatro están respaldados por diferentes observaciones palinológicas (Marchant et al. 2002, Wilee et al. 2001, Wille et al. 2000, Helmens et al. 1996, Hooghiemstra & Ran 1994, Hooghiemstra & Van der Hammen 1993, Helmens & Kuhry 1986), dentro de las cuales se destacan observaciones de polen proveniente de *Quercus* como un elemento dominante durante el Holoceno (van der Hammen & Hooghiemstra 2003).

En términos generales la presente tesis presenta un panorama biogeográfico y filogeográfico considerable del género *Quercus* en en Neotrópico. Ambos capítulos biogeográficos (Capítulo Uno y Cuatro) presentan evidencia de la importancia que ha tenido la dinámica de variables físicas como la geografía y el clima tanto en la estructura de las comunidades de los encinos en el Neotrópico a nivel latitudinal (escala amplia), como a nivel intra-específico en un gradiente altitudinal (escala local). Por su parte, los resultados obtenidos en la caracterización filogeográfica de las cinco especies Neotropicales del género *Quercus* (Capítulos Dos, Tres y Cinco) sugieren que aún ante la presencia de patrones biogeográficos comunes para el género *Quercus*, la respuesta de las especies por separado a cambios en las condiciones geológicas (p. e. eventos tectónicos y volcánicos) y climáticas históricas (p. e. fluctuaciones durante el Cuaternario) pueden diferir aún entre especies co-distribuidas. Estas diferencias observadas sugieren que además de considerar los diferentes escenarios geológicos y las diferencias ecológicas de las especies que determinan el flujo génico (como su distribución altitudinal y fenología) para entender los patrones filogeográficos y demográficos históricos de las especies del género *Quercus*, es fundamental tener un marco filogenético que permita evaluar si las diferencias en los tiempos de diversificación y especiación para los encinos Neotropicales explican las diferencias observadas en los tiempos de expansión poblacional, diversidad genética y estructura filogeográfica. La presencia de expansiones poblacionales recientes, cambios en la distribución durante periodos glaciales e interglaciales, evidencia de colonizaciones múltiples y re-colonización, podrían evidenciar procesos de re-distribución de poblaciones durante el Cuaternario como lo describen Bennet et al. (2012), mientras que procesos como diversificación, divergencia de linajes y especiación posiblemente se presentaron durante etapas previas (Bennet et al. 2012) como el Plioceno, tal como lo sugieren Bagley & Jhonson (2014) para otros taxa Neotropicales.

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Anexo

CHICAGO JOURNALS

How Are Oaks Distributed in the Neotropics? A Perspective from Species Turnover, Areas of Endemism, and Climatic Niches
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HOW ARE OAKS DISTRIBUTED IN THE NEOTROPICS? A PERSPECTIVE FROM SPECIES TURNOVER, AREAS OF ENDEMISM, AND CLIMATIC NICHES

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Premise of research. The most important diversity hot spot of genus *Quercus* (Fagaceae) in America is situated in southern Mexico. From this area down to the Colombian Andes, oak species diversity decreases considerably, but the pattern of species distribution and turnover has not been analyzed. This study aimed at determining geographical patterns of species turnover, species distribution, and endemism for Neotropical *Quercus* species.

Methodology. Occurrence records for 58 oak species belonging to the *Quercus* and *Lobatae* sections were obtained. Patterns of species turnover were determined by comparing species composition among latitudinal/longitudinal units. Areas of endemism were determined using weighted networks. The potential distribution of oak species was determined using ecological niche models. Finally, a principal component analysis was used to identify changes in the oak species' ecological niche across areas.

Pivotal results. The species composition analysis indicated that the Tehuantepec Isthmus, the Nicaraguan Depression, and the Panamanian Isthmus represent species turnover points. Nine areas of endemism were recovered, distributed through mountainous ranges from Mexico to Costa Rica. Most of these areas were delimited by the species turnover points detected. Ecological niche modeling indicated that the turnover points represent areas with low climatic suitability for most oak species and represent discontinuities in the distribution of *Quercus*. Niche comparisons suggest niche differentiation among species distributed in different areas of endemism or on opposite sides of turnover points.

Conclusions. The results indicate that the Tehuantepec Isthmus, the Nicaraguan Depression, and the Panamanian Isthmus have acted as important barriers to the dispersal of oak species, influencing species diversity, biogeographic patterns, and niche divergence.

Keywords: biogeography, distribution, diversity, Neotropical trees, *Quercus*.

Online enhancements: appendix tables.

Introduction

The area between the tropics of Mexico and the Colombian Andes is characterized by a high biological diversity and includes three of the most important global biodiversity hot spots (Mesoamerica, Chocó/Darién, and the Tropical Andes; Myers et al. 2000). This area is very important biogeographically and in terms of conservation of biodiversity due to various reasons, including (i) it represents a transition zone between the Neotropical biota and the Nearctic biota (Morrone 2010); (ii) within this area, a great number of animal and plant taxa have experienced processes of diversification, extinction, and migration (Gutiérrez-García and Vázquez-Domínguez 2013); and (iii) this area is undergoing exceptional loss of habitat (Myers et al. 2000).

