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*I wish I could divulge to you the sorrows of my heart,
and tell how I feel at times
that my heart will break by the oppression of my ignorance.*

S. Chandrasekhar

*If it were required to know the position of the fruit-dots
or the character of the indusium
nothing could be easier than to ascertain it
but if it is required that you be affected by ferns
that they amount to anything, signify anything, to you
that they be another sacred scripture and revelation to you
helping to redeem your life
this end is not so easily accomplished*

HD Thoreau

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INTRODUCCIÓN

Los patrones actuales de biodiversidad son el resultado de una larga y compleja historia evolutiva. Indudablemente, los más recientes cambios geológicos y climáticos han tenido un impacto importante sobre la distribución y abundancia de las especies (Darwin 1859; Hewitt 2004; Avise 2007; Rull 2011). Sin embargo, las causas principales de la gran diversidad de especies en regiones tropicales permanece como una de las preguntas principales para la biología evolutiva. En particular, el Neotrópico es una de las regiones con mayor diversidad de especies. Para explicar esta diversidad se han propuesto muchos mecanismos y teorías, pero aún no existe un síntesis satisfactoria (Hughes *et al.* 2013) debido a que la diversificación de especies ha sido propiciada por diversos factores actuando a escalas espacio-temporales distintas (Gentry 1982; Rull 2011).

Originalmente, se creía que la alta biodiversidad presente en estas regiones estaba asociada a eventos geológicos (*e.g.*, orogenia de los Andes) y a una lenta acumulación de especies debido a la estabilidad climática de los bosques tropicales (Gentry 1982; Bush *et al.* 2011; Rull 2011; Cadena *et al.* 2012). Sin embargo, la mayor parte de los bosques Neotropicales ha estado sujeta a cambios drásticos de temperatura y precipitación durante los últimos millones de años, lo cual ha llevado a plantear que un gran número de especies puede ser el producto de eventos recientes de diversificación (Gentry 1982; Van der Hammen 1974; Bush *et al.* 2011; Rull 2011; Hughes *et al.* 2013). Particularmente, los últimos dos millones de años han sido un periodo de elevada inestabilidad climática, asociada a una historia de repetidos ciclos glaciales que han alterado globalmente la distribución de las especies (Avise 2009; Carnaval *et al.* 2009; Stewart *et al.* 2010; de Mello-Martins 2011). Estos ciclos involucraron la re-organización masiva de los sistemas climáticos globales (Otto-Bliesner *et al.* 2009; Hessler *et al.* 2010), lo cual impactó en gran medida la composición y funcionamiento de los ecosistemas terrestres (Kohfeld & Harrison 2000; Hessler *et al.* 2010; Correa-Metrio *et al.* 2013).

La mayoría de los estudios sobre diversificación en el Neotrópico, han intentado buscar un mecanismo general para explicar la extraordinaria diversidad de esta región (Gentry 1982; Rull 2011; Hughes *et al.* 2013; Ornelas *et al.* 2013; Smith *et al.* 2014). La

diversidad neotropical ha sido afectada de manera importante tanto por los ciclos de cambio climático del Pleistoceno, como por los eventos geológicos ocurridos durante el Neogeno (Bryson *et al.* 2010; Smith *et al.* 2014). En este sentido, parece poco claro a que grado los repetidos cambios climáticos y la historia geológica del Neotrópico han contribuido a la gran diversidad de especies presente en los bosques tropicales. Asimismo, la diversificación en el Neotrópico parece haber ocurrido de manera diferencial en biomas distintos, lo cual hace necesario una aproximación bioma por bioma para el estudio de la diversidad en el Neotrópico (Hughes *et al.* 2013). Sin embargo, muchos biomas neotropicales permanecen relativamente olvidados en este esfuerzo (*e.g.*, bosques de niebla).

El aspecto temporal de la diversificación neotropical sigue siendo una pregunta abierta, ¿es ésta producto de una lenta acumulación de especies? ¿o de una reciente diversificación? Más allá de su aspecto temporal (*i.e.*, acumulación constante de especies vs. diversificación reciente), ésta es una pregunta sobre los factores que promueven la diversificación de especies (Rull 2011; Hughes *et al.* 2013). De acuerdo con Rull (2005), los mecanismos y factores asociados a la diversificación serían muy distintos si ésta hubiera ocurrido antes o durante los últimos millones de años. Los cambios geográficos mayores ocurridos en el Neotrópico (*i.e.*, orogenia de Los Andes, cierre del Istmo de Panamá) han sido considerados como factores principales en la divergencia de especies, ligado principalmente a procesos de diferenciación alopátrida (Ornelas *et al.* 2013; Smith *et al.* 2014). Estos procesos deberían haber generado patrones espaciales y temporales de diferenciación congruentes entre distintas especies. No obstante, algunos estudios han observado una marcada incongruencia temporal entre los patrones de divergencia de especies de plantas y animales (Ornelas *et al.* 2013; Smith *et al.* 2014). De esta manera, una hipótesis alternativa es que los cambios geográficos mayores en el Neotrópico produjeron ambientes geográficamente complejos en los cuales ocurrieron eventos de diferenciación (Smith *et al.* 2014), asociados principalmente a fluctuaciones en los intervalos de distribución de las especies asociados a los ciclos de cambio climático del Pleistoceno.

Los patrones geográficos y topográficos en el Neotrópico han permanecido relativamente sin cambios durante los últimos millones de años (Bush *et al.* 2011; Rull 2011). Durante el mismo periodo, se cree que los cambios ambientales pronunciados asociados a los repetidos ciclos glaciales del Pleistoceno han tenido impactos evolutivos importantes (Bush *et al.* 2011; Rull 2011; Hughes *et al.* 2013). Por ejemplo, diversos estudios han sugerido que los ciclos glaciales del Pleistoceno, acoplados a la compleja topografía de las regiones de montaña en el Neotrópico, fueron eventos importantes en la historia evolutiva de biomas alpinos (*e.g.*, *Lupinus* (Fabaceae), Hughes & Eastwood 2006; *Puya* (Bromeliaceae), Jabaily & Sytsma 2013) y selvas neotropicales (*e.g.*, *Astrocaryum* (Arecaceae), Roncal *et al.* 2013; Chrysobalanaceae, Bardon *et al.* 2013; *Inga* (Fagaceae), Richardson *et al.* 2001; *Chrysophyllum* (Sapotaceae), Dick *et al.* 2013). Por otro lado, algunos linajes de árboles de bosques tropicales semiáridos muestran patrones de diversificación anteriores al Pleistoceno (*e.g.*, Pennington *et al.* 2004; De-Nova *et al.* 2011), lo que indica que alguna proporción significativa de la diversidad neotropical se estableció antes de este periodo.

Los ciclos glaciales del Pleistoceno indudablemente causaron cambios significativos en la distribución de los bosques tropicales (Haffer 1969; Bush *et al.* 2011), provocando cambios importantes en los procesos de diferenciación en estas especies (Darlington 1957; Haffer 1969; Avise & Walker 1998; Jaramillo *et al.* 2008). Los procesos involucrados en la dinámica geográfica de expansión y retracción de las especies en respuesta a estos cambios climáticos incluyen extinción, migración y deriva (Hewitt 2004; Thiel-Egenter *et al.* 2011). Se ha propuesto que en muchas situaciones estos factores probablemente afectaron de manera paralela a la distribución de la variación genética y a la riqueza de especies (Vellend 2005; Thiel-Egenter *et al.* 2011). En este contexto, entender los mecanismos micro-evolutivos que conducen a la divergencia genética es esencial para comprender el efecto de los ciclos glaciales sobre la diversificación de las especies (Vellend 2005). Sin embargo, el efecto de estos cambios sobre los patrones de diversidad genética de las especies permanece como una de las preguntas fundamentales para la genética evolutiva (Manel & Holderegger 2013).

En regiones templadas, la evidencia paleoecológica ha demostrado que, durante el último de estos ciclos glaciales (130,000 años), el avance de las capas de hielo polar y el descenso en la temperatura global tuvieron un efecto dominante sobre las especies, desplazándolas a latitudes menores con condiciones climáticas favorables (Comps *et al.* 2001; Heuertz *et al.* 2004; Hewitt 2004; Soltis *et al.* 2006; Vendramin *et al.* 2008; Stewart *et al.* 2010). Las consecuencias genéticas de estos desplazamientos latitudinales ha sido extensamente estudiado, lo cual ha llevado a tener un claro entendimiento sobre los patrones de distribución de la diversidad genética en especies templadas (Soltis *et al.* 2006; Provan & Bennett 2008; Sommer & Zachos 2009). Más aún, se ha propuesto que que los patrones de distribución de la diversidad genética en especies de plantas son congruentes con los patrones diversidad de especies (Thiel-Egenter *et al.* 2011). En este sentido, los ciclos glaciales ocurridos en el Pleistoceno pueden explicar la congruencia entre estos dos niveles de la diversidad biológica (Vellend 2005; Thiel-Egenter *et al.* 2011).

En especies tropicales, no obstante, el efecto de los cambios de distribución asociados a los ciclos glaciales permanece relativamente poco estudiado y comprendido (Manel & Holderegger 2013; Pauls *et al.* 2013). En las montañas tropicales, las zonas altitudinales fueron repetidamente comprimidas y expandidas verticalmente durante periodos fríos y calientes, respectivamente (Hooghiemstra & Van der Hammen 1998, 2004; Bush *et al.* 2011). Se ha propuesto que esto llevó a la intermitente fragmentación y conexión de poblaciones de especies tropicales de montaña (Haffer 1969; Jaramillo *et al.* 2008). Los efectos de los ciclos glaciales en los ecosistemas tropicales de montaña parecen ser más complejos que esto, debido principalmente a la alta heterogeneidad climática y topográfica (Hessler *et al.* 2010; Hughes *et al.* 2013) y dinámicas demográficas complejas (*i.e.*, supervivencia *in situ*, Ornelaz & González 2014). En este contexto, la historia ambiental de los trópicos no se ha resuelto, a pesar de la importancia de los sistemas tropicales para comprender y predecir los patrones generales de cambio climático y como éste altera la distribución de las especies (Bush *et al.* 2011).

CICLOS GLACIALES Y LOS BOSQUES NEOTROPICALES

En los últimos 40 años, diversos estudios se han enfocado en descifrar el carácter y alcance de los ciclos de cambio climático ocurridos durante el Pleistoceno en regiones tropicales (Haffer 1969; Bartlett & Barghoorn 1973; Prance 1982; Bush *et al.* 2011). Estudios paleoecológicos, particularmente para el Neotrópico, han proveído evidencia sobre modificaciones de la vegetación producto de estos cambios climáticos (Bartlett & Barghoorn 1973; Bush *et al.* 2011). En su estudio seminal de 1961, Van der Hammen hizo dos inferencias básicas acerca del cambio climático en el Neotrópico durante el Último Máximo Glacial (UMG). La primera, sobre el descenso generalizado de la temperatura (ca. -4.5°C), acompañado de un desplazamiento pendiente abajo de los bosques de montaña, ha sido ampliamente aceptada (Bush *et al.* 2011; Ramírez-Barahona & Eguiarte 2013). Sin embargo, la segunda, donde se propone que los periodos fríos (glaciales) estuvieron caracterizados por un aumento en la precipitación y los periodos cálidos (inter-glaciales) por condiciones de aridez, ha sido debatida (Bush *et al.* 2011; Ramírez-Barahona & Eguiarte 2013).

Debido a la complejidad de los sistemas de circulación atmosféricos-oceánicos que afectan las regiones tropicales (Hessler *et al.* 2010), los cambios en los niveles de precipitación para el UMG no son homogéneos a lo largo de distintas regiones neotropicales (Figura 1). Algunas regiones permanecieron relativamente húmedas (*e.g.*, Los Andes, Golfo de México), mientras que otras sufrieron reducciones significativas de la precipitación (*e.g.*, sureste brasileño, centro de México; Ramírez-Barahona & Eguiarte 2013). Sin embargo, muchos registros paleoecológicos dentro de una misma región muestran evidencia aparentemente contradictoria sobre los patrones de precipitación y la estabilidad de los bosques tropicales durante el UMG. En este contexto, la interpretación de un último periodo glacial en el Neotrópico con un clima más seco (o más húmedo) ha sido ampliamente debatida (Baker *et al.* 2001, 2003; Mourguiart & Ledru 2003; Ramírez-Barahona & Eguiarte 2013). En algunos casos, este debate ha resultado de distintas interpretaciones de secuencias palinológicas y sedimentarias (Urrego *et al.* 2005). En otros casos, el carácter contradictorio de los registros paleoecológicos se ha visto acentuado por distintas interpretaciones del efecto de la precipitación anual y su estacionalidad sobre el

establecimiento y continuidad de los bosques tropicales (Colinvaux *et al.* 2000; Bush & Silman 2004; Bush & de Oliveira 2006).

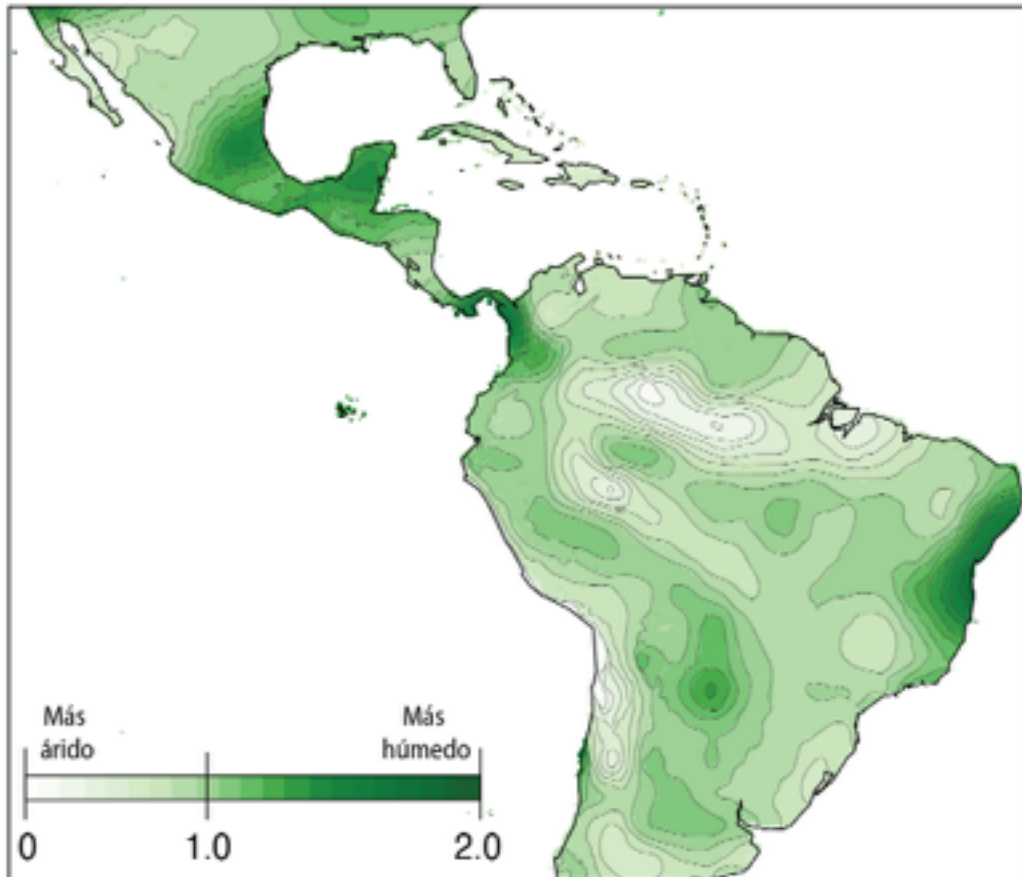


Figura 1. Precipitación anual en el Neotrópico durante el Último Máximo Glacial (UMG) estandarizada con respecto a los niveles actuales de precipitación anual. Los datos de precipitación para el UMG se obtuvieron a partir del modelo de circulación CCSM (Community Climate System Model; Kiehl & Gent 2004).

En particular, se ha propuesto que la distribución de los bosques húmedos tropicales ha presentado fluctuaciones marcadas como respuesta a la aridificación del clima (*i.e.*, reducción en la precipitación anual) ocurrida durante el UMG (Haffer 1969; Toledo 1982; Haffer & Prance 2001; Ornelas *et al.* 2010; de Mello-Martins 2011). No obstante, este modelo de refugios ha sido altamente cuestionado y, por el contrario, se ha sugerido un UMG con condiciones constantes de humedad (Colinvaux *et al.* 2000; Bush & Silman 2004; Bush *et al.* 2009; Gutiérrez-Rodríguez *et al.* 2011; Ramírez-Barahona & Eguiarte 2013). Este escenario predice, a diferencia del modelo de refugios, la existencia de bosques más extensos y continuos, los cuales serían capaces de albergar poblaciones

más grandes y con niveles de conectividad mayores. En este sentido, la dinámica poblacional esperada bajo un escenario de bosques continuos es muy distinta a la esperada para bosques reducidos a regiones de refugio. La distinción de las consecuencias genéticas y demográficas de estos cambios ambientales es esencial para comprender y predecir los patrones de respuesta de las especies al cambio climático (Valencia *et al.* 2010; Bush *et al.* 2011). Por tanto, es esencial estudiar la dinámica genética y demográfica de especies de bosque de niebla bajo un marco analítico que permita poner a prueba estas hipótesis contrastantes (Ramírez-Barahona & Eguiarte 2013).

BOSQUES DE NIEBLA

Para el propósito del presente trabajo, se definen los bosques de niebla como aquellos bosques cuyas características fisonómicas y florísticas se encuentran ligadas a los altos niveles de humedad presentes a lo largo del año, principalmente en forma de niebla (Brujinzeel & Hamilton 2000; Foster 2001; Mulligan & Burke 2005). En la actualidad, estas condiciones climáticas únicas se encuentran restringidas a intervalos altitudinales definidos (Figura 2a), por lo que los bosques de niebla tienen una distribución fragmentada a lo largo de las principales cadenas montañosas (Luna-Vega *et al.* 1999; Foster 2001; Mulligan & Burke 2005). La presencia constante de altos niveles de humedad resulta en climas con escasa o nula estacionalidad (Foster 2001).

Los bosques de niebla americanos presentan niveles excepcionalmente altos de diversidad y endemismo, con muchas de sus especies ocupando intervalos altitudinales estrechos (Terborgh 1977; Luna-Vega *et al.* 2001; Foster 2001; Bush *et al.* 2011). Las plantas epífitas (*e.g.*, bromelias, orquídeas, helechos, musgos) son un elemento característico de los bosques de niebla, donde representan aproximadamente 25% del total de especies de plantas presentes (Foster 2001). Más allá de su importancia florística, las epífitas tienen un papel fundamental para mantener el balance hídrico de los bosques de niebla (Richardson *et al.* 2000; Foster 2001). Por medio de la denominada precipitación horizontal (*i.e.*, niebla), estas especies capturan importantes cantidades de agua

equivalentes al 5-20% de la precipitación neta anual, aunque pueden alcanzarse niveles del 100% (Figura 2b). Este aporte de humedad está íntimamente relacionado con los niveles de agua disponibles para las plantas herbáceas y arbóreas. El suelo de los bosques de niebla rara vez muestra déficit de agua (Figura 2b), incluso en periodos de sequía (Foster 2001), por ser abundantes las plantas con potenciales de evapotranspiración elevados (*e.g.*, helechos, Page 2002).

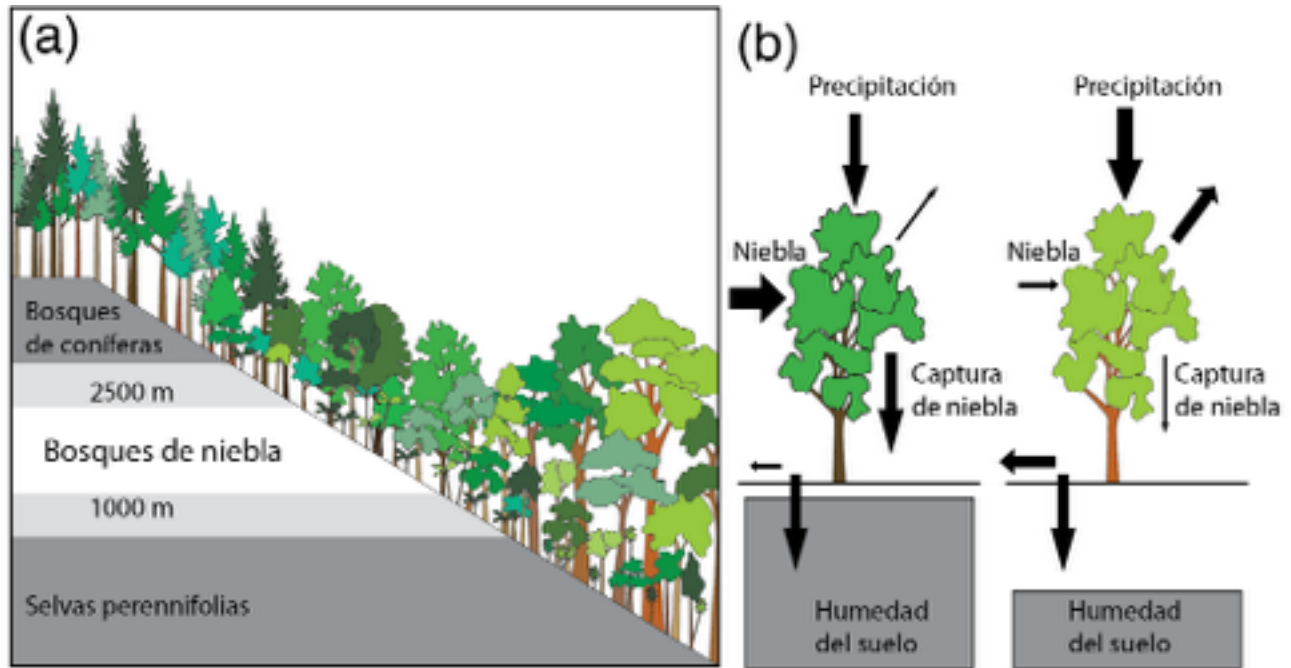


Figura 2. (a) Intervalo altitudinal de los bosques de niebla en México y Centro América (modificado de Brujinzeel & Hamilton 2000). (b) Balance hídrico de los bosques de niebla (izquierda) comparado con el balance hídrico de las selvas perennifolias (derecha). El tamaño de las flechas indica el flujo relativo de humedad en el sistema (modificado de Foster 2001).

Los atributos climáticos y biológicos de los bosques de niebla hacen que sean sistemas extremadamente sensibles al cambio climático (Pounds *et al.* 1999; Still *et al.* 1999; Foster 2001; Rojas-Soto *et al.* 2012; Ponce-Reyes *et al.* 2013). En general, se ha propuesto que las comunidades tropicales no se desplazan en bloque a nuevas áreas en respuesta a los cambios climáticos, debido a que éstas se componen de distintas especies que, probablemente, responden al cambio climático de manera individual (Bradubury 1997; Collevatti *et al.* 2013; Montade *et al.* 2014). Sin embargo, las especies de bosque de niebla probablemente muestren respuestas paralelas debido a la presencia de nichos

climáticos similares y altamente restringidos (Lessa *et al.* 2003; Soltis *et al.* 2006; Carnaval *et al.* 2009). Una manera de poder cuantificar el grado restricción de los nichos climáticos y, de esta manera, evaluar la cohesión ecológica y evolutiva de estas comunidades, es a través del análisis de la historia evolutiva de especies de bosque de niebla.

HELECHOS ARBORESCENTES

Existen más de 10,000 especies de helechos vivientes (Smith *et al.* 2008), las cuales en su mayoría son bióticamente independientes para su reproducción (Page 2002). Por lo tanto, la historia evolutiva de la gran mayoría de especies parece estar influenciada por tres factores principales: 1) el sistema de apareamiento; 2) la disponibilidad de hábitat; 3) y la dispersión de esporas (Barrington 1993; Haufler 2002; Wolf *et al.* 2001; Page 2002). La biología reproductiva única de los helechos, con dos fases de vida libre (*i.e.*, gametofito y esporofito), parece limitar seriamente la ecología y la distribución de las especies (Soltis & Soltis 1990; Wolf *et al.* 2001; Page 2002).

La fase gametofítica es probablemente la etapa con el mayor índice de mortalidad, debido a bajas tasas de germinación y establecimiento de individuos (Bernabé *et al.* 1999; Page 2002). El establecimiento de la fase gametofítica determina el sitio general de origen de la planta adulta, aún cuando las condiciones ecológicas no sean óptimas para su desarrollo (Page 2002). En la reproducción gametofítica la fertilización requiere de la presencia de agua para el movimiento de las gametas masculinas, lo cual limita los sitios y tiempos donde puedan existir películas de agua entre gametofitos. La dispersión por medio de esporas haploides ha sido considerada como una fuerza dominante en la evolución de los helechos, debido principalmente al tamaño pequeño y a la gran cantidad de esporas que se producen (Wolf *et al.* 2001; Labiak *et al.* 2014). Estos dos aspectos se traducen en un alto potencial de dispersión, lo cual explica que los helechos tengan una mayor representación en islas oceánicas y niveles de endemismo más bajos que las plantas con semilla (Smith 1972; Kramer 1993; Geiger & Ranker 2005). Debido a la fragilidad de la fase gametofítica y la dispersión de las esporas, se cree que la preferencia

de hábitat en los helechos, y por lo tanto su distribución, refleja condiciones climáticas y otros factores abióticos (Barrington 1993; Wolf *et al.* 2001; Page 2002).

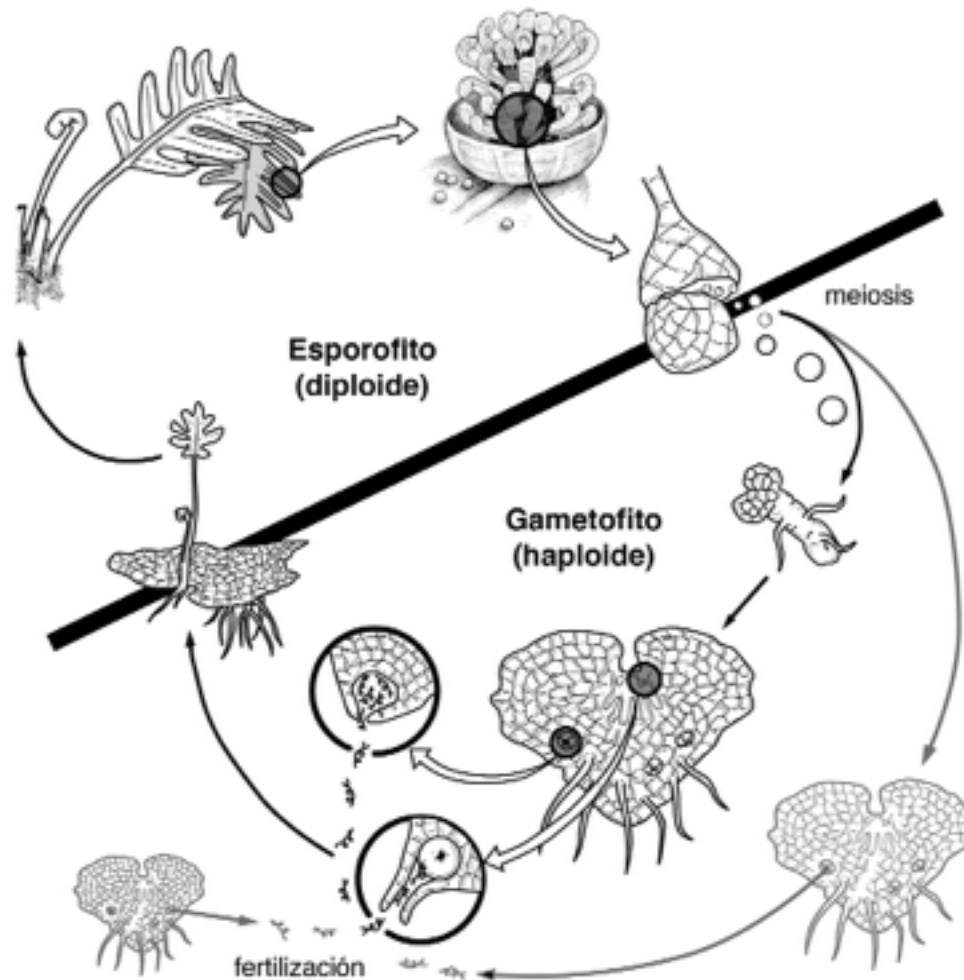


Figura 3. Ciclo de vida general de los helechos. El ciclo de vida de los helechos está dado por la alternancia entre una fase diploide (esporofito) y una haploide (gametofito). Por lo general, la fase dominante del ciclo es el esporofito, el cual produce grandes cantidades de esporas que son dispersadas por viento. Las esporas germinan y se desarrollan, dando lugar a la fase gametofítica en donde se producen gametos masculinos (anterozoides) y femeninos (óvulos). El ciclo de vida se cierra con la fertilización, dando lugar a un nuevo esporofito diploide. Existen tres tipos de fertilización: 1) fertilización inter-gametofítica (cruzada), 2) auto-fertilización inter-gametofítica, 3) auto-fertilización intra-gametofítica.

Las características biológicas y ecológicas únicas de los helechos tienen implicaciones biogeográficas importantes (Page 2002). El entendimiento de su ciclo de vida (Figura 3) y

el sistema reproductivo es crítico para comprender los procesos evolutivos y ecológicos que operan en estas especies. Asimismo, los requerimientos ambientales de la fase gametofítica y la necesidad de ‘regresar’ al agua para reproducirse son factores determinantes en la evolución de los helechos (Page 2002). No obstante, la dominancia de la fase esporofítica en el ciclo de vida ha llevado a pensar que dicha fase tiene el mayor impacto sobre los patrones de distribución de las especies (Wolf *et al.* 2001; Page 2002). Particularmente, la dispersión de las esporas y la tolerancia climática de los esporofitos han sido considerados como dos de los factores más importantes que afectan la ecología y evolución en los helechos (Tryon 1971, 1972; Conant 1983; Wolf *et al.* 2001; Page 2002; Wild & Gagnon 2005; Kreier & Schneider 2006; Haufler 2007; Watkins *et al.* 2007; Korall & Pryer 2014). La mayoría de los estudios poblacionales se han enfocado en especies templadas, por lo que es necesario comprender los patrones genéticos y ecológicos en helechos tropicales, para así clarificar los patrones y procesos involucrados en la evolución de este grupo de plantas.

El orden Cyatheaales es el segundo grupo más diverso de helechos vivos, con +660 especies que se distribuyen principalmente en regiones tropicales (Large & Braggins 2004; Smith *et al.* 2008). De las ocho familias reconocidas dentro del orden (Figura 4), Cyatheaceae es por mucho la más diversa (+600 especies), siendo ésta un elemento conspicuo de los bosques tropicales de montaña alrededor del mundo (Tryon 1971; Foster 2001; Large & Braggins 2004; Ramírez-Barahona *et al.* 2011). La característica distintiva de la familia Cyatheaceae es la presencia del hábito arborescente, con especies que pueden alcanzar 20 metros de altura (Large & Braggins 2004), y la presencia de escamas que cubren el meristemo apical y la base de las frondas (Conant 1983; Lehnert 2006a, 2006b; Korall *et al.* 2007). El conocimiento sobre la familia ha avanzado considerablemente, particularmente sobre las relaciones filogenéticas a nivel genérico (*e.g.*, Conant *et al.* 1994; Korall *et al.* 2006, 2007; Janssen *et al.* 2008; Smith *et al.* 2008; Bystriakova *et al.* 2011; Korall & Pryer 2014) y la descripción de nuevas especies (Lehnert 2003, 2006a, 2006b, 2009a, 2009b, 2011; Lehnert *et al.* 2013; Janssen & Rakotondrainibe 2006, 2007, 2008; Moran *et al.* 2008; Labiak & Matos 2009).

