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Efectos de la fragmentación del hábitat en la diversidad y estructura

genética de poblaciones de *Quercus castanea* Née, en la cuenca de

Cuitzeo, Michoacán.

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Por medio de la presente me permito informar a usted que en la reunión ordinaria del Comité Académico del Posgrado en Ciencias Biológicas, celebrada el día 22 de agosto del 2011, se acordó poner a su consideración el siguiente jurado para el examen de DOCTORA EN CIENCIAS de la alumna **HERRERA ARROYO MARÍA LUISA** con número de cuenta **503005298**, con la tesis titulada: "Efectos de la fragmentación del hábitat en la diversidad y estructura genética de poblaciones de *Quercus castanea* Née, en la cuenca de Cuitzeo, Michoacán", bajo la dirección del Dr. Alberto Ken Oyama Nakagawa.

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Resumen

La fragmentación del hábitat representa una de las principales causas de pérdida de diversidad, incluyendo diversidad y estructura genética de las poblaciones perturbadas. Otra consecuencia importante de la transformación del hábitat, es la generación de árboles aislados, los cuales aún cuando permanecen inmersos en matrices de vegetación degradadas conforman estructuras claves para el mantenimiento y recuperación de los ecosistemas. En México se estima que aproximadamente 167 000 ha de bosque templado se pierden al año, siendo Michoacán, uno de los estados en los cuales se registran las más altas tasas de fragmentación y deforestación. En la cuenca de Cuitzeo actualmente se han registrado más de 1200 fragmentos de bosque de encino de diferentes tamaños y con diferente grado de perturbación. Dada la magnitud de este proceso de fragmentación, esta investigación tuvo como objetivo evaluar el efecto de la fragmentación sobre la diversidad y estructura genética en poblaciones de *Quercus castanea* en la cuenca de Cuitzeo, Michoacán y de manera particular, determinar el impacto de la fragmentación antropogénica reciente sobre la diversidad genética y el flujo génico mediado por semillas, así como determinar la diversidad genética y el movimiento de genes a través de polen en poblaciones fragmentadas y árboles aislados. Se colectaron diferentes poblaciones de *Quercus castanea* en la cuenca de Cuitzeo en fragmentos de distinto tamaño, y además se colectaron árboles aislados. Para cada una de las poblaciones se colectaron individuos adultos y plántulas, mismas que se mantuvieron congeladas a -70° C hasta su procesamiento en el laboratorio. Posteriormente se extrajo el ADN y se utilizaron marcadores moleculares de microsatélites de cloroplasto y nucleares para evaluar los parámetros de diversidad y flujo génico. Los resultados de este trabajo se encuentran divididos en dos capítulos:

1. Seed mediated connectivity among fragmented populations of *Quercus castanea* in a Mexican landscape. Este capítulo documenta una gran diversidad haplotípica de cloroplasto de *Q. castanea* en la zona de estudio. En total se encontraron 70 haplotipos de cloroplasto, de los cuales 53 fueron compartidos entre plántulas y adultos. Estos haplotipos generaron valores de diversidad promedio intrapoblacional $h_s = 0.61$ para los individuos adultos y $h_s = 0.63$ para las plántulas. También se documentó una correlación positiva y significativa entre el número de haplotipos y el tamaño de fragmento y marginalmente significativa entre el h_s y el tamaño de fragmento, para las plántulas. También se encontró una mayor movilidad de semillas en poblaciones de adultos que en aquellas semillas que dieron origen a las plántulas, y mayor dispersión a larga

distancia, en adultos, en comparación con las plántulas. En general, el grado de conectividad entre poblaciones fue significativamente menor en poblaciones de plántulas que en adultos. Es posible evidenciar un impacto negativo de la fragmentación del hábitat sobre la diversidad y conectividad genética.