Oak species (*Quercus*: Fagaceae) are widely distributed within the Neotropics, ranging from the north of the Mexican Transition Zone (MTZ; sensu Morrone and Márquez 2001) down to the south of the Colombian Andes. This genus has high levels of diversity in the southeastern United States. However, the greatest oak species diversity occurs in the mountains of southern Mexico (Nixon 2006), although Valencia-Ávalos (2004) pointed out that the central and northeastern regions

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of the country also bear considerable numbers of species. In Central America, although oak species richness is lower than in Mexico, *Quercus* occupies the second place of importance in terms of richness in the montane forests.

The taxonomy and species richness of *Quercus* L. in the American territory have been continuously revised topics over the years, particularly for the Mexican region (Nixon 1997; Valencia-Ávalos 2004). The reviews on the subject indicate that the total number of species in America (distributed in North America, Central America, and the northern portion of South America) is around 220, distributed from Canada and the United States with 4 and 90 species, respectively, through Mexico with approximately 161 species (Valencia-Ávalos 2004), Central America with about 40 (Nixon 2006), and Colombia, where there is only a single species, *Q. humboldtii* (Pulido et al. 2006).

Nixon (2006) described these changes in the richness of *Quercus* and the variation in phenotypic characteristics associated with the latitudinal range of Neotropical oak species. These patterns represent an interesting topic considering geological and biological processes in Mexico and Central America, including (i) the formation of a transition zone between the Nearctic biota and the Neotropical biota as a result of the lifting of the Panamanian Isthmus (Panama, Costa Rica, and southern Nicaragua) and (ii) geological activity at morphotectonic provinces such as the Trans-Mexican Volcanic Belt and the Tehuantepec Isthmus in Mexico, the Nicaraguan Depression, and the mountains of Costa Rica. The first event caused a bidirectional biotic exchange that occurred from the Eocene to the present, reaching a peak during the Pliocene and the Quaternary (Webb 1991). During this period, specifically at 470 ka BP, Nearctic elements such as *Quercus* appeared in the fossil record at the north of the Andes (Van't Veer and Hooghiemstra 2000). The second set of events promoted a new landscape configuration, the product of a constant and recent volcanic and geologic activity that could have favored the expansion of various lineages in a latitudinal gradient, the formation of barriers to dispersal, and local diversification and extinction processes (Cavender-Bares et al. 2011; Ornelas et al. 2013).

Patterns of change in species diversity and species composition for Neotropical *Quercus* species have been considered obvious. Most of the studies examining the diversity of oak communities have focused on describing or commenting on the effect of altitudinal gradients on community composition and structure (Kappelle 1996; Kappelle and Uffelen 2006; Luna-Vega et al. 2006) and on the relationship between the diversity of oak species and elevation and/or latitude at the regional level (e.g., Valencia Ávalos 2004). Recently, Torres-Miranda et al. (2011, 2013) described the biogeography of the *Lobatae* section in Mexico and Central America. These studies identified as centers of species richness, with high levels of endemism, the north of the Sierra Madre Oriental and the southern foothills of Jalisco (both located in Mexico) and proposed to redesign the protected-areas system to ensure the conservation of the *Lobatae* key elements. However, the distribution patterns for the whole *Quercus* genus within the Neotropics have not been analyzed, particularly in regard to how species composition changes across the region.

Recent advances in the development of spatial analysis tools, geographic information systems, species distribution modeling, and the availability of information on the occurrence of species at both global (e.g., Global Biological Information Facility) and regional (e.g., Biological Information System [SIB], Colombia; Biodiversity National Institute [INBio], Costa Rica; National Commission for Knowledge and Use of Biodiversity [CONABIO], Mexico) databases allow us to apply various analyses of distribution patterns in terms of ecology and biogeography from local to regional scales. Fortunately, the genus *Quercus* is significantly represented in these databases and herbarium collections, a fact that allowed us to propose a broadscale analysis. Therefore, we have set as the main goal of this article to analyze distribution patterns of Neotropical *Quercus* species using macroecological and biogeographic approaches, including (i) the identification of areas of particularly high species turnover across the Neotropics, (ii) the determination of areas of endemism for the genus and their possible relationship with the areas of species turnover, (iii) the evaluation of climatic niche differences across areas of endemism, and (iv) the modeling of the potential distribution and *Quercus* species co-occurrence patterns.

Material and Methods

Study Area

The study area (fig. 1) was delimited to the north by the MTZ sensu Morrone and Márquez (2001) and Morrone (2001, 2006) and to the south by the Colombian Andes. These edges encompass the distribution area of most of the oak species within the Neotropics.

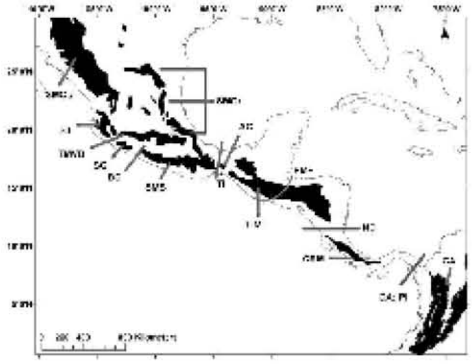

Fig. 1 Study area and its principal geological elements. TMVB = Trans-Mexican Volcanic Belt; SMOc = Sierra Madre Occidental; SMOr = Sierra Madre Oriental; SJ = Serranías de Jalisco; SG = Serranías de Guerrero; SMS = Sierra Madre del Sur; AC = Altos de Chiapas; TI = Tehuantepec Isthmus; TIM = Trans-Isthmian Mountains; PMF = Polochic-Motagua Fault; ND = Nicaraguan Depression; CRM = Costa Rican mountains; PI = Panamanian Isthmus; DAR = Darién region; CA = Colombian Andes. Black areas represent mountainous systems (>1000 m asl).