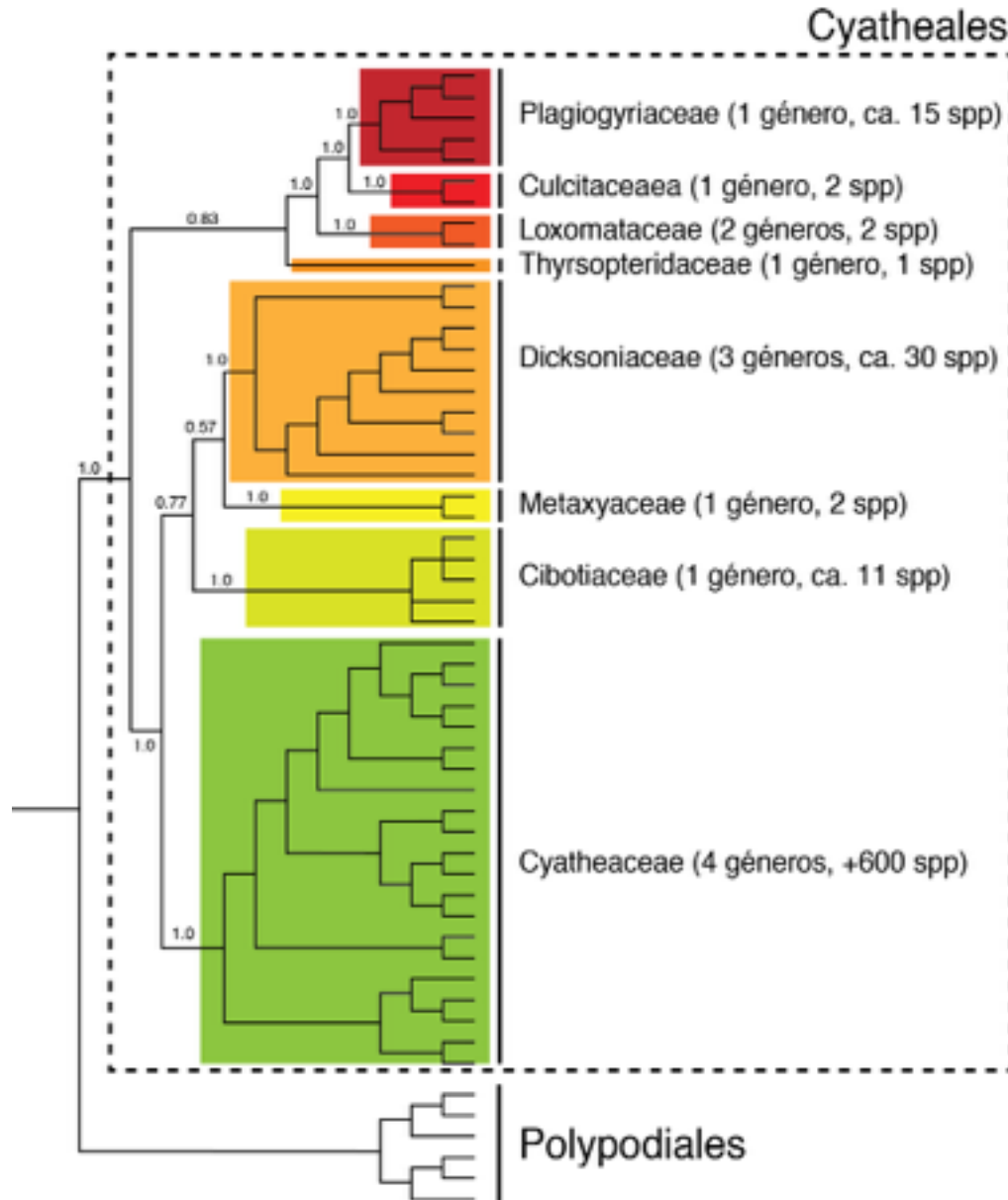


Figura 4. Filogenia para el orden Cyatheaales tomada de Korall *et al.* (2006). El árbol filogenético se obtuvo a partir del consenso de mayoría (50%) de un análisis bayesiano. Los valores de soporte (probabilidad posterior) se muestran únicamente para los nodos internos del árbol filogenético. Para cada familia se muestra el número de géneros y especies de acuerdo a Smith *et al.* (2008).

Aún cuando existen importantes estudios evolutivos y ecológicos para la familia (Hallé 1966; Tryon 1971; Conant & Cooper-Driver 1980; Conant 1990; Soltis *et al.* 1991; Bernabé *et al.* 1999; Arens & Sánchez-Barcaldo 2000; Janssen *et al.* 2008; Mehlreter & García-Franco 2008; Bystriakova *et al.* 2011a, b; Korall & Pryer 2014), no se conocen detalles

ecológicos, genéticos ni biológicos que parecen ser importantes para la comprensión de los patrones biogeográficos y evolutivos. La falta de estudios en la familia se ve acentuada por la incertidumbre sobre el número y la distribución de especies. Para el Neotrópico, se pueden reconocer 254 especies de Cyatheaceae, las cuales muestran niveles de provincialismo y endemismo mayores a los observados en otros grupos de helechos (Tryon 1971, 1972; Tryon & Gastony 1975; Conant 1983; Christenhusz 2011; Lehnert 2011a; Ramírez-Barahona & Luna-Vega 2014). Asimismo, la diversidad de Cyatheaceae en el Neotrópico se concentra en regiones de montaña con una alta proporción de bosques de niebla (Ramírez-Barahona *et al.* 2011; Figura 5), debido a que la mayoría de las especies son intolerantes a periodos prolongados de sequía o frío (Velíz & Vargas 2006; Lehnert 2011a).

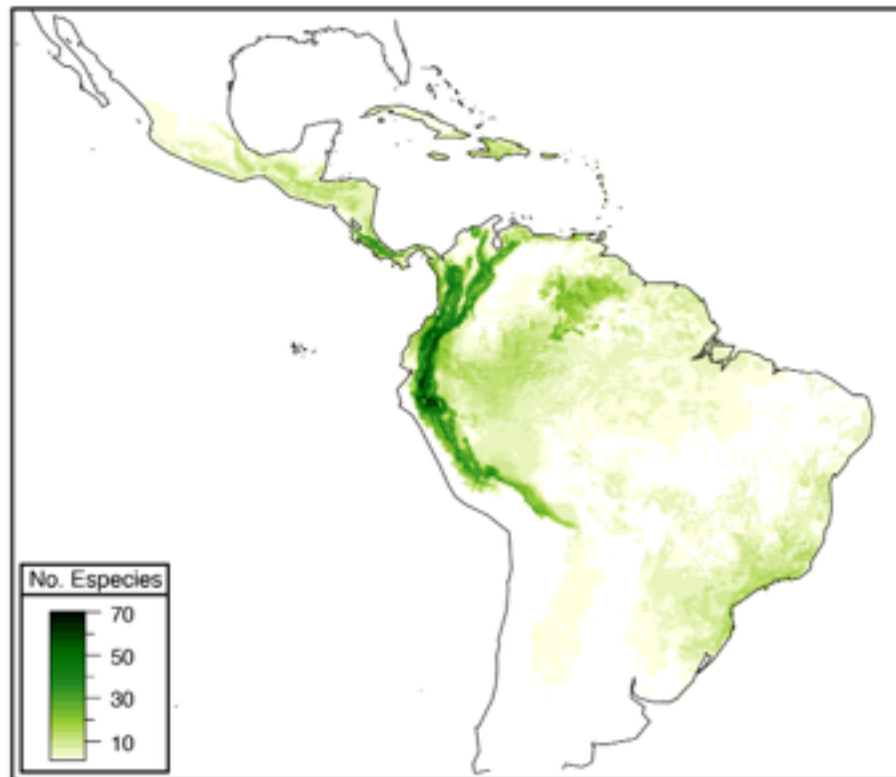


Figura 5. Riqueza de especies para la familia Cyatheaceae en el Neotrópico. La riqueza se calculó a partir de modelos de distribución construidos con Maxent (Phillips *et al.* 2006) a partir de ocurrencias para 165 especies (65% del total en el Neotrópico). La presencia de cada especie se actualizó a partir de los datos de Ramírez-Barahona *et al.* (2011) y de datos adicionales para Brasil (www.gbif.org, consultada en febrero 2014), Las Antillas (Conant 1983; Tryon 1971) y otras regiones del Neotrópico (Lehnert 2011a, 2011b, 2014).

Los helechos están poco representados en estudios de genética evolutiva, a pesar de su potencial como modelos para explorar las respuestas evolutivas de las plantas a cambio ambientales o geográficos (Vogel *et al.* 1999; Trewick *et al.* 2002; Sheperd *et al.* 2007; Hunt *et al.* 2009). En particular, las características biológicas y ecológicas de Cyatheaceae hacen de estas especies un sistema ideal para estudiar la historia evolutiva de los bosques tropicales de montaña. Por un lado, los patrones de distribución en estos helechos apuntan hacia probables eventos de diversificación asociados a la historia geológica del Neotrópico. Los principales linajes de Cyatheaceae surgieron durante el Cretácico tardío (ca. 90-65 millones de años; Janssen *et al.* 2008; Korall & Pryer 2014), sugiriendo que los principales eventos geológicos en el Neotrópico fueron posibles mecanismos de diversificación. Por otro lado, se espera que, debido a la afinidad de la mayoría de las especies por bosques húmedos de montaña, los cambios climáticos asociados a los ciclos glaciales del Pleistoceno hayan tenido un efecto importante sobre los patrones de diversificación en estas especies.

OBJETIVOS Y ESTRUCTURA DE LA TESIS

En el presente trabajo se pretende evaluar el efecto de los ciclos glaciales ocurridos durante el Pleistoceno sobre los patrones y niveles de diversidad genética en especies de bosque de niebla, utilizando a una especie de helechos arborescentes (*Alsophila firma*) como sistema de estudio. En el primer capítulo, se presenta una revisión de registros paleoecológicos para el Neotrópico y se proponen dos posibles modelos sobre la dinámica demográfica y diversificación genética en especies de bosque de niebla durante el último periodo glacial (Ramírez-Barahona & Eguiarte 2013). En el segundo capítulo se presenta un estudio sobre los patrones geográficos de diversidad genética y la historia demográfica del helecho arborescente *Alsophila firma* (Ramírez-Barahona & Eguiarte 2014a). En éste se estudiaron dichos aspectos de la historia evolutiva de la especie y se evaluó su congruencia con los cambios de distribución inferidos para el bosque de niebla durante el último periodo glacial (ca. 15,000 - 130,000 años). En el tercer capítulo se presenta un estudio sobre la estructura genética espacial dentro de dos poblaciones de *A. firma* (Ramírez-Barahona & Eguiarte 2014b). Este trabajo permitió realizar inferencias sobre algunos aspectos de su biología reproductiva (e.g., dispersión, sistema de apareamiento), los cuales son altamente relevantes para la comprensión de los patrones genéticos observados entre poblaciones. De acuerdo a los resultados obtenidos en estos estudios, se discute la posible dinámica geográfica de los bosques de niebla y su relación con la historia demográfica inferida para especies que habitan estos bosques. Asimismo, se discute la relación que guardan los patrones de estructuración genética en *A. firma* con la dinámica genética y demográfica en esta especie. Por último, se proponen posibles líneas de investigación para profundizar en el conocimiento de los patrones de diversificación en especies de helechos arborescentes.

CICLOS GLACIALES Y EL BOSQUE DE NIEBLA

RAMÍREZ-BARAHONA & EGUIARTE (2013)

Resumen.— Los cambios en los niveles de precipitación durante el Último Máximo Glacial (UMG) han sido propuestos como un factor importante que ha afectado a las especies neotropicales. Sin embargo, el carácter e intensidad de esos cambios permanece en debate. En esta revisión se propone una aproximación para probar hipótesis paleoecológicas contrastantes por medio de los efectos demográficos y genéticos predichos para especies de los bosques de niebla neotropicales. Se revisaron 48 registros paleoecológicos abarcando el UMG en el Neotrópico, los cuales muestran evidencia contrastante acerca de los cambios de precipitación ocurridos durante este periodo. Varios registros paleoecológicos dentro de la misma región muestran evidencia aparentemente contrastante sobre los niveles de precipitación y la estabilidad de los bosques. A partir de estos datos se proponen y delinean dos escenarios demográficos/genéticos para especies de bosque de niebla basados en regímenes de precipitación opuestos: la hipótesis de los refugios áridos y la de los bosques húmedos. Se buscaron estudios que investigaran la estructura genética poblacional de taxa de bosques de niebla y otros bosques de montaña, y se compararon sus resultados con los dos escenarios propuestos. Los pocos estudios moleculares disponibles muestran evidencia genética insuficiente sobre la predominancia de aridez glacial en el Neotrópico. Dada la información paleoecológica ambigua, deben utilizarse datos sobre la estructura genética de especies de bosque de niebla para probar explícitamente las consecuencias genéticas de hipótesis paleoecológicas contrastantes.

The role of glacial cycles in promoting genetic diversity in the Neotropics: the case of cloud forests during the Last Glacial Maximum

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Keywords

Climate change, historical demography, Last Glacial Maximum, paleoecology, phylogeography, population genetics, refugia.

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Abstract

The increasing aridity during the Last Glacial Maximum (LGM) has been proposed as a major factor affecting Neotropical species. The character and intensity of this change, however, remains the subject of ongoing debate. This review proposes an approach to test contrasting paleoecological hypotheses by way of their expected demographic and genetic effects on Neotropical cloud forest species. We reviewed 48 paleoecological records encompassing the LGM in the Neotropics. The records show contrasting evidence regarding the changes in precipitation during this period. Some regions remained fairly moist and others had a significantly reduced precipitation. Many paleoecological records within the same region show apparently conflicting evidence on precipitation and forest stability. From these data, we propose and outline two demographic/genetic scenarios for cloud forests species based on opposite precipitation regimes: the dry refugia and the moist forests hypotheses. We searched for studies dealing with the population genetic structure of cloud forest and other montane taxa and compared their results with the proposed models. To date, the few available molecular studies show insufficient genetic evidence on the predominance of glacial aridity in the Neotropics. In order to disentangle the climatic history of the Neotropics, the present study calls for a general multi-disciplinary approach to conduct future phylogeographic studies. Given the contradictory paleoecological information, population genetic data on Neotropical cloud forest species should be used to explicitly test the genetic consequences of competing paleoecological models.

Introduction

The Pleistocene glacial periods have been regarded as major factors influencing the geographical distribution, demographic dynamics, and patterns of genetic diversity of species (Comes and Kadereit 1998; Haffer and Prance 2001; Hewitt 2004; Soltis et al. 2006; Stewart et al. 2010). Paleoecological and molecular studies have provided abundant data concerning the vegetation changes resulting from these glacial cycles (Hewitt 2004; Stewart et al. 2010). The main bulk of research has been focused on northern latitudes, particularly on taxa inhabiting Europe and North America (e.g., Comes and Kadereit 1998; Petit et al. 2003; Skrede et al. 2006; Soltis et al. 2006; Waltari et al. 2007; Wang et al. 2009). There is a consensus view that the advance of ice sheets and temperature descent

through Last Glacial Maximum (LGM, ~23–18 kyr BP) had a major impact on the distribution of species in these regions. Temperate species persisted through this period in isolated southern refugia where climatic conditions were less extreme (Hewitt 2004; Soltis et al. 2006; Provan and Bennett 2008). Thus, the cold glacial pulses have been regarded as main forces driving the divergence of populations.

The Neotropics is regarded as having an extremely diverse flora and fauna (Gentry 1982; Kier et al. 2009). Specifically, the mountainous regions have been considered as exceptionally rich centers of biodiversity and endemism (Kier et al. 2009). Given their complex topography and habitat heterogeneity, there is likely to be a high genetic diversity within most montane species and signals of the genetic and evolutionary process that

occurred as a consequence of the Pleistocene glacial cycles (Haffer 1969; Gentry 1982; Jaramillo-Correa et al. 2008). There have been attempts to explicitly uncover the effects of these glacial periods on the genetic diversity of Neotropical montane species (e.g., Aguirre-Planter et al. 2000; Jaramillo-Correa et al. 2008; Kosciński et al. 2008; Carnaval et al. 2009; Hensen et al. 2011). In spite the availability of paleoecological data for many Neotropical regions, the available phylogeographic studies seldom use these data to generate explicit predictions on the genetic structure of extant populations. Thus, there is still considerable ambiguity regarding the effect of glacial periods on the genetic structure of Neotropical montane species.

Several authors have proposed the refugia theory as the underlying model for glacial and postglacial population dynamics of tropical species during the LGM (Haffer 1969; Van der Hammen and Hooghiemstra 2000; Haffer and Prance 2001; Mourguiart and Ledru 2003). The basic tenet of the tropical refugia theory is that the cooler glacial climates were also characterized by a drastic reduction in precipitation. The widespread aridity during glacial times caused tropical forests to be compressed, fragmented, and isolated into distinct forest patches separated by intervening non-forest vegetation (Haffer 1969; Toledo 1982; Carnaval and Moritz 2008; Carnaval et al. 2009; de Mello-Martins 2011).

Some authors have questioned the pervasiveness of this suggested aridity during the LGM and therefore the validity of the refugia model (Colinvaux et al. 2000; Colinvaux and de Oliveira 2001; Baker et al. 2003). These authors support Van der Hammen's (1961) inference that "the most important cold phases were wet phases at the same time and the warmer phases dry". Contrary to the refugia scenario, the supporters of this view claim that precipitation was not significantly reduced during the LGM. Consequently, tropical forests had stable and continuous distribution during glacial periods (Farrera et al. 1999; Hostetler and Mix 1999; Caballero et al. 2010).

The cloud forests are a suitable system to investigate the direction of change in precipitation during the LGM and its effects on tropical montane plant species because they are extremely vulnerable to changes in the hydrological cycle (Foster 2001). The cloud forests are characterized by persistent cloud immersion, which directly influences the moisture availability by a constant input of large amounts of water as horizontal precipitation (Foster 2001). The cloud forests are some of the most diverse Neotropical forest communities, characterized by the considerable number and proportion (10–30%) of endemic species (Gentry 1982; Foster 2001; Luna-Vega and Magallón 2010).

Most of the proposed refugia for Neotropical lowland species lie within mountain regions that are currently

cloud forest areas (Prance 1982). This is the direct result of aridity displacing forest species into mid-elevations where the humidity would be maintained better than in the lowlands. Paleoecological data indicate that Neotropical cloud forests were subjected to down-slope migration in response to glacial cooling (Colinvaux et al. 1996; Urrego et al. 2005; Caballero et al. 2010; Valencia et al. 2010). Thus, under cool-arid conditions cloud forests would have been subjected to opposing forces and consequently be compressed into distinct refugia (Hooghiemstra and Van der Hammen 2004). Given the debate on glacial aridity, it remains unsubstantiated whether cloud forests were contracted or not into forest refugia during the LGM.

The knowledge on precipitation during the LGM, and hence on the distribution of Neotropical forests, is largely based on limited and conflicting paleoecological data. In this sense, this review presents an approach to test the proposal of widespread glacial aridity by way of its expected genetic effects on cloud forest species. To achieve this, in the first section we present a review of several paleoecological studies representing 48 records throughout tropical America. Based on these data, in the second section we propose two demographic scenarios for cloud forests based on opposite precipitation regimes: the dry refugia and the moist forests hypotheses. Then we outline the basic genetic consequences of these models as expectations for the glacial dynamics of cloud forest species. Finally, within the framework of these criteria, in the last section we present a brief overview of some molecular studies available for cloud forest species and other montane taxa.

Literature search

In the first section, we focused our survey on paleoecological data encompassing the LGM for the Neotropics. To achieve this, we performed a reference search on key articles on the topic (e.g., Van der Hammen 1961, 1974; Haffer 1969; Prance 1982; Bush and Colinvaux 1990; Bradbury 1997). The final review included studies covering 48 paleoecological sites throughout the Neotropics (Fig. 1).

In the last section, we searched for relevant articles dealing with the intra-specific phylogeography of cloud forest taxa on the Web of Knowledge database (<http://apps.webofknowledge.com>), using the following search terms combinations: cloud forests* AND phylogeography*. This resulted in only two studies dealing with plant species, both in Mexico and upper Central America. Given the paucity of information, we included other relevant studies for Neotropical montane forest taxa in the discussion.

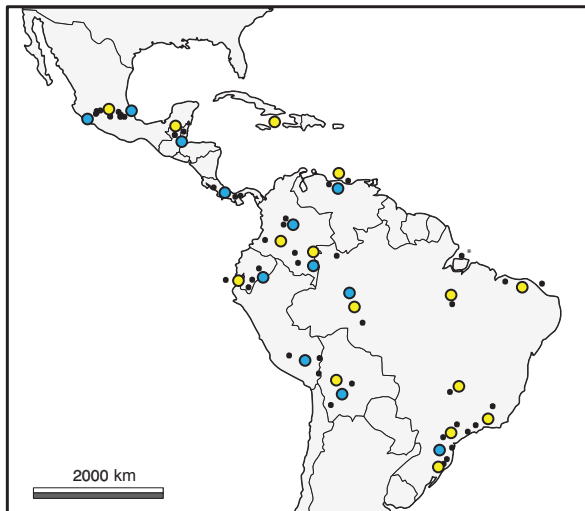


Figure 1. Map of the Neotropics with the geographical location of the paleoecological records reviewed (black dots) and the inferred humidity conditions during the LGM (colored circles). Blue and yellow circles represent moist and dry conditions, respectively.

The LGM in the Neotropics

In his seminal work of 1961, Van der Hammen made two basic inferences about climate change in the Neotropics during the LGM. The first inference on the nature of temperature descent, along with the subsequent down-slope displacement of montane forests, has been widely accepted. On the contrary, there is an ongoing debate surrounding Van der Hammen's second inference that the most important cold periods were characterized by increased humidity and that warmer periods were dry. In this context, the next section addresses the main inferences on precipitation during the LGM drawn from paleoecological data in tropical America (Table 1; Fig. 1).

Mexico, Central America, and the Caribbean

Caballero et al. (2010) recorded a 1000–1500 m lowering of glaciers in Central Mexico. From this, the authors interpreted an 8°C cooling. The glacier descent was less pronounced in Central Mexico than near the coasts. This suggests that Central Mexico was dryer than the Pacific and Gulf coasts. Accordingly, the dominance of grass pollen in the records and reduced lake levels reflect an overall cool and dry climate. This climate favored the down-slope migration of montane forests species (e.g., *Pinus*, *Quercus*, *Alnus*). Many of these taxa, particularly those from cloud forests, probably inhabited areas of wet microclimates immersed in a matrix of dry conditions (Caballero et al. 2010).

Table 1. Main observations and inferred humidity conditions during the LGM for Neotropical regions based on several paleoecological records. Only references to the most relevant papers are included for each region (see text for an extended reference list).

| Region | Humidity conditions | Authors |
|-----------------------|---------------------|----------------------------------------|
| Central Mexico | Dry | Caballero et al. (2010) |
| Upper Central America | Dry | Anselmetti et al. (2006) |
| Upper Central America | Moist | Mueller et al. (2010) |
| Lower Central America | Moist | Colinvaux et al. (1996) |
| Northern Andes | Moist | Valencia et al. (2010) |
| Northern Andes | Dry | Mourguiart and Ledru (2003) |
| Amazonia | Dry | Van der Hammen and Hooghiemstra (2000) |
| Amazonia | Moist | Colinvaux et al. (2000) |
| SE Brazil | Dry | Behling (2002) |

Moreover, the studies of Leyden et al. (1993), Bradbury (1997), Anselmetti et al. (2006), and Metcalfe et al. (2009) suggested a reduction in wet season precipitation for upper Central America and Mexico. Bradbury (1997) observed dominant grasslands in records around the Caribbean basin, indicative of cooler and dryer climate. The dominance of pollen from *Pinus*, *Cupressus*, *Juniperus*, *Myrica*, and *Poaceae* is taken as evidence of enhanced dry conditions (Leyden et al. 1993; Bradbury 1997). Accordingly, Anselmetti et al. (2006) observed an 87% reduction in the water volume of Lake Peten-Itza, Guatemala. This reduction has also been observed in New River Lagoon, Belize (Metcalfe et al. 2009) and Lake Quexil, Guatemala (Leyden et al. 1993).

It has been suggested that the summer precipitation regime was collapsed, resulting in arid conditions extending over much of Mexico, Central America, and the Caribbean (Hostetler and Mix 1999; Metcalfe et al. 2000). However, Metcalfe et al. (2000) suggested that Central Mexico remained cool and moist. According to the authors, winter precipitation was enhanced and evaporation was reduced by the southward position of the Laurentide ice sheet. This would have resulted in less seasonality and prevailing wet conditions even with reduced wet season precipitation (Hostetler and Mix 1999). In line with these observations, Hodell et al. (2008) and Mueller et al. (2010) suggested that there was no effective decrease in humidity in upper Central America. The records from Lake Peten-Itza showed a rich clay deposit and a pollen diagram dominated by *Pinus*, *Quercus*, and some mesic elements such as *Liquidambar* and *Alnus*. These records are indicative of a montane forest community thriving in lower elevations under cool and moist conditions (Hodell et al. 2008; Bush et al. 2009). The down-slope migration of montane taxa has also been

observed in pollen records from Panama (Bush and Colinvaux 1990; Colinvaux et al. 1996). These authors showed that montane taxa (e.g., *Ericaceae*, *Quercus*, *Ilex*, *Thalictrum*, *Symplocos*, *Ranunculus*, *Magnolia*, *Alnus*, *Podocarpus*, *Weinmannia*) were growing 500–800 m lower than their present range.

The analyses of González et al. (2008) from the Cariaco basin in northern Venezuela also support the lowering of montane forest taxa in response to cooling. These taxa, including cloud forest elements (e.g., *Ilex*, *Juglans*, *Podocarpus*, *Cyatheaceae*) increased in abundance at lower elevations over cooler periods, suggesting stable humidity conditions. However, González et al. (2008) point out that dry seasonal forests also showed an expansion during these periods. This last result led the authors to support a cool and dry climate extending throughout the Caribbean basin. The proposed reduced precipitation hindered the down-slope expansion of cloud forests, which were displaced into discrete areas with stable humidity conditions (González et al. 2008; Caballero et al. 2010).

The Andes, Amazonia, and southeastern Brazil

As initially proposed by Van der Hammen (1961), montane forests in the Colombian Andes grew 1000–1500 m down-slope of their present range (Van der Hammen 1974; Bush et al. 1990; Paduano et al. 2003; Hooghiemstra and Van der Hammen 2004; Brunschön and Behling 2009; Hillyer et al. 2009; Valencia et al. 2010). The displacement of montane vegetation and the observed glacier descent of 500–900 m (Hostetler and Mix 1999) have been used to infer a 4.5°C cooling.

The montane forests around Lake Pacucha (southern Peru) were replaced by grassland vegetation (Valencia et al. 2010). Other records confirm the replacement of montane forests by grassland vegetation throughout the Andes (Bush et al. 1990; Behling et al. 1998; Baker et al. 2003). Accordingly, the records from Lake Consuelo (southern Peru), located 1700 m lower than Lake Pacucha, show the down-slope migration and expansion of montane taxa (Bush et al. 2004; Urrego et al. 2005). The vegetation around Lake Consuelo had upper montane taxa being dominant (e.g., *Alnus*, *Hedyosmum*, *Myrsine*, *Podocarpus*, *Symplocos*, *Vallea*, and *Weinmannia*), which were subsequently displaced upslope during the Holocene and are today largely absent around Lake Consuelo. Taken together, these records show an expansion of montane taxa into lower elevations during the LGM. This is taken as evidence of prevailing cool and moist conditions in the region.

The prevalence of montane forests throughout the Andes (i.e., Colombia, Ecuador, Peru, Bolivia) and the

inferred moist conditions have been confirmed by the high percentage of fresh water diatoms and low levels of carbonates in sediment cores from Lake Titicaca, Peru (Baker et al. 2001a). These authors inferred that Lake Titicaca was deep, fresh, and overflowing. In this context, the oscillations between gypsum and clay layers at Salar de Uyuni (southern Bolivia) indicate that precipitation was not reduced in the Bolivian and Peruvian Andes during the LGM (Baker et al. 2001b).

In spite the stable precipitation inferred for the Andes, some records from lowland regions in Amazonia show dominant grasslands during the LGM, which were later replaced by modern rain forests (Van der Hammen 1974; Heusser and Shackleton 1994; Hooghiemstra and Van der Hammen 1998, 2004; Van der Hammen and Hooghiemstra 2000). These records support reduced precipitation and prevailing dry conditions throughout the lowlands of the Amazon basin. In accordance, Van der Hammen and Hooghiemstra (2000) suggested a 45% reduction in precipitation. In turn, Hooghiemstra and Van der Hammen (2004) suggested that the elevational range of montane forests was compressed as much as 50%. As expected, the low abundance of forest elements indicates that tropical forests contracted and were less continuous during this arid period (Heusser and Shackleton 1994; Behling et al. 1998; Hessler et al. 2010).

However, other records from lowland Amazonia do not show a replacement of rain forest by savanna-like vegetation. Colinvaux et al. (2000) and Colinvaux and de Oliveira (2001) showed that forest cover remained constant in the lowlands of western Amazonia, with significant incursions of montane taxa such as *Alnus*, *Podocarpus*, *Ilex*, and *Hedyosmum*. Accordingly, sedimentary records from the Amazon fan detect an increase of montane taxa during the LGM (Hoorn 1997). These records do not show an overrepresentation of grass pollen, which would otherwise be indicative of savanna-like vegetation expanding throughout Amazonia (Hoorn 1997; Colinvaux et al. 2000; Colinvaux and de Oliveira 2001).

In the highlands of southeastern Brazil there is evidence of the dominance of grassland taxa and of a poor representation of montane elements (Ledru et al. 1996; Behling 1997). The records also show the occurrence of montane forest species in the lowlands of the Brazilian Atlantic coast (Behling 1997, 2002). This altitudinal displacement implies a cooler than present climate. The extension of open grassland vegetation has been taken as evidence of aridity extending throughout the region (Behling 2002; Ledru et al. 2007; Hessler et al. 2010). Ledru (1993) and Ledru et al. (1996) inferred cooler and humid conditions before and after the LGM in central Brazil. These conditions are reflected in the dominance of montane forest taxa, such as *Araucaria*, *Podocarpus*, *Drymis*, and *Cyathea*,

before and after this period. In addition, Ledru (1993) and Ledru et al. (1996) observed a sedimentary hiatus during the LGM, which is taken as evidence of extreme aridity. However, the same authors proposed that seasonality in precipitation was reduced as a result of the northward migration of the polar front, which would have augmented the humidity during the dry season.

Summary of the paleoecological data during the LGM

From the above review we observe that precipitation changes during the LGM were not homogenous across the Neotropics. Some regions remained fairly moist (e.g., the Andes) and others had a significantly reduced precipitation (e.g., southeastern Brazil). This is not surprising given the complex nature of the atmospheric-oceanic circulation system over the Neotropics (Hessler et al. 2010). However, many paleoecological records within the same geographical region show apparently conflicting evidence on forest stability during the LGM, mainly resulting from contrasting interpretations of pollen and sedimentary sequences (Urrego et al. 2005). Furthermore, the conflicting evidence has been enhanced by differing ideas on the effect of reduced precipitation on the continuity of tropical forests. Some authors claim that the reduction in wet season precipitation has a minor effect on the continuity of forest cover compared with the overall change in precipitation seasonality (Colinvaux et al. 2000; Bush and Silman 2004; Bush and de Oliveira 2006).

Cloud forests during the LGM

In the absence of conclusive paleoecological data, analyzing the genetic structure of cloud forest plant species can be used to assess the two models of precipitation. Given their high hydrological vulnerability, even the most conservative estimate of precipitation reduction would result in significant changes in the demography and distribution of these species. Molecular studies that explicitly test the genetic structure of cloud forests species can provide some insight into the climatic history of Neotropical forests and thus serve to further corroborate the paleoecological data.

The next section gives a brief description of the expected genetic consequences of the dry refugia hypothesis, which are subsequently compared with those expected under the moist forests model (Table 2; Fig. 2). The proposed consequences are largely based on the vast number of studies undertaken for temperate taxa (e.g., Bennett, 1985; Comes and Kadereit 1998; Haffer and Prance 2001; Knowles 2001; Hewitt 2004; Williams et al. 2004; Schönswetter et al. 2005; Soltis et al. 2006; Carstens

Table 2. Demographic and genetic consequences for cloud forest taxa predicted by the two precipitation models for the Neotropics during the Last Glacial Maximum: the dry refugia and the moist forests models.

| Model | Demographic and genetic consequences |
|----------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <i>Dry refugia</i> | <p>Re-colonization and demographic expansion from small population sizes.</p> <p>Loss of genetic diversity and marked genetic structuring of populations.</p> <p>Species with concordant patterns of isolation and divergence between refugial lineages.</p> |
| <i>Moist forests</i> | <p>Range expansion and population connectivity in the lowlands.</p> <p>Upslope range fragmentation and little to no demographic expansion.</p> <p>Increased genetic diversity resulting from spatial heterogeneity and diffuse genetic structuring of populations.</p> <p>Less likely phylogeographic concordance across species.</p> |

and Knowles 2007; Bennett and Provan 2008; Holderegger and Thiel-Egenter 2009; Stewart et al. 2010).

The dry refugia model

The dry refugia hypothesis was originally proposed and subsequently debated for lowland tropical forests, mainly in the Amazon (Haffer 1969). The present model is a direct extrapolation of this hypothesis, relying in the fact that the proposed refugia were located at mid-elevations in mountain regions with stable temperature and humidity conditions (Haffer 1969; Toledo 1982; Burnham and Graham 1999; Haffer and Prance 2001; Hooghiemstra and Van der Hammen 2004; Bush and de Oliveira 2006). Under this scenario, the cloud forests were displaced and compressed into refugia by the opposing effects of aridity and cooling. Subsequently, these populations would expand and re-colonize the species former range at the onset of more humid and warm conditions (Fig. 2).