2. Gene flow interruption in a recently human-modified landscape: the value of isolated trees for the maintenance of genetic diversity in *Quercus castanea* Née (Fagaceae). Este capítulo muestra altos valores de diversidad genética tanto en adultos como en las progenies en poblaciones fragmentadas y en árboles aislados. El análisis de la estructura genética en poblaciones fragmentadas mostró que los adultos no presentan diferenciación genética entre poblaciones, mientras que las plántulas sí. Las poblaciones de adultos y progenies mostraron un significativo exceso de homócigos, para los árboles aislados. Las poblaciones de progenies presentaron una diferenciación genética significativa, a diferencia de las poblaciones fragmentadas, éstos no presentaron valores significativos de endogamia. Poblaciones fragmentadas observaron un mayor número de donadores de polen con respecto a los árboles aislados. Todas las poblaciones mostraron ser altamente exocruzadas.

A partir de la información generada con marcadores nucleares y de cloroplasto, es posible concluir que a pesar de que estos procesos de fragmentación del hábitat son relativamente recientes, pudimos observar que existen importantes consecuencias negativas en las poblaciones afectadas. Hasta el momento los patrones de diversidad genética poblacional no han sido fuertemente afectados, caso contrario a lo que ocurrió con el flujo génico y la conectividad genética entre poblaciones las cuales mostraron ser parámetros mucho más susceptibles a la fragmentación.

Palabras clave: Fragmentación del hábitat, árboles aislados, *Quercus*, Diversidad genética, conectividad genética.

Abstract

Habitat fragmentation is a significant threat to the maintenance of biodiversity, including genetic diversity and genetic structure. In addition, scattered trees are prominent features in modified landscapes, despite their low density, they have been considered as keystone structures to different ecological processes on ecosystem recovery. In Mexico, approximately 167 000 ha of temperate forests are lost per year. Michoacán is one of states with the highest deforestation and fragmentation rates. Currently, the Cuitzeo basin has more than 1200 oak forest fragments of different size. Due to the magnitude of this fragmentation process, the main goal of this study was to evaluate the effect of forest fragmentation on genetic diversity and genetic structure in *Quercus castanea* populations in the Cuitzeo basin. The particular goals were to determine the impact of recent forest fragmentation on seed and pollen mediated genetic diversity and gene flow and in fragmented populations and isolated trees. We collected different *Quercus castanea* populations throughout the range of the species in the Cuitzeo basin, from different size fragments and collected isolated trees too. From each population, we sampled young leaves from adults and seedlings, they were frozen (-70°C) until processing. After DNA extracting, we used nuclear and chloroplast microsatellites markers to analyze genetic diversity and gene flow.

Results are integrated into two chapters.

1. Seed mediated connectivity among fragment populations of *Quercus castanea* in a Mexican landscape. This analysis revealed extremely high chloroplast DNA haplotype diversity. We found 70 chloroplast haplotypes, 53 shared between seedlings and adults and average haplotype diversity values of $h_s = 0.61$ for adults and $h_s = 0.63$ for seedlings. Seedlings showed a positive and significant correlation between haplotype number and fragment size, and a marginally significant correlation for h_s and fragment size. Genetic connectivity was lower in seedling than in adult populations. These results demonstrate a negative effect of forest fragmentation over genetic diversity and connectivity.

2. Gene flow interruption in a recently human-modified landscape: the value of isolated trees for the maintenance of genetic diversity in *Quercus castanea* Née (Fagaceae). This analysis found high genetic diversity parameters in both, adult and offspring populations, as well as in fragmented populations and isolated trees. Genetic structure was not significant in adult populations but significant in offspring populations. Adult and seedlings from isolated trees showed an excess of homozygotes,

and were genetically structured. Fragmented populations did not show significant inbreeding values. Fragmented populations observed an increased number of pollen donors than isolated trees. All populations were highly outcrossed.

From the chloroplast and nuclear information we may conclude that even though habitat fragmentation processes are relatively recent, there are significant consequences on affected populations. Until now, genetic diversity has not been highly affected, but gene flow patterns are significantly affected, thus this gene flow and connectivity parameters were more susceptible to habitat fragmentation, the effects observed on these parameters are much more significant.