Species Studied and Occurrence Records

The oak species distributed in the study area (table A1; tables A1–A3 available online) were defined using taxonomical databases (Flora Mesoamericana Project, Missouri Botanical Garden; available at http://www.tropicos.org/Project/FM) and reviews of the genus (Valencia Ávalos 2004; Morales 2010). Geographical information was compiled using oak species presence records reported on INBio (available at http://atta.inbio.ac.cr), the Missouri Botanical Garden Tropicos data sets (available at http://www.tropicos.org/Home.aspx), and data published by Herbario del Instituto de Ecología, A.C., MEXU/Tipos de Plantas Vasculares, Catálogo de Autoridad Taxonómica del Género *Quercus*, Fagaceae en México, Herbarium of the New York Botanical Garden, and Instituto Alexander von Humboldt through the GBIF data portal (available at http://www.data.gbif.org). This information was complemented with our own database, compiled from specimens deposited in several Mexican herbaria and field observations.

Sections *Quercus* and *Lobatae* differ in their distribution (*Lobatae* reaches the Colombian Andes, while the southernmost distribution limit for *Quercus* is in Panama) and phenological traits (e.g., annual acorn maturation vs. biannual acorn maturation in white oaks and red oaks, respectively). In view of such differences, turnover and niche distribution analyses were performed considering the genus *Quercus* as the study unit but also for both sections separately.

Species Turnover

The study area was divided separately in two ways: in rectangles of a latitudinal degree of height spanning from 0° to 30°N and in rectangles of a longitudinal degree of width from −117° to −75°W. Similar subdivisions have been used in studies of avifauna in humid montane forests along the Neotropical region (Sánchez-González and Navarro-Sigüenza 2009). Species turnover was calculated using the dissimilarity index (βsim) proposed by Lennon et al. (2001) for each latitudinal or longitudinal rectangle relative to its upper/lower or left/right neighbors for the whole genus and separately for the *Quercus* and *Lobatae* sections. This turnover index quantifies the relative magnitude of species gain and loss relative to the minimum value of species richness. Therefore, it allows identifying changes in the composition or species richness in relation to the unit with the lower richness value. The mean and standard deviation (SD) were estimated for 1-βsim values across all study units. Units showing 1-βsim values beyond 1 SD of the mean were considered as areas of atypically high species turnover.

Areas of Endemism

In order to determine the geographical association of the Neotropical oak species in terms of co-occurrence patterns, the network analysis method (NAM; Dos Santos et al. 2008, 2012) was employed. NAM uses species' punctual records directly and is different from the traditional procedures (such as the ones described by Szumik et al. 2004) in which sympatry is determined by overlapping species using grids of square cells of an arbitrary size delimited a priori. As with NAM no grids or polygons are needed; the uncertainty about the appropriate dimension of study units is not a major topic (Dos Santos et al. 2012). After creating sympatry networks, NAM identifies clusters of cohesively sympatric species that are simultaneously allopatric with other clusters. NAM was implemented using the software SyNet 2.0 (available at http://www.cran.r-project.org; Dos Santos et al. 2008), which is an add-on package for the statistical software R, using standard parameters. Once the cleavogram was obtained, branches were selected using the backward search, as our interest was to recover the smallest sympatry areas. To evaluate whether, besides barriers to dispersal, niche divergence resulting from local adaptation has also played a role in delimiting the areas of endemism, we conducted niche comparisons among the groups of species that defined the areas of endemism. Climatic data associated with species records were subjected to a principal component analysis (PCA) and plotted to visualize climatic variation across the identified areas.

Ecological Niche Modeling (ENM)

ENM was used to define environmental affinities among areas where *Quercus* species are present and to identify the location of possible gaps in their distribution. For this goal, we used the maximum entropy algorithm implemented in MAXENT, version 3.3.3a (Phillips et al. 2006), using default parameters. In order to avoid overfitting due to correlation between climatic variables, within the distribution range of each oak species, between 500 and 2500 random points were calculated, and the values corresponding to the 19 climatic variables reported by Hijmans et al. (2005) were extracted at a 30-arc second (~1-km) spatial resolution (available at http://www.worldclim.org). For the data of each species, correlation matrices were calculated among all 19 variables, and from each pair of highly correlated variables ($r > 0.7$), the more specific variable was discarded. Additionally, the soil type (FAO-UN digital soil map of the world; available at http://www.fao.org) and elevation variables were also considered as scenopoetic data to construct the ENMs (table A2).

Considering that not all oak species had a sufficient number of records, only 43 species were used for this analysis (species over 30 occurrences). Occurrence records of each species were filtered altitudinally and latitudinally by comparison with reported distribution information (following Valencia Ávalos 2004; Morales 2010). In order to decrease possible effects of spatial autocorrelation due to proximity and aggregation of records, we used only points separated by more than 0.1 decimal degrees with respect to their nearest neighbors. Finally, ENMs were restricted to the biogeographic provinces where oak species are present in order to avoid including possible climatically suitable areas that *Quercus* species have not occupied historically (e.g., the Yucatán Peninsula).