Given the small size and isolation of the refugial populations, genetic drift would cause the average probability of identity by descent to increase within these populations. This would be reflected in allele sorting and the fixation of different alleles in separate refugia (Vendramin et al. 2008; Sommer and Zachos 2009). Populations from different refugia would thus become differentiated from each other, accumulate new mutations and would likely show reciprocal monophyly (Petit et al. 2003; de Mello-Martins 2011). In addition, gene flow between isolated populations from different refugia, while possible, would be limited (Schoville et al. 2011). Extant lineages

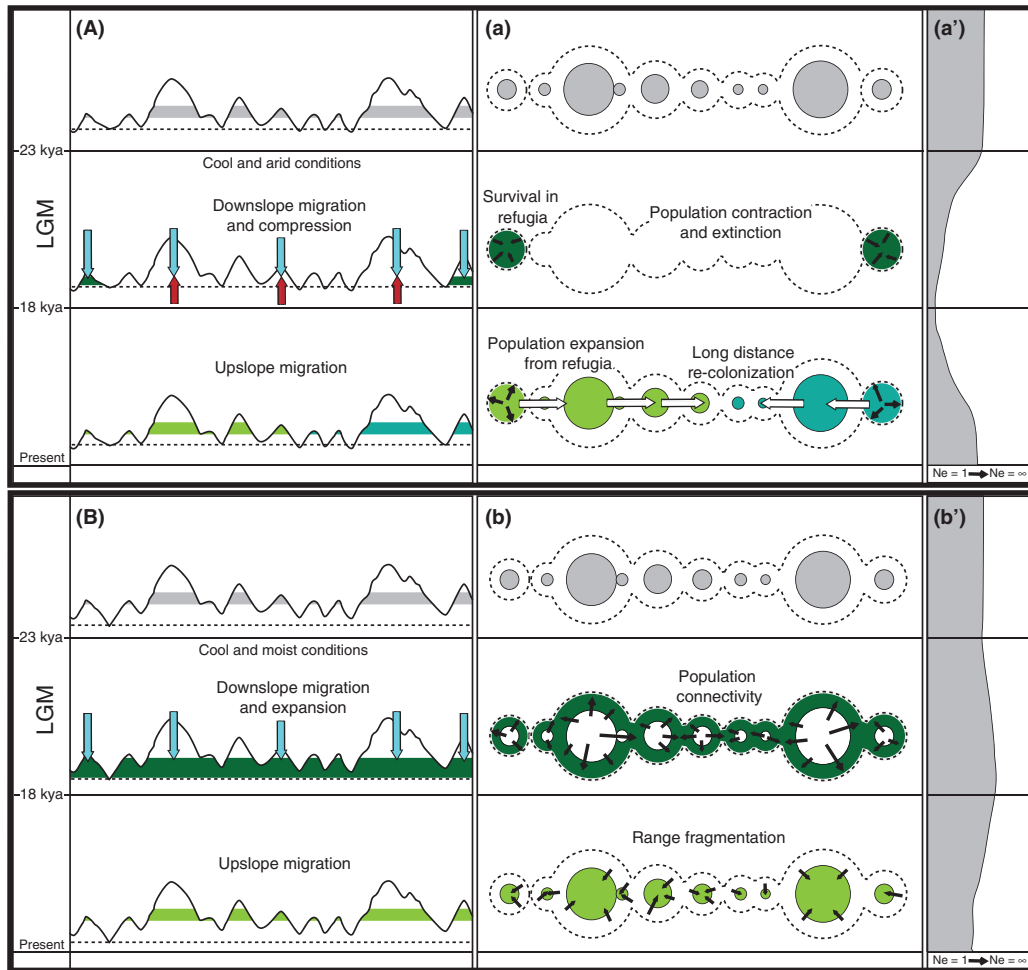


Figure 2. Models of the distributional and demographic dynamics of cloud forests during the Last Glacial Maximum (LGM, ca. 23–18 kya). (A) Dry refugia model. (B) Moist forests model. The distribution and abundance of cloud forests are represented in a cross-section (A, B), the corresponding aerial view (a, b) and a graphic of the idealized population change over time (a', b'). From top to bottom: the distribution of cloud forests before (gray), during (dark green), and after (light green/blue) the LGM. Blue and red arrows indicate the direction of displacement resulting from cooling and aridity, respectively (A, B). Black solid arrows indicate the direction and magnitude of population growth and white arrows indicate the direction of re-colonization (a, b). The dashed line represents minimum altitude level attained by the down-slope migration during the LGM.

originated in separate refugia would be significantly divergent and therefore show significant genetic differentiation (Knowles 2001; Heuertz et al. 2004). Ultimately, the observed levels of genetic differentiation would be dependent on the extent of former refugia populations and on the levels of past gene flow between them (Soltis et al. 2006).

The demographic contraction and subsequent expansion away from refugial populations would have a marked effect on the frequency distribution of allele differences in extant populations (Rogers and Harpending 1992). After expansion, the distinct refugial lineages would consist of one or few common alleles and would accumulate an

excess of rare alleles separated by few mutational steps. Thus, a unimodal distribution of allele differences (mismatch distribution) and a star-shaped allele genealogy are expected for expanding refugial lineages (Slatkin and Hudson 1991; Rogers and Harpending 1992).

The refugial scenario would also have profound effects on the geographical distribution of alleles. In general, the re-colonization out of refugia would involve repeated long-distance dispersal over relatively large geographical areas. Therefore, this process would be characterized by repeated founder effects and by the subsequent loss of allelic richness (Comps et al. 2001; Hewitt 2004; Excoffier et al. 2009). The occurrence of founder effects would be

negatively associated with the dispersal ability of species (Petit et al. 1997, 2003; Lessa et al. 2003; Tremetsberger et al. 2003; Bialozyt et al. 2006; Soltis et al. 2006; Excoffier et al. 2009; Callens et al. 2011). Thus, depending on the dispersal ability of species and the time elapsed, the re-colonization out of refugia would result in the decline of genetic diversity away from refugial populations and in a high differentiation of the most recently colonized populations (Slatkin 1993; Austerlitz et al. 1997; Lessa et al. 2003; Tremetsberger et al. 2003; Heuertz et al. 2004; Hewitt 2004; Ehrich et al. 2007). Additionally, as the common alleles present in refugia would be the more likely source of re-colonization, these would automatically become frequent in the founding populations and therefore would become widespread following colonization. On the other hand, the rare alleles would accumulate around refugial populations, resulting in a high probability of geographical clustering of these alleles (Comps et al. 2001; Hewitt 2004).

The loss of genetic diversity during re-colonization and, in many cases the secondary contact between different refugial lineages, would break down the pattern of isolation by distance even in species with dispersal mechanisms that should promote such a pattern (Slatkin 1993; Petit et al. 2003; Caetano et al. 2008; Provan and Bennett 2008; Sommer and Zachos 2009). However, the colonization dynamics out of single refugia could also result in isolation by distance because genetic differentiation reaches the equilibrium state of isolation by distance relatively quickly (Slatkin 1993).

Finally, species with similar niches would show concordant signals of isolation and divergence between refugia populations (Lessa et al. 2003; Soltis et al. 2006; Carnaval et al. 2009). There is clear evidence that taxa respond individually to climate change, but most of the responses of montane taxa appear not to be entirely independent due to cross-correlations among plant niches (Lessa et al. 2003; Heuertz et al. 2004; Hooghiemstra and Van der Hammen 2004; Williams et al. 2004). Ultimately, the genetic variation in refugia and the dispersal ability of each species would have a direct effect on the geographical distribution of genetic diversity of extant populations and thus affect the power to detect phylogeographical concordance among species (Heuertz et al. 2004; Excoffier et al. 2009; Sommer and Zachos 2009).

The moist forests model

The moist forests hypothesis contends that changes in precipitation had a minor effect on the continuity of forest cover (Colinvaux et al. 2000; Bush and Silman 2004; Bush and de Oliveira 2006; Bush et al. 2009). The unchanging humidity conditions favored down-slope

range expansion, little to no demographic growth, and population connectivity during the cold glacial periods, followed by the fragmentation into high altitude populations during warm interglacials (Fig. 2) (Ledig et al. 2002; Lessa et al. 2003; Jaramillo-Correa et al. 2008; Hensen et al. 2011; Schoville et al. 2011).

The sorting of lineages and demographic bottlenecks would be less likely to occur in continuous and large populations (Sommer and Zachos 2009). Although gene flow would homogenize genetic variation in an expanded moist forest, it is likely that divergent lineages pre-dating the last glacial would be present in extant populations and show a marked differentiation (Avice and Walker 1998). However, contrary to the refugia model, the divergent lineages would have an overall wide distribution and show no geographic structuring.

In general, genetic diversity would be favored and preserved throughout the species range by habitat heterogeneity and significant gene flow between populations. After fragmentation, extant populations would be composed of basically the same set of common alleles, resulting in a diffuse genetic structure (Caetano et al. 2008; Sommer and Zachos 2009; Schoville et al. 2011). The extended populations would be composed of several common alleles that would show a more complex genealogy than expected under the dry refugia model. The distribution of allele differences (mismatch distribution) would not fit a unimodal distribution (Rogers and Harpending 1992). In addition, as expected by mutation, rare alleles would be randomly produced over the species range and thus have low probability of clustering (Comps et al. 2001).

Under the moist forests model, the re-colonization process would basically consist of geographically localized altitudinal migration. In this respect, repeated founder effects are less likely to occur in up-slope mountain colonization because postfounding immigration would be highly probable (Hewitt 2004; Ehrich et al. 2007; Hensen et al. 2011). Thus, species with prevailing altitudinal migration would not show any clear geographical gradient of genetic diversity (Tremetsberger et al. 2003). On the other hand, the connectivity between populations would result in the development of a pattern of isolation by distance (Slatkin 1993). This kind of pattern arises with geographically restricted dispersal, resulting in the accumulation of local genetic differences and an increase in genetic differentiation with distance.

Genetic consequences

We propose that the climatic history of Neotropical cloud forests can be investigated by some of the basic genetic and demographic consequences of the dry refugia and

moist forests models (Table 2). In order to successfully assess these models, the proposed consequences represent minimal requirements that can be easily tested with the classic tools of population genetics.

We acknowledge that the above-mentioned consequences do not represent the only testable outcomes of the range dynamics of species during the last glacial period. One possible differing consequence of the two models would be the timing of divergence between lineages. Yet, no models of divergence during glacial maxima have been developed specifically for cloud forest species. A good starting hypothesis would be that under the dry refugia model, divergence events for most loci would trace back to the LGM. However, given the different dynamics of molecular evolution across species, we believe is best to test the refugia hypothesis in terms of the demographic events accompanying the glacial cycles.

In any case, a multi-disciplinary approach including paleodistributional or paleoclimatological data would be more fruitful. Thus, the application of more sophisticated theoretical and analytical tools would be useful in testing the two models (Bayesian and coalescent inference: Ho and Shapiro 2011; colonization simulations: Bialozyt et al. 2006; paleodistribution and spatial modeling: Chan et al. 2011).

Traditionally, uniparentally inherited DNA (chloroplast, mitochondria) has been used to describe geographical patterns of genetic diversity. Nevertheless, the use of multiple genetic markers from different genomes would be advantageous to evaluate distinct attributes of the genetic structure and diversity of populations. In all cases, marker resolution would be the limiting factor when studying the demographic history of species. The choice of markers should be done bearing in mind relevant attributes of the molecular dynamics of distinct markers in particular taxa (e.g., the rate of mutation, the mode of inheritance, neutrality).

Cloud forest phylogeography: testing the models

For the cloud forests, still there are virtually no genetic data for testing the above predictions (Ornelas et al. 2010; Gutiérrez-Rodríguez et al. 2011). Moreover, there are only a handful studies that directly test the genetic proposals of the refugia theory in Neotropical montane taxa. The next section briefly reviews some phylogeographic studies in order to highlight the lack of consensus regarding the glacial dynamics of Neotropical montane species (Table 3) (Velo-Antón et al. 2007; Caetano et al. 2008; Carnaval and Moritz 2008; Carnaval et al. 2009; Barker et al. 2011; de Mello-Martins 2011; Twyford et al. 2012).

Table 3. Supported model of demographic and genetic dynamics during the Last Glacial Maximum for the studied cloud forest and montane species in the Neotropics. (cpDNA = chloroplast DNA sequences, mtDNA = mitochondrial DNA sequences, nDNA = nuclear DNA microsatellites).

| Region | Species | Model |
|-----------------------------------------|-----------------------------------------------------------------------------------|--------------|
| <i>Mexico and upper Central America</i> | | |
| | <i>Podocarpus matudae</i> (cpDNA) (Ornelas et al. 2010) | Moist forest |
| | <i>Palicourea padifolia</i> (cpDNA) (Gutiérrez-Rodríguez et al. 2011) | Dry refugia |
| | <i>Begonia heracleifolia</i> (cpDNA) (Twyford et al. 2012) | Moist forest |
| <i>The Antilles (Puerto Rico)</i> | | |
| | <i>Eleutherodactylus portoricensis</i> (mtDNA, nDNA) (Barker et al. 2011) | Dry refugia |
| | <i>E. coqui</i> (mtDNA) (Velo-Antón et al. 2007) | Dry refugia |
| <i>Brazilian Atlantic forests</i> | | |
| | Vertebrates (mtDNA, distribution modeling) (Carnaval and Moritz 2008) | Ambiguous |
| | <i>Hypsiboas</i> spp. (mtDNA, distribution modeling) (Carnaval et al. 2009) | Dry refugia |

Ornelas et al. (2010) analyzed the phylogeographical structure of chloroplast DNA (cpDNA) haplotypes of *Podocarpus matudae* (Podocarpaceae) in cloud forests of Mexico and upper Central America. Their results appear to support the dry refugia hypothesis and its demographic consequences. The authors found a high genetic differentiation among populations and several common alleles with widespread distributions in distinct regions. In this context, the different common alleles are present in populations located around Toledo's (1982) proposed refugia. However, there is no evidence of range expansions, except in one population, and the separation of populations predates the LGM.

In contrast, Gutiérrez-Rodríguez et al. (2011) explicitly tested the existence of refugia against a scenario of more continuous forest cover during the LGM. They used data on cpDNA haplotypes of *Palicourea padifolia* (Rubiaceae), a species inhabiting cloud forests in Mexico and upper Central America. There was significantly high population differentiation between populations within Toledo's (1982) refugia. The authors also found evidence of demographic expansions in distinct lineages. Thus, Gutiérrez-Rodríguez et al. (2011) support a history of rapid population growth from ancestral populations with small size. However, the authors do not support Toledo's forest refugia for the region and instead advocate in favor of

more widespread refugial populations. The authors do not use paleoecological data and thus it remains unclear whether their results fully reject the dry refugia model or only contradict the geographical location and extent of the proposed refugia.

Twyford et al. (2012) analyzed the genetic structure of two species of *Begonia* (*B. heracleifolia* and *B. nelumbiifolia*, Begoniaceae) in Mexico and upper Central America. *B. nelumbiifolia* is a moist-adapted species living in altitudes from 70 to 1200 m, whereas *B. heracleifolia* is adapted to more seasonal conditions with an altitude range of 300–1700 m (Twyford et al. 2012). Populations of the latter species are characterized by a high genetic differentiation with significant isolation by distance. The authors tested for the genetic structuring of populations within the refugial regions proposed by Toledo (1982), but found that most of the variation was present within populations. Twyford et al. (2012) give no support to Toledo's refugia and instead propose the *in situ* survival of *B. heracleifolia*. In this respect, Twyford et al. (2012) argue that the monomorphic nature of *B. nelumbiifolia* is the result of population bottlenecks. Accordingly, their results appear to fit the history of expanding aridity during the LGM and partially support the existence of refugia, albeit not Toledo's refugia. However, the authors do not provide any demographic tests to corroborate their results and there is no reference to paleoecological data.

Barker et al. (2011) analyzed the genetic structure of the frog *Eleutherodactylus portoricensis*, a species living in cool-moist forests above 600 m in Puerto Rico. The authors found high genetic differentiation and reciprocal monophyly between mitochondrial (mtDNA) and nuclear DNA (nDNA) lineages occupying separate mountain ranges. In this respect, the authors claim that populations from distinct mountain ranges were continually isolated during cold periods, including the LGM, as a result of enhanced dryness. The authors performed several demographic tests with inconsistent results, but in general there are no signs of population changes during the last glacial period. In conclusion, Barker et al. (2011) support that populations of *E. portoricensis* have a long-history of isolation prompted by the enhanced glacial aridity recorded for the Caribbean region (González et al. 2008).

These results are comparable to those of Velo-Antón et al. (2007). These authors analyzed the genetic structure of the frog *E. coqui* in Puerto Rico using mtDNA, a species that inhabits a wide variety of habitats and occupies the complete altitude continuum (0–1300 m, Velo-Antón et al. 2007). As in *E. portoricensis*, populations of *E. coqui* are characterized by a deep genetic differentiation and reciprocal monophyly between lineages from distinct mountain ranges. In this respect, Velo-Antón et al. (2007) observed a unimodal mismatch distribution that, together

with other demographic analyses, showed a history of sudden population decline and subsequent demographic expansion over the last 30 000 years.

Carnaval and Moritz (2008) and Carnaval et al. (2009) explicitly tested the dry refugia hypothesis in the Atlantic rain forests of Brazil by implementing paleodistribution modeling of the Atlantic Forest biome to infer climatically stable areas (refugia) since the LGM. Carnaval and Moritz (2008) then compared the location of the refugial areas with previous mtDNA sequence data for several vertebrate taxa from the Atlantic forest: opossums (*Marmosops incanus*; *Metachirus nudicaudatus*), the Atlantic tree rat (*Phyllomys pattoni*), lizards (*Gymnodactylus darwini*), and the three-toed sloth (*Bradypus variegatus*). In the case of *B. variegatus* and *G. darwini*, the authors observed higher levels of genetic diversity in inferred refugial populations than in populations outside refugia. In addition, these two species showed evidence of population expansions in accordance with the postulated history of refugia. Carnaval and Moritz (2008) did not find congruence between genetic and paleoecological data in all species. On the contrary, Carnaval et al. (2009) did find congruence between the inferred region of habitat stability and mtDNA sequence variation in three frog species: *Hypsi-boas albomarginans*, *H. semilineatus* and *H. faber*.

These results are further supported by de Mello-Martins (2011), who used genetic data on vertebrates from the Atlantic forests of Brazil: howler monkey (*Alouatta guariba*), pitivers (*Bothrops*), the lesser wood creeper (*Xyphorhynchus fuscus*), vampire bat (*Desmodus rotundus*), and frogs (*Hypsi-boas*; *Rhinella crucifer*). Accordingly, de Mello-Martins (2011) inferred a more constant demographic history of the refugia populations compared with the recently colonized southern populations. Thus, at least for some species of the forests of the Brazilian Atlantic coast, the genetic consequences of refugia clearly agree with the paleoecological data and support the expansion of arid conditions over the LGM.

The direct test of the refugia theory has also been applied for seasonally dry tropical forests (Caetano et al. 2008; Poelchau and Hamrick 2011). Contrary to the case of cloud forests, the dryer conditions lead to a more continuous distribution of dry tropical forests during the LGM (Prado and Gibbs 1993; Pennington et al. 2009). In this context, Caetano et al. (2008) analyzed the populations of *Astronium urundeuva* (Anacardiaceae) in eastern South America, a species confined to seasonally dry tropical forests (Caetano et al. 2008). Based on cpDNA and nDNA, the authors inferred the existence of three groups of populations (southeast, central, and northwest) with a high genetic differentiation. The two extreme groups showed a positive correlation between genetic and geographical distance, indicative of isolation by distance. On

the contrary, the central group showed a sign of admixture and did not have any spatial genetic structure. The authors inferred a range expansion from the two extreme groups into the central region and thus supported the more continuous distribution of these forests during dry glacial periods.

Summary of the genetic patterns

It is clear that further genetic analyses are needed to establish the validity of the refugia hypothesis for cloud forests species. The inconsistency between studies can be the result of a differential response of species to climate change. The congruence among the inferred glacial dynamics of different species may be dependent on their distribution, ecology, or phylogenetic relationship. However, this inconsistency can also be the result of the difference in the analytical framework of distinct studies.

In this respect, the few molecular studies show little refinement in explicitly testing the genetic expectations of the dry refugia model and thus provide ambiguous evidence regarding the effect of widespread aridity on cloud forest taxa. Most of these studies do not incorporate available paleoecological data in their discussions. So far, the most detailed tests of the dry refugia model have relied on the direct comparison of the geographical location of proposed refugia and genetic data. Although valuable, the direct geographical inspection of proposed refugia is by itself inconclusive because these proposals are based on limited and inaccurate paleoecological data. This highlights the difficulty in pinpointing the exact location of former refugia without incorporating sufficient paleoecological data in phylogeographic studies.

The above discussion focuses on the genetic outcomes of the last glacial period and in doing so assumes that the genetic imprints of this period have overridden the effects of past glaciations. As observed in these studies, it is highly probable that the genetic divergence of many species pre-dates the last glacial period. However, the demographic events accompanying the LGM would have affected the distribution and frequency of mutations that occurred in the more distant past.

Conclusions

The present study is not an exhaustive review of the literature. This review is intended to highlight the need of a general framework for conducting future studies on Neotropical phylogeography (Fig. 3). Depending on the geographical location and ecology of species, we strongly recommend a case-by-case examination of more detailed paleoecological, geographical, and ecological aspects of each species. The present review highlights the need for

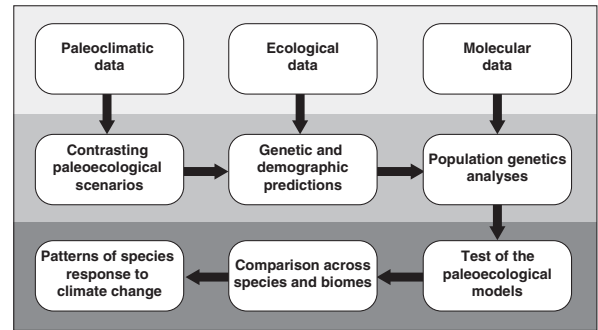


Figure 3. Proposed general framework for conducting phylogeographic studies on Neotropical species based on the integration of paleoclimatic, ecological, and molecular data.

more detailed multi-disciplinary investigations to disentangle the climatic history of Neotropical forest communities. Most importantly, we strongly support the use of explicit alternative hypothesis in the statistical evaluation of the phylogeographic history of Neotropical forest species (Knowles 2001; Avise 2009; Hu et al. 2009; Hickerson et al. 2010; Brown and Knowles 2012; Dawson 2012). The genetic outcomes and hypotheses herein discussed represent a good starting point to achieve this purpose.

The climatic history of the Neotropics might be more complicated than stated herein. We are aware that other hypotheses might be more fitting to the data (e.g., micro-refugia; Rull 2009) and that the alternative scenarios might not be mutually exclusive. The dry refugia and moist forests models are valid first approximations that are prone to further modification. Ultimately, there would be a continuum of species responses to climate change lying in between these two extremes, with particular patterns of genetic diversity being dependent on particular biological and historical attributes of species.

Further genetic studies should deal with co-distributed species, in particular those living within ecologically narrow forest communities, such as cloud forests. This might prove useful in uncovering general ecological response patterns of species to climate change, particularly precipitation. There is need of detailed molecular studies of Neotropical cloud forest taxa that include species with distinct ecologies and that represent different lineages. A first requirement would be to undertake broad scale latitudinal and altitudinal surveys and test them against paleoecological data. This can provide evidence of multiple refugial lineages and diversity gradients. If there is evidence of refugia, studies on the genetic structure can be conducted on finer spatial scales to evaluate the location and extent of these forest refugia. On a wider level, comparative research across species from different biomes, such as seasonally dry forests and deserts, is needed to determine the universality of the refugia hypothesis.

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Biographies

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Santiago Ramírez Barahona is a PhD student under the advisory of LEE and is mainly interested in the ecology, biogeography, and evolution of cloud forest species in the Neotropics.

Conflict of interest

None declared.

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DEMOGRAFÍA HISTÓRICA

RAMÍREZ-BARAHONA & EGUIARTE (2014A)

Resumen.— Los bosques de niebla han sufrido cambios significativos en su distribución durante los últimos 130,000 años. Para probar si estos cambios en la distribución de los bosques de niebla pueden explicar los patrones de diversidad genética en especies características de estos, se evaluó cómo los cambios en la distribución de estos bosques predicen los patrones de diversidad genética en el helecho arborescente *Alsophila firma* (Cyatheaceae). Se colectaron 204 individuos en 16 localidades en México y se obtuvieron secuencias para cinco microsatélites de *cpDNA* y un gen nuclear. Se utilizaron modelos de distribución para predecir la distribución histórica de los bosques de niebla durante el último periodo glacial utilizando dos modelos paleoclimáticos: CCSM (Community Climate System Model) y MIROC (Model for Interdisciplinary Research on Climate). Se realizó una correlación entre la estabilidad temporal de los bosques y la diversidad genética y se realizó un análisis ABC para probar dos escenarios demográficos. Los cambios observados en la distribución de los bosques de niebla durante los últimos 130,000 años han afectado la distribución de la variación genética en *A. firma*. La elevada diversidad genética en áreas con un alta estabilidad ambiental se debe, probablemente, al aumento en los tamaños poblacionales y a una mayor conectividad entre poblaciones. De acuerdo a la expansión de los bosques de niebla predicho por CCSM, los datos genéticos apoyan un escenario de crecimiento poblacional hace ca. 100,000 años, seguido de una divergencia poblacional hace ca. 20,000 años. Sin embargo, una dinámica poblacional de expansión de micro-climas puede reconciliar la estabilidad del bosque de niebla predicha por MIROC y los patrones genéticos observados. La elección del modelo paleoclimático tiene impactos substanciales en las inferencias sobre los patrones genéticos y demográficos. El uso de hipótesis paleoclimáticas alternativas, junto con modelos de distribución de biomas enteros, pueden proveer un marco analítico común para evaluar la cohesión histórica de las comunidades tropicales.



ORIGINAL
ARTICLE



Changes in the distribution of cloud forests during the last glacial predict the patterns of genetic diversity and demographic history of the tree fern *Alsophila firma* (Cyatheaceae)

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ABSTRACT

Aim We investigated changes in distribution of cloud forests during the last 130 kyr, and tested whether these changes explain the spatial patterns of genetic diversity of the tree fern *Alsophila firma* (Cyatheaceae), a species restricted to this habitat.

Location Mexican cloud forests.

Methods We sampled 204 individuals from 16 localities. Genetic data consisted of DNA sequences for five chloroplast microsatellites and one nuclear gene. We used distribution modelling to predict the historical distribution of cloud forests during the last glacial period, using two palaeoclimate models: the Model for Interdisciplinary Research on Climate (MIROC) and the Community Climate System Model (CCSM). We tested the correlation between temporal cloud forest stability and genetic diversity and used an approximate Bayesian computation (ABC) framework to test two plausible demographic scenarios.

Results The range fluctuations observed for cloud forests during the last 130 kyr are key factors affecting the distribution of genetic variation in *A. firma*. Increased genetic diversity in areas with high temporal environmental suitability is probably the result of increased population sizes and higher interpopulation connectivity. In accordance with the expansion of cloud forests predicted by CCSM, the genetic data supported the scenario of a population expansion occurring c. 110 ka, followed by population divergence c. 20 ka. However, population dynamics involving expansion of suitable microclimates could reconcile the stability of cloud forests predicted by MIROC and the observed genetic patterns.

Main conclusions The predicted changes in the distribution of cloud forests were congruent with the population genetics of *A. firma*. However, the choice of palaeoclimate model has a substantial impact on the inferences drawn from the observed genetic and demographic patterns. The use of alternative palaeoclimate hypotheses and biome modelling can provide a common analytical framework for evaluating the historical cohesiveness of forest communities.

Keywords

CCSM, Central America, distribution modelling, landscape genetics, MIROC, Palaeoclimate, phylogeography, refugia, tropical forests.

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INTRODUCTION

The effect of climate change on genetic diversity is one of the key questions in evolutionary genetics (Manel & Holderegger, 2013). Palaeoecological and genetic evidence

has shown that, during the last glacial period, advancing ice sheets and cooling have had an overarching effect on temperate species, displacing these species to isolated southern locations with favourable climatic conditions (Heuertz *et al.*, 2004; Hewitt, 2004; Soltis *et al.*, 2006). Despite the

importance of tropical ecosystems for understanding global climate change (Bush *et al.*, 2011), the effects of glacial climate on tropical montane species remain poorly understood.

The most intuitive outcome of glacial cooling in the tropics, corroborated by palaeoecological data, is the downslope displacements of montane species by several hundred metres (Hooghiemstra & van der Hammen, 1998; Colinvaux *et al.*, 2000; Haffer & Prance, 2001; Jaramillo-Correa *et al.*, 2008; Carnaval *et al.*, 2009; Caballero *et al.*, 2010). This displacement would have increased the area occupied by the species, creating opportunities for population expansion and increased gene flow. However, more complex population dynamics are expected, mainly because of the high climatic and topographical heterogeneity of tropical regions (Bush *et al.*, 2011). The distribution of tropical species was also probably affected by changing precipitation regimes (Bush *et al.*, 2011; Ramírez-Barahona & Eguiarte, 2013).

In the Neotropics, palaeoecological evidence indicates significant changes in precipitation during glacial periods (e.g. Colinvaux *et al.*, 1996, 2000; Caballero *et al.*, 2010; Bush *et al.*, 2011). The ubiquity of such changes has been the subject of intense debate, which has centred on the effect of variation in precipitation on the establishment of tropical forest communities (Hooghiemstra & van der Hammen, 1998; Colinvaux *et al.*, 2000; Haffer & Prance, 2001; Bush & de Oliveira, 2006). Given the ambiguity of palaeoecological data for the last glacial period in the Neotropics (Ramírez-Barahona & Eguiarte, 2013), information on the genetic diversity of species, combined with palaeoclimate modelling, is needed to infer the effect of past climatic changes on tropical forests (Richards *et al.*, 2007).

Ecological niche modelling, or distribution modelling, can be used to estimate present and past species distributions (Elith *et al.*, 2011; Peterson *et al.*, 2011), and thus some studies have explicitly coupled this approach with phylogeography to associate patterns of population structure and forest stability (e.g. Carnaval *et al.*, 2009; Ornelas *et al.*, 2010, 2013; Poelchau & Hamrick, 2011). The estimated distributions, however, can also be compared with population size changes, which can improve inferences about the factors affecting the levels of genetic diversity and the demography of populations (Carstens & Richards, 2007; Carnaval *et al.*, 2009; Brown & Knowles, 2012).

A comparison between species-specific distribution models has been advocated as a suitable approach because of the uncorrelated responses of species to climate change and the effects of biotic interactions (Martínez-Meyer *et al.*, 2004; Collevatti *et al.*, 2013). However, this approach is not without limitations and cross-species comparisons are not straightforward because the estimated probability of presence inferred from the models ultimately depends on the sampling effort and the abundance of the target species (Peterson *et al.*, 2011). In this context, distribution modelling of entire biomes, although questioned (Collevatti *et al.*, 2013), can be used to overcome these difficulties (VanDerWal *et al.*, 2009). This approach has been used successfully to predict the

responses of individual species to climate change, especially when these species are highly restricted to particular biomes (Carnaval *et al.*, 2009; Freedman *et al.*, 2010).

In this study, changes in the distribution of the cloud forests inferred from distribution modelling were compared with population genetic data for the tree fern *Alsophila firma* (Baker) D.S. Conant (Cyatheaceae). The demographic dynamics of this species should be related to changes in the distribution of cloud forest biomes because of a high sensitivity to changing precipitation and temperature (Conant, 1983; Foster, 2001; Mickel & Smith, 2004). We used distribution modelling to describe the historical distribution of cloud forests using the two palaeoclimate models available for the last glacial period: the Model for Interdisciplinary Research on Climate (MIROC; Hasumi & Emori, 2004) and the Community Climate System Model (CCSM; Kiehl & Gent, 2004). Our specific objectives were to: (1) quantify the genetic diversity within populations and correlate this with forest suitability during the last glacial period; (2) evaluate environmental and geographical drivers of population genetic structure and differentiation; and (3) investigate whether the genetic data are consistent with the demographic size changes predicted by the two palaeoclimate models.

MATERIALS AND METHODS

Sampling

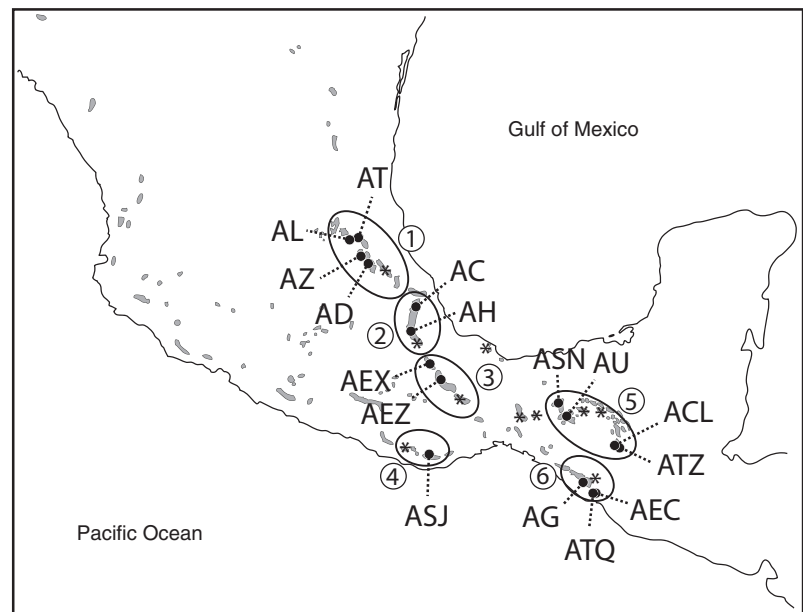
Alsophila firma is distributed throughout the cloud forests of Mexico and Central America, and is highly sensitive to cold and drought (Conant, 1983; Mickel & Smith, 2004). It is a long-lived species with an estimated mean generation time of 10 years (Mehltreter & García-Franco, 2008) and a predominantly out-crossing breeding system (Soltis *et al.*, 1991). As with most ferns, *A. firma* has no known co-evolved herbivores and is biotically independent for its dispersal and reproduction (Page, 2002).

Samples were collected from 204 individuals from 16 localities distributed throughout the range of *A. firma* in Mexico (Fig. 1; see Appendix S1 in Supporting Information). These samples represented a subset of 25 potential sampling localities identified from herbarium records. In many localities tree ferns were absent, as a consequence of recent deforestation and direct tree fern exploitation (S. Ramírez-Barahona, pers. obs.). Once collected, leaf tissue was stored at -20°C until further processing.