Key words: Habitat fragmentation, isolated trees, genetic diversity, genetic connectivity.

CAPÍTULO I

1. INTRODUCCIÓN

Como resultado del uso del ambiente para fines antropogénicos y de disturbios naturales en la dinámica de los ecosistemas, se han generado importantes cambios en la distribución y configuración espacial de los hábitats naturales, de tal manera que la cantidad y calidad de hábitat disponible para las comunidades naturales de plantas y animales cada vez es mas restringida, ya que los ambientes cada día se encuentran más fragmentados (Young *et al.*, 1996; Ottewell *et al.*, 2009; Lander *et al.*, 2010).

Estos cambios en el paisaje ocasionados por la pérdida de hábitat y fragmentación son un punto muy importante a evaluar en el campo de la biología de la conservación (Ferreira *et al.*, 2013).

El crecimiento de las poblaciones humanas y en consecuencia, el incremento en el uso de los recursos utilizados, ejerce una fuerte presión en cuanto al mantenimiento y conservación de dichos recursos naturales, de manera que la transformación en el uso de las tierras a través de aclareos, forestaría, pastoreo, urbanización, etc. ha generado importantes consecuencias en los ecosistemas, entre ellas la pérdida de especies así como la pérdida de las relaciones o interacciones entre las especies que permanecen (Vitousek *et al.*, 1997; Segelbacher *et al.*, 2010; Ferrerira *et al.*, 2013).

La fragmentación del hábitat puede definirse como un proceso por medio del cual un área continua es transformada en un gran número de pequeños fragmentos separados de otros por una matriz de hábitat distinto al original (Fahrig, 2003), o simplemente como una disrupción en la continuidad del hábitat (Lord & Norton, 1990). La fragmentación del hábitat es una de las características que representan a los sistemas naturales en la actualidad (Laurance, 2004).

La transformación en los ecosistemas, generalmente no es completa, usualmente da como resultado un mosaico de parches antropogénicos y nativos en donde los procesos bióticos y abióticos son alterados en diferente grado (Nason y Hamrick, 1997; Fahrig, 2003; Lander *et al.*, 2010).

Además de la pérdida de hábitat, el proceso de fragmentación resulta en otros efectos, éstos son el incremento en el número de fragmentos remanentes, disminución en el tamaño de los mismos, así como el incremento en el grado de aislamiento entre los fragmentos generados, lo cual crea discontinuidades en recursos y en condiciones ambientales. (Fahrig, 2003; Segelbahr *et al.*, 2010).

Otro de los efectos generados por la remoción del hábitat es la presencia de árboles aislados. Los árboles aislados son una de las características más comunes en los paisajes fragmentados, estos árboles persisten en matrices de vegetación degradadas, mismas que en algún momento formaron parte de un paisaje continuo (Manning *et al.*, 2006; Lander *et al.*, 2010).

Diferentes estudios han demostrado que estos árboles aislados son estructuras clave dentro de los ecosistemas actuales debido a su efecto en el funcionamiento de los mismos, ya que actúan como puentes en el movimiento de genes entre los diferentes fragmentos (Hobee *et al.*, 2007; Herrera y García, 2009; Lander *et al.*, 2010).

A pesar de su baja densidad, los árboles aislados poseen un importante papel en el sostenimiento de diferentes procesos ecológicos, como el mantenimiento de hábitat para la biota silvestre, protección contra la erosión y la desertificación y además de su relevancia en la producción de semillas para el restablecimiento de la vegetación (Manning *et al.*, 2006; Ottewell *et al.*, 2009; Fisher *et al.*, 2010). Sin embargo, debido al pobre reclutamiento que se lleva a cabo en áreas con degradación, estos individuos pueden ser completamente perdidos en un lapso de 90-180 años (Gibbons *et al.*, 2008).