ENMs were implemented using the bootstrap resampling method with 50 replicas. From the initial data set, 30% of the presence records were used as a subrun to calculate various estimates of quality, and the remaining 70% were used to run the models. Models were initially evaluated with a threshold-independent method, the receiver operating characteristic curve analysis, to determine model quality. As a threshold-dependent method, we implemented the intrinsic omission rate, using the cumulative value of 10%. This threshold was selected considering that databases could include several erroneous occurrence

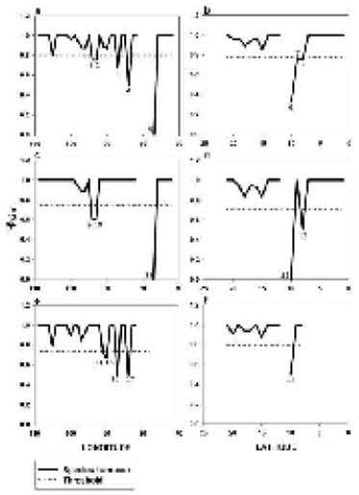

Fig. 2 Neotropical *Quercus* species turnover patterns. The horizontal dotted lines represent the threshold of similitude values beyond the expected variation. *a*, Longitudinal turnover pattern for the whole genus. *b*, Latitudinal turnover pattern for the whole genus. *c*, Longitudinal turnover pattern for red oaks (sect. *Lobatae*). *d*, Latitudinal turnover pattern for red oaks (sect. *Lobatae*). *e*, Longitudinal turnover pattern for white oaks (sect. *Quercus*). *f*, Latitudinal turnover pattern for white oaks (sect. *Quercus*). Numbers indicate the turnover points (for geographical location abbreviations, see fig. 1) as follows: 1, 2 = TI; 3 = PMF; 4 = ND; 5 = PI; 6 = ND; 7 = CRM; 8 = PI; 9, 10 = ND; 11 = PI; 12 = ND; 13 = PI; 14, 15 = AC; 16 = PMF; 17, 18 = ND.

records even after extensive depuration processes. Using the sum of the binomial outputs generated, considering the threshold rule mentioned above, we identified the areas with a low number of potentially co-occurring species (low-suitability areas) that separate regions with a high number of co-occurring species (high-suitability areas). Additionally, the values of the climatic variables used to build the ENMs were compared among low- and high-suitability areas using an analysis of variance implemented in R to identify the most important climatic factors that limit the distribution of oak species.

Results

Species Turnover

Species turnover values exceeding the defined threshold of 1 SD of the 1-βsim mean value were observed in both latitudinal gradients and longitudinal gradients at the whole-genus level and also for the *Quercus* and *Lobatae* sections separately (fig. 2). Species composition changes (figs. 2*a*, 2*b*, 3*A*, 3*B*) at the genus level were observed in the Tehuantepec Isthmus (TI), the Motagua-Polochic fault (MPF), the Nicaraguan Depression (ND), and the Panamanian Isthmus (PI).

Red oak species (*Quercus* sect. *Lobatae*; figs. 2*c*, 2*d*, 3*C*, 3*D*) showed a similar pattern of turnover areas. The TI appears as the first turnover area, followed by the ND and finally the PI. Species turnover values for *Quercus* sect. *Quercus* (figs. 2*e*, 2*f*, 3*E*, 3*F*) indicated that the limit between the Altos de Chiapas (AC) and the Trans-Isthmian Mountains (TIM) is the area where the first atypically high species turnover occurs. At the MPF region, there is a second area of species turnover where several species with wide latitudinal distributions reach their southern distribution limit. Finally, the ND is the last turnover area for white oaks. The PI did not appear as a turnover area, as no white oak species reach the Colombian Andes.

Areas of Endemism

Nine network partitions or units of co-occurrence (UCs) were recovered in the cleavogram derived through NAM. These are shown in figure 4: a, supported by four species (*Q. martinezii*, *Q. nixoniana*, *Q. salicifolia*, and *Q. uxoris*) distributed in the north of the Sierra Madre del Sur (SMS), Serranías de Guerrero, and Serranías de Jalisco (fig. 4*A*); b, supported by three species (*Q. deserticola*, *Q. frutex*, and *Q. rugosa*) distributed in the Sierra Madre Occidental (SMOc),

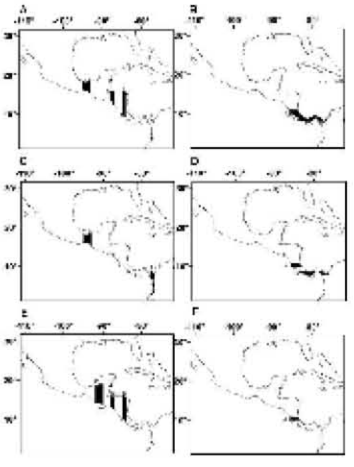

Fig. 3 Geographical location of the main turnover points for *Quercus* species. Black bars represent the longitudinal and latitudinal units where marked species turnover occurs (see also fig. 2). *A*, Longitudinal turnover pattern for the whole genus. *B*, Latitudinal turnover pattern for the whole genus. C, Longitudinal turnover pattern for red oaks (sect. *Lobatae*). *D*, Latitudinal turnover pattern for red oaks (sect. *Lobatae*). *E*, Longitudinal turnover pattern for white oaks (sect. *Quercus*). *F*, Latitudinal turnover pattern for white oaks (sect. *Quercus*). Numbers correspond to those shown in fig. 2 for values of 1-βsim and correspond to the following areas (for geographical location abbreviations, see fig. 1): 1, 2 = TI; 3 = PMF; 4 = ND; 5 = PI; 6 = ND; 7 = CRM; 8 = PI; 9, 10 = ND; 11 = PI; 12 = ND; 13 = PI; 14, 15 = AC; 16 = PMF; 17, 18 = ND.