Molecular data

Total DNA was extracted following standard cetyltrimethylammonium bromide (CTAB) protocols (Doyle & Doyle, 1990). We amplified four chloroplast intergenic regions with a combined length of 2045 bp (*atpB-rbcL*, *rbcL-accD*, *trnL-trnF*, *matK-rps16*) and a 600-bp fragment of the nuclear gene *gapCp* (Appendix S1). All markers were amplified and

Figure 1 Geographical distribution of sampled localities for *Alsophila firma* (Cyatheaceae) superimposed onto the distribution of cloud forests (grey) in Mexico (CONABIO, 1999). Black circles represent localities with sampled individuals and black stars represent localities where no individuals could be found. Ellipses show the groups of populations used in the analysis of molecular variance (AMOVA) and approximate Bayesian computation (ABC) analyses. (1) Sierra Madre Oriental north, (2) Sierra Madre Oriental south, (3) Sierra Norte de Oaxaca, (4) Sierra Madre del Sur, (5) Altos de Chiapas and (6) Soconusco.



subjected to direct cycle sequencing (High Throughput Genomic Unit, University of Washington, Seattle, WA, USA) and polymorphisms were identified by direct chromatogram inspection using CONSED (Gordon *et al.*, 1998). Genotyping errors were assessed in 20 randomly chosen samples by independent rounds of amplification and sequencing. Unique sequences for *gapCp* and each chloroplast intergenic region were deposited in GenBank (KJ438879–KJ438932).

Chloroplast sequences are completely monomorphic, restricting our analyses to five mono-nucleotide microsatellites (cpSSRs) located within the amplified regions. To evaluate homoplasy by mutation in these markers, linkage disequilibrium was evaluated to search for a random association between allelic states across loci. As the chloroplast genome is non-recombinant, the confirmation of linkage disequilibrium between loci should indicate that homoplasy by mutation is negligible. We constructed cpSSR haplotypes for each sampled individual and calculated the number of haplotypes (h) and haplotype diversity (Hd) per population using DNASP 5 (Librado & Rozas, 2009).

For *gapCp*, single nucleotide polymorphisms (SNPs) were used to reconstruct gene alleles for each individual using the program PHASE 2.1 (Stephens *et al.*, 2001), which implements Bayesian statistics to infer allelic phase from genotype data. Five independent runs of the program were performed to check for consistency of results using the default parameters. For the *gapCp* allele data, allelic richness (A) and gene diversity (Hs) were calculated using the HIERFSTAT package in R (Goudet, 2011). Nucleotide diversity (π) was estimated using DNASP 5. We estimated rarefied measures of *gapCp* allelic richness (A_R) and cpSSR haplotype richness (h_R) for a standardized sample size of five individuals (10 alleles for the nuclear data). All statistical analyses were performed in R.

Data analysis

Distribution modelling

The distribution of cloud forests for the present day, Last Glacial Maximum (LGM; 21 ka) and Last Interglacial (LIG; 130 ka) was predicted using MAXENT 3.3 (Phillips *et al.*, 2006). Cloud forest occurrence data were retrieved from the geographical distribution of vegetation types in Mexico (CONABIO, 1999) and represented 175 unique localities. No occurrence data from Central America were used because of nomenclatural issues. The models were built from 12 climate layers (see Appendix S2) with a 30-arc second resolution obtained from the WorldClim database (Hijmans *et al.*, 2005). To avoid data redundancy, we used only the variables that measured precipitation and temperature on a quarterly basis. Layers for the LGM were obtained from two different palaeoclimate models (CCSM and MIROC) that simulate climatic conditions for the LGM but give differing predictions because of different initial configurations (Braconnot *et al.*, 2007). Distribution models were built with 20 replicates using the default settings and a random 20% of the occurrence records were selected for model validation. The mean area under the receiver operating characteristic curve (AUC) across replicate runs was 0.9827. The present-day distribution model for cloud forest was compared with one constructed for *A. firma* using the same settings as above. This allowed us to quantify the proportion of overlap between models and assess the fidelity of *A. firma* for cloud forest environments (Appendix S2).

The elevation of the predicted areas of cloud forests was characterized by extracting data from 2000 random pixels with values greater than the average 10-percentile training presence threshold (0.313). Differences between distribution

models were evaluated using a Wilcoxon rank sum test. The two palaeoclimate models were characterized in terms of mean annual temperature and annual precipitation using 5500 random points where cloud forests were predicted to occur by any model (Appendix S2). Statistical analyses were handled in R.

Genetic diversity and differentiation

Genetic diversity was expected to be positively correlated with temporal habitat suitability, resulting from more stable population sizes (Brown & Knowles, 2012). Indices of temporal environmental suitability were calculated from the present-day and past distribution models. For each time-specific distribution model, we calculated the spatial autocorrelation (Moran's I) for every pixel and multiplied it by the corresponding original model value. Two indices were calculated for CCSM and MIROC separately, by subtracting the corresponding spatial autocorrelation values for subsequent time periods: $T_1 = \text{LGM} - \text{present}$; $T_2 = \text{LIG} - \text{LGM}$. Because the calculation was done from past to present, negative values indicated increasing suitability. Linear regressions were performed using T_1 and T_2 as predictor variables and measures of genetic diversity as response variables. Three monomorphic populations (AZ, AG and AU; Appendix S1) were omitted from these analyses.

The geographical range of *A. firma* spans several biogeographical regions, which raises the possibility that populations form spatially restricted clades. An analysis of molecular variance (AMOVA) was performed using ARLEQUIN 3.1 (Excoffier *et al.*, 2005) to determine how genetic variation was partitioned among groups of populations, among populations and among individuals within populations. Six groups of populations were defined according to the distribution of cloud forests (Luna-Vega *et al.*, 1999): (1) Sierra Madre Oriental north, (2) Sierra Madre Oriental south, (3) Sierra Norte de Oaxaca, (4) Sierra Madre del Sur, (5) Altos de Chiapas, and (6) Soconusco (Fig. 1).

Other potential environmental drivers of genetic structure were considered: geographical distances between populations; the current distribution of cloud forest; and the distribution of cloud forests during the LGM (CCSM and MIROC). We used a causal modelling approach using partial Mantel testing to identify which of these variables were positively correlated with genetic differentiation (Cushman *et al.*, 2006, 2013; Manel & Holderegger, 2013). The performance of the competing hypotheses was assessed based on whether significant correlations were found between each variable and genetic differentiation, independently of the other variables. We calculated matrices of geographical distance (km) and least-cost environmental distances between populations. Least-cost distances represent paths between pairs of populations that minimize the resistance between them along a given environmental gradient. The distribution models for the present day and LGM were used as layers of habitat connectivity, where high values of the model represented pixels with a low resistance to gene

flow (Ortego *et al.*, 2012). Pairwise genetic differentiation was estimated with D' using the `MMOD` package in R (Winter, 2012), which is an index recommended for highly polymorphic loci (Jost, 2008). We tested for significant Mantel partial correlations with 10,000 permutations. Spatial data and statistical analyses were handled in R.

Demographic history

Changes in the geographical area covered by cloud forests were expected to influence the population sizes of *A. firma*. Based on the predictions of CCSM and MIROC, we investigated whether the genetic data were consistent with demographic expansion or stability. For this, we implemented mismatch distribution analyses, which are robust in identifying recent demographic size changes without using genealogical information (Rogers & Harpending, 1992; Heurtz *et al.*, 2004). The distribution of mismatches is usually multimodal in populations with stable size, reflecting the stochastic shape of gene trees, but it is usually unimodal in populations that have experienced a recent demographic expansion (Rogers & Harpending, 1992). The observed mismatch distributions were compared with those expected under a pure demographic expansion as implemented in ARLEQUIN 3.1 (Excoffier *et al.*, 2005). We calculated the time to the expansion t using the parameter $\tau = 2\mu t$ (Rogers & Harpending, 1992), where μ is the mutation rate. To our knowledge, no estimates of mutation rates for nuclear genes or chloroplast microsatellites are available for fern species. Thus we assumed lower and upper bounds for these rates: 2×10^{-9} and 2×10^{-8} for *gapCp* (Wolfe *et al.*, 1987), 1.0×10^{-5} and 3.0×10^{-5} for the cpSSRs (Provan *et al.*, 1999).

Two plausible demographic scenarios for *A. firma* were compared using the approximate Bayesian computation (ABC) statistical framework (Beaumont *et al.*, 2002; Beaumont, 2010). We used DIYABC 2.0 (Cornuet *et al.*, 2014) to generate 5 million simulated datasets for the cpSSRs and *gapCp*. DIYABC uses coalescent-based simulations, with model parameters drawn from prior distributions, to generate datasets with the same number of gene copies and loci as the observed data. For each simulated dataset, the program estimates summary statistics that are compared with those estimated from the observed data. The parameters used in each demographic scenario were based on a qualitative examination of the predictions of the two palaeoclimate models (Fig. 2; see Appendix S3). A first scenario (*expanded*) consisted of a demographic expansion of an ancestral population t_2 generations ago and a subsequent divergence into six present-day groups of populations t_1 generations ago. A second scenario (*stable*) consisted of an initial divergence of the six groups of populations at t_2 , with no significant changes in population size thereafter (Appendix S3). The posterior probability of scenarios was estimated using a weighted polychotomous logistic regression on the 1% of simulated datasets closest to the observed data (Cornuet *et al.*, 2008; Fontaine *et al.*, 2013). For the best-supported scenario, point

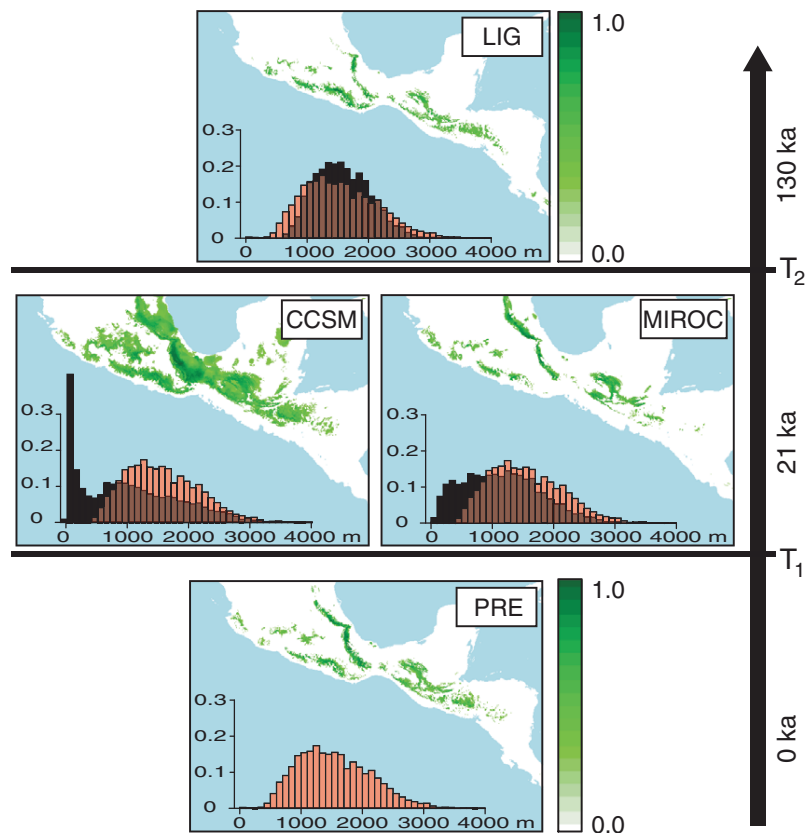


Figure 2 Predicted distribution of cloud forests in Mexico and Central America obtained for the present day (PRE), the Last Glacial Maximum (CCSM and MIROC; 21 ka) and the Last Interglacial (LIG; 130 ka). Frequency histograms show the elevation in metres above sea level of 2000 random points extracted from the distribution models. Red bars correspond to the frequency histograms of the present-day model and black bars show the frequency histograms of the corresponding past-distribution model. The arrow indicates the time-scale used in the approximate Bayesian computation (ABC), going backwards.

estimates for temporal and demographic parameters were estimated by local linear regression on the 1% of simulations closest to the observed data (Beaumont *et al.*, 2002; Cornuet *et al.*, 2008). To assess the confidence in model choice (Robert *et al.*, 2011), we estimated type I and type II error rates by simulating 500 pseudo-observed datasets (PODs) under each scenario (Appendix S3).

RESULTS

Distribution modelling

Compared with the present-day model, the two palaeoclimate models indicated that the elevational range of cloud forests should have been significantly displaced downslope ($P < 0.05$) during the LGM but not during the LIG (Fig. 2). However, the models also suggested that the cloud forests were not fully displaced downslope, but rather their distribution expanded into lowland regions. Although the two palaeoclimate models showed similar elevational shifts, they provided differing results on the past range of cloud forests (Fig. 2). CCSM predicted a considerably wider distribution of cloud forests during the LGM (349% of the present-day distribution), whereas MIROC predicted a slightly reduced distribution during the same period (95%). Compared with the LIG, CCSM predicted a significantly wider distribution for the LGM (446% of the LIG distribution), whereas MIROC predicted a more stable distribution (104%). The two palaeo-

climate models predicted cooler conditions during the LGM compared with present-day conditions. Furthermore, MIROC showed a reduced annual precipitation for the LGM, whereas CCSM showed an increased precipitation for the same time period (Appendix S2).

The comparison of biome and species models showed that *A. firma* was strictly limited to the cloud forest biome. Most of predicted distribution of *A. firma* (81%) lay within cloud forest environments, and only 19% of the cloud forests were not inhabited by *A. firma* (Appendix S2).

Genetic diversity and temporal suitability

We estimated measures of genetic diversity in populations of *A. firma* and tested for a positive correlation between these and indices of temporal cloud forest suitability (T_1 and T_2). We found 21 *gapCp* alleles, with a mean nucleotide diversity (π) of 0.00168 and a gene diversity (H_s) of 0.56 (Fig. 3a, Appendix S1). Nucleotide diversity (π) was positively correlated with T_2 (LIG–LGM) temporal suitability under CCSM ($R^2 = 0.281$, $P < 0.05$; Fig. 4a) but not under MIROC. The standardized allelic richness (A_R) was also positively correlated with T_2 under CCSM ($R^2 = 0.6211$, $P < 0.05$; Fig. 4b) but not under MIROC.

We recovered 42 cpSSR haplotypes, with a mean H_d of 0.96 (Fig. 3b, Appendix S1). An outlier test identified one population (AEX) that had atypically low cpSSR diversity values. By removing this population, measures of genetic

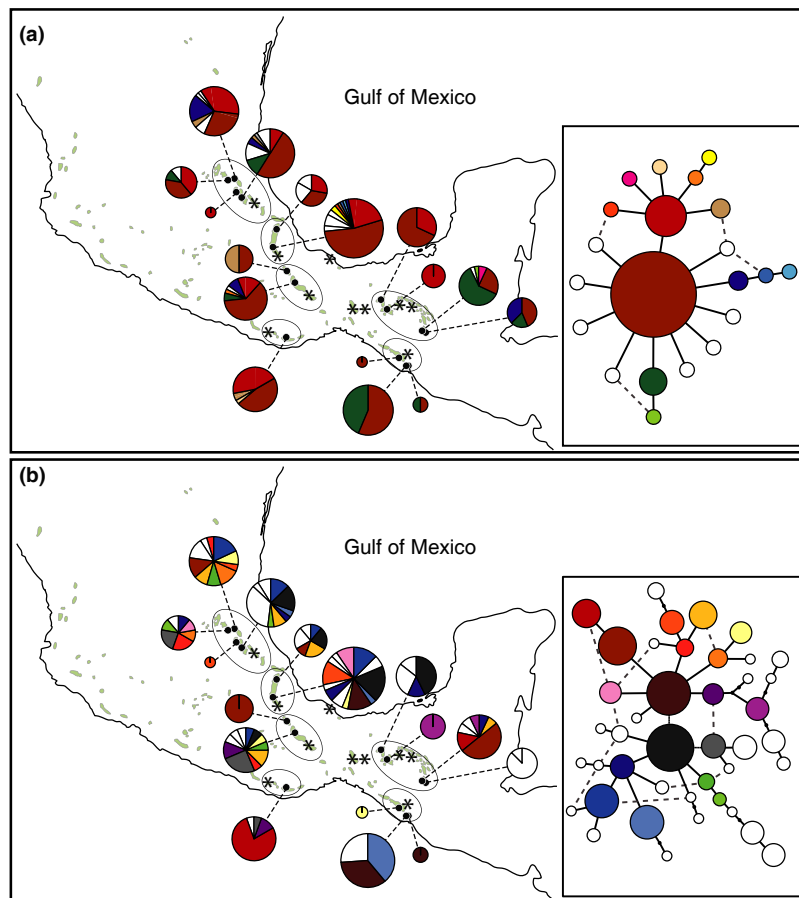


Figure 3 Geographical distribution and statistical parsimony network for (a) the nuclear gene *gapCp* alleles and (b) the chloroplast microsatellite (cpSSRs) haplotypes in *Alsophila firma* (Cyatheaceae) from cloud forest populations in Mexico. Pie charts represent alleles/haplotypes found in each population. The size of sections in the pie charts is proportional to the number of individuals with a given allele/haplotype. Colours indicate different alleles/haplotypes (white represents alleles/haplotypes that are only present in the corresponding population). Black circles represent localities with sampled individuals and black stars represent localities where no individuals could be found. Ellipses show the groups of populations used in the analysis of molecular variance (AMOVA) and approximate Bayesian computation (ABC) analyses. See Appendix S1 for diversity estimates per population.

diversity, H_d and h_R , showed an increase in diversity with increasing T_2 temporal suitability, as predicted by CCSM ($R^2 = 0.326$, $P < 0.05$; $R^2 = 0.436$, $P < 0.05$; Fig. 4c,d) but not by MIROC. The same trends of increasing diversity were recovered by including AEX, but these were not statistically significant.

Measures of genetic diversity were not correlated with sample size ($P > 0.05$). No significant correlations were found between any measure of genetic diversity and T_1 (LGM–present) environmental suitability, using either CCSM or MIROC. Linkage disequilibrium between loci was confirmed in *gapCp* and the cpSSRs, suggesting that homoplasy by mutation was negligible.

Genetic structure and differentiation

Allopatric divergence between the six cloud forest regions could not explain the geographical patterns of genetic variation in *A. firma* (Fig. 3). The AMOVA showed that most of the genetic variation was attributed to differences among individuals within populations (cpSSRs 71.08%, *gapCp* 79.65%), followed by differences among populations within groups (cpSSRs 28.91%, *gapCp* 12.76%) and by differences among groups of populations (cpSSRs 2.22%, *gapCp* 7.6%). Similar results were obtained by grouping populations

into east and west of the Isthmus of Tehuantepec (data not shown).

We used partial Mantel testing to evaluate other factors that could possibly influence pairwise genetic differentiation, including geographical distance, current habitat connectivity and past habitat connectivity. All variables showed significant correlation with pairwise genetic differentiation between populations. However, the expectations of positive partial correlations did not hold, indicating that these variables could not explain, by themselves, genetic differentiation. There was weak support for past connectivity affecting genetic differentiation, although this was subject to the palaeoclimate model (Table 1). For *gapCp* differentiation, geographical distance appeared to be independent of past connectivity under MIROC ($r = 0.21$, $P < 0.01$) but not under CCSM. Similarly, present-day connectivity appeared to be independent of past connectivity under MIROC ($r = 0.22$, $P < 0.05$) but not under CCSM. For the cpSSRs, partial correlations supported past connectivity under MIROC but not under CCSM (Table 1).

Demographic history

We investigated whether the genetic data were consistent with demographic expansion or stability, as predicted by

Figure 4 Linear relationship between the temporal cloud forest stability in Mexico during the Last Interglacial (LIG)– Last Glacial Maximum (LGM) transition (T_2 , environmental suitability) predicted by CCSM, and measures of genetic diversity in the studied populations of *Alsophila firma* (Cyatheaceae). For the nuclear gene fragment *gapCp*, (a) nucleotide diversity (π) and (b) rarefied allelic richness (A_R) are shown. For the chloroplast microsatellites (cpSSRs), (c) haplotype diversity (Hd) and (d) rarefied haplotype richness (H_R) are shown. Significant adjusted R^2 coefficients ($P < 0.05$): (a) 0.281, (b) 0.281, (c) 0.621, (d) 0.436. The width of the points indicates sample size.

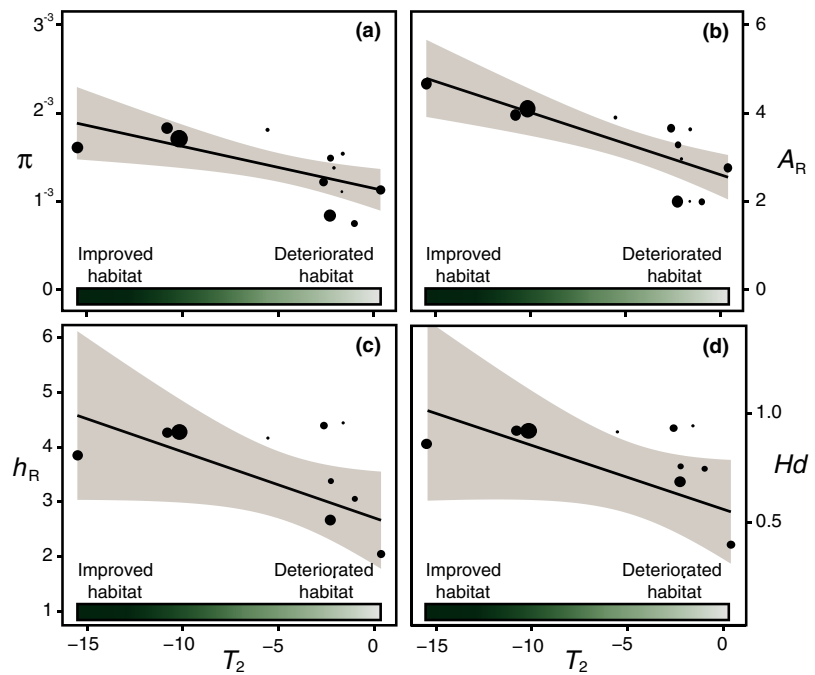


Table 1 Results from causal modelling using partial Mantel tests on the relationship between pairwise genetic differentiation and environmental/geographical distances for *Alsophila firma* (Cyatheaceae) in Mexican cloud forests.

| | Adjusted Mantel r | | | |
|---------------------------------|---------------------|------|--------|------|
| | <i>gapCp</i> | | cpSSRs | |
| D' geographical | 0.36 | *** | 0.16 | * |
| D' geographical present day | 0.09 | n.s. | -0.07 | n.s. |
| D' geographical MIROC | 0.21 | *** | -0.19 | n.s. |
| D' geographical CCSM | -0.06 | n.s. | -0.55 | n.s. |
| $D' \sim$ present | 0.37 | *** | 0.22 | ** |
| D' present day geographical | 0.10 | n.s. | 0.17 | * |
| D' present day MIROC | 0.22 | ** | -0.123 | n.s. |
| D' present day CCSM | 0.05 | n.s. | -0.02 | n.s. |
| $D' \sim$ MIROC | 0.30 | ** | 0.31 | ** |
| D' MIROC geographical | -0.003 | n.s. | 0.32 | *** |
| D' MIROC present day | -0.04 | n.s. | 0.25 | * |
| $D' \sim$ CCSM | 0.38 | *** | 0.25 | *** |
| D' CCSM geographical | 0.12 | n.s. | 0.57 | *** |
| D' CCSM present day | 0.11 | n.s. | 0.12 | n.s. |

Statistical significance of the adjusted partial Mantel correlation coefficients: *** $P = 0.01$; ** $P = 0.05$; * $P = 0.1$; n.s., not significant.

D' , pairwise genetic differentiation; *geographical*, isolation by geographical distance; *present day*, isolation by present-day environmental connectivity; *MIROC*, isolation by past environmental connectivity using the Model for Interdisciplinary Research on Climate (MIROC); *CCSM*, isolation by past environmental connectivity using the Community Climate System Model (CCSM). cpSSRs, chloroplast microsatellites; *gapCp*, a nuclear gene fragment.

CCSM and MIROC, respectively. Mismatch distribution analyses and an ABC framework were used to compare two demographic scenarios for *A. firma*. Mismatch distributions

for *gapCp* and cpSSRs were unimodal ($P_R > 0.05$), with a significant fit to the curves expected under demographic expansion ($P_{ssd} > 0.05$) (Appendix S3). The timing of the expansion was subject to the imprecision of the mutation rates. As a result, the minimum and maximum times to the expansion calculated from τ varied considerably (47.38–473.8 ka for *gapCp*; 55.73–167.2 ka for the cpSSRs).

The posterior probability of the *expanded* scenario, which assumed a demographic expansion followed by population divergence, was significantly higher than that of the *stable* scenario, which assumed early population divergence and no demographic changes (Table 2). Based on the performance of the models, we were able to distinguish between these two scenarios. For *gapCp* and cpSSRs, the *expanded* scenario was correctly identified in 97.2% and 98.2% of the 500 simulated PODs, respectively (Table 2). Accordingly, 4.2% and 12.6% of the 500 PODs simulated under the *stable* scenario were incorrectly identified as the *expanded* scenario. Point estimates for the temporal (t_2 and t_1) parameters under the *expanded* scenario are provided in Table 2. Using a mean generation time of 10 years for *A. firma* (Mehlreter & García-Franco, 2008), t_2 and t_1 were estimated to be c. 110 ka and 20 ka, respectively. The composite θ parameter ($N\mu$) for *gapCp* calculated for the expanded populations was 0.103, whereas the estimate for the present-day populations was 0.0035. Assuming a constant mutation rate, these estimates indicated a significant increase in population size during the last glacial period.

DISCUSSION

We tested how the estimated past distribution dynamics of cloud forests can explain the distribution of genetic variation

Table 2 Confidence values in model choice and estimated time parameters from the approximate Bayesian computation (ABC) analysis performed with `DIYABC` for *Alsophila firma* (Cyatheaceae) in Mexican cloud forests.

| Scenario | <i>gapCp</i> | | cpSSRs | |
|--------------------------------------------|-------------------------|-------------------------|------------------------|-------------------------|
| | Expanded | Stable | Expanded | Stable |
| Posterior probability | 0.8776 (0.8719, 0.8833) | 0.1224 (0.1167, 0.1282) | 0.938 (0.9347, 0.9413) | 0.0620 (0.0587, 0.0653) |
| Model performance (%) | | | | |
| PODs exp. | 97.2 | 2.8 (type I error) | 98.2 | 1.8 (type I error) |
| PODs sta. | 4.2 (type II error) | 95.8 | 12.6 (type II error) | 87.4 |
| Outlying summary statistics ($P < 0.05$) | 7 | 16 | 0 | 2 |
| Time parameters | | | | |
| Divergence (t_1) | 2,200 (1,220–3,300) | – | 2,020 (993–3,150) | – |
| Expansion (t_2) | 11,200 (7,730–14,400) | – | 10,800 (7,560–14,300) | – |

Posterior probabilities are given with 95% confidence intervals. PODs exp., proportion of cases in which each scenario was selected using 500 pseudo-observed datasets simulated under the expanded scenario; PODs sta., proportion of cases in which each scenario was selected using 500 pseudo-observed datasets simulated under the stable scenario. Mean parameter estimates are given with the 5% and 95% quantiles. Time parameters are given in generations (see Appendix S3 for prior distributions and relative mean bias of parameters). cpSSRs, chloroplast microsatellites; *gapCp*, a nuclear gene fragment.

and the demographic history of the tree fern *A. firma*. Based on the species' low climatic tolerance (Conant, 1983; Mickel & Smith, 2004), levels of genetic diversity and population size changes were expected to be closely associated with the temporal changes in the distribution of cloud forests. These associations were tested under two palaeoclimate models (MIROC and CCSM), which gave strikingly different predictions of the distribution of cloud forests during the LGM.

CCSM's predictions support cycling retractions of cloud forests into highland regions during interglacial periods, followed by expansion into the lowlands during glacial periods (Colinvaux *et al.*, 2000; Bush *et al.*, 2011). MIROC's predictions also support cycling elevational displacements of cloud forests but with no range expansion during glacial periods, perhaps because of decreased precipitation levels (Hooghiemstra & van der Hammen, 1998; Haffer & Prance, 2001). The distinct predictions of both models are probably a reflection of the differing precipitation levels predicted by both models, which appear to be more accentuated in areas with high precipitation levels (Poelchau & Hamrick, 2011). The different predictions between MIROC and CCSM highlight the fact that model misspecification can lead to significant biases in the estimation of past distributions (Poelchau & Hamrick, 2011; Collevatti *et al.*, 2013). The validity of alternative past distribution models should thus be evaluated using palaeoecological data (Martínez-Meyer *et al.*, 2004). Unfortunately, palaeoecological data are scarce for the current study region, especially in coastal areas, where cloud forests are predicted to have migrated downslope during the LGM (Ramírez-Barahona & Eguiarte, 2013). Some palaeoecological records support CCSM's predictions for the LGM (e.g. Panama, Colinvaux *et al.*, 1996; Central Mexico, Caballero *et al.*, 2010; Guatemala, Bush *et al.*, 2011), but the climatic conditions inferred from these records are debatable and, thus, some of these data can also be used to support MIROC (Ramírez-Barahona & Eguiarte, 2013).

The mismatch distributions and the ABC analyses give support to a population expansion occurring *c.* 110 ka, followed by population divergence starting at the end of the LGM (*c.* 20 ka). Increasing the number of nuclear markers should increase the accuracy of the ABC analyses (Beaumont, 2010) and the potential to test more detailed hypotheses on the demography and divergence of populations. The duality of the results obtained with the two alternative palaeoclimate models pose some interesting questions for the interpretation of the demographic size changes observed in *A. firma*. The inferred demographic expansion mirrors the range expansion of cloud forests during the LGM as predicted by CCSM. The results of MIROC, however, can explain the observed demographic changes by invoking expansion through sites with suitable microclimates (e.g. gallery forests; Freedman *et al.*, 2010). Nonetheless, allopatric divergence across groups of populations (Ornelas *et al.*, 2010, 2013; Ruiz-Sanchez & Ornelas, 2014), which appeared as the main prediction of MIROC, was not supported by the genetic data.

Allopatric divergence is not an important factor promoting genetic structure in the focal species, as the six groups of populations have little or no genetic structuring between them. Our interpretation follows that of Luna-Vega *et al.* (1999), that the current lack of differentiation among populations is probably the result of past connectivity followed by the recent fragmentation of cloud forests. Some phylogeographical studies (Ornelas *et al.*, 2010, 2013) have suggested that glacial refugia in cloud forest species might not be as important for lineage divergence as previously thought. Here we show that glacial refugia, which predict population size reductions and isolation, are not consistent with the demographic history and genetic structure of *A. firma*. Thus the role of glacial refugia in modulating changes in the distribution of cloud forest should be reconsidered.

The role of environmental drivers of genetic differentiation is critically important to understand and predict species' responses to climate change (Sexton *et al.*, 2014). In the

present case, geographical and environment distances (past and present) appear to influence the observed pairwise genetic differentiation. The influence of the environment on gene flow is common in many species and is probably the result of widespread dispersal and poor adaptation to suboptimal habitats (Sexton *et al.*, 2014). Past environmental conditions also appear to be a good predictor of genetic differentiation across populations (Ortego *et al.*, 2012; Epps *et al.*, 2013). As observed in *A. firma*, this time lag is probably the result of the relatively long generation times and large population sizes inferred during the LGM. However, the effect of past landscapes has been shown to disappear quickly with high rates of long-distance dispersal (Landguth *et al.*, 2010; Ortego *et al.*, 2012), which are common in tree ferns (Korall & Pryer, 2014). Given the ambiguity of our results, we acknowledge that the factors analysed may not provide optimal estimates of resistance to gene flow.

Areas of temporal climatic stability during the last glacial period have been recognized as having the most genetically diverse populations (Carnaval *et al.*, 2009; Knowles & Alvarado-Serrano, 2010; Ortego *et al.*, 2012). Genetic diversity in *A. firma* is greater within populations that were subjected to improving habitat suitability during the LIG–LGM transition. This pattern is probably the result of larger population sizes and increased connectivity between populations (Knowles & Alvarado-Serrano, 2010; Ortego *et al.*, 2012). However, temporal suitability during the LIG–LGM transition can only account for a portion of the variance in genetic diversity, which might result from differences between the distribution of cloud forest biome and that of *A. firma*. Missing environmental data (i.e. variables or spatial resolution) in the cloud forest models may hinder their efficiency to infer correctly changes in distribution (Peterson *et al.*, 2011). Nevertheless, the concerted response of *A. firma* to the changes in the distribution of cloud forests probably reflects the stenotopic character of the species' climatic niche (Conant, 1983).

The probable niche overlap of cloud forests species should result in concordant genetic signals of population size changes (Lessa *et al.*, 2003; Soltis *et al.*, 2006; Carnaval *et al.*, 2009). Ornelas *et al.* (2013) showed a temporal incongruence in the times of intraspecific lineage divergence in several cloud forest species, highlighting the complex evolutionary history of these forests. Nonetheless, evidence of population expansions in some of these species has been uncovered, although with inconsistent results [*Podocarpus matudae* Lundell, Ornelas *et al.*, 2010; *Palicourea padifolia* (Willd. ex Roemer & Schultes) C.M. Taylor & Lorence, Gutiérrez-Rodríguez *et al.*, 2011; *Liquidambar styraciflua* L., Ruiz-Sanchez & Ornelas, 2014]. The distribution dynamics predicted for the cloud forest biome could be used to test explicitly the congruency between demographic histories across species using a common analytical framework (Carstens & Richards, 2007; Richards *et al.*, 2007).