Teóricamente estos cambios asociados a la degradación del hábitat tienen importantes implicaciones para los individuos, las poblaciones y los ecosistemas en general.

1.1. Consecuencias ecológicas de la fragmentación

La fragmentación representa una de las principales causas de pérdida de la diversidad biológica, afecta la abundancia y distribución de las especies, puede modificar la estructura genética de poblaciones de plantas y animales, todo esto con consecuencias potencialmente negativas para la persistencia de las mismas (Fahrig, 2003; Keyghobadi *et al.*, 2005; Ferreira *et al.*, 2013).

1.1.1. Incremento de bordes

En el aspecto ecológico, una de las consecuencias críticas de la fragmentación del hábitat es el incremento desproporcionado de los bordes en los fragmentos (Saunders *et al.*, 1991; Laurance 2004). Este incremento en los bordes, altera las condiciones microclimáticas (evapotranspiración, humedad, temperatura, retención de agua en el suelo, entre otras) y más aún, las relaciones bióticas, generando de ésta manera importantes repercusiones en el reclutamiento de nuevos individuos de la vegetación nativa, (Benitez-Malvido y Kossmann-Ferraz, 1999; Bruna, 1999; Kolb y Diekmann, 2005)

1.1.2. Polinización y dispersión de semillas

Uno de los patrones más frecuentemente observados en diferentes estudios es que las reducciones en el hábitat y la proximidad a los bordes puede influenciar estados críticos para las plantas, particularmente en lo que se refiere a polinización y reclutamiento (Jules, 1998), ya que algunas poblaciones de polinizadores y dispersores pueden declinar, desaparecer o modificar su composición, abundancia y actividad (Johnson *et al.*, 2004; Ferreira *et al.*, 2013).

En especies que dependen del viento y gravedad para la polinización y dispersión, como son la mayor parte de las especies de árboles de bosque templado, el incremento en las distancias entre fragmentos o individuos disminuye de manera directa la interconexión entre éstos (Young *et al.*, 1996; Knapp *et al.*, 2001; Sork *et al.*, 2002) por lo que este grupo de especies puede ser afectado de manera negativa por el aislamiento o disrupción de la conectividad de las poblaciones (Kolb y Diekmann, 2005).

Sin embargo, los procesos de fragmentación y pérdida de hábitat no necesariamente impiden el movimiento de los polinizadores entre los fragmentos (Sork y Smouse, 2006), incrementándose incluso en algunas ocasiones el flujo de polen (Lander *et al.*, 2010).

1.2. Consecuencias genéticas de la fragmentación

1.2.1. Pérdida de diversidad genética

En un conjunto de poblaciones que han sufrido fragmentación, la demografía y la dinámica de los genes, depende de los patrones de dispersión de polen y semillas (Jaquiéry *et al.*, 2010), la disminución en la variación genética puede depender de la interacción entre la magnitud del proceso de fragmentación y la estructura genética previa a estos procesos (Nason y Hamrick, 1997), además de la importancia relativa de los cuellos de botella poblacionales inducidos por la fragmentación, los subsecuentes ciclos de deriva génica, efectos fundador y los niveles de flujo génico entre las distintas poblaciones remanentes (Dow y Ashley, 1998a; Vakkari *et al.*, 2006; Jaquiéry *et al.*, 2010).

Teóricamente puede haber una disminución de la diversidad genética. Ésta puede llevarse a cabo en dos formas: por un lado, reducciones en tamaño poblacional al

momento de la fragmentación provocan una pérdida de riqueza alélica, generándose cuellos de botella donde permanece solo una pequeña parte del “pool” génico original (Young *et al.*, 1996). Siguiendo a esta pérdida inicial de variación, las poblaciones remanentes pueden permanecer pequeñas y aisladas por varias generaciones continuas, lo que puede llevar a una pérdida de alelos por deriva génica así como también se puede favorecer el incremento en la diferenciación poblacional (Young *et al.*, 1996).