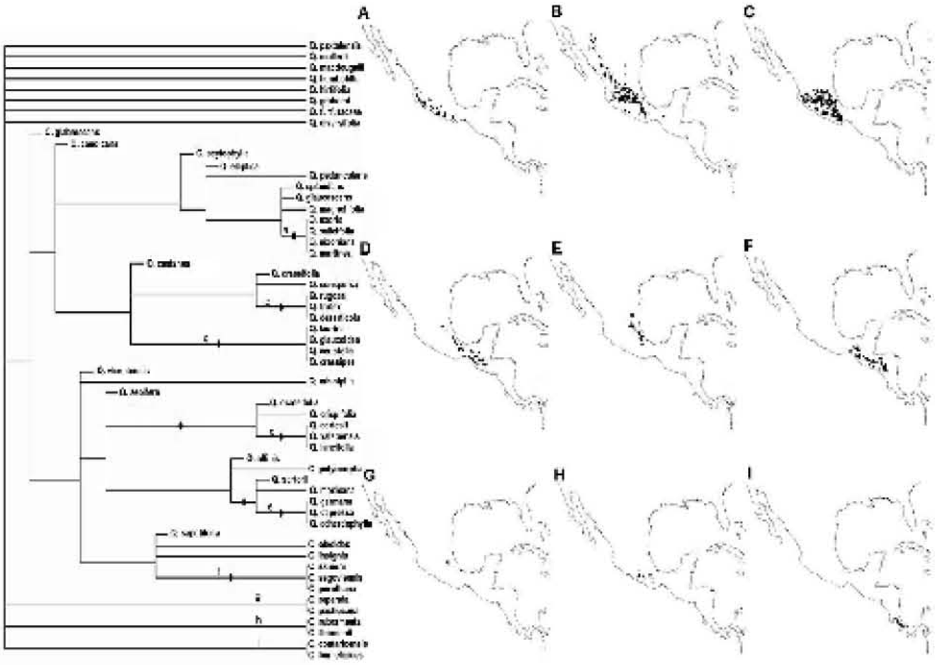

Fig. 4 Cleavogram representing units of co-occurrence (or areas of endemism) for Neotropical oak species estimated using the network analysis method. Letters from a to i indicate each of the identified units of co-occurrence, and the maps on the right side indicate the geographical distribution of the species groups that constitute each unit of co-occurrence.

Trans-Mexican Volcanic Belt (TMVB), and Altos de Chiapas (AC; fig. 4*B*); c, supported by four species (*Q. acutifolia*, *Q. crassipes*, *Q. glaucoides*, and *Q. laurina*) distributed in the TMVB, southern Sierra Madre Oriental (SMOr), and SMS (fig. 4*C*); d, supported by three species (*Q. cortesii*, *Q. lancifolia*, and *Q. xalapensis*) distributed in the SMC, southern SMOr, and north of the TIM (fig. 4*D*); e, supported by three species (*Q. acherdophylla*, *Q. depressa*, and *Q. germana*) distributed in the SMOr (fig. 4*E*); f, supported by three species (*Q. segoviensis*, *Q. skinerii*, and *Q. purulhana*) distributed in the TIM (fig. 4*F*); g, supported by two species (*Q. pachucana* and *Q. repanda*) distributed in the eastern TMVB (fig. 4*G*); h, supported by two species (*Q. liebmanii* and *Q. rubramenta*) distributed in the SMS (fig. 4*H*); i, supported by two species (*Q. bumelioides* and *Q. costaricensis*) distributed in the Costa Rican mountains (fig. 4*I*). It can be observed that the distribution of all the UCs were delimited at least at one edge by the turnover points described above as follows: UCs a, b, c, e, and h are delimited by the TI at the south; UC d is delimited by the MPF at the south; UC f is delimited by the TI at the north and the ND at the south; and UC i is delimited by the ND at the north and the PI at the south. The PCA (table 1; fig. 5) indicated that groups of species that defined most of the UCs have partially overlapping climatic niches. In particular, UCs b and c have wide and overlapping climatic envelopes that also contain the relatively narrower envelopes of UCs g and h. A second recognizable group was formed by UCs a, e, and f. Finally, UCs d and i seem to be the most distinct in terms of their climatic niches.

ENM

The models for all the species evaluated showed a good performance (AUC values >0.89). For most oak species, the climatic variables with the highest influence on the ENMs were annual mean precipitation, temperature seasonality, temperature annual range, annual precipitation, and precipitation seasonality (table A1). The map with the sum of the models for all individual species shows the gaps in the *Quercus* species distribution (fig. 6*A*). In the northern part of the studied region, high levels of *Quercus* species co-occurrence are observed in mountainous areas of central and southern Mexico, particularly, the TMVB and the SMS. The Balsas River Depression is an area with low presence of *Quercus* species that separates the TMVB and the SMS. The TI constitutes a clear gap in the distribution of *Quercus* species. From Guatemala to Nicaragua, the TIM stands out as the area with the highest *Quercus* species overlapping, while the lowlands of eastern Nicaragua and