The present analyses of the cloud forest biome have potential drawbacks inherent to distribution modelling (Elith *et al.*, 2011; Peterson *et al.*, 2011), such as simplistic distribu-

tion models resulting from missing relevant environmental factors (e.g. soils and mist; Foster, 2001). Our predicted present-day distribution of cloud forests appears to be more restricted than the one obtained by Rojas-Soto *et al.* (2013), who combined species-specific models to define the distribution of cloud forests. These differences are probably related to the presence of outlier species occurrences within suitable microclimates, which otherwise remain unseen because of the lack of spatial resolution of the climatic data. Ultimately, this and other concerns associated with cross-species model comparison give biome models some advantages over sum-of-species models (VanDerWal *et al.*, 2009; Elith *et al.*, 2011; Peterson *et al.*, 2011).

The drastic differences observed between the present-day and past distributions of cloud forests indicate that they probably underwent dramatic range fluctuations in relatively short periods of time (Lessa *et al.*, 2003; Carstens & Richards, 2007; Bush *et al.*, 2011). These fluctuations, occurring over the last 100 kyr, appear to be key factors affecting the distribution of genetic variation of species (Carnaval *et al.*, 2009; Gutiérrez-Rodríguez *et al.*, 2011). However, there is the need to obtain a clearer picture on how changing climate exerts its effects on the population genetics of species. Co-distributed species do not necessarily respond uniformly to the climatic forces that have shaped the distribution of the ecosystem (Carstens & Richards, 2007; Collevatti *et al.*, 2013). Although distribution modelling of entire biomes has this potential drawback (Collevatti *et al.*, 2013), this approach can provide a common framework for performing comparative studies. As shown here, distribution modelling of cloud forests can be used to predict the patterns of genetic diversity and demographic history of species restricted to these forests. Furthermore, biome modelling can be used to assess the degree of ecological and temporal cohesiveness of these forest communities, which is critically important in understanding and predicting the response of species to climate change.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Primer sequences and measures of genetic variation per population.

Appendix S2 Supplementary methods and results for the distribution modelling.

Appendix S3 Supplementary methods and results for the demographic analyses.

BIOSKETCHES

Santiago Ramírez-Barahona is a PhD student supervised by L.E.E. and is interested in the ecology, biogeography and evolution of cloud forest species, particularly ferns.

Luis E. Eguiarte is Professor of Evolutionary Ecology and Population Genetics at the Institute of Ecology of the National Autonomous University of Mexico (UNAM). His current research projects include the study of the evolution, ecology and conservation genetics of several groups of Neotropical plants, and he has made several contributions to these subjects.

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ESTRUCTURA GENÉTICA ESPACIAL

RAMÍREZ-BARAHONA & EGUIARTE (2014B)

Resumen.— La presencia de estructura genética espacial (EGE) en plantas con semilla ha sido conectada con algunos atributos biológicos de las especies, como el sistema de apareamiento y la forma de vida. Sin embargo, se sabe poco acerca de la EGE en helechos, los cuales son únicos entre las plantas terrestres por tener un ciclo de vida con alternancia de fases. En este estudio se investigó la EGE en dos poblaciones de *Alsophila firma* (Cyatheaceae) combinando análisis de agregación espacial y de auto-correlación genética espacial con cinco microsatélites de cloroplasto y un gen nuclear. Se compararon los patrones observados con aquellos estimados para otras especies de plantas con semillas y de helechos. Las poblaciones de *A. firma* mostraron una marcada EGE con agregación espacial de individuos, un alta diversidad clonal y ausencia de autofertilización. Comparada con la EGE de especies de plantas con semilla analizadas a la fecha, la EGE en especies de helechos es más alta. Contrario a las expectativas, el sistema de apareamiento cruzado, la dispersión por viento de las esporas y la forma de vida arborescente, no se traducen en una débil o nula EGE. En los helechos, la EGE probablemente se ve afectada por el ciclo de vida con dos fases de vida libre. En este sentido, la biología reproductiva de los helechos parece ser más compleja de lo que se había asumido (*i.e.*, autofertilización, compensación reproductiva). Esto implica que la EGE en helechos está probablemente siendo afectada por factores que no son evidentes a partir del estudio de las plantas con semilla.

Spatial genetic analyses reveal strong genetic structure within two populations of the outcrossing tree fern *Alsophila firma* (Cyatheaceae)

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Running title: Spatial genetic structure in tree ferns

ABSTRACT

The development of spatial genetic structure (SGS) in seed plants has been linked to several biological attributes of species, such as breeding system and life form. However, little is known about SGS in ferns, which are unique among land plants in having an alternating life cycle. We combined spatial aggregation statistics and spatial genetic autocorrelation analyses using five chloroplast microsatellites and one nuclear gene to investigate SGS within two populations of the outcrossing tree fern *Alsophila firma* (Cyatheaceae). We assessed how the observed patterns compare to those estimated for other seed plants and ferns. Populations of *A. firma* exhibited strong SGS, spatial clustering of individuals, substantial clonal diversity and no inbreeding. SGS in ferns appears to be higher than in most seed plants analyzed to date. Contrary to expectations, an out-crossing breeding system, wind dispersal and an arborescent life form, do not translate into weak to no SGS. In ferns, SGS is most likely being affected by the unique life cycle with two free-living life stages. The reproductive biology of ferns appears to be more complex than previously thought. This implies that SGS in ferns is being affected by some factors which are not evident from the study of flowering plants.

Keywords: arborescence - clonality - dispersal - fine scale genetic structure - inbreeding - population genetics - pteridophytes

INTRODUCTION

Spatial genetic structure (SGS) in plant species can be defined as the non-random spatial distribution of genotypes within populations. The presence of SGS is tightly linked to key evolutionary and ecological processes, such as dispersal, inbreeding, mating system, local adaptation and population dynamics (Smouse & Peakall, 1999; Ng, Lee & Koh, 2004; Chung, Nason & Chung, 2011; Pandey & Rajora, 2012). Population genetics theory predicts that in sexual species with restricted dispersal, a patchy distribution of genotypes will quickly develop within populations (Loiselle *et al.*, 1995; Smouse & Peakall, 1999; Dutetch *et al.*, 2002; Epperson, 2005, 2007). The localised dispersal of sexually produced propagules has been considered one of the main processes generating SGS (Heywood, 1991; Loiselle *et al.*, 1995; Vekemans & Hardy, 2004; Clark-Tapia *et al.*, 2005; Domínguez *et al.*, 2005; Epperson, 2005), and so the strength of SGS has generally been negatively correlated with the dispersal capability of species (Vekemans & Hardy, 2004; Epperson, 2007).

Spatial genetic autocorrelation methods have been widely used to identify correlations among genotypes and locations of individuals (Loiselle *et al.*, 1995; Vekemans and Hardy, 2004). Despite the crucial role of propagule dispersal, SGS in plants is highly dependent on other demographic and biological factors, such as the extent and frequency of sexual and asexual reproduction (van Rossum *et al.*, 2004; Clark-Tapia *et al.*, 2005; Dutetch *et al.*, 2008; Chung *et al.*, 2011; Pandey & Rajora, 2012). These analyses can reveal the existence of relevant underlying ecological processes, such as clonal propagation (Dutetch *et al.*, 2008) and habitat heterogeneity (Kalisz *et al.*, 2001). Such processes would not only affect the levels of SGS, but would also affect the spatial aggregation of individuals (Kalisz *et al.*, 2001; Wiegand & Moloney, 2004). Thus, spatial clustering analyses can be used to evaluate hypotheses about specific processes affecting SGS and thus lead to more robust inferences (Wiegand & Moloney, 2004; Chung *et al.*, 2011). In particular, asexual propagation can effectively increase SGS at short distances (Alberto *et al.*, 2005; Dutetch *et al.*, 2008). However, this would depend on the spatial extent at which clonal growth occurs and the frequency of clone intermingling (Charpentier, 2002). For plants with mixed sexual and

asexual reproduction, incorporating both aspects of reproduction is essential to make a correct inference about patterns of gene dispersal and inbreeding within populations (Dutetch *et al.*, 2008). The need of evaluating these effects is further accentuated in species with cryptic asexual reproduction, where evidence of clonal propagation is limited.

Although factors affecting SGS are well understood, most studies have almost exclusively focused on seed plants, especially angiosperms. In general, these studies have shown that herbaceous and selfing species tend to have stronger SGS than arboreal and out-crossing species, respectively (Dutetch *et al.*, 2002; Ng *et al.*, 2004; Vekemans & Hardy, 2004; Pandey & Rajora, 2012). To fully understand these correlations, the widespread presence of SGS should be investigated in other groups of plants with different biological attributes. Besides flowering plants, ferns are a group that comprises species with different life forms and breeding systems. Ferns are unique among land plants in having a complex life cycle with two free-living life stages (*i.e.*, gametophyte, sporophyte), which has been considered as a main factor affecting their molecular evolution (Klekowski, 1973; Korall, Schuettpeiz & Pryer, 2010; Chung & Chung, 2013) and, therefore, is probably a major factor affecting SGS in these plants. To date few studies of SGS have focused on ferns species, all of which are terrestrial and acaulescent (*Pteris multifida*, Murakami *et al.*, 1997; *Dryopteris aemula*, Jiménez *et al.*, 2010; *Cyrtomium falcatum*, Chung & Chung, 2013). Based on the wind-dispersion of spores, fern populations were expected to show weak to no SGS. However, except for Jiménez *et al.* (2010), authors have found strong SGS associated to localized spore dispersal. Other factors, such as life form and breeding system, should also be linked to SGS in ferns.

The present work explores SGS within two populations of the tree fern *Alsophila firma* (Baker) D.S. Conant (Cyatheaceae) in Mexico. This is a rare species that occurs mainly as a riparian species in montane cloud forests from Mexico to Panama, with stems that can reach 15 meters in height. Some populations of *A. firma* have been characterised as out-crossing (Soltis, Soltis & Smith, 1991) and, as most tree ferns, with a copious production of wind-dispersed spores (Conant, 1983). Clonal growth is absent or unknown in American species of *Alsophila* (Conant, 1983), although it has been documented in other species of tree ferns (Hallé, 1966; Lenhert, 2011). More recently, however, anecdotal observations of clonality

occurring within 60-80 cm have been made for *A. firma* (Mehlreter and García-Franco, 2008), although clonal frequency remains unknown. To date, molecular markers for tree ferns are scant to non-existent, with few molecular studies being conducted in the family Cyatheaceae (*e.g.*, Korall *et al.*, 2010; Wang & Guan, 2011), all of which have used chloroplast DNA sequences. Here, we developed markers for *cpDNA* microsatellites (*cpSSRs*) and for the nuclear gene *gapCp* to investigate SGS in *A. firma*. More specifically, we (1) combined spatial aggregation statistics and spatial genetic autocorrelation analyses to address the existence of SGS in populations of this tree fern; (2) assessed how the observed patterns compare to SGS estimated for seed plants and ferns; and (3) discuss how the strong SGS estimated for ferns might be affected by their unique life-cycle, inbreeding and clonal growth.

MATERIALS AND METHODS

Sampling and mapping

Sample sites were located within two cloud forest patches in central-eastern Mexico. The first site (Huatusco, Veracruz: AH) is located on a private biological reserve comprising ca. 200 ha of cloud forest at 1370 m of elevation (96.9907° W, 19.1906° N). The second site (Tlanchinol, Hidalgo: ATL) is found within an unprotected cloud forest patch of ca. 20,000 ha at 1460 m of elevation (98.6321° W, 20.9826° N). In each population, all identifiable individual stems (AH: 115, ATL: 102) were tagged and mapped along a 200 m section of a forest trail running parallel to a stream (Fig. 1). The location of individual stems was carefully mapped using the distance (m) and compass bearings between individuals, thus obtaining the relative spatial coordinates of each individual.

Leaf tissue samples were taken for each individual stem from fully expanded fronds and stored at - 80°C. For each sampled individual, total DNA was extracted following CTAB protocols (Doyle and Doyle, 1990). As a result of the poor quality of leaf tissue for some samples, PCR amplification of all markers was successful only for 74 out of 115 and 99 out of 102 individuals from AH and ATL, respectively.

Molecular data

Chloroplast DNA. Four chloroplast intergenic regions (*atpB-rbcL*, *rbcL-accD*, *trnL-trnF*, *matK-rps16*) were amplified using standard PCR protocols and primers (Taberlet *et al.*, 1991; Ebihara *et al.*, 2003; Korall *et al.*, 2007; Supporting Information Table S1). Due to the lack of single nucleotide polymorphisms, we restricted our analyses to five mono-nucleotide microsatellites (*cpSSRs*) located within these regions, which were subjected to direct cycle sequencing (High Throughput Genomic Unit, University of Washington, Washington). Length polymorphism was assessed by direct chromatogram inspection with Consed (Gordon, Abajian & Green, 1998). Independent rounds of amplification and sequencing using 20 randomly chosen individuals were performed to assess genotyping errors. Homoplasmy resulting from mutation was evaluated by testing for linkage disequilibrium using the ‘genetics’ (Warnes *et al.*, 2012) package in R 2.15.2 (R Core Team, 2012). Sequences of the four intergenic sequences were deposited in GenBank: KJ438908-KJ438932.

Nuclear DNA. Initial amplification of the nuclear *gapCp* gene (*glyceraldehyde-3-phosphate dehydrogenase*; Schuettpeitz *et al.* (2008) yielded three different sized products [(*short* (ca. 700); *medium* (ca. 900); *large* (ca. 1300 bp)]. For three individuals, all copies were purified using the QIAquick Gel Extraction Kit (QIAGEN, Netherlands) and subjected to direct cycle sequencing. The resulting sequences were manually aligned to other known *gapCp* sequences of ferns (Schuettpeitz *et al.*, 2008): *Adiantum malenasium* (EU551254, EU551253), *Cheilanthes covillei* (EU551268, EU551263), *Pteris vittata* (EU551448, EU551443) and *Dryopteris marginalis* (EU551409, EU551411). Only the medium copy (*gapCp-M*) could be successfully aligned. Specific primers for *gapCp-M* (Supporting Information Table S1) were designed to amplify an intron of this gene copy. For each individual, products from independent rounds of amplification were sequenced using forward and reverse primers separately. Single nucleotide polymorphisms were identified by direct chromatogram inspection with Consed (Gordon *et al.*, 1998). Independent rounds of amplification and sequencing using 20 randomly chosen polymorphic individuals were undertaken to check for

genotyping errors. Linkage disequilibrium between loci was evaluated using the ‘genetics’ package in R. Sequences were deposited in GenBank: KJ438879-KJ438907.

Data analysis

Point pattern analysis. Point pattern analyses were performed using the univariate O-ring statistic of Wiegand & Moloney (2004), implemented in the software Programita (Wiegand, 2004). The $O(r)$ function describes the expected number of points (individuals) at a distance r from an arbitrary point. Compared to other functions, such as Ripley’s K , the $O(r)$ utilizes concentric rings, which has the advantage of isolating specific distance classes and detecting spatial clustering at a given distance r (Wiegand & Moloney, 2004), making it directly comparable to SGS. The significance of the spatial clustering is typically evaluated by comparison against a null model with complete spatial randomness (CSR) using multiple Monte Carlo simulations (Wiegand & Moloney, 2004).

These analyses were carried out on the total number of individual stems (AH: 115 and ATL: 102), with an observation window defined by a 10 meter buffer around individuals. This ensured that the CSR simulations were directly comparable to the observed clustering. An edge correction was applied, where the number of points in an incomplete ring was divided by the proportion of the ring that lied within the observation window (Wiegand, 2004). The number of points within each ring was maximized by setting the ring width to 2 meters, reducing the raggedness of the function. Confidence intervals (%95) for the CSR model were obtained from 999 replicates. Finally, we tested for the effects of reduced sample size by conducting the same analysis on a reduced dataset containing only those individuals that were successfully genotyped (74 in AH and 99 in ATL).

Genetic diversity. The nuclear SNP genotypes were used to reconstruct gene alleles for each individual using the program Phase 2.1 (Stephens, Smith & Donnelly, 2001), which implements Bayesian statistics to infer allelic phase from genotype data. The program was set to run with the default parameters: 100 iterations, with 100 burn-in and a thinning interval

of 1. Five independent runs were performed to check for consistency of results. Using the allele data for each individual (*i.e.*, one locus), deviations from Hardy-Weinberg Equilibrium (HWE) and the following measures of genetic variation were calculated using the ‘hierfstat’ (Goudet, 2011) package in R: observed heterozygosity (H_o), gene diversity (H_s) and inbreeding coefficient (F_{IS}).

Clonal assignment of individual stems was based on the probability of the joint occurrence of *cpSSRs* haplotypes and *gapCp-M* alleles. The probability that two individuals share the same genomic combinations by chance (p_{gen}) was calculated as the product of the frequencies of the corresponding nuclear alleles and the *cpSSRs* haplotype (Parks & Werth, 1993). Individuals having identical genomic combinations with $p_{gen} < 0.05$ were assigned to the same clone. Identical genomic combinations were partitioned into several clones if the corresponding stems formed clusters separated by more than 10 meters. In this case, we calculated the probability that a pair of genetically identical individuals forming distinct spatial clusters be separate clones (p_{se}) as $1 - (1 - p_{gen})^G$, where G is the number of different clones identified (Parks & Werth, 1993).

Following Alberto *et al.* (2005), clonal diversity (R) was measured as $(G-1)/(N-1)$, where G and N are the number of distinct clones and individuals analyzed, respectively. After counting the number of copies of the same clone, the mean inter-*ramet* distance (\pm SE) for each clone was calculated using the ‘stats’ (R Core Team, 2012) and ‘spatstat’ (Baddeley & Turner, 2005) packages in R. Differences between populations in these statistics were assessed using Wilcoxon rank sum tests as implemented in the ‘stats’ package in R.

Spatial autocorrelation analyses. We assessed SGS within populations using spatial autocorrelation analyses as implemented in SpaGeDi (Hardy & Vekemans, 2002). All analyses were performed using Loiselle *et al.* (1995) kinship coefficient (R_L), which performs well under different levels of inbreeding (Vekemans & Hardy, 2004). For each population, autocorrelation analyses were performed separately on the *cpSSRs* haplotype and *gapCp-M* allele data sets by considering only individual pairs belonging to different clonal categories. To

estimate how clonal growth affects SGS, we performed autocorrelation analyses using all pairs of sampled individuals stems.

Kinship coefficients were computed for 20 distance intervals (maximum distance in meters) that were arbitrarily defined in order to maximize the number of pair-wise comparisons. Significance of R_L at each distance interval and the inbreeding coefficient (F_{IS}) were obtained after 10,000 random permutations of individuals among locations and genes among individuals, respectively. Significant SGS was evaluated by testing the regression slope of R_L on the logarithm of distance (b_R) with 10,000 replicates. To assess the consistency of SGS between populations and loci, 95% confidence intervals (CI) around b_R were approximated as $b_R \pm 1.96 \text{ s.e.}$, using the *s.e.* obtained from the regression analysis. Finally, SGS was quantified using the Sp statistic, $-b_R / (1 - F_{(1)})$, where $F_{(1)}$ is the mean coefficient for the first distance interval. The Sp statistic is robust to the arbitrary setting of the first distance class (Vekemans & Hardy, 2004).

RESULTS

Point pattern analysis. The $O(r)$ function showed that individuals in both populations have significant spatial aggregation at short distances (Fig. 2). Compared to AH, the ATL population showed a higher O -ring statistic at the first distance interval and a more rapid decrease of clustering with increasing distance. Individuals from AH showed significant spatial clustering extending larger distances than individuals from ATL (Fig. 2). This indicates that, in general, individuals from ATL form more restricted and dense clusters than individuals from AH. The tests performed with the reduced datasets showed that the reduction in sample size in AH produced a less intensive clustering, but did not affect the spatial patterns of clustering (data not shown).

Genetic diversity. The five *cpSSRs* were in linkage disequilibrium, which confirms the absence of significant levels of homoplasmy due to mutation in these highly variable markers. From these data, 24 *cpSSRs* haplotypes were recognised for the species, of which 15 and 16 were

found in AH and ATL, respectively (Supporting Information Table S2). Measures of haplotype diversity were similar for both populations: 0.88 (AH) and 0.91 (ATL) (Table 1).

The nuclear SNPs were also under linkage disequilibrium. The most likely reconstruction of allelic phase in the nuclear gene resulted in 25 alleles for the species. Populations had 15 (AH) and 11 (ATL) alleles, which produced 18 and 22 distinct genotypes that were in HWE. Populations shared only one *gapCp-M* allele, which was the single most common allele in AH (Supporting Information Table S2). ATL had a more even distribution of allele frequencies than AH. Nuclear genetic diversity was high across the two studied populations: mean observed heterozygosity (H_o) was 0.38 and mean gene diversity (H_s) was 0.36 (Table 1). Inbreeding coefficients (F_{IS}) ranged from - 0.071 to - 0.044, but were not significantly different from zero.

In both populations, most individuals sharing the same genomic combinations were identified as the likely result of clonal propagation, rather than sexual reproduction ($p_{gen} < 0.05$). In AH, however, nine individuals showed a higher probability of sharing the same genotype by chance ($p_{gen} > 0.05$). These individuals formed two spatial clusters separated by more than 10 meters and thus were assigned as distinct clones ($p_{se} > 0.05$). In ATL, the two genomic combinations with the highest $p_{se} (> 0.01)$ showed spatial clusters separated by more than 20 meters and thus were considered as distinct clones ($p_{se} > 0.05$). Both populations had substantial levels of clonal diversity: 0.563 and 0.439 for AH and ATL, respectively (Table 1). Given the numbers of nuclear markers used, a low power to detect sexually produced identical genotypes is possible, especially in AH, where there is a single most common *gapCp-M* allele (Supporting Information Table S2).

The frequency distribution of the number of *ramets* per clone showed that ca. 50% of clones were unique in both populations (Supporting Information Fig. S1). The proportion of clones occurring more than twice was greater in ATL than in AH, where the most spread clones comprised six and nine individual stems. The Wilcoxon test failed to detect significant differences between populations in this proportion ($W=827.5$, $p < 0.473$). The observed clones were more spatially restricted in ATL than in AH (Supporting Information Fig. S1). The mean inter-*ramet* distance (\pm s.e.) was 223.41 ± 16.43 cm in ATL and 174.11 ± 9.64 cm in AH. The

Wilcoxon test indicated that on average, inter-*ramet* distances in AH were greater than in ATL ($W=4700$, $p < 0.00235$).

Spatial autocorrelation. Analyses considering only individual pairs belonging to different clones, resulted in a negative linear relationship between kinship coefficients and the logarithm of distance in all analyses for both populations (Fig. 3). Permutation tests showed that regression slopes were significantly different from zero in all analyses (Table 2). Overall, steeper regression slopes were observed in the ATL population, which were significantly distinct from the slopes obtained for the AH population. The *gapCp-M* gene had a weaker SGS than the *cpSSRs* (Table 2), with the regressions slopes for *cpSSRs* being significantly steeper than those for *gapCp-M*. This trend, however, was not observed in AH. For this population, the *cpSSRs* and *gapCp-M* had similar SGS, with overlapping regression slopes.

The *Sp* statistics indicated a strong SGS in both populations, with a mean *Sp* value of 0.127 and 0.036 for the *cpSSRs* and *gapCp-M*, respectively (Table 2). Clonal propagation had a significant effect on SGS, effectively increasing the levels of structuring in both populations at short distances. The analyses performed on all sampled individuals indicated a more pronounced SGS when repeated genomic combinations were considered, considerably increasing the mean *Sp* statistic in both *cpSSRs* and *gapCp-M* (data not shown).

DISCUSSION

We evaluated the presence of SGS in populations of the tree fern *Alsophila firma*. Based on the wind-dispersal of spores, out-crossing breeding system and arborescent life form, populations were expected to exhibit low to non-significant SGS. On the contrary, significant negative correlation between kinship coefficients and increasing distance was observed, where individuals within short distances were genetically more similar than expected by chance. Populations also exhibited significant spatial clustering of individuals, substantial clonal diversity and no inbreeding.

Consistent with the observed SGS patterns in flowering plants (Vekemans & Hardy 2004), the tree fern *A. firma* showed stronger SGS than acaulescent ferns. However, the generality of such a comparison is limited by the scarcity of fern species analyzed to date [*Pteris multifida*, Murakami *et al.* (1997); *Dryopteris aemula*, Jiménez *et al.* (2010); *Cyrtomium falcatum*, Chung & Chung (2013)]. Furthermore, the results of two of these studies are not conclusive as to SGS in ferns populations. The absence of SGS in *D. aemula* may be the result of a history of recent colonization of populations (Jiménez *et al.*, 2010), whereas the contribution of sexual reproduction to SGS in *P. multifida* was not confidently evaluated (Murakami *et al.* 1997). It is important to consider the frequency of clonality to make reasonable comparisons of SGS between species, because this process should have marked effects on SGS (van Rossum *et al.*, 2004; Clark-Tapia *et al.*, 2005). The higher SGS observed by analyzing all sampled individuals of *A. firma* shows the potential bias that clonality has on estimates of SGS.

Chung & Chung (2013) compared their results to the data compiled by Vekemans & Hardy (2004) for flowering plants, concluding that *C. falcatum* has one of the highest estimates of SGS observed to date. We observed that the mean *Sp* estimate for *gapCp-M* (0.036) in *A. firma* is comparable to that of herbaceous angiosperms with animal-pollination and gravity-dispersed seeds (Vekemans & Hardy 2004). From the 26 trees considered by Vekemans & Hardy (2004), only two showed stronger SGS than *A. firma*, *Vouacapoua americana* (Caesalpiniaceae) in French Guiana (Dutetch *et al.*, 2002) and *Helicteres brevispira* (Malvaceae) in Brazil (Franceschinelli & Kesseli, 1999), both with limited dispersal of seeds, substantial spatial clustering and inbreeding (Dutetch *et al.*, 2002; Franceschinelli & Kesseli, 1999). *A. firma* also shows stronger SGS than populations of the conifers *Pinus pinaster* (Pinaceae) in Spain (De Lucas *et al.*, 2009) and *Thuja occidentalis* (Cupressaceae) in eastern North America (Pandey & Rajora, 2012). In these species, large continuous populations had weak to no SGS, whereas small and fragmented populations had almost a two-fold increase in SGS (De Lucas *et al.* 2009; Pandey & Rajora 2012). Even the small, fragmented and inbred populations of these species showed lower SGS than *A. firma*.

Assuming that dispersal has an overarching effect on SGS (Epperson, 2005), the present results indicate that spore dispersal is probably limited within fern populations (Peck,

Peck & Farrar, 1990). Korall & Pryer (2014) suggested that tree ferns have very low rates of long-distance dispersal events in time. Accordingly, spores of *A. firma* are known to have a low viability (< 10%, Bernabé, Williams-Linera & Palacios-Ríos, 1999) and thus, although wind-dispersed, can only travel relatively short distances (Peck *et al.*, 1990). Anecdotal observations of clonal growth have been made in *A. firma* (Mehltreter & García-Franco 2008) and other species of *Alsophila* and *Cyathea* (Hallé, 1966; Lenhart 2011). The two processes—clonality and limited dispersal—are probably related. For instance, in taxa with limited seed (spore) fertility, population fitness would be augmented by an increase of clonal propagation (Winkler & Fischer, 2001). A negative correlation between the fertility of spores and clonal growth has been proposed for tree ferns in general (Hallé, 1966), which could partly explain the observed SGS. To investigate the widespread presence of this correlation, clonal growth and fertility need to be quantified in other species.

Differences in SGS among populations might be a reflection of a sampling bias caused by missing *gapCp-M* genotypes for individuals in AH. This is not surprising because kinship coefficients are highly dependent on sampling (Vekemans & Hardy, 2004; Epperson, 2005, 2007). However, the population with a higher intensity of clustering (ATL) shows a more pronounced SGS, suggesting that spatial clustering might be affecting SGS (Dutetch *et al.*, 2002). The aggregated distribution of individuals can be the result of limited spore dispersal, but also result from spatial heterogeneity in the distribution of sites with favorable conditions for establishment and maturation (Kalisz *et al.*, 2001), particularly of the fragile gametophytic stage (Peck *et al.*, 1990; Bernabé *et al.*, 1999). In this context, the complex life cycle of ferns, with two alternating free-living life stages might impose serious limits to the effective dispersal of genes within populations (Chung & Chung, 2013). We are aware that the analysis of SGS without reference to demographic information might fail to observe significant patterns of structuring (Soltis *et al.*, 1991; De Almeida-Vieira *et al.*, 2002; Ng *et al.*, 2004; De Lucas *et al.*, 2009). The study of the spatial genetics and demography of the two life stages of ferns would be highly valuable to deepen our understanding of the causes of SGS in these plants. This assessment, however, would be limited by the difficulty of studying natural population of gametophytes, mainly resulting from highly similar gametophyte morphologies across species (Conant, 1990).

Ferns are unique among land plants in having a life cycle that involves the alternation between two free-living stages (Page, 2002). The breeding mechanisms involved in this alternation may hold the key to understand the observed patterns of SGS in ferns. For example, the asymmetry between the dispersal of spores and that of male gametes (Page, 2002) might be affecting SGS (Chung & Chung, 2013). In seed plants, an asymmetry in the dispersal distances between seeds and pollen has been associated with significant SGS within populations (Kalisz *et al.*, 2001). In ferns, *cpDNA* and *nDNA* would be equally dispersed by haploid spores. Assuming the maternal inheritance of *cpDNA* (Guillon & Raquin, 2000), *nDNA* would be further dispersed by the movement of male gametes, which should result in a lowering of SGS in nuclear genes compared to *cpDNA*. In *A. firma*, the lower SGS for *gapCp-M* than for *cpSSRs*, might be indicative of the effects of male gamete dispersal on SGS. On the contrary, in fern species with apogamy, such as *C. falcatum* (Matsumoto, 2003), the production of sporophytes is independent of fertilization and, therefore, male gamete dispersal should not contribute to SGS. This can explain the differences in SGS between the acaulescent *C. falcatum* and the tree fern *A. firma*. However, it is possible that the SGS observed in one single nuclear locus of *A. firma*, might be the result of a stochastic process and thus uninformative about breeding systems. Although we believe this to be unlikely because of the congruency of SGS between *cpSSRs* and *gapCp-M*, data on additional nuclear loci would be valuable.

Besides dispersal asymmetry, other factors might be partly responsible for SGS in ferns, such as developmental, physiological or genetic characteristics of gametophytes (*e.g.*, hormonal suppression of male gamete production; Page, 2002). One interesting possibility stems from the fact that strong spatial aggregation, as observed in *A. firma*, should increase the frequency of fertilization between close relatives, which in turn would generate inbreeding depression (Charpentier, 2002; Dutetch *et al.*, 2002; Porcher & Lande, 2005; Chung & Chung, 2013). However, populations of *A. firma* showed no inbreeding (Soltis *et al.*, 1991). Most ferns have gametophytes with multiple archegonia that can be simultaneously fertilized (polyembryony), creating an opportunity for a selective process of embryos with the higher levels of heterozygosity (Klekowski, 1973). This would reduce the apparent selfing rates, even within inbred populations, and effectively attenuate the adverse effects of inbreeding

depression (Porcher & Lande, 2005). This process can explain the presence of tree fern populations with no heterozygote deficiency but with significant spatial clustering and elevated SGS. Further research on the genetics of gametophyte fertilization are needed to corroborate this hypothesis.

The present analyses suggest that fern species appear to have unexpectedly high SGS. The successful assessment of the mechanisms behind SGS in ferns, however, needs to incorporate the simultaneous study of the ecology and genetics of the two free-living stages. To better understand the processes governing SGS in ferns, it is also necessary to conduct more extensive comparative studies that consider differences in life-history traits among species. The reproductive biology of ferns appears to be more complex than previously thought (Korall & Pryer 2013). Contrary to expectations, an out-crossing breeding system, the wind dispersal of spores and an arborescent life form, do not translate into random SGS within fern populations (Chung & Chung 2013). The alternating life cycle, with two free-living life stages, might impose limits to the effective dispersal of genes within populations, which are not evident from the study of SGS in seed plants.