La pérdida de variación genética, a largo plazo puede reducir la capacidad de las poblaciones para responder a cambios por presiones de selección ambiental, incrementando el riesgo de extinción (Ledig *et al.*, 2001; Lowe *et al.*, 2005), por lo que se espera que las poblaciones perturbadas experimenten mayor susceptibilidad a las plagas y enfermedades (Petit *et al.*, 2002; Lowe *et al.*, 2005).

1.2.2. Endogamia

Por otro lado, el apareamiento de individuos emparentados o endogamia, puede incrementar los niveles de homocigosidad (Sherwin y Moritz, 2000; Frankham *et al.*, 2002) y a largo plazo puede resultar en una disminución de la adecuación, es decir, depresión por endogamia (Keller y Waller, 2002). La endogamia puede ser medida como la probabilidad de que dos alelos sean idénticos por descendencia (Ellstrand, 1992). Las poblaciones naturales contienen cierta cantidad de alelos deletéreos (la mayoría de ellos parcialmente recesivos) en frecuencias muy bajas (Keller y Waller, 2002). Como resultado de la fragmentación, los individuos tienden a ser más endogámicos, lo cual podría disminuir la viabilidad y la fecundidad debido a depresión por endogamia (Couvét, 2002).

En poblaciones pequeñas, los alelos recesivos deletéreos tienden a acumularse debido a los apareamientos entre individuos emparentados. Esto puede llevar a fijar determinada carga genética por procesos de deriva génica. La exposición a la selección

natural de esta carga genética puede eliminar estos alelos recesivos deletéreos, sin embargo muchas poblaciones no pueden evitar la endogamia (Keller y Waller, 2002).

1.2.3. Modificación de los patrones de flujo génico

El flujo génico es un proceso mediante el cual determinados alelos (genes) o individuos (genotipos) son intercambiados entre poblaciones separadas geográficamente (Dow y Ashley, 1998b). Éste, es un factor muy importante en la dinámica evolutiva de poblaciones naturales, ya que moldea la organización de la diversidad genética dentro y entre poblaciones a través de la dispersión de genes mediante dos vehículos distintos, polen y semillas (Dow & Ashley, 1998a).

Desde el punto de vista de la biología de la conservación, el flujo génico es esencial para mantener la conectividad genética entre paisajes (Segelbacher *et al.*, 2010).

La consecuencia evolutiva mas conocida del flujo génico es que homogeniza la estructura genética de la población, es decir, actúa en contra del efecto de la deriva génica (Keyghobadi *et al.*, 2005). Por el contrario, el flujo génico restringido conduce a la subestructuración de las poblaciones, a tamaños efectivos reducidos dentro de las poblaciones y a la diferenciación genética interpoblacional debido a procesos de selección natural y deriva génica (Dow & Ashley, 1998b).

Desde la perspectiva de un organismo, la discontinuidad en su distribución a través de habitats favorables pueden llevar a la reducción de la conectividad entre poblaciones fragmentadas (Segelbacher *et al.*, 2010), esta continuidad funcional entre las poblaciones, es críticamente importante para el intercambio y flujo de genes.

La fragmentación y el aislamiento de las poblaciones reducen el flujo de genes entre éstas, lo que frecuentemente tiene implicaciones adversas para la conservación (Young & Merriam, 1994; Sherwin y Moritz, 2000; White *et al.*, 2002; Lowe *et al.*,

2005), generalmente afecta los patrones de apareamiento al disminuir el número de parejas disponibles, por lo que tamaños de vecindario pequeños, pueden llevar a la endogamia (Eckert *et al.*, 2010; Llorens *et al.*, 2012). Aunque no necesariamente la fragmentación provoca disminución del flujo génico (White *et al.*, 2002; Dick *et al.*, 2003; Lander *et al.*, 2010).

La dispersión y el flujo de polen no son independientes del contexto ambiental, por tanto, es muy importante evaluar como los cambios en el paisaje, por ejemplo, la fragmentación del hábitat pueden influenciar estos procesos (Smouse *et al.*, 2001).