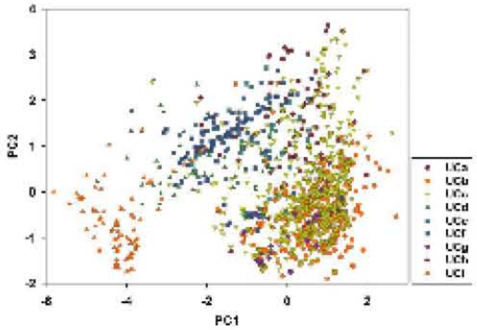

Fig. 5 Principal component (PC) analysis showing ecological niche envelopes for groups of species constituting the nine units of co-occurrence.

the ND configure an important gap of the genus distribution. In Costa Rica, the higher species concentration is observed in the Talamanca Mountains and part of the Pacific lowlands. Finally, species concentration decreases in the territory corresponding to the PI and the lowlands of northwestern Colombia. The map reflects a marked reduction in the number of species co-occurring from southern Mexico to the Colombian Andes. The maximum values of predicted number of co-occurring species observed for the different countries are distributed as follows: 18 species in Mexico, 10 species in Guatemala, 9 species in Honduras, 7 species in Nicaragua, 6 species in Costa Rica, and 3 species in Panama.

Section *Lobatae* (fig. 6*B*) showed a larger number of co-distributed species in central and southern Mexico than section *Quercus*. From southern Nicaragua down to the Colombian Andes, *Lobatae* species appear considerably restricted to the mountainous regions of Costa Rica, but in the Colombian Andes, a single species (*Q. humboldtii*) has a very broad distribution with a wide altitudinal range (between 800 and 3500 m). For section *Quercus* (fig. 6*C*), species co-occurrence values are higher in the central TMVB and the SMS, followed by the area from Guatemala down to northern Nicaragua. Costa Rica and Panama exhibit only three and two white oak species, respectively. The observed distribution of sections *Quercus* and *Lobatae* species showed that areas such as TI, ND, and PI are not suitable habitats for *Quercus* species. Both the PCA and ANOVA comparisons between low-suitability areas and high-suitability areas indicated highly significant differences in annual mean temperature, temperature seasonality, temperature annual range, annual precipitation, and precipitation seasonality (table 2; fig. 7; see table A3 for PCA details).

Discussion

Studies on the turnover patterns of *Quercus* species in the Neotropics have focused mainly on altitudinal patterns (Gentry 2001; Kappelle 2006; Kappelle and Van Uffelen 2006). Our study considered latitudinal units and also longitudinal units in order to complement the current knowledge about the distribution patterns of Neotropical *Quercus*. Most of the previous studies on oak distribution have highlighted not only the change in oak species diversity from southern Mexico to Colombia but also the fact that mountainous regions corresponding to southern Mexico are important diversity hot spots for the *Quercus* genus in America (Valencia Ávalos 2004; Nixon 2006; Torres-Miranda et al. 2011, 2013).

Kappelle (2006) suggested that the distribution patterns of oaks can be explained by the geological and climatic history of the American continent and the evolution of its flora. Recent phylogeographic evidence from *Quercus* species (Cavender-Bares et al. 2011) and other taxa (for a detailed per-species description, see Gutiérrez-García and Vázquez-Domínguez 2013; Ornelas et al. 2013) indicates that intra- and interspecific processes such as divergence, speciation, and migration coincide with historical geological and climatic features of Central America. Our results, based on biogeographical and macroecological approaches, suggest that most of the areas identified as barriers to gene flow for different taxa are also important *Quercus* species turnover points, areas of climatic discontinuities, and boundaries for areas of endemism. Below we provide a detailed discussion of the patterns found at each region.

Central and Southern Mexico

This region is particularly interesting due to the high number of co-occurring oak species and the presence of several UCs limited at the south by the TI. Both the TMVB and the southern

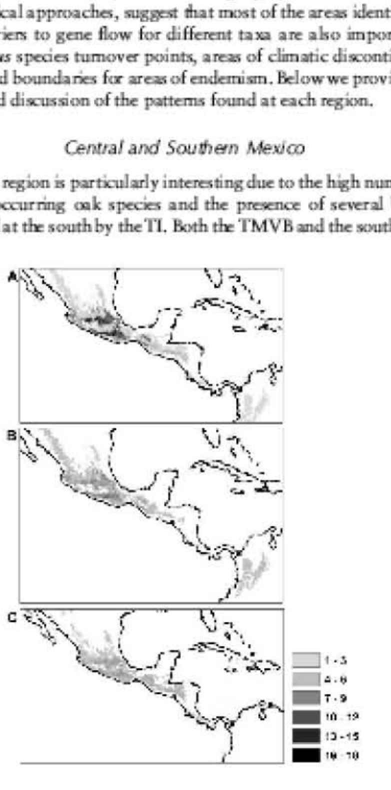

Fig. 6 Potential co-occurrence patterns for Neotropical *Quercus* species determined using ecological niche modeling. *A*, Distribution of the potential number of co-occurring species for the whole *Quercus* genus. *B*, Distribution of the potential number of co-occurring species for red oaks (sect. *Lobatae*). *C*, Distribution of the potential number of co-occurring species for white oaks (sect. *Quercus*). The scale of grays indicates the number of co-occurring species.