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TABLES

Table 1. Genetic diversity using nuclear (*gapCp-M*) and chloroplast microsatellites (*cpSSRs*) and statistics of clonal extent in the two studied populations of the tree fern *Alsophila firma* (AH: Huatusco; ATL: Tlanchinol). *n*: number of individuals; *a*: number of alleles; *H_O*: observed heterozygosity; *H_S*: gene diversity (haplotype diversity); *F_{IS}*: inbreeding coefficient; *G*: number of genets (unique genets); *R*: clonal diversity; *IRD*: inter-ramet distances. *IRD* are given in centimeters (\pm standard error).

| | <i>n</i> | <i>a</i> | <i>H</i> | <i>H</i> | <i>F</i> | <i>G</i> | <i>R</i> | <i>IRD</i> |
|----------------|----------|----------|----------|----------|----------|----------|----------|--------------------|
| AH | 74 | | | | | 41 (23) | 0.563 | 223.41 \pm 16.43 |
| <i>gapCp-M</i> | | 15 | 0.33 | 0.31 | -0.071 | | | |
| <i>cpSSRs</i> | | 15 | - | 0.88 | - | | | |
| ATL | 99 | | | | | 44 (24) | 0.439 | 174.11 \pm 9.64 |
| <i>gapCp-M</i> | | 11 | 0.42 | 0.40 | -0.044 | | | |
| <i>cpSSRs</i> | | 16 | - | 0.91 | - | | | |

Table 2. Statistics of spatial genetic structure using nuclear (*gapCp-M*) and chloroplast microsatellites (*cpSSRs*) in the two studied populations of the tree fern *Alsophila firma* (AH: Huatusco; ATL: Tlanchinol). Regression slopes (*b_R*) are given with \pm standard error and 95% confidence intervals. *Sp*_{mean}: mean *Sp* statistic across populations.

| | <i>gapCp-M</i> | | <i>cpSSRs</i> | |
|-----------|-----------------------------------------|-----------------------------------------|-----------------------------------------|-----------------------------------------|
| | AH | ATL | AH | ATL |
| <i>b</i> | -0.024 \pm 0.0042 (-0.032, -0.016) | -0.036 \pm 0.0032 (-0.042, -0.030) | -0.042 \pm 0.0064 (-0.055, -0.029) | -0.095 \pm 0.0038 (-0.102, -0.087) |
| <i>Sp</i> | 0.026 | 0.041 | 0.068 | 0.186 |
| <i>Sp</i> | 0.036 | | 0.127 | |

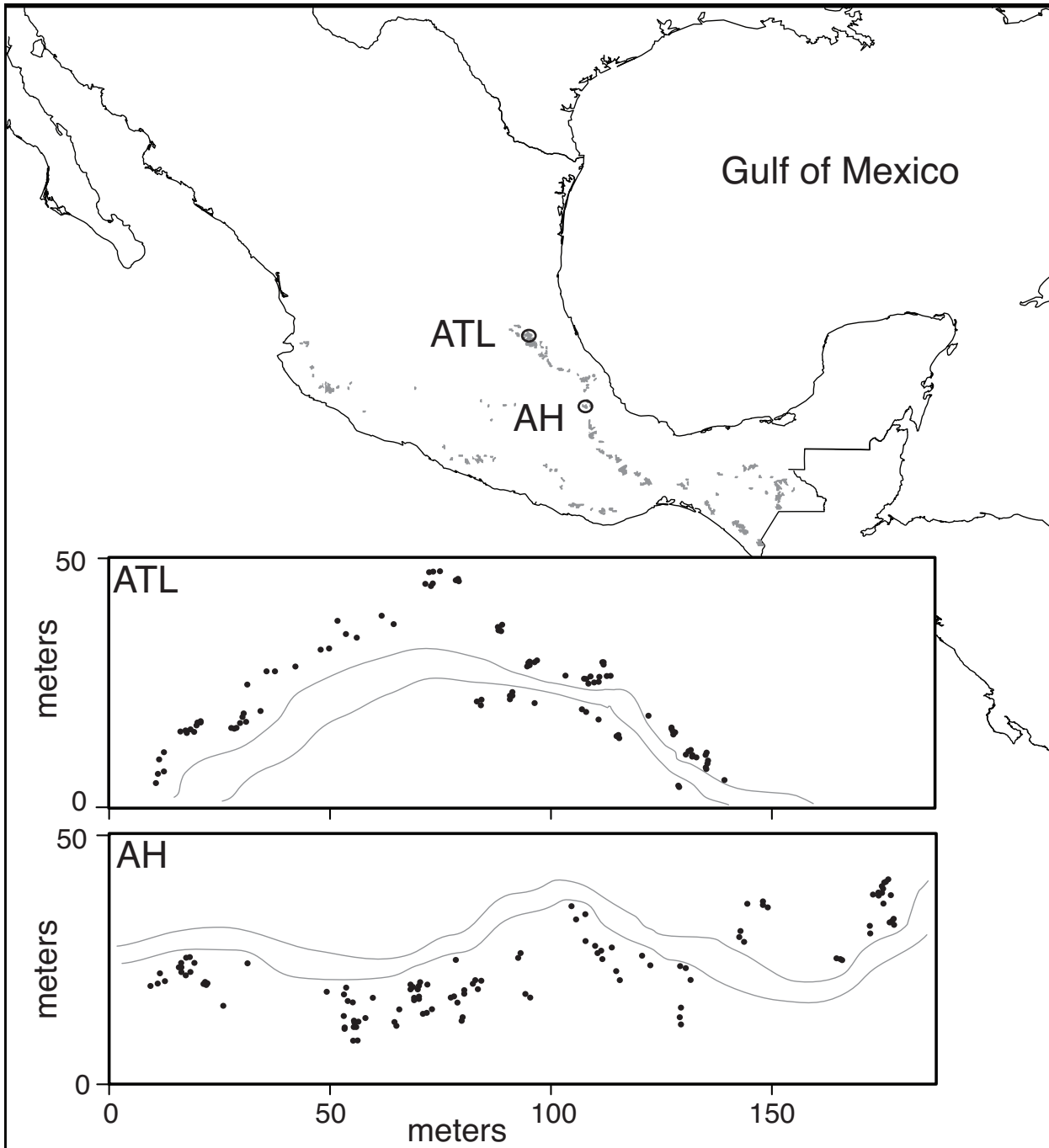


Figure 1. Spatial distribution of individual stems within the two studied populations of the tree fern *Alsophila firma* (AH and ATL). Map showing geographical location of populations in Mexico (open circles) and the distribution of cloud forest patches (grey).

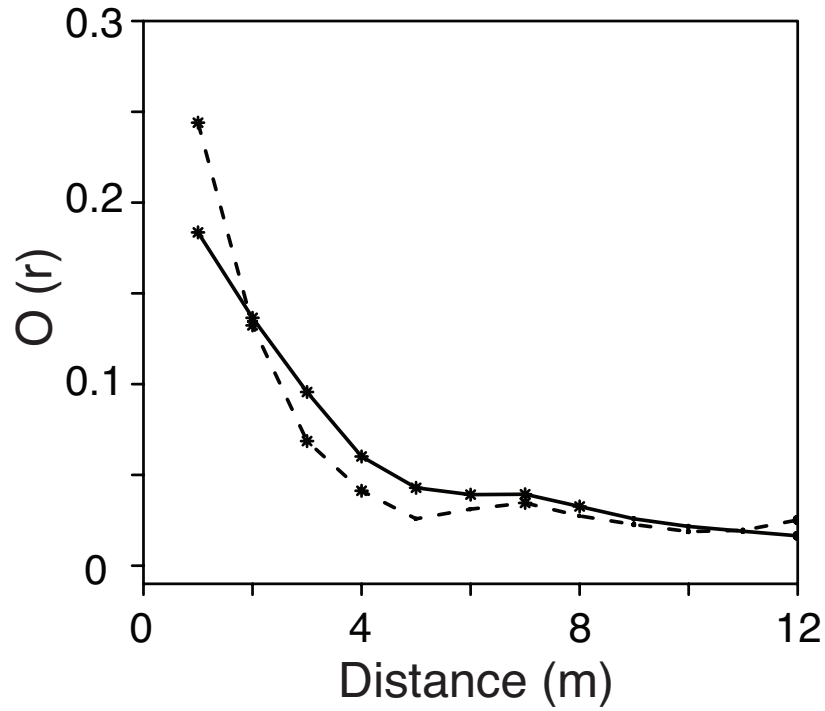


Figure 2. Spatial aggregation (*O*-ring statistic) of individuals in two studied populations of the tree fern *Alsophila firma* (AH = solid line; ATL = dashed line). Black stars, distance classes where spatial aggregation was significantly different from the model of complete spatial randomness (CSR).

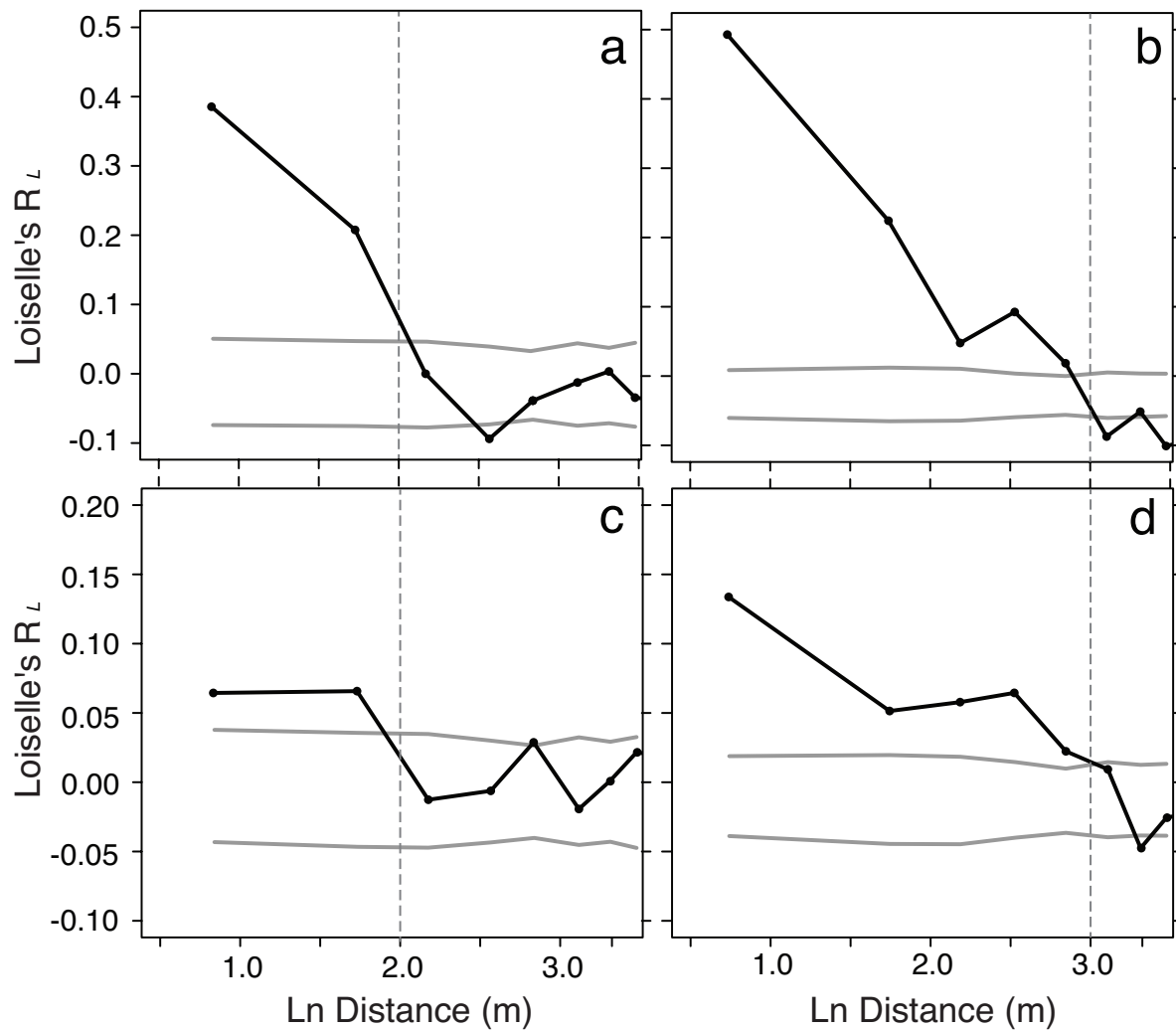


Figure 3. Spatial correlograms of estimated Loiselle's kinship coefficient (R_L) over the natural logarithm of distance (metres) for nuclear *gapCp-M* (a, c) and chloroplast haplotypes (b, d) in two studied populations of the tree fern *Alsophila firma*: AH (a, b) and ATL (c, d). Closed circles, mean values of R_L for corresponding distances; grey lines, the upper and lower bounds of 95% confidence envelopes constructed for the null hypothesis of $R_L = 0$; dashed lines, distance at which R_L becomes non-significant: ≈ 7.4 m for AH (a, b) and ≈ 20.1 m for ATL (c, d).

DISCUSIÓN

En el presente trabajo se estudiaron los patrones geográficos de diversidad genética y la historia demográfica del helecho arborescente *Alsophila firma* en los bosques de niebla en México (Ramírez-Barahona & Eguiarte 2014a). Se estudió como la distribución histórica estimada para los bosques de niebla explica la distribución de la variación genética y la demografía histórica de *A. firma*. Debido a la poca tolerancia climática de esta especie (Conant 1983; Mickel & Smith 2004), los niveles de diversidad genética y los cambios demográficos mostraron una fuerte asociación con los cambios temporales en la distribución de los bosques de niebla. Estas asociaciones fueron exploradas usando dos modelos paleoclimáticos para el último máximo glacial: MIROC (Model for Interdisciplinary Research on Climate; Hasumi & Emori 2004) y CCSM (Community Climate System Model; Kiehl & Gent 2004), los cuales produjeron predicciones muy distintas sobre la distribución de los bosques de niebla durante este periodo (*i.e.*, estabilidad vs. expansión).

Paralelamente, se estudiaron los patrones de estructura genética espacial (EGE) dentro de dos poblaciones de *A. firma* (Ramírez-Barahona & Eguiarte 2014b). Se probaron predicciones sobre los efectos que la dispersión, la forma de vida y el sistema de apareamiento tienen sobre los patrones de EGE en esta especie. Se esperaba que las poblaciones de *A. firma* mostraran una estructuración baja o nula, debido principalmente a la dispersión por viento de las esporas, a un sistema de apareamiento exogámico y a la forma de vida arborescente. Por el contrario, se describió la presencia de una marcada estructura genética espacial a escala fina (*i.e.*, transectos de 200 m) en estas poblaciones, donde se observó que los individuos espacialmente más cercanos eran genéticamente más similares de los esperado al azar. Asimismo, las poblaciones no mostraron evidencia de endogamia, tuvieron una distribución agregada de individuos y un alta frecuencia de reproducción clonal.

DEMOGRAFÍA HISTÓRICA EN LOS BOSQUES DE NIEBLA

La comprensión de los mecanismos que dieron origen a la gran diversidad de especies tropicales, especialmente en el Neotrópico, ha sido uno de los grandes retos de la biología evolutiva (Gentry 1982; Manel & Holderegger 2013). Los patrones geográficos y topográficos en el Neotrópico han permanecido relativamente sin cambios durante los últimos millones de años (Bush *et al.* 2011; Rull 2011). Durante el mismo periodo, los cambios climáticos asociados a los repetidos ciclos glaciales del Pleistoceno han tenido impactos evolutivos importantes (Bush *et al.* 2011; Rull 2011; Hughes *et al.* 2013). No obstante, el efecto de los cambios climáticos sobre las poblaciones de especies tropicales permanece como una pregunta fundamental para la genética evolutiva (Manel & Holderegger 2013). En este contexto, la dinámica demográfica y los patrones de distribución de la variación genética son altamente relevantes para comprender los procesos de diversificación de las especies tropicales (Vellend 2005; Stuessy *et al.* 2014).

La ocupación temporal de un mismo bioma por parte de especies de un linaje (conservadurismo de bioma) tiene un papel significativo en la evolución de éstas (Eisehardt *et al.* 2013; Hughes *et al.* 2013; Olmstead 2013). Asimismo, se ha sugerido que radiaciones recientes (con una gran riqueza de especies) están restringidas ecológica y geográficamente dentro de biomas específicos (Olmstead 2013). En este sentido algunos biomas pueden considerarse como meta-comunidades evolutivas (Eisehardt *et al.* 2013; Hughes *et al.* 2013), en las cuales varios factores (*e.g.*, migración, deriva) estarían afectando paralelamente la diversidad de especies y la variación genética (Hubbell 2001; Vellend 2005; Thiel-Egenter *et al.*, 2014). Los patrones de diversidad y distribución de los helechos arborescentes son congruentes con estas ideas, siendo un grupo con un alta riqueza de especies y un nicho climático altamente conservado (Bystriakova *et al.* 2011b). La gran mayoría de las especies de Cyatheales, específicamente aquellas en el continente americano, muestran una elevada afinidad por los ambientes de bosque de niebla (Ramírez-Barahona *et al.* 2011). El análisis comparativo de la distribución geográfica, nicho climático y genética de poblaciones de especies de helechos arborescentes puede

ayudar a comprender la cohesión ecológica y evolutiva de los bosques de niebla (Stuessy *et al.* 2014), y como estos han sido afectados por los ciclos glaciales del Pleistoceno.

En regiones templadas, el descenso en la temperatura durante el último periodo glacial generó congruencia en los patrones geográficos de variación genética entre distintas especies (Heuertz *et al.* 2004; Hewitt 2004; Soltis *et al.* 2006). La historia de los bosques tropicales parece mucho más complicada que la de sus contrapartes templados. En los trópicos, el descenso en la temperatura en regiones de gran elevación llevó, en muchos casos, a procesos de diversificación en muchos linajes (*e.g.*, plantas, Hughes & Eastwood 2006; aves, Weir 2006; anfibios, Hutter *et al.* 2013). Sin embargo, la temperatura no tuvo un impacto tan preponderante en otras comunidades tropicales debido a la presencia de otros factores ambientales (*e.g.*, precipitación, topografía) que afectaron la distribución de las especies (Colinvaux *et al.* 2000; Bush & de Oliveira 2006; Bush *et al.* 2011). Más aún, los procesos de diversificación genética en el Neotrópico parecen haber ocurrido de manera distinta en biomas diferentes (*e.g.*, bosques tropicales áridos, selvas tropicales húmedas, bosques de niebla), producto de respuestas diferenciales a eventos de cambio climático. Esto hace necesaria una aproximación bioma por bioma para el estudio de la diversidad en el Neotrópico (Hughes *et al.* 2013).

Los bosques de niebla neotropicales han tenido una historia evolutiva compleja, acoplada a diversos procesos geográficos y de cambio climático (Luna-Vega *et al.* 1999; Ornelas *et al.* 2013). Estos bosques han mostrado un gran dinamismo geográfico durante los periodos glaciales de intenso cambio climático, lo que probablemente ha tenido una gran influencia sobre los patrones de variación genética y demografía de las especies (Bryson *et al.* 2010; Cadena *et al.* 2012; Ramírez-Barahona & Eguiarte 2013, 2014a; Smith *et al.* 2014). No obstante, la dinámica demográfica de las especies en respuesta a estos cambios en la distribución de los bosques debe estar fuertemente ligada al grado de especialización ecológica. Específicamente, los cambios en la distribución del bosque de niebla afectarán a las especies en función del grado de especialización ecológica por el hábitat de bosque de niebla. En particular, el modelo de refugios glaciales no parece ajustarse a los patrones de estructuración filogeográfica ni a los procesos demográficos en varias especies de bosque de niebla (Ornelas *et al.* 2013; Ornelas & González 2014;

Ramírez-Barahona & Eguiarte 2014a). Estos resultados sugieren que la importancia de los refugios glaciales en generar divergencia en estos bosques ha sido sobreestimada (Ornelas *et al.* 2010, 2013). De manera paralela, la teoría de refugios, que predice aislamiento y reducciones demográficas, no es consistente con la historia demográfica de algunas especies que habitan estos bosques (Ramírez-Barahona & Eguiarte 2014a). La relevancia del modelo de refugios glaciales para comprender la dinámica geográfica de los bosques de niebla debe ser reconsiderada.

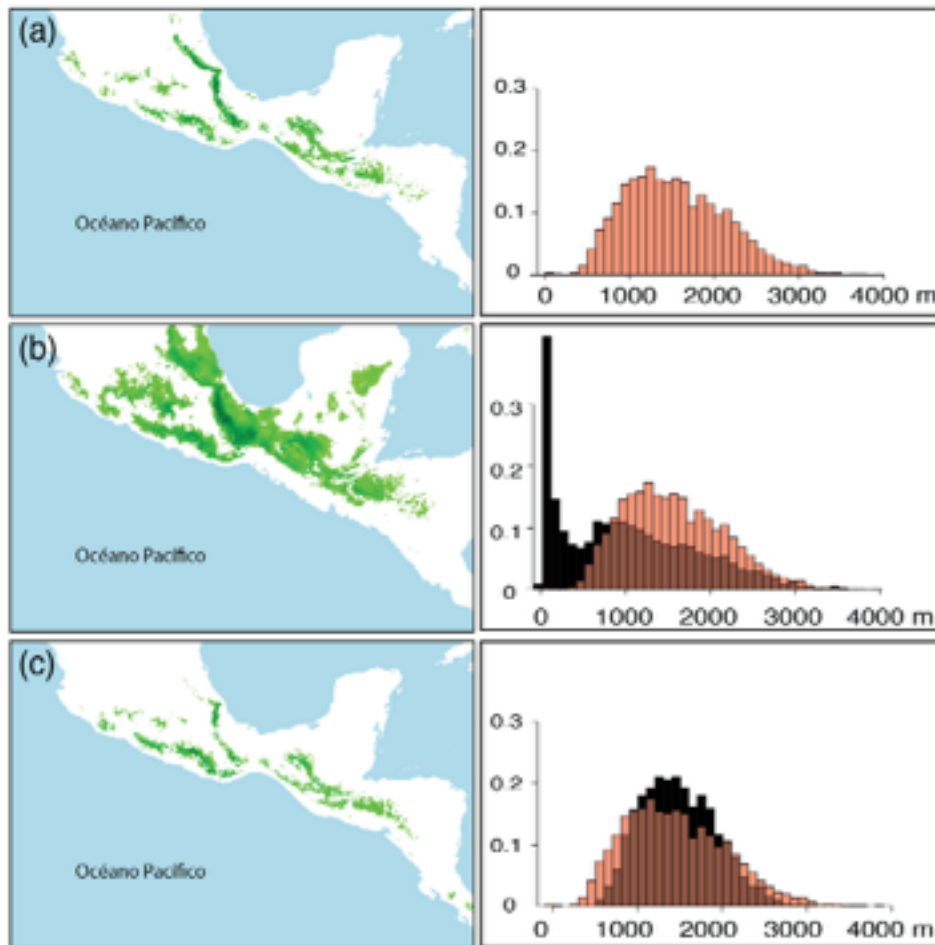


Figura 6. Modelos de distribución construidos con Maxent (Phillips *et al.* 2006) para los bosques de niebla en México y América Central para (a) el presente, (b) el Último Máximo Glacial (UMG, 21,000 años) y (c) el Último Interglacial (130,000 años). La distribución para el UMG se predijo bajo el modelo de circulación CCSM (Community Climate System Model; Kiehl & Gent 2004). Histogramas de frecuencias muestran la elevación en metros (m) de 2,000 puntos aleatorios extraídos de los modelos de distribución. Las barras rojas muestran la elevación del modelo al presente y las barras negras muestran la elevación del modelo al pasado correspondiente: (b) el UMG y (c) el Último interglacial.

Las marcadas diferencias observadas entre la distribución actual y pasada de los bosques de niebla (Figura 6) indican que estos probablemente estuvieron sujetos a importantes fluctuaciones en su distribución en periodos de tiempo relativamente cortos (Lessa et al. 2003; Carstens & Richards 2007; Bush *et al.* 2011). Específicamente, las fluctuaciones ocurridas durante los últimos 130,000 años parecen ser factores principales que afectaron la distribución de la variación genética de las especies de estos bosques (Carnaval *et al.* 2009; Gutiérrez-Rodríguez *et al.* 2011). Existe una congruencia entre los cambios de distribución de los bosques de niebla y los patrones de distribución de la variación genética de especies restringidas a estos (Ramírez-Barahona & Eguiarte 2014a). Probablemente, esta congruencia sea una faceta del paralelismo que existe entre los patrones de distribución de la diversidad genética y los de la diversidad de especies (Vellend 2005; Thiel-Egenter *et al.* 2011).

Los resultados de Ramírez-Barahona & Eguiarte (2014a) apoyan una historia para los bosques de niebla de retracciones hacia tierras altas durante periodos inter-glaciales, seguidas de eventos de expansión hacia tierras bajas durante los periodos glaciales (Figura 6) (Colinvaux *et al.* 2000; Bush *et al.* 2011). No obstante, algunas de las predicciones para los bosques de niebla (Ramírez-Barahona & Eguiarte 2014a) no apoyan una expansión de estos durante los periodos glaciales, debido probablemente a una baja en los niveles de precipitación (Van der Hammen 1961; Hooghiemstra & Van der Hammen 1998; Haffer & Prance 2001). Las distintas predicciones entre modelos paleoclimáticos son un reflejo de las diferencias en los niveles de precipitación predichos (Figura 7), las cuales parecen estar más acentuadas en áreas con niveles altos de precipitación (Poelchau & Hamrick 2011).

Mas allá de las diferencias en precipitación, distintos modelos paleoclimáticos conllevan interpretaciones distintas sobre los procesos demográficos inferidos a partir de datos genéticos. De acuerdo a Ramírez-Barahona & Eguiarte (2014a), bajo el modelo CCSM (Community Climate System Model; Kiehl & Gent 2004) los bosques de niebla sufrieron una expansión geográfica significativa durante el UMG (349% de la distribución actual; Figura 6), lo cual se ve reflejado en un crecimiento demográfico en *A. firma* durante este periodo. Por el contrario, bajo el modelo MIROC (Model for Interdisciplinary Research

on Climate; Hasumi & Emori 2004) los bosques de niebla se mantuvieron relativamente constantes (95% de la distribución actual), con lo cual se tiene un crecimiento demográfico en *A. firma* en ausencia de una expansión geográfica de estos bosques (Ramírez-Barahona & Eguiarte 2014a). En este caso, una posible interpretación sobre la crecimiento demográfica incluye procesos de expansión a través de áreas con microclimas propicios. Estos resultados resaltan la dificultad para analizar y comprender la historia evolutiva de comunidades tropicales, como los bosques de niebla, en ausencia de datos paleoecológicos detallados. En este sentido, Ornelas *et al.* (2013) observaron que varias especies restringidas a los bosques de niebla en Mesoamérica comparten un patrón de diferenciación poblacional asociado a la presencia de barreras biogeográficas (*e.g.*, Istmo de Tehuantepec). No obstante, estos patrones de divergencia intraespecífica no tienen una congruencia temporal entre especies, es decir, los eventos de divergencia entre poblaciones han ocurrido en distintos periodos de tiempo (Ornelas *et al.* 2013; Smith *et al.* 2014).

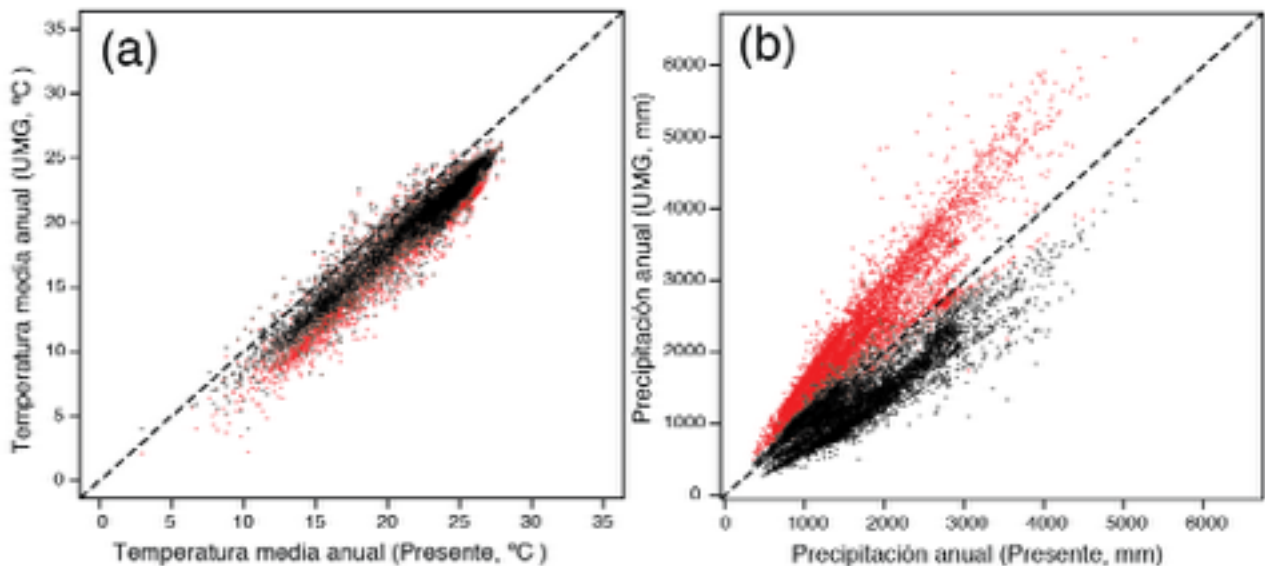


Figura 7. (a) Temperatura media anual y (b) precipitación anual en el presente y durante el Último Máximo Glacial (UMG, 21,000 años) para 5,500 puntos aleatorios en México y Centro América. Los modelos paleoclimáticos CCSM (Community Climate System Model; Kiehl & Gent 2004) y MIROC (Model for Interdisciplinary Research on Climate; Hasumi & Emori 2004) están representados por puntos rojos y negros, respectivamente.

A pesar de la historia evolutiva compleja de los bosques de niebla, se ha encontrado evidencia de expansiones demográficas en varias especies características de estos (Tabla 1), aunque con resultados inconsistentes probablemente relacionados a la imprecisión de las tasas de mutaciones utilizadas. Existe evidencia de procesos de expansión demográfica en *Liquidambar styraciflua* (Ruiz-Sánchez & Ornelas 2014) y en *Moussonia depeana* (Ornelas & González 2014), pero no proveen datos sobre el tiempo en el que pudo ocurrir este evento. Ornelas *et al.* (2010) encontraron evidencia de expansión poblacional en una sola población de *Podocarpus matudae*. A partir de los análisis de mismatch de Ornelas *et al.* (2010) y utilizando una tasa de mutación de 3×10^{-8} - 3×10^{-9} mutaciones/sitio/año [una tasa más alta que la reportada por Wolfe *et al.* (1987) para regiones codificantes de cpDNA], el tiempo a la expansión resulta en 46 - 460 mil años. De manera similar, Gutiérrez-Rodríguez *et al.* (2011) reportaron un tiempo de expansión para *Palicourea padifolia* de 0.5 - 1.5 millones de años, utilizando las tasas originales de Wolfe *et al.* (1987) para regiones codificantes de cpDNA (*i.e.*, 3×10^{-9} - 1×10^{-9} mutaciones/sitio/año). Sin embargo, estas tasas probablemente sean muy conservadoras para ser aplicadas en regiones intergénicas de cpDNA. Utilizando la tasa menos conservadora de 3×10^{-8} - 3×10^{-9} mutaciones/sitio/año, el tiempo calculado a la expansión es de 26-260 mil años. Así mismo, Ramírez-Barahona & Eguiarte (2014a), utilizando la misma metodología, pero aplicando tasas de mutación de 3×10^{-5} - 1×10^{-5} mutaciones/sitio/año reportadas para microsatélites de cpDNA (Provan *et al.* 1999), calcularon un tiempo a la expansión en *Alsophila firma* de 55-167 miles de años (para el nDNA, el tiempo a la expansión fue de 47-470 miles de años, utilizando una tasa de 2×10^{-8} - 2×10^{-9} mutaciones/sitio/año). Análisis demográficos más detallados en esta última especie indican que probablemente la expansión poblacional comenzó hacia el final del último periodo inter-glacial (ca. 110,000 años).

Las especies de bosque de niebla han respondido significativamente a los ciclos glaciales, con cambios en su distribución y tamaños poblacionales, probablemente como consecuencia de nichos climáticos altamente restringidos (Ramírez-Barahona & Eguiarte 2014a). La evidencia genética parece indicar que todas las expansiones ocurrieron en un periodo de 50-500 kya (Tabla 1). Sin embargo, la congruencia temporal de las

expansiones debe ser tomada con precaución, debido a la incertidumbre en las tasas de mutación utilizadas. Asimismo, la dinámica geográfica de los bosques de niebla puede no ajustarse a los datos genéticos de especies particulares en función de la complejidad de su dinámica demográfica y algunos factores biológicos de éstas, como la dispersión y la estructura genética dentro de las poblaciones. Por lo tanto, es necesario realizar un análisis más detallado para probar explícitamente la hipótesis de una respuesta concertada de las especies como consecuencia de los cambios en la distribución del bosque de niebla. En este sentido, modelar la dinámica geográfica de biomas enteros puede proveer un marco analítico común para realizar estudios comparados.

Tabla 1. Tiempos estimados a la expansión poblacional en especies de bosque de niebla inferidos a partir de análisis de distribución de diferencias pareadas (mismatch) utilizando datos genéticos de *cpDNA*.

| Especie | Tiempo a la expansión (miles de años) | | Referencia |
|--------------------------------|------------------------------------------|--------|------------------------------------------|
| | Mínimo | Máximo | |
| <i>Liquidambar styraciflua</i> | ? | ? | Ruiz-Sánchez & Ornelas (2013) |
| <i>Moussonia depeana</i> | ? | ? | Ornelas & González (2014) |
| <i>Podocarpus matudae</i> | 46 | 460 | Ornelas <i>et al.</i> (2010) |
| <i>Palicourea padifolia</i> | 26 | 260 | Gutiérrez-Rodríguez <i>et al.</i> (2011) |
| <i>Alsophila firma</i> | 47 | 470 | Ramírez-Barahona & Eguiarte (2014a) |

Las especies co-distribuidas no necesariamente responderán uniformemente al las fuerzas climáticas que han moldeado la distribución del ecosistema (Carstens & Richards 2007; Collevatti *et al.* 2013). Las respuestas individuales de las especies pueden ser comparadas con los cambios en el bioma completo, con lo cual se puede analizar el grado de similitud ecológica entre especies y la cohesión temporal de las comunidades tropicales. Los análisis de Ramírez-Barahona & Eguiarte (2014a) y los datos de otros estudios (Tabla 1), indican que los cambios predichos en la distribución de los bosques de niebla parecen ser congruentes con la expansión demográfica de algunas especies restringidas a este hábitat. En última instancia, la respuesta de las especies a los cambios de distribución del bioma dependerán del grado de especialización ecológica de éstas. En

el caso particular de *A. firma*, esta especie tiene un alta afinidad por los ambientes de bosque de niebla (Figura 8), lo cual probablemente se ve reflejado en su dinámica demográfica. En este sentido, la superposición del nicho ecológico entre especies de un bioma particular se reflejaría en estas señales genéticas y demográficas que son congruentes con los cambios de distribución de dicho bioma (Lessa et al. 2003; Soltis et al. 2006; Carnaval *et al.* 2009). Sin embargo, las comunidades tropicales actuales se encontraban desintegradas bajo las condiciones climáticas del último periodo glacial, donde las especies formaban comunidades sin un análogo en el presente (Ledru 1993; Bradubury 1997; Valencia *et al.* 2010; Collevatti *et al.* 2013; Montade *et al.* 2014). Algunos estudios paleoecológicos recientes han descrito bosques tropicales que permanecieron estables durante periodos de cambio climático incluso cuando cambió su composición de especies (Montade *et al.* 2014).