1.3. Deforestación y fragmentación de bosques en México

En México, existe actualmente un acelerado proceso de deterioro y pérdida de los recursos forestales. El cambio de uso de suelo es un factor que ha afectado grandes extensiones de bosques, convirtiéndose estos territorios forestales en ranchos de ganadería extensiva principalmente, y en menor grado hacia un uso agrícola (Masera *et al.*, 1997). A pesar de las grandes incertidumbres que se tienen sobre las tasas precisas de deforestación y degradación forestal, se estima que la pérdida anual de bosques es de 668,000 ha/año, de las cuales 167,000 ha corresponden a los bosques templados y 501,000 son de bosque tropical. De manera que las tasas de deforestación son realmente altas: 2%/año para bosques tropicales y 0.64%/año para bosques templados (Masera *et al.*, 1997). Aproximadamente, el 80 % de la deforestación está concentrado en las zonas Centro y Sureste del país, siendo Michoacán el estado en donde se registran las tasas más altas deforestación 1.8%/año para bosques templados por lo que la pérdida de bosque se estima en mas de 50,000 ha/año (Masera *et al.*, 1997; Bocco *et al.*, 2001).

1.4. El género *Quercus* en México

El género *Quercus* (Fagaceae) es uno de los grupos de plantas forestales más importantes en nuestro país. Debido al gran número de especies y endemismos que posee, México ha sido considerado como centro de diversificación del género para América (Rzedowski, 1978; Nixon, 1993). De acuerdo con Nixon (1993), en México están representadas tres de las cuatro secciones del subgénero *Quercus*: *Quercus*, *Lobatae* y *Protobalanus*. Las estimaciones más recientes de riqueza de encinos indican que en México existen 161 especies, de las cuales 81 pertenecen a la sección *Quercus*, 76 a la sección *Lobatae*, y la sección *Protobalanus* está representada con 4 de las 5 especies que la conforman (Valencia, 2004). De éstas, 109 especies son endémicas.

Respecto al aprovechamiento de los bosques de encino, cabe mencionar que en general, los encinares son bastante explotados tanto a escala local como a escala regional, aunque muy poco a nivel industrial. Localmente la madera de encinos se emplea para construcciones, fabricación de muebles, postes y muchos otros usos, pero sobre todo como combustible, bien sea para transformarla en carbón o utilizarla directamente como leña (Rzedowski, 1978; Aguilar, 2010). Además, extensas superficies de encinares han sido transformadas a pastizales para ganadería y terrenos de cultivo (Rzedowski, 1978). Adicionalmente, la urbanización, la eliminación de áreas boscosas, la fragmentación, así como el pobre reclutamiento de nuevos individuos debido en gran parte a presiones por ganadería y a la competencia de las plántulas con malezas introducidas, amenazan la permanencia de poblaciones no solamente de los encinos, también de muchas otras especies de plantas (Knapp *et al.*, 2001).

Los encinos en la Cuenca de Cuitzeo, en el Estado de Michoacán son utilizados principalmente para la elaboración de carbón vegetal y leña. La principal actividad de

muchos pueblos en la Cuenca es la producción de carbón vegetal, y una de las especies más utilizadas para este fin es *Quercus castanea* (Aguilar, 2010)

2. SISTEMA DE ESTUDIO

2.1. Especie de estudio.

Quercus castanea Née (Lobatae). Pertenece a la serie Castaneae. Son árboles de hasta 18 m de altura, ocasionalmente pueden ser arbustos (Trelease, 1924).

Como todas las especies de encinos, son polinizados por el viento, poseen exocruzamiento obligado, la dispersión se lleva a cabo por la gravedad, algunas aves y roedores (Sork *et al.*, 2002; Schondube *et al.*, 2010). En general, el periodo de vida para los encinos es muy largo, algunas especies pueden llegar a vivir más de 400 años (Kaul, 1985).