Table 1

Principal Component (PC) Analysis for Climatic Niche Variation among the Groups of Species Constituting the Nine Units of Co-Occurrence

Variables	PC1	PC2	PC3
Annual mean temperature	−.20	.90	.34
Temperature seasonality	.45	−.08	.70
Temperature annual range	.55	−.01	.19
Annual precipitation	−.50	−.09	.26
Precipitation seasonality	.43	.41	−.52
Percentage explained	58.97	19.58	13.72
Cumulative percentage	58.97	78.56	92.28
Top variable loadings	Temperature annual range	Annual mean temperature	Temperature seasonality

SMOr have been recovered as regions with high levels of endemism in several similar analyses performed for groups such as birds and mammals (Corona et al. 2007; Vargas et al. 2008). For the TMVB, Escalante et al. (2009) reported low levels of mammal endemism, proposing a review of the importance of the area as the limit between the Nearctic region and the Neotropical region. However, our analysis suggests an important role of the TMVB on the history of oak species distribution, reflected in the presence of three UCs (b, c, and g) within this area. This morphotectonic province is located between latitudes 17°30′ and 20°25′N and longitudes −96°20′ and −105°20′W and spans from coast to coast, presenting a wide variety of climatic zones (Ferrusquía-Villafranca 1993), which may have allowed the establishment of different oak species with different climatic requirements or niches. Recent studies have also highlighted the importance of the TMVB as an area of high haplotype diversity and endemism within particular oak species (González-Rodríguez et al. 2004).

The SMOr and the SMOc are also key areas for the distribution and endemism of oak species. There was evidence of niche divergence between the central/western Mexico species groups (UCs a and b) and the central/eastern Mexico species group (UCs d and e; table 1; fig. 5), which, added to the limited dispersal ability of the oaks, may have limited their migration to other regions. Both the SMOr and the SMOc are characterized by elevations ranging from 200 to 3000 m asl and a heterogeneous physiographic landscape (Ferrusquía-Villafranca 1993). Climatically, the SMOc is more stable, and elevation seems to be the more important variable that defines the province (Ferrusquía-Villafranca 1993). Meanwhile, the SMOr exhibits important climatic factors that probably also influenced the distribution patterns observed. During the winter, polar air currents called nortes are spread over the eastern coast of Mexico through the SMOr, bringing heavy rains in the eastern slope (Metcalfe et al. 2000). This precipitation regime could have allowed the colonization of habitats by different oak species, considering that areas with high levels of precipitation usually have high oak diversity levels (such as the humid montane oak forests; Luna-Vega et al. 2006).

TI

Species composition analysis identified the TI as an area of species turnover, but only for red oaks (section *Lobatae*) and not for white oaks (section *Quercus*). This can be explained by the fact that there are several white oak species distributed through the isthmus lowlands (e.g., *Q. oleoides*) or on both sides of the TI (e.g., *Q. corrugata*, *Q. insignis*, and *Q. lancifolia*). On the contrary, several red oaks are found only to the west of the TI (e.g., *Q. acutifolia*, *Q. crassipes*, *Q. laurina*, and *Q. salicifolia*). ENMs showed that, to the east of the TI, there are areas with a higher potential number of co-occurring species than are actually observed, suggesting that some species distributed to the west of the TI could have found climatically suitable areas but probably failed to disperse across this barrier. In fact, the NAM analysis identified several UCs (a, b, c, e, g, f, and h; fig. 4) that have a geographical distribution delimited to the south by the presence of the TI. Interestingly, a PCA based on climatic variables for the different UCs showed that several geographical units separated by the TI, such as UCs b and c in comparison to UCs f and i, also differ from each other climatically.

These differences in distribution between red oak species and white oak species may be due to the ecological differences between the two species groups. For example, apparently a higher proportion of white oak species are able to predominate in drier regions, where red oaks normally seem to not develop well (Nixon 1993). Other differences between red oak species and white oak species relate to seed dormancy (Struve 1998), which may be important in determining the dispersal patterns of the

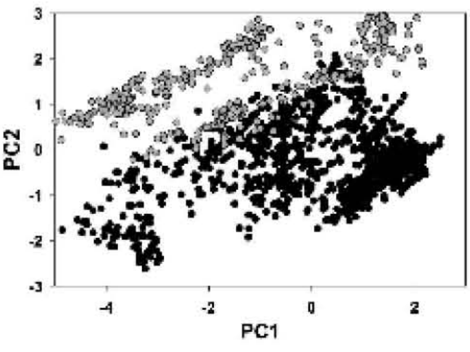

Fig. 7 Principal component (PC) analysis showing climatic differences between the areas with low suitability (barriers, gray dots) and high suitability (highlands, black dots) for the presence of *Quercus* species.

Table 2

One-Way ANOVA Comparing the Values of the Five Climatic Variables with the Largest Influence on the Prediction of the Distribution of Oak Species between High- and Low-Suitability Areas

	Mean	SE	*F*	*P*
Annual mean temperature			3728.34	<.001
Optimal-suitability areas	16.23	.05		
Low-suitability areas	25.86	.14		
Temperature seasonality			39.44	<.001
Optimal-suitability areas	14.72	.1		
Low-suitability areas	12.99	.25		
Temperature annual range			191.04	<.001
Optimal-suitability areas	19.58	.08		
Low-suitability areas	16.50	.2		
Annual precipitation			305.34	<.001
Optimal-suitability areas	1324.25	13.12		
Low-suitability areas	1938.16	32.58		
Precipitation seasonality			47.98	<.001
Optimal-suitability areas	86.83	.32		
Low-suitability areas	80.83	.8		

seeds. It is possible that a combination of these and other traits has to some extent influenced the distribution of both sections. The effect of the TI as a barrier to oak species distribution was also reported by Torres-Miranda et al. (2013) considering only red oak (section *Lobatae*) species. However, it is also true that some roles for local adaptation cannot be discarded since the niche comparison tests indicated significant niche divergence among the species groups that constituted the UCs at both sides of the TI.