Los resultados de Montade *et al.* (2014) proveen un ejemplo de como especies particulares pueden persistir localmente incluso bajo frecuentes re-organizaciones de la comunidad. Este patrón probablemente esté más acentuado en muchas especies de los bosques de niebla (*e.g.*, *Moussonia deppeana*, Ornelas & González 2014), lo que se traduciría en una distribución geográfica y una historia demográfica similar. Como demostraron Ramírez-Barahona & Eguiarte (2014a), el conservadurismo de nicho en una especie se verá reflejado en cambios en su distribución que le permiten seguir los cambios en la disponibilidad climática (Wiens *et al.* 2010; Razgour *et al.* 2013). El conservadurismo de nicho, inferido a través de estudios filogenéticos, se ve confirmado mediante la validación de la dinámica paleoclimática utilizando datos genéticos (Cordellier & Pfenninger 2009; Razgour *et al.* 2013). En este sentido, los resultados de Ramírez-Barahona & Eguiarte (2014b) pueden ser vistos como una corroboración de la existencia de un nicho climático altamente conservado en Cyatheaceae (Bystriakova *et al.* 2011b).

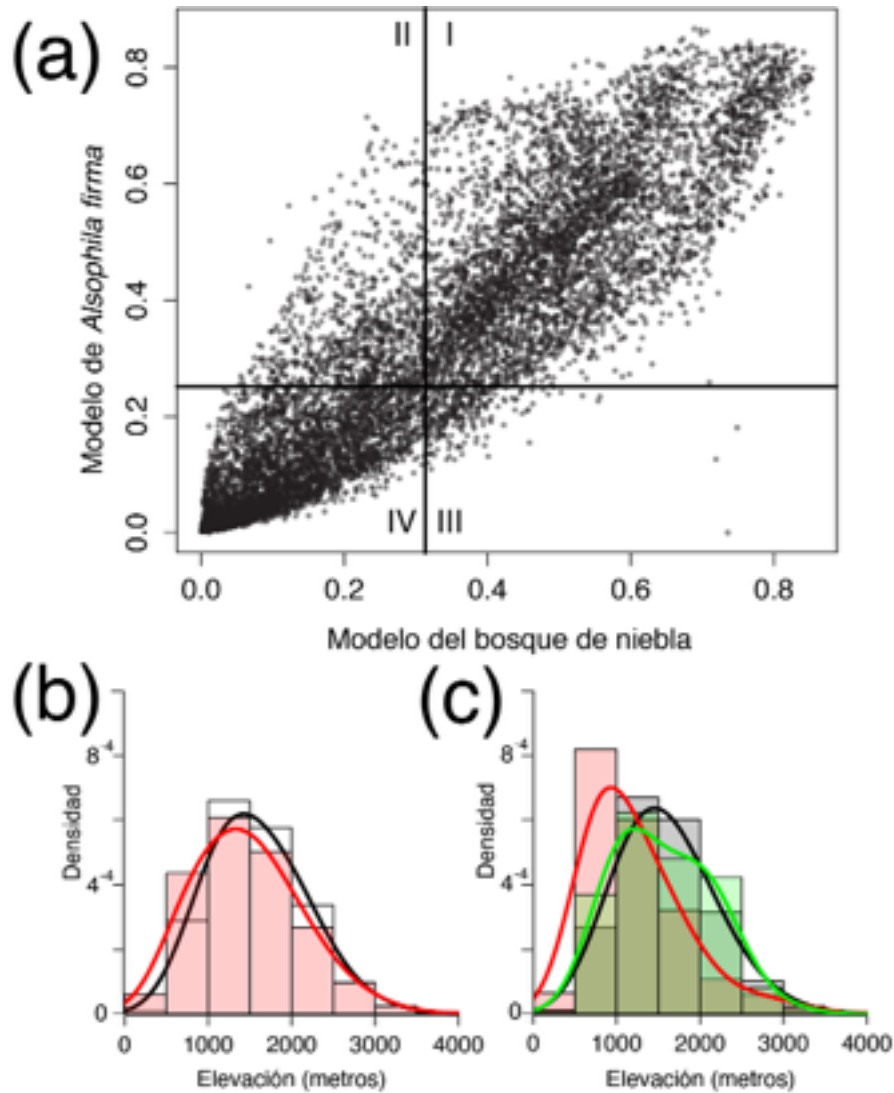


Figure 8. (a) Gráfica de dispersión mostrando los valores para cada pixel predichos por los modelos de nicho ecológico para bosque de niebla y *Alsophila firma* dentro del área de estudio (México y América Central) construidos con Maxent (Phillips *et al.* 2006). Solamente se muestran los valores para 50,000 pixeles aleatorios. El umbrales de presencia para los modelos se muestran con las líneas vertical (bosque de niebla) y horizontal (*A. firma*). (b) Densidad de probabilidad para la elevación (metros) predicha por los modelos para el bosque de niebla (negro) y *A. firma* (rojo). (c) Densidad de probabilidad para el cuadrante I (negro), II (rojo) y III (verde) de la gráfica de dispersión. Cuadrante I: distribución de *A. firma* dentro del bosque de niebla; cuadrante II: distribución de *A. firma* fuera del bosque de niebla; cuadrante III: bosques de niebla no ocupados por *A. firma* (ver Apéndice 2 para una descripción de la metodología utilizada).

ESTRUCTURA ESPACIAL Y EL CICLO DE VIDA DE LOS HELECHOS

La comprensión de los mecanismos y patrones de diversificación genética requieren de un buen entendimiento de la biología reproductiva, genética poblacional y patrones de dispersión de las especies (Broquet & Petit 2009). En particular, el flujo génico es crucial para la genética de poblaciones porque afecta de manera significativa la frecuencia alélica dentro y entre poblaciones. De la misma manera, la dispersión es el factor que media el aislamiento entre poblaciones y, por consecuencia, los procesos de diversificación de las especies. El estudio de los patrones espaciales de variación genética pueden proveer información sobre los mecanismos que regulan la diversidad de especies (Stuessy *et al.* 2006, 2014).

La estructura genética espacial (EGE) en plantas se puede definir como la distribución espacial no aleatoria de genotipos dentro de una población. La presencia de patrones marcados de EGE está íntimamente relacionada con procesos evolutivos y ecológicos de gran importancia, como dispersión, sistema de apareamiento, adaptación local y dinámicas demográficas (Wright 1943; Smouse & Peakall 1999; Chung *et al.* 2004; 2011; Ng *et al.* 2004; Pandey & Rajora 2012). La gran mayoría de los estudios de EGE en plantas se han realizado con angiospermas, mostrando que las especies herbáceas y con auto-fertilización tienden a tener niveles más altos de EGE que especies leñosas y con fertilización cruzada, respectivamente (Heuertz *et al.* 2004; Ng *et al.* 2004; Vekemans & Hardy 2004; Dutetch *et al.* 2005; Harata *et al.* 2012; Pandey & Rajora 2012). El estudio de EGE a escala fina dentro de poblaciones naturales de helechos puede ayudar a comprender los mecanismos que regulan su sistema de apareamiento.

A la fecha se han realizado pocos estudios de EGE en especies de helechos (*i.e.*, *Pteris multifida*, Murakami *et al.* 1997; *Dryopteris aemula*, Jiménez *et al.* 2010; *Cyrtomium falcatum*, Chung & Chung 2013). Debido a la dispersión por viento de las esporas, se espera que la mayoría de las poblaciones de helechos muestren débil o nula EGE. Excepto por Jiménez *et al.* (2010), todos los estudios en helechos han encontrado una marcada EGE, lo que sugiere que la alta capacidad de dispersión de estas plantas no se traduce en una distribución aleatoria de los genotipos dentro de las poblaciones. Los

resultados de Ramírez-Barahona & Eguiarte (2014b) sugieren que la EGE en *A. firma*, al igual que en *C. falcatum*, es uno de los estimados más elevados en especies de plantas estudiadas (Vekemans & Hardy 2004). Estos resultados indican que los helechos parecen en general tener niveles de EGE más elevados que las plantas con semilla (Chung & Chung 2013). ¿Pero a qué se debe la inesperadamente alta EGE observada en poblaciones de helechos?

Los helechos son únicos entre las plantas terrestres por tener un ciclo de vida que incluye una alternancia entre dos fases de vida libre, lo cual ha sido considerado como uno de los factores más importantes que afecta la evolución de estas especies (Klekowski 1973; Korall *et al.* 2010; Chung & Chung 2013). Los mecanismos de apareamiento involucrados en esta alternancia de generaciones parecen ser claves en el establecimiento de los patrones de variación genética en poblaciones naturales de helechos. El análisis de la EGE dentro de poblaciones de helechos permite hacer inferencias sobre el sistema de apareamiento de las especies (Haufler 2002). Por ejemplo, en plantas con semilla, la asimetría entre la dispersión del polen y las semillas ha sido asociada con niveles significativos de EGE (Hamrick & Nason 1996; Kalisz *et al.* 2001). En general, la dispersión del polen es elevada y la de las semillas está limitada [*e.g.*, *Helicteres brevispira* (Malvaceae), Franceschinelli & Kesseli 1999], lo cual genera una fuerte EGE dentro de las poblaciones. En el caso contrario, cuando la dispersión de polen está limitada y no así la de las semillas [*e.g.*, *Fraxinus excelsior* (Oleaceae), Heuertz *et al.* 2003), no se genera EGE dentro de las poblaciones.

La asimetría entre la dispersión de las esporas y los gametos masculinos (Page 2002) puede estar afectando la EGE en helechos (Chung & Chung 2013). El *cpDNA* y el *nDNA* se dispersarían de igual manera por medio de las esporas haploides, pero asumiendo herencia materna del *cpDNA* (Guillon & Raquin 2000), sólo el *nDNA* será dispersado por el movimiento de los gametos masculinos. Esto resultaría en una reducción de la EGE en *nDNA* comparado con el *cpDNA*. En *A. firma*, la EGE más baja en el *nDNA* que en el *cpDNA* puede ser un indicador del efecto de la dispersión de los gametos masculinos (Ramírez-Barahona & Eguiarte 2014b). Por el contrario, en especies con apogamia (*i.e.*, producción de esporofitos en ausencia de fertilización), como *C. falcatum* (Matsumoto

2003), la producción de esporofitos es independiente de la fertilización y por lo tanto, la dispersión de gametos masculinos no contribuye a la EGE. La presencia de apogamia en *C. falcatum* puede explicar los niveles más elevados de EGE en esta especie que los observados en poblaciones del helecho arborescente *A. firma*.

Además de la asimetría en la dispersión, otros factores asociados al sistema de apareamiento pueden estar afectando la EGE en poblaciones de helechos. En *A. firma*, una posibilidad interesante surge de la fuerte agregación espacial de individuos, la cual debería incrementar la frecuencia de fertilización entre parientes y por lo tanto acentuar los niveles de endogamia (Holsinger 1987; Husband & Schemske 1996; Chung & Epperson 2000; Charpentier 2002; Dutetch *et al.* 2002; Porcher & Lande 2005; Chung & Chung 2013). Sin embargo, las poblaciones de *A. firma* no muestran evidencia de endogamia (Soltis *et al.* 1991). Más allá de los mecanismos fisiológicos y morfológicos para evitar la endogamia (Soltis & Soltis 1990; Page 2002), existen otros que impiden (o reducen) los niveles de autofertilización (Klekowski 1973). La mayoría de los helechos tienen gametofitos con múltiples arquegonios que pueden ser fertilizados simultáneamente (*i.e.*, poli-embrionía), creando una oportunidad para un proceso selectivo de aquellos embriones con los niveles más altos de heterocigosis (Klekowski 1973). Incluso en poblaciones con un alta frecuencia de endogamia, este proceso selectivo reduciría las tasas aparentes de autofertilización y mitigaría los efectos de la endogamia (Porcher & Lande 2005). No obstante, para probar esta hipótesis se necesitan estudios que analicen de manera directa la fertilización de gametofitos y los procesos genéticos involucrados en este proceso.

La inferencia de una dispersión limitada de esporas en *A. firma* está apoyada por la observación de una baja viabilidad de esporas en esta especie (< 10%, Bernabe *et al.* 1999). La presencia de una baja viabilidad está probablemente relacionada con la frecuencia de reproducción clonal en *A. firma* (Mehltreter & García-Franco 2008; Ramírez-Barahona & Eguiarte 2014b). En especies con una fertilidad limitada, la adecuación poblacional se incrementa por la presencia de propagación clonal frecuente (Winkler & Fischer 2001). En este sentido, ha sido propuesta para los helechos arborescentes en general una relación negativa entre la fertilidad de las esporas y la propagación clonal (Hallé 1966), lo que podría explicar parcialmente los elevados niveles de EGE observados

en *A. firma*. Sin embargo, la presencia de clonalidad y bajas tasas de fertilidad deben ser investigadas en otras especies de helechos para evaluar la universalidad de esta relación.

En los helechos se ha propuesto una relación entre el sistema de apareamiento y los niveles de dispersión entre poblaciones (Soltis & Soltis 1990; Soltis *et al.* 1991). Asimismo, también se ha propuesto una relación entre el sistema de apareamiento y la diferenciación genética entre poblaciones (Soltis *et al.* 1991; Haufler 2002). De acuerdo a los autores, especies con fertilización cruzada obligada tendrían tasas más bajas de dispersión a grandes distancias en comparación con especies con niveles significativos de autofertilización (Soltis & Soltis 1990). En general, especies con fertilización cruzada tienden a mantener niveles altos de variación dentro de sus poblaciones y niveles bajos de diferenciación entre poblaciones (Soltis & Soltis 1987, 1988; Wolf *et al.* 2001). Por otro lado, especies con sistemas de apareamiento donde predomina la auto-fertilización, tienden a mostrar niveles reducidos de variación dentro de sus poblaciones y una conspicua estructuración poblacional (Soltis & Soltis 1986, 1987; Murakami *et al.* 1997; Hauk & Haufler 1999; Haufler 2002). En una especie con fertilización cruzada, el establecimiento de un esporofito migrante dentro de una nueva población (o área) se ve limitada por la presencia de otros individuos espacialmente cercanos para que se lleve a cabo la fertilización entre gametofitos (Soltis & Soltis 1987, 1990). La necesidad de dos o más gametofitos parece ser bastante común en los helechos homospóricos, lo cual puede limitar seriamente el flujo genético entre poblaciones incluso con un alta movilidad de esporas (Soltis & Soltis 1990).

En este contexto, se espera que especies con niveles significativos de autofertilización muestren mayores tasas de migración y menor diferenciación genética entre poblaciones (Wolf *et al.* 2001; Page 2002). Por ejemplo, poblaciones de *A. firma* en Costa Rica muestran niveles altos de diferenciación poblacional (F_{ST} no reportada, Soltis & Soltis 1990), lo cual se relaciona con una dispersión limitada de esporas y un sistema de fertilización cruzada en esta especie (Conant 1978; Soltis *et al.* 1991). Por el contrario, los resultados de Ramírez-Barahona & Eguiarte (2014a) muestran que las poblaciones de *A. firma* en México tienen niveles bajos de diferenciación poblacional, los cuales deberían estar relacionados con niveles elevados de dispersión y de autofertilización. Sin embargo,

la distribución no aleatoria de genotipos dentro de poblaciones de *A. firma* (Ramírez-Barahona & Eguiarte 2014b) confirma la presencia de una dispersión limitada de esporas y un sistema de fertilización cruzada en esta especie. Parece claro que *A. firma* tiene niveles reducidos de dispersión, lo cual se apoya por la frecuencia de reproducción clonal (Ramírez-Barahona & Eguiarte 2014b), la baja viabilidad de las esporas (Bernabe *et al.* 1999) y la presencia de altos niveles de EGE dentro de las poblaciones (Ramírez-Barahona & Eguiarte 2014b). Entonces, ¿a qué se debe la baja diferenciación poblacional observada entre poblaciones? A diferencia de las poblaciones analizadas en Costa Rica (Soltis & Soltis 1990; Soltis *et al.* 1991), la baja diferenciación genética mostrada en las poblaciones mexicanas de esta especie tiene que ser el resultado de otros procesos de homogeneización de la variación genética, como las expansiones demográficas y los cambios en la conectividad entre poblaciones generados por los cambios en la distribución del bosque de niebla (Ramírez-Barahona & Eguiarte 2014a).

Barrington (1993) se preguntaba cuáles eran los atributos biológicos distintivos de los helechos comparados con los grupos más comúnmente estudiados de angiospermas y animales vertebrados. En este contexto, los patrones de diferenciación entre poblaciones de helechos se han explicado, generalmente, en términos de su alta capacidad de dispersión (Tryon 1971; Peck *et al.* 1990; Wolf *et al.* 2001; Perrie & Brownsey 2005, 2007; Sheperd *et al.* 2009). Por ejemplo, se ha sugerido que la diferenciación en Cyatheales, particularmente en América, es el resultado principal de procesos de diferenciación acoplados a la dispersión a grandes distancias (Tryon 1971; Conant 1983). La otra cara de este argumento predice que en ausencia de límites a la dispersión, los helechos no mostrarán patrones significativos de diferenciación poblacional.

Los resultados del presente trabajo muestran que la relación entre fertilización, dispersión y variación genética parece no ser tan obvia (Ramírez-Barahona & Eguiarte 2014a, b). El ciclo de vida de los helechos parece ser mucho más complicado de lo que se creía (Korall & Pryer 2014), lo cual conducirá a una re-interpretación de los patrones biogeográficos y genéticos en estas especies. La complejidad del sistema de apareamiento de los helechos, y sus consecuencias genéticas (*e.g.*, EGE marcada), complica la comprensión de la historia evolutiva de estas especies. La presencia de una

elevada EGE sugiere que la baja diferenciación genética observada entre poblaciones es el resultado probable de procesos demográficos y no de una elevada tasa de dispersión. En este sentido, el papel de la dispersión ha sido sobrevalorado al momento de explicar la historia evolutiva de las especies (Korall & Pryer 2014; Ramírez-Barahona & Luna-Vega 2014). El aislamiento histórico, promovido por eventos climáticos, parece estar involucrado en la generación de los patrones actuales de distribución de las especies. La importancia de la dispersión para la comprensión de la historia evolutiva de estas especies se ve acentuada por la relación que existe entre los patrones de diversidad a varios niveles de organización (Hubbell 2001; Vellend 2005; Vellend *et al.* 2014). La evaluación de los patrones y niveles de dispersión en los helechos arborescentes en escalas microevolutivas tiene consecuencias importantes para la comprensión de los patrones de diversificación de estas especies.

PERSPECTIVAS

Las Cyatheaceae, al igual que otros linajes de plantas tropicales (Gentry 1982; Bardon et al. 2013; Hughes *et al.* 2013; Jabaily & Sytsma 2013), parecen ser vestigios de una flora que evolucionó antes del Mioceno y que diversificó posteriormente como resultado de los eventos geológicos y climáticos ocurridos durante el Neogeno (Janssen *et al.* 2008; Korall & Pryer 2014). Esto se presenta como el escenario ideal para evaluar los principales mecanismos responsables de la diversificación de especies en el Neotrópico. Mediante el estudio de las relaciones filogenéticas dentro de la familia se han observado patrones biogeográficos interesantes (Korall & Pryer 2014). Por ejemplo, Lehnert (2011) observó que las especies americanas de *Cyathea* relacionadas con el grupo de *C. decurrens* (*i.e.*, grupo de Australasia) están concentradas en el norte de América del Sur y están completamente ausentes del sureste de Brasil. A nivel regional, las especies del grupo de *C. platylepis* están restringidas a las tierras altas (tepuís) de las Guayanas (Lehnert 2011a), patrón de distribución que comparten con el grupo *Hymenophyllopsis* (= *Cyathea*, Christenhusz 2011). Asimismo, ca. 50% de las especies americanas de *Alsophila* están concentradas en Las Antillas (Conant 1983; Ramírez-Barahona *et al.* 2011). Sin embargo, las posibles causas de muchos de estos patrones no pueden ser estudiados a detalle por la falta de resolución taxonómica a nivel infra-genérico, debido en gran parte a la poca representatividad de especies (Lehnert 2011a).

La divergencia observada dentro de grupos americanos de *Alsophila*, *Cyathea* y *Sphaeropteris* sugieren que su radiación ocurrió recientemente (Janssen *et al.* 2008). Al parecer, estos tres grupos evolucionaron durante el Cretácico superior (ca. 145-65 ma) y han tenido radiaciones recientes durante el Neogeno, probablemente acopladas a ciclos de cambio climático (Janssen *et al.* 2008). Sin embargo, muy pocas especies han sido incluidas en los estudios filogenéticos de la familia Cyatheaceae. Estudiar cómo han respondido estas especies a los cambios climáticos puede llevar a un mejor entendimiento de los procesos de diversificación en regiones tropicales. Los resultados de Ramírez-Barahona & Eguiarte (2014a) muestran que existe una estrecha relación entre los cambios

demográficos en *Alsophila firma* y los cambios en la distribución del hábitat que ocupan. Pero, ¿qué pasa con otras especies de helechos arborescentes?

Las características geográficas y ecológicas de las especies de Cyatheaceae permiten probar la congruencia de la respuesta demográfica de estas especies a los cambios climáticos. Por un lado, la independencia biótica para la reproducción de la mayoría de las especies de helechos permite evaluar de manera más sencilla las respuestas y patrones compartidos entre especies. Se espera que especies distintas muestren patrones demográficos acoplados a la historia climática particular de la región que habitan (e.g., expansión en México y el norte de América Central, aislamiento y fragmentación en el sur de América Central). Por otro lado, los análisis de Bystrakova *et al.* (2011b) muestran que las Cyatheaceae han tenido un alto conservadurismo de nicho a lo largo de su historia evolutiva. El estudio comparado de los patrones de estructuración filogeográfica en especies con amplia distribución también puede ayudar a comprender algunos de los patrones biogeográficos observados en la familia. Por ejemplo, la distribución de pares de especies hermanas en América Central muestra una diferenciación entre el norte de América Central/México y el sur de América Central (i.e., Costa Rica y Panamá): e.g., *Sphaeropteris horrida* (norte) vs. *S. brunei* (sur); *C. tuerkheimii* (norte) vs. *C. divergens* (sur); *C. austroamericana* (norte) vs. *C. multiflora* (sur). También hay evidencia de patrones de variación morfológica a lo largo del intervalo de distribución en muchas especies con amplia distribución [e.g., *Loxsomopsis pearcei* en el Neotrópico (Lehnert *et al.* 2001)]. Adicionalmente, existen casos de posibles procesos de divergencia recientes en especies insulares [e.g., *C. alfonsiana* en la Isla de Cocos, Costa Rica (Lehnert 2011); *C. weatherbyana* en las Islas Galápagos, Ecuador (Lehnert 2011); *A. phlebodes* en Indonesia (Lehnert *et al.* 2013); *C. sechellarum* en las Islas Seychelles (Janssen & Rakotondrainibe 2008)].

Muchos linajes Neotropicales han estado sujetos frecuentemente a otras formas de diversificación además del aislamiento geográfico (Evans *et al.* 2009; Löwenberg-Neto *et al.* 2011; Eiserhardt *et al.* 2012; Michelangeli *et al.* 2013). La presencia de barreras geográficas a la dispersión no es necesaria para la generación de nuevas especies o variedades (Graham *et al.* 2004; Eiserhardt *et al.* 2012; Hughes *et al.* 2013). En este

sentido, el estudio de la distribución geográfica de especies Cyatheaceae sugiere que existe un efecto significativo de la altitud y del tipo de suelo en delimitar especies y variedades (Conant 1983; Janssen & Rakotondaribe 2007, 2008; Lehnert 2009, 2011, 2014; Lehnert *et al.* 2013; Noben & Lehnert 2013; Tabla 2). Las diferencias altitudinales, edáficas y morfológicas observadas en Cyatheaceae lleva a pensar que probablemente éstas tengan elevadas tasas de especiación ecológica.

Tabla 2. Intervalo altitudinal y distribución de algunas especies cercanamente emparentadas (o variedades) en el Neotrópico, África y Australasia.

| Especie | Intervalo altitudinal | Distribución | Referencia |
|------------------------------------------------|-----------------------|------------------------------------------------------------------------------------------|-----------------|
| <i>Alsophila (Neotrópico)</i> | | | |
| <i>A. firma</i> | 800 - 2000 | México a Panamá, Ecuador | Conant (1983) |
| <i>A. erinaceae</i> | 800 - 2000 | Costa Rica y Panamá, Los Andes (Venezuela a Bolivia) | |
| <i>A. cuspidata</i> | 0 - 800 | Costa Rica y Panamá, Los Andes (Venezuela a Bolivia), Amazonia (Guyana Francesa, Brasil) | |
| <i>Cyathea (Neotrópico)</i> | | | |
| <i>C. farinosa</i> | 1000 - 2500 | Venezuela (Cordillera de la Costa) | Lenhert (2014) |
| <i>C. gibbosa</i> | 900 - 1500 | Los Andes (Colombia a Bolivia) | |
| <i>C. delgadii</i> (morfo típico) | 1000 - 2000 | Centro América, Los Andes (Colombia a Bolivia), Mata Atlántica (Brasil) | Lenhert (2011b) |
| <i>C. delgadii</i> (morfo pubescente) | 0 - 900 | Amazonia (Brasil, Suriname, Guyana) | |
| <i>C. patens</i> | 1900 - 2700 | Los Andes (Venezuela a Perú) | Lenhert (2011b) |
| <i>C. nephele</i> | 2600 - 3600 | Los Andes (Perú) | |
| <i>C. meridensis</i> var. <i>meridensis</i> | 1200 - 2500 | Los Andes (Venezuela a Ecuador) | Lenhert (2009b) |
| <i>C. meridensis</i> var. <i>nana</i> | 2500 - 2900 | Los Andes (Ecuador y Perú) | |
| <i>C. meridensis</i> var. <i>objecta</i> | 2500 - 2600 | Los Andes (Ecuador y Perú) | |

| Especie | Intervalo altitudinal | Distribución | Referencia |
|--------------------------------------------------|-----------------------|-----------------------------------------|---------------------------------|
| <i>Alsophila</i> (África) | | | |
| <i>C. emilei</i> var. <i>emilei</i> | 500 - 1200 | Madagascar (Norte y Centro) | Janssen & Rakotodrainibe (2008) |
| <i>C. emilei</i> var. <i>dauphinensis</i> | 50 - 500 | Madagascar (Sur) | |
| <i>C. decrescens</i> var. <i>decrescens</i> | 300 - 1200 | Madagascar (Norte y Centro) | Janssen & Rakotodrainibe (2008) |
| <i>C. decrescens</i> var. <i>manongarivensis</i> | 1300 - 1699 | Madagascar (Norte) | |
| <i>C. decrescens</i> var. <i>cristata</i> | 100 - 900 | Madagascar (Sur) | |
| <i>C. decrescens</i> var. <i>quadrata</i> | 900 - 1000 | Madagascar (Centro) | |
| <i>Gymnosphaera</i> (Australasia) | | | |
| <i>A. hornei</i> | 100 - 1400 | Indonesia, Nueva Guinea a Islas Solomón | Lehnert <i>et al.</i> (2013) |
| <i>A. olivacea</i> | 1400 - 2000 | Nueva Guinea (Norte) | |
| <i>A. phlebodes</i> | 1850 - 1950 | Indonesia | |
| <i>A. ramispinoides</i> | 1000 - 1500 | Indonesia | |

Entender los mecanismos micro-evolutivos que conducen a la divergencia genética es esencial para comprender los procesos de diversificación de las especies. En este contexto, el uso de recursos genómicos para estudiar la variación genética en especies hermanas puede ayudar a comprender de manera más detallada los procesos de diferenciación en este grupo de plantas (Freedman *et al.* 2010; Eckblom & Galindo 2011; Eaton & Ree 2013; Rothfels *et al.* 2013; Vellend *et al.* 2014). La comprensión de estos procesos requiere de un entendimiento detallado de los mecanismos de aislamiento reproductivo entre individuos. El estudio de la distribución espacial de variantes genómicas dentro y entre poblaciones puede llevar a una mejor comprensión de los mecanismos reproductivos involucrados en la alternancia de fases en el ciclo de vida de los helechos.

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APÉNDICE 1.

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SUPPORTING INFORMATION

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Table S1 Geographical location and measures of genetic diversity for the sampled populations of *Alsophila firma* (Cyatheaceae).

| Population code | Longitude | Latitude | Elevation (m) | <i>n</i> | <i>gapCp</i> | | | cpSSRs | | | |
|-----------------|-----------|----------|---------------|----------|--------------|----------|-----------|----------|----------|-----------|----------|
| | | | | | π | <i>A</i> | <i>Hs</i> | <i>A</i> | <i>h</i> | <i>Hd</i> | <i>h</i> |
| AH | -96.9907 | 19.1906 | 1370 | 32 | 0.00171 | 11 | 0.68 | 4.1 | 13 | 0.92 | 4.3 |
| AT | -98.6321 | 20.9826 | 1471 | 22 | 0.00183 | 7 | 0.81 | 4.0 | 10 | 0.92 | 4.3 |
| AL | -98.6428 | 21.0278 | 1507 | 9 | 0.00154 | 4 | 0.73 | 3.6 | 7 | 0.94 | 4.4 |
| AC | -96.9810 | 19.4719 | 1285 | 9 | 0.00181 | 4 | 0.78 | 3.9 | 6 | 0.92 | 4.2 |
| AD | -98.2135 | 20.3240 | 1687 | 22 | 0.00161 | 8 | 0.72 | 4.7 | 9 | 0.86 | 3.9 |
| AZ | -98.6158 | 20.6320 | 1985 | 1 | 0 | 1 | 0.00 | – | 1 | 0.00 | – |
| ASJ | -96.5208 | 15.9830 | 1493 | 18 | 0.00113 | 4 | 0.69 | 2.8 | 4 | 0.40 | 2.0 |
| AU | -92.7672 | 16.9336 | 1785 | 5 | 0 | 1 | 0.00 | 1.0 | 1 | 0.00 | 1.0 |
| ASN | -92.9593 | 17.2130 | 1856 | 14 | 0.00075 | 2 | 0.45 | 2.0 | 4 | 0.75 | 3.1 |
| ATZ | -91.6738 | 16.0778 | 1523 | 8 | 0.00138 | 3 | 0.66 | 3.0 | 2 | 0.25 | 1.6 |
| ACL | -91.6813 | 16.1060 | 1590 | 14 | 0.00149 | 5 | 0.58 | 3.3 | 7 | 0.76 | 3.4 |
| ATQ | -92.0910 | 15.0949 | 1823 | 23 | 0.00084 | 3 | 0.51 | 2.0 | 3 | 0.69 | 2.7 |
| AEC | -92.0738 | 15.0817 | 1516 | 2 | 0.00111 | 2 | 0.50 | 2.0 | 2 | 0.00 | 1.0 |
| AG | -92.6548 | 15.4130 | 675 | 1 | 0 | 1 | 0.00 | – | 1 | 0.00 | – |
| AEX | -96.8598 | 18.1662 | 1426 | 7 | 0.00179 | 2 | 0.50 | 2.0 | 1 | 0.00 | 1.0 |
| AEZ | -96.3496 | 17.6268 | 1562 | 17 | 0.00122 | 6 | 0.61 | 3.7 | 11 | 0.93 | 4.4 |
| Total | | | | 204 | 0.00168 | 21 | 0.56 | | 42 | 0.96 | |

n, number of sampled individuals; elevation in metres above sea level; π , nucleotide diversity; *A*, allelic richness; *A_R*, rarefied allelic richness; *Hs*, gene diversity; *h*, number of haplotypes; *Hd*, haplotype diversity;

h_R , rarefied number of haplotypes. A_R and h_R were only estimated for populations with more than five individuals.