Q. castanea se encuentra ampliamente distribuida en la Sierra Madre Occidental, Eje Neovolcánico Transversal, Sierra Madre del Sur y en algunas localidades de la Sierra Madre Oriental (Valencia, 2004). En Michoacán se distribuye prácticamente a lo largo de la cordillera Neovolcánica, incluyendo manchones en la parte central y este de la depresión del Río Lerma, así como en la parte central de la Sierra Madre del Sur, en un intervalo altitudinal de los 1450 a 2600 msnm (Bello & Labat, 1987) (Fig. 1).

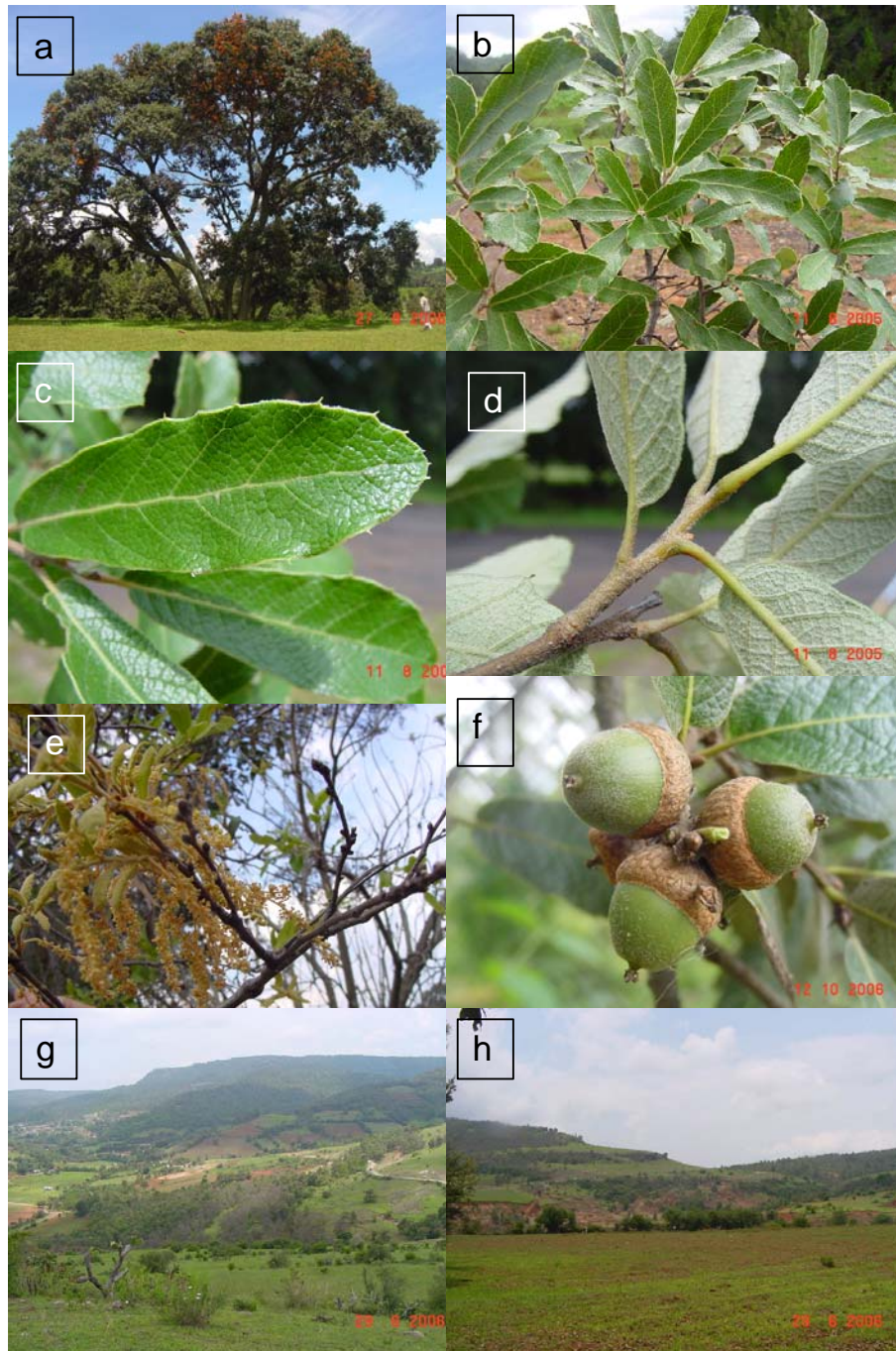


Fig. 1. *Quercus castanea*: a) vista general del árbol, b) ramilla con hojas, c) hoja mostrando las aristas, d) detalle del indumento de ramillas y peciolo, e) inflorescencias masculinas, f) semillas, g) y h) paisajes antropogénicos después de la transformación del bosque para la agricultura y la ganadería.

2.2. Sitio de estudio

La cuenca del lago de Cuitzeo se localiza dentro del Sistema Volcánico Transversal, entre los 19°30' y 20°05' latitud norte y 100°35' y 101°30' longitud Oeste (Fig. 2), posee una superficie de 4026 km², (cifra calculada a partir de la definición y digitalización del parteaguas de la cuenca), la mayor parte de la superficie de la cuenca se encuentra dentro del estado de Michoacán, aunque también algunos municipios de Guanajuato forman parte de la misma (Lopez *et al.*, 2006; Mendoza *et al.*, 2006).



Fig. 2. Localización de la Cuenca del Lago de Cuitzeo (tomado de López-Granados 2006).

Los tipos de vegetación mas comunes en la cuenca son: bosque de oyamel, bosque de pino, bosque de encino, bosque mesófilo de montaña, bosque tropical

caducifolio, matorral subtropical, bosque espinoso, bosque de galería, pastizal, entre otros (Lopez *et al.*, 2006).