Geologically speaking, the TI has several characteristics that could explain the above-mentioned patterns. It is formed by two tectonic subprovinces of Chiapas called the Central Depression and the Pacific Coastal Plain. Both correspond to areas from 0 to 1000 m asl (Ferrusquía-Villafranca 1993). The TI is characterized by a sudden change in elevation between central and southern Mexico that can represent a barrier for the dispersal of oak species, especially considering their seed dispersal is largely mediated by gravity. Similarly, by separating tropical ecosystems from those with higher Nearctic influence, the TI represents an important turnover point for the distribution of species. Interestingly, this area has been reported in several phylogeographic studies as an area separating different haplotype lineages and/or impeding gene flow in different periods (Ornelas et al. 2013 and references therein).

AC and North TIM

Although areas of co-occurrence of a high number of species are not present to the east/south of the TI, there are two other major species turnover points: the AC and the north of the TIM (located in Chiapas and Guatemala). These locations represent sites where species composition of the white oak section changes significantly and the limit to the distribution of UCs formed by species that cross through the TI. Apparently, several species made it through the TI, but not all of them continued their migration southward, reaching their southern limit at the Motagua-Polochic system. The region between the TI and the Motagua-Polochic system is a tremendously heterogeneous area, as the portion within the Mexican territory is characterized by discontinuous sierras and a series of transverse straight rivers, tributaries of the Río Grande de Chiapas (Ferrusquía-Villafranca 1993), while the Guatemalan portion is defined by the tectonic boundary between the North American plate and the Caribbean Plate in Guatemala, a region that consists of a complex system of large-scale faults that separate blocks with contrasting geological features (Ortega-Obregón et al. 2008).

ND

Volcanic activity in southern Nicaragua is largely a product of the interaction of the Caribbean Plate with the Cocos Plate. Particularly, the ND is characterized by a chain of Quaternary volcanoes, recent volcanic activity, volcaniclastic sediments, the presence of Lake Managua and Lake Nicaragua, and being surrounded by a discontinuous group of prominent faults (Arengi and Hodgson 2000). These events, along with the formation of the Cordillera de Guanacaste in Costa Rica (0.6 Ma), led to important climate changes (particularly in Costa Rica; Van Wyk de Vries et al. 2007), which may have molded the distribution patterns of the oak species (Cavender-Bares et al. 2011). This is particularly true for species that constitute the UC i, which are distributed in the Costa Rican mountains and exhibited a well-differentiated group in the ecological space with respect to the UCs distributed to the north of the ND (fig. 5).

The peaks of volcanic activity in the region could also have led to changes in the distribution of species and favored the isolation of populations. Likewise, volcanic activity may have determined a significant barrier to dispersal of species that are distributed through the mountain ranges. This case is particularly clear for the ND, which reports not only volcanic activity but also a significant change in elevation that limits the distribution of the predominant species in the mountainous areas. Based on their analysis of red oak distribution, Torres-Miranda et al. (2013) also suggested that the ND may have played a role as an important barrier. Our results indicate that the ND has also had an effect on the distribution of the white oaks and on the diversification of the genus as a whole. In the case of other biological groups, phylogenetic and phylogeographic analyses have reported this area as a major feature determining genetic and biogeographic patterns (Gutiérrez-García and Vázquez-Domínguez 2013).

Costa Rican Mountains and PI

The southern border of Costa Rica represents the southernmost barrier to the migration of genus *Quercus* into the Colombian Andes. This region has low oak species diversity and also a low number of potentially co-occurring species as indicated by the ENMs. This area is known as Boca del Toro (boundary between Costa Rica and Panama) and defines the end of the mountainous region as well as the start of the Panamanian lowlands. Boca del Toro is also recognized as an important area that has influenced the current distribution patterns of several species of amphibians (Crawford et al. 2005; Wang et al. 2008). Finally, in the Darién region (border between Panama and Colombia), the last important point of oak species turnover was identified. Even considering that Costa Rican and Panamanian mountains show a small number of oak species, the Darién region is crucial in order to understand the distribution of the genus *Quercus* in the Neotropics. Important facts such as its recent geological origin, landscape heterogenity, climatic contrasts (particularly between the Caribbean and Pacific slopes), and proximity to the Andean region should have determined the arrival of the oaks into the Colombian Andes, where *Q. humboldtii* is a key element of the montane ecosystems between 800 and 3500 m asl (Pulido 2006; Fernández-M 2007).

Conclusions

This study is the first to analyze the changes in the oak species composition throughout the Neotropics. We found that there are different regions that have acted as barriers to species dispersal, influencing the composition of forest communities by limiting the number of species that colonized southward areas and probably impacting speciation processes as well. The ENMs also supported the role of these barriers by indicating that some areas in Central America could potentially harbor a higher number of species than is actually observed. These barriers are the TI, the Motagua-Polochic system, the ND, and the PI. According to the ENMs, these areas are regions with low climatic suitability for oak species that also define the borders of the endemism areas identified.

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