Table S2 Primer names and sequences used to analyse the spatial genetic structure in two populations (AH and ATL) of the tree fern *Alsophila firma*.

| DNA region | Primer | Sequence (5'–3') | Author |
|-------------------|-------------|------------------------------------|----------------------------------|
| <i>rbcL-atpB</i> | ESRBCL26R* | GCT TTA GTC TCC GTT TGT GGT GAC AT | Korall <i>et al.</i> (2007) |
| | ATPB703R* | CCA ATG ATC TGA GTA ATS TAT CC | Korall <i>et al.</i> (2007) |
| <i>accD-atpB</i> | RBCL1187F* | GGA ACY TTG GGA CAT CCT TGG | Korall <i>et al.</i> (2007) |
| | ACCD816R* | CCA TGA TCG AAT AAA GAT TCA GC | Ebihara <i>et al.</i> (2003) |
| | accDSSRAF† | ATT GGC TGC CGC CTG CGA GGT ATG | This study |
| | accDSSRAR† | ACC CCG AGT TCA ATA GCC CA | This study |
| | accDSSRBF† | AAA GCT CCG ATC AAT CCA CCG A | This study |
| | accDSSRBR† | GAG CAC ATG GTT TCG AGC CGT | This study |
| <i>trnL-trnF</i> | TRNLC* | CGA AAT CGG TAG ACG CTA CG | Taberlet <i>et al.</i> (1991) |
| | TRNFF* | ATT TGA ACT GGT GAC ACG AG | Taberlet <i>et al.</i> (1991) |
| | trnLFSSRAF† | AGT GAC AGC TCC CAG ATT CA | This study |
| | trnLFSSRAR† | CTA ACC GTG TAG AAT GGG ACT CT | This study |
| <i>matK-rps16</i> | matKF1‡ | AGT AAC CCC TCA AGC ATC GA | This study |
| | rps16R1‡ | CGT TTC TTC ACG AAG AGG TGG | This study |
| <i>gapCp</i> | ESGAPCP8F1 | ATY CCA AGY TCA ACT GGT GCT GC | Schuettpeiz <i>et al.</i> (2008) |
| | ESGAPCP11R1 | GTA TCC CCA YTC RTT GTC RTA CC | Schuettpeiz <i>et al.</i> (2008) |
| <i>gapCp-M</i> | GAPCPM1F | TGG GCT GTT GGC CCT CAC AA | This study |
| | GAPCPM1R | TCT GCA GCC AAC CAA AGG CAA CAG | This study |

*Primers used only for amplification and sequencing of intergenic sequences.

†Primers specifically designed to amplify chloroplast (cp) DNA microsatellites from *A. firma* sequences.

‡Primers specifically design to amplify cpDNA microsatellites from the complete chloroplast genomes of *Alsophila spinulosa* (Gao *et al.*, 2009).

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APÉNDICE 2.

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SUPPORTING INFORMATION

Changes in the distribution of cloud forests during the last glacial predict the patterns of genetic diversity and demographic history of the tree fern *Alsophila firma* (Cyatheaceae)

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Supplementary methods

A comparison was made between the species and the cloud forest biome models. A database of occurrence records for *Alsophila firma* (Cyatheaceae) in Mexico and upper Central America was obtained from herbarium records. This database was corrected manually using available monographic information for the species (Conant, 1983; Mickel & Smith, 2004), eliminating those records outside the reported distribution of the species (i.e. probable referencing errors) and those lying outside the reported elevational range for the species (i.e. probable misidentification of species). We had a final list of 36 occurrences that could be confidently ascribed to *A. firma*. These records were used to construct a MAXENT distribution model using the same settings as those used for the modelling of the cloud forests (Table S3, Fig. S1; see main text for model settings).

For each grid in the map, we plotted the values predicted by the biome model against those predicted by the species model (Fig. S2a). We divided this plot into four quadrants according to the presence thresholds defined for each model (10 percentile training threshold): 0.2541 for *A. firma* and 0.313 for the cloud forests. These quadrants represented: (I) pixels predicted by both the biome and species models (i.e. *A. firma* distribution within cloud forests); (II) pixels predicted by the species but not by the biome model (i.e. distribution of *A. firma* outside cloud forests); (III) pixels predicted by the biome but not by the species model (i.e. cloud forests not occupied by *A. firma*); and (IV) pixels not predicted by any model (i.e. background area). We calculated the proportion of the

area within each quadrant to give a more detailed account of the congruence between models, which can be seen as measuring the habitat fidelity of *A. firma*. Finally, we extracted elevation data for all the pixels in order to visualize differences in elevation across models and between quadrants (Fig. S2b,c).

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Table S3 List of the 12 bioclimatic variables with a 30-arc second resolution used in the distribution modelling, obtained from the WorldClim online database (Hijmans *et al.*, 2005).

| Abbreviation | Variable |
|--------------|-----------------------------------------|
| BIO1 | Annual mean temperature |
| BIO4 | Temperature seasonality |
| BIO8 | Mean temperature of the wettest quarter |
| BIO9 | Mean temperature of the driest quarter |
| BIO10 | Mean temperature of the warmest quarter |
| BIO11 | Mean temperature of the coldest quarter |
| BIO12 | Annual precipitation |
| BIO15 | Precipitation seasonality |
| BIO16 | Precipitation of the wettest quarter |
| BIO17 | Precipitation of the driest quarter |
| BIO18 | Precipitation of the warmest quarter |
| BIO19 | Precipitation of the coldest quarter |

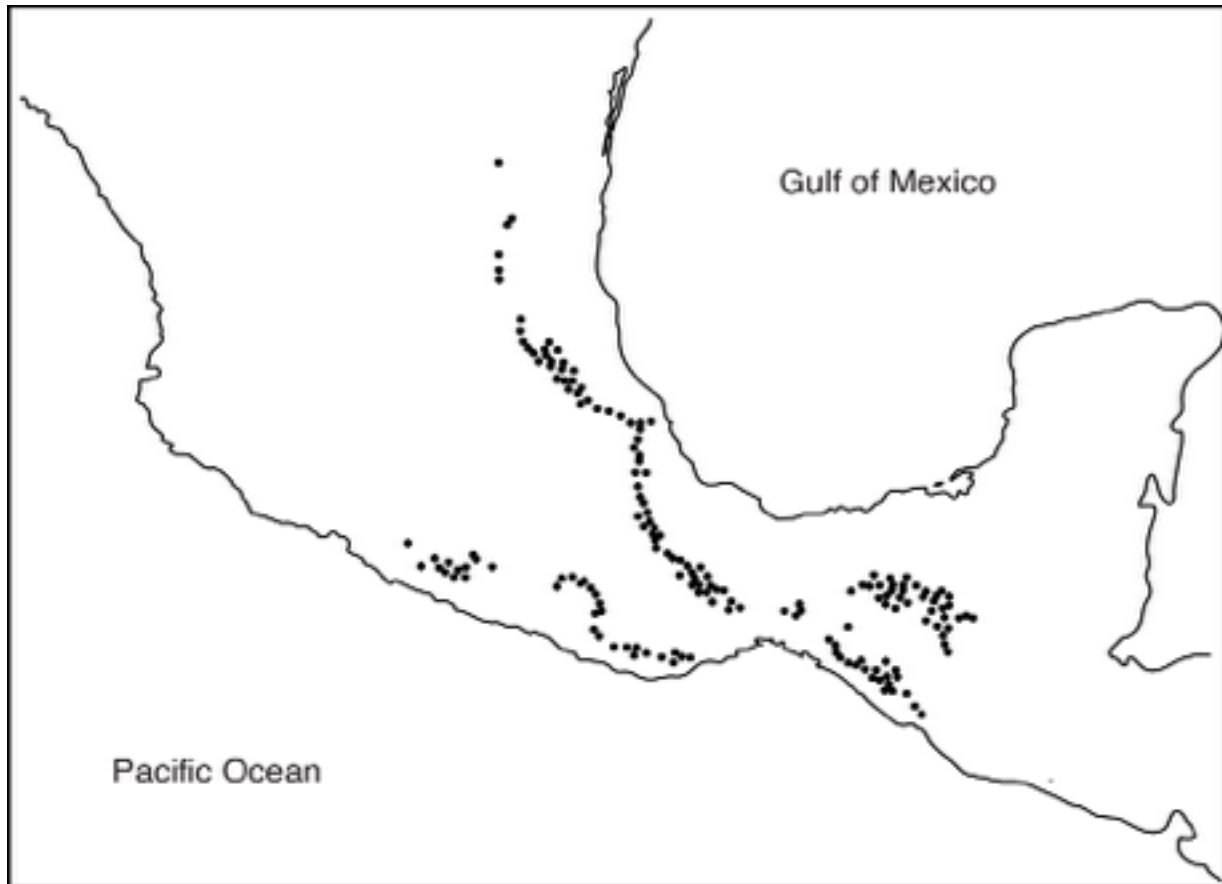


Figure S1 Map showing the geographical position (black circles) of the 175 localities of Mexican cloud forests used in the distribution modelling.

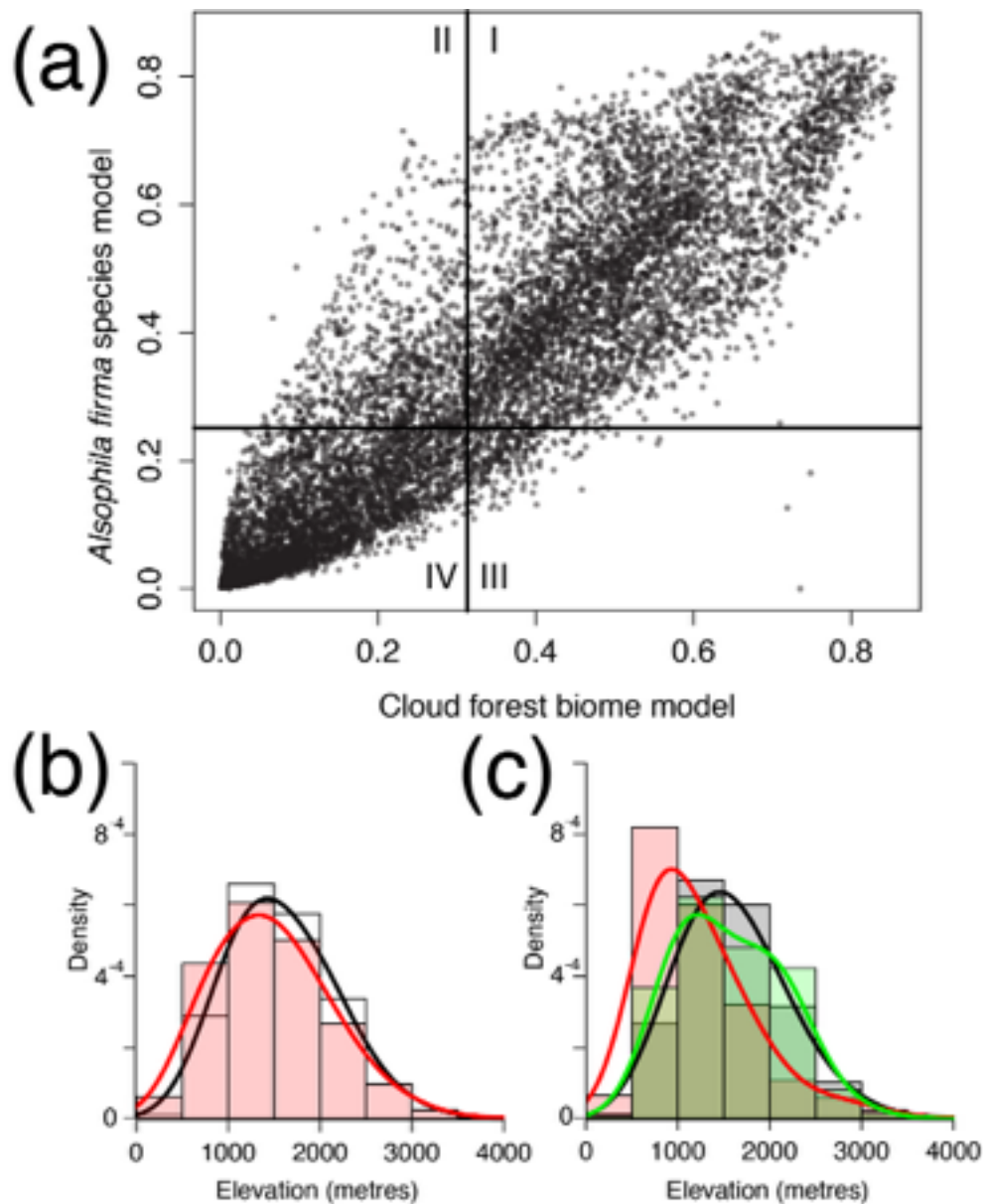


Figure S2 (a) Scatter plot showing the MAXENT values for each pixel within the study area (Mexico) predicted by the cloud forest biome and the *Alsophila firma* models. For graphical purposes we only plotted a random sample of 50,000 pixels. (b) Histogram showing the probability densities of the elevation of the cloud forest model (black) and the *A. firma* model (red). (c) Histogram showing the probability densities of the elevation for quadrants I

(black), II (red) and III (green). See Supplementary methods for details on quadrant definition.

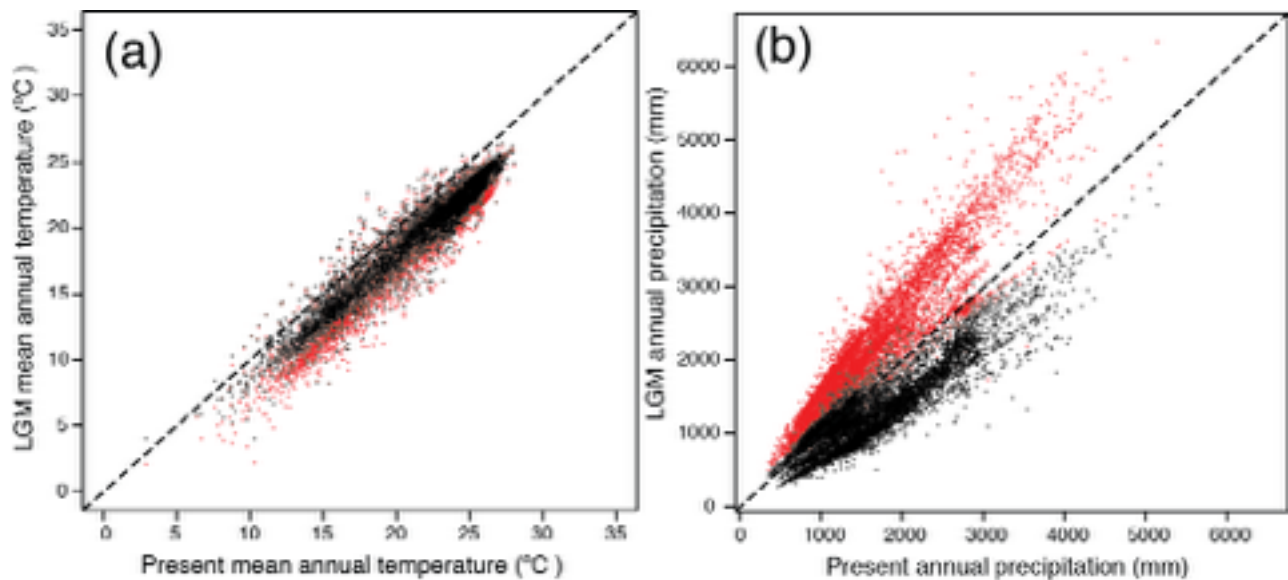


Figure S3 Scatter plots of (a) annual precipitation and (b) mean annual temperature for the present-day and the Last Glacial Maximum (LGM) extracted from 5500 random points in Mexico and Central America. The CCSM (Community Climate System Model) and MIROC (Model for Interdisciplinary Research on Climate) palaeoclimate models are represented by red and black points, respectively.

APÉNDICE 3.

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SUPPORTING INFORMATION

Changes in the distribution of cloud forests during the last glacial predict the patterns of genetic diversity and demographic history of the tree fern *Alsophila firma* (Cyatheaceae)

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Supplementary methods

A total of 33 summary statistics were calculated for the nuclear gene fragment *gapCp*. For each group of populations of *Alsophila firma* (Cyatheaceae), the number of alleles, mean pairwise differences, variance of pairwise differences and genetic differentiation (F_{ST}) were calculated in the simulations. Groups of populations are the same as the ones used for the analysis of molecular variance (AMOVA): (1) Sierra Madre Oriental north, (2) Sierra Madre Oriental south, (3) Sierra Norte de Oaxaca, (4) Sierra Madre del Sur, (5) Altos de Chiapas, and (6) Soconusco (Fig. 1). A total of 27 summary statistics were calculated for the chloroplast microsatellites (cpSSRs). For each population, mean number of alleles, mean genic diversity and genetic differentiation (F_{ST}) were calculated in the simulations. The prior distributions for the time parameters were defined to encompass the temporal intervals of the environmental data: the Last Interglacial (LIG; c. 115–130 ka) and the Last Glacial Maximum (LGM; c. 18–25 ka). The mutation rates used in the simulations were the same as the ones used in the mismatch distribution analyses.

Parameter estimation and model check procedure

The posterior probability of each scenario was estimated by a weighted polychotomous logistic regression on the 1% of simulated datasets closest to the observed, after a linear discriminant analysis on summary statistics. This regression was performed with the proportion of datasets from each scenario closest to the observed data as the dependent variable, and the differences between the observed and simulated summary statistics as

the independent variable. The intercept of this regression was taken as the point estimate of the posterior probability of the scenario under consideration.

For the best supported scenario, the posterior probability distribution of time and demographic parameters were estimated, after a logit transformation, by local linear regression on the 1% of simulations closest to the observed data. We generated 500 simulated datasets to assess the bias of the mean parameter estimates, after a logit transformation of parameters. The bias in the estimation of parameters was measured with the average relative bias as calculated in DIYABC (Cornuet *et al.*, 2014); this is calculated as the difference between the simulated point estimate and the true value, divided by the true value, averaged over the 500 simulated datasets. True parameter values for the simulations were fixed to the mean point estimate obtained from the posterior probability.

We evaluated the ability of approximate Bayesian computation (ABC) analysis to discriminate between scenarios by estimating type I and type II error rates among 500 simulated datasets generated under each scenario. Finally, 10,000 pseudo-observed datasets were simulated from the posterior under the best-supported scenario in order to evaluate whether this model could successfully reproduce the observed data. The results from this test showed that the best supported scenario (*expanded*) could not confidently estimate seven summary statistics: (1) the number of haplotypes in group 6 (simulated > observed); (2) mean pairwise differences in group 5 (simulated > observed); (3) variance in pairwise differences in group 5 (simulated > observed); (4) F_{ST} between groups 1 and 2 (simulated < observed); (5) F_{ST} between groups 1 and 4 (simulated < observed); (6) F_{ST} between groups 4 and 5 (simulated > observed); (7) F_{ST} between groups 5 and 6 (simulated > observed).

REFERENCE

Cornuet, J.M., Pudlo, P., Veyssier, J., Dehne-Garcia, A., Gautier, M., Leblois, R., Marin, J.M. & Estoup A. (2014) DIYABC v2.0: a software to make approximate Bayesian computation inferences about population history using single nucleotide polymorphisms, DNA sequence and microsatellite data. *Bioinformatics*, **30**, 1187–1189.

Table S4 Prior distributions for model parameters in DIYABC.

| | Prior distributions | |
|----------------|---------------------|------------------|
| | <i>gapCp</i> | cpSSRs |
| n_1 | ND (100–100,000) | ND (50–50,000) |
| n_2 | ND (100–100,000) | ND (50–50,000) |
| n_3 | ND (100–100,000) | ND (50–50,000) |
| n_4 | ND (100–100,000) | ND (50–50,000) |
| n_5 | ND (100–100,000) | ND (50–50,000) |
| n_6 | ND (100–100,000) | ND (50–50,000) |
| n_7 | ND (100–30) | ND (50–15) |
| t_1 | ND (10–4000) | ND (10–4000) |
| t_2 | ND (7000–15,000) | ND (7000–15,000) |
| Mutation rates | UD (2 | UD (2 |

n_1 , population size of group 1; n_2 , population size of group 2; n_3 , population size of group 3; n_4 , population size of group 4; n_5 , population size of group 5; n_6 , population size of group 6; n_7 , population size of the ancestral expanded population; t_1 , time (in generations) to the divergence of the six groups of populations under the expanded scenario; t_2 , time (in generations) to the expansion of the ancestral population under the expanded scenario; t_2 , time (in generations) to the divergence of the six groups of populations under the stable scenario (see scenarios in Fig. S5). UD, uniform distribution; ND, normal distribution; cpSSRs, chloroplast microsatellites; *gapCp*, a nuclear gene fragment.

Table S5 Parameters and mean relative bias estimated in DIYABC.

| | <i>gapCp</i> | | | cpSSRs | | |
|-------|--------------|-----------------|---------------|------------|-----------------|---------------|
| | True value | Simulation mean | Relative bias | True value | Simulation mean | Relative bias |
| n | 23,500 | 40,580 | 0.726 | 31,500 | 29,870 | -0.054 |
| n | 39,300 | 48,580 | 0.236 | 30,100 | 28,890 | -0.040 |
| n | 81,100 | 101,100 | 0.247 | 56,200 | 70,150 | 0.248 |
| n | 63,300 | 52,870 | -0.164 | 33,100 | 71,800 | 1.169 |
| n | 34,000 | 47,820 | 0.406 | 20,400 | 24,760 | 0.213 |
| n | 60,600 | 52,290 | -0.137 | 15,300 | 22,480 | 0.469 |
| n | 10,300,000 | 10,000,000 | -0.028 | 4,960,000 | 4,976,000 | -0.131 |
| t_1 | 2200 | 2013 | -0.085 | 2020 | 1754 | 0.003 |
| t_2 | 11,200 | 10,940 | -0.022 | 10,800 | 10,750 | -0.005 |

n_1 , population size of group 1; n_2 , population size of group 2; n_3 , population size of group 3; n_4 , population size of group 4; n_5 , population size of group 5; n_6 , population size of group 6; n_7 , population size of the ancestral expanded population; t_1 , time (in generations) to the divergence of the six groups of populations under the expanded scenario; t_2 , time (in generations) to the expansion of the ancestral population under the expanded scenario; t_2 , time (in generations) to the divergence of the six groups of populations under the stable scenario (see scenarios in Fig. S5). cpSSRs, chloroplast microsatellites; *gapCp*, a nuclear gene fragment.

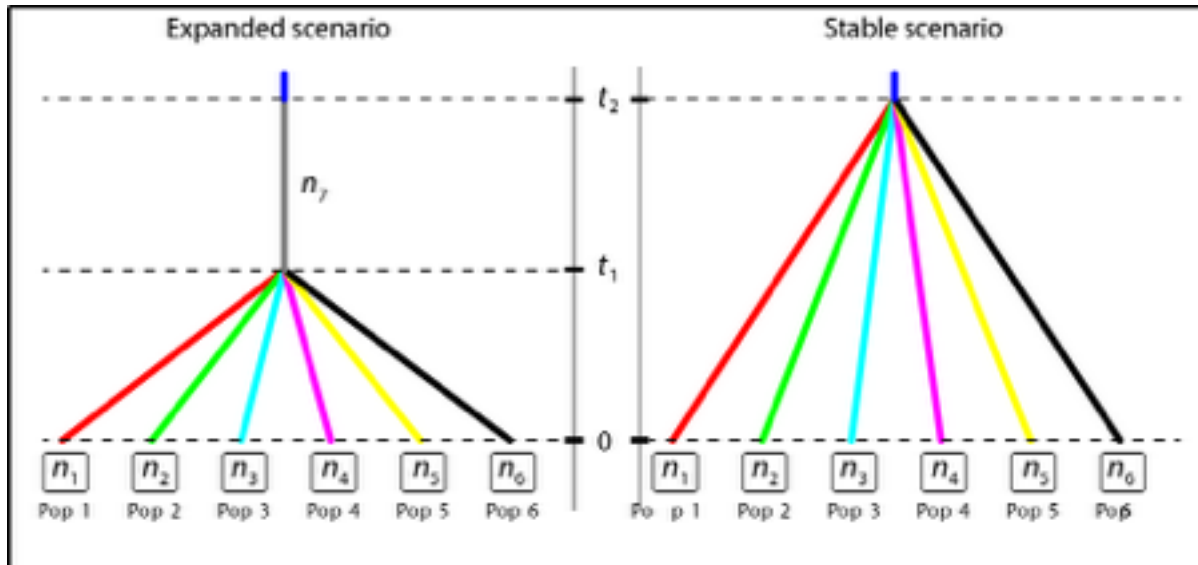


Figure S4 Pictures of the two demographic scenarios simulated in DIYABC. Pop, population. n_1 , population size of group 1; n_2 , population size of group 2; n_3 , population size of group 3; n_4 , population size of group 4; n_5 , population size of group 5; n_6 , population size of group 6; n_7 , population size of the ancestral expanded population; t_1 , time (in generations) to the divergence of the six groups of populations under the expanded scenario; t_2 , time (in generations) to the expansion of the ancestral population under the expanded scenario; t_2 , time (in generations) to the divergence of the six groups of populations under the stable scenario (see prior and posterior distribution of parameters in Tables S4 and S5).

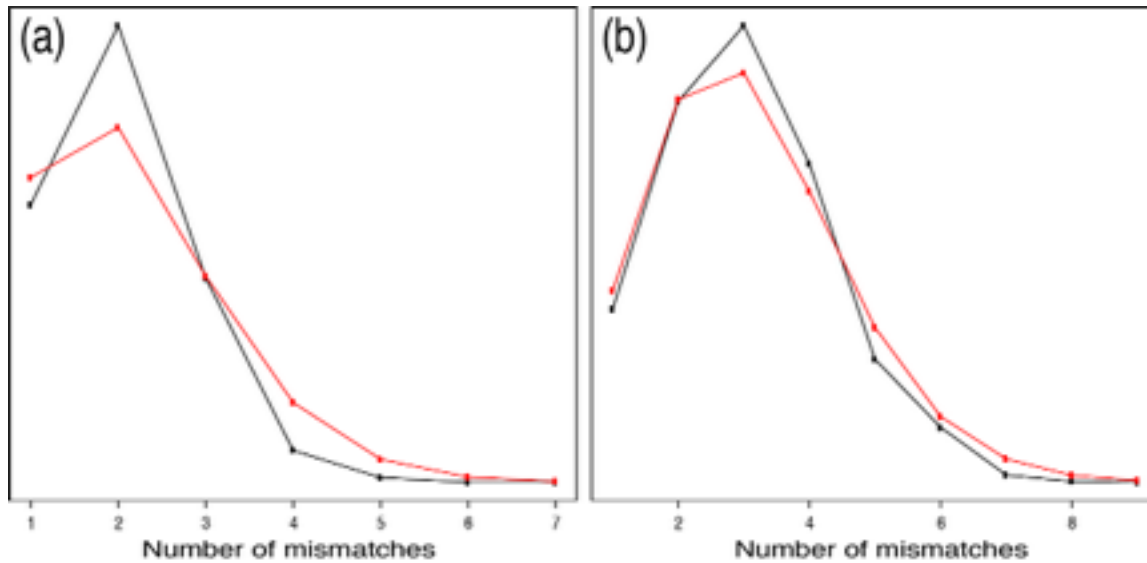


Figure S5 Mismatch distributions for (a) the nuclear gene *gapCp* and (b) the chloroplast microsatellites (cpSSRs) in the tree fern *Alsophila firma*. Black lines represent the observed distribution of mismatches and red lines represent the expected distribution under a pure demographic expansion.

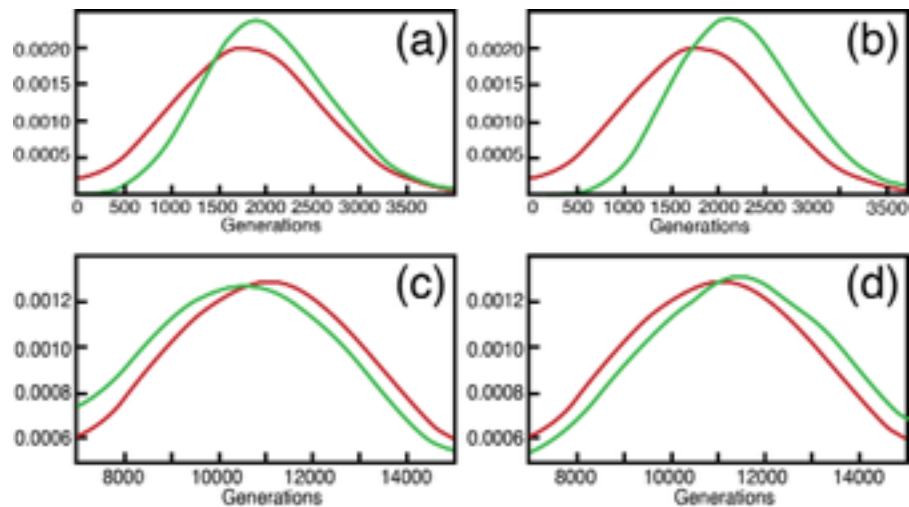


Figure S6 Prior and posterior probability distribution of parameters calculated in DIYABC for the (a, c) chloroplast microsatellites (cpSSRs) and (b, d) *gapCp* data. (a, b) Time of divergence (t_1) and (c, d) time of expansion (t_2) in generations before present.

APÉNDICE 4.

Botanical Journal of the Linnean Society

SUPPORTING INFORMATION

Spatial genetic analyses reveal strong genetic structure within two populations of the outcrossing tree fern *Alsophila firma* (Cyatheaceae)

Santiago Ramírez-Barahona and Luis E. Eguiarte

Table S1. Primer names and sequences used to analyze the spatial genetic structure in two populations of the tree fern *Alsophila firma* (AH and ATL).

| DNA region | Primer | Sequence (5'-3') | Author |
|------------|-----------|---------------------------------------|--------------------------|
| rbcL-atpB | ESRBCL26R | GCT TTA GTC TCC GTT TGT GGT GAC AT | Korall et al. (2007) |
| | ATPB703R | CCA ATG ATC TGA GTA ATS TAT CC | Korall et al. (2007) |
| accD-atpB | RBCL1187F | GGA ACY TTG GGA CAT CCT TGG | Korall et al. (2007) |
| | ACCD816R | CCA TGA TCG AAT AAA GAT TCA GC | Ebihara et al. (2003) |
| | accDSSRAF | ATT GGC TGC CGC CTG CGA GGT ATG | This study. |
| | accDSSRAR | ACC CCG AGT TCA ATA GCC CA | This study. |
| | accDSSRBF | AAA GCT CCG ATC AAT CCA CCG A | This study. |
| | accDSSRBR | GAG CAC ATG GTT TCG AGC CGT | This study. |

| DNA region | Primer | Sequence (5'-3') | Author |
|------------|-------------|-----------------------------------|------------------------------|
| trnL-trnF | TRNLC | CGA AAT CGG TAG ACG CTA CG | Taberlet et al. (1991) |
| | TRNFF | ATT TGA ACT GGT GAC ACG AG | Taberlet et al. (1991) |
| | trnLFSSRAF | AGT GAC AGC TCC CAG ATT CA | This study. |
| | trnLFSSRAR | CTA ACC GTG TAG AAT GGG ACT CT | This study. |
| matK-rps16 | matKF1 | AGT AAC CCC TCA AGC ATC GA | This study. |
| | rps16R1 | CGT TTC TTC ACG AAG AGG TGG | This study. |
| gapCp | ESGAPCP8F1 | ATY CCA AGY TCA ACT GGT GCT GC | Schuettpelz et al. (2008) |
| | ESGAPCP11R1 | GTA TCC CCA YTC RTT GTC RTA CC | Schuettpelz et al. (2008) |
| gapCp-M | GAPCPM1F | TGG GCT GTT GGC CCT CAC AA | This study. |
| | GAPCPM1R | TCT GCA GCC AAC CAA AGG CAA CA | This study. |

^a Primers used only for amplification and sequencing of intergenic sequences.

^b Primers specifically designed to amplify sequences.

Table S2. Nuclear and chloroplast haplotype frequencies per population in two populations of the tree fern *Alsophila firma* (AH and ATL). The absolute numbers of haplotypes are given in parentheses. Shared haplotypes between populations are indicated in bold.

| <i>Allele</i> | <i>gapCp-M</i> | | <i>Haplotype</i> | <i>cpSSRs</i> | |
|---------------|------------------|------------------|------------------|------------------|------------------|
| | AH | ATL | | AH | ATL |
| 1 | | 0.14 (28) | 1 | 0.01 (1) | 0.16 (16) |
| 2 | 0.58 (86) | 0.28 (56) | 2 | 0.24 (18) | 0.03 (3) |
| 3 | | 0.03 (6) | 3 | 0.03 (2) | 0.04 (4) |
| 4 | | 0.06 (11) | 4 | 0.01 (1) | 0.01 (1) |
| 5 | | 0.30 (59) | 5 | 0.05 (4) | |
| 6 | | 0.01 (1) | 6 | 0.05 (4) | 0.10 (10) |
| 7 | | 0.05 (10) | 7 | 0.07 (5) | |
| 8 | | 0.01 (1) | 8 | 0.18 (13) | 0.03 (3) |
| 9 | | 0.06 (12) | 9 | 0.11 (8) | |
| 10 | | 0.04 (7) | 10 | 0.09 (7) | 0.06 (6) |
| 11 | | 0.04 (7) | 11 | 0.04 (3) | 0.09 (9) |
| 12 | 0.01 (1) | | 12 | 0.01 (1) | |
| 13 | 0.13 (19) | | 13 | 0.03 (2) | |
| 14 | 0.05 (7) | | 14 | 0.01 (1) | |
| 15 | 0.05 (8) | | 15 | 0.04 (3) | |
| 16 | 0.01 (1) | | 16 | 0.01 (1) | |
| 17 | 0.01 (2) | | 17 | | 0.11 (11) |
| 18 | 0.03 (4) | | 18 | | 0.04 (4) |
| 19 | 0.01 (1) | | 19 | | 0.05 (5) |
| 20 | 0.03 (5) | | 20 | | 0.01 (1) |

| <i>Allele</i> | <i>gapCp-M</i> | | <i>Haplotype</i> | <i>cpSSRs</i> | |
|---------------|----------------|-----|------------------|---------------|-----------|
| | AH | ATL | | AH | ATL |
| 21 | 0.03 (5) | | 21 | | 0.10 (10) |
| 22 | 0.02 (3) | | 22 | | 0.01 (1) |
| 23 | 0.01 (2) | | 23 | | 0.14 (14) |
| 24 | 0.01 (1) | | 24 | | 0.01 (1) |
| 25 | 0.01 (1) | | | | |