Respecto al bosque de encino, debido al alto grado de alteración (Fig. 3) que se presenta a causa de la tala y los incendios, más de 1200 fragmentos de diferentes tamaños y con diferentes grados de desarrollo se encuentran al interior de la cuenca, por lo que es difícil determinar si se trata de vegetación primaria o secundaria. Además, no se tienen datos exactos sobre el tiempo que ha transcurrido posterior a la fragmentación. Sin embargo, los propietarios de los bosques relatan que hace aproximadamente 50 a 100 años ocurrió esta transformación del bosque. *Mendoza et al.*, (2011) señala que la degradación del bosque en la cuenca de Cuitzeo se incrementó en la década de los 80's.

3. PREGUNTAS DE INVESTIGACIÓN

Esta tesis se plantea las siguientes preguntas:

1. ¿Se ha reducido la conectividad vía semillas entre poblaciones de *Quercus castanea* en la cuenca de Cuitzeo, Michoacán como resultado de la fragmentación del hábitat?
2. ¿Puede la diversidad genética y el flujo génico a través de polen ser afectados por procesos contemporáneos de fragmentación de hábitat? y
- 3.-¿Cuál es el papel de los árboles aislados en este proceso de fragmentación del hábitat?

4. HIPÓTESIS

Las siguientes hipótesis se pusieron a prueba:

Las poblaciones actuales de *Quercus castanea* históricamente han mantenido conectividad genética a través de semillas.

Los procesos actuales de fragmentación de hábitat no afectan la diversidad genética de cloroplasto.

Respecto al movimiento de genes a través de polen, se espera encontrar diferencias en los patrones de apareamiento, es decir, el número de padres que polinicen a los árboles madre en los fragmentos de menor tamaño será diferente al número de padres que polinizan a los árboles madre en los fragmentos de mayor tamaño.

5. OBJETIVOS

Objetivo general:

Evaluar el efecto de la fragmentación sobre la diversidad y estructura genética en poblaciones de *Quercus castanea* en la cuenca de Cuitzeo, Michoacán.

Objetivos específicos:

Determinar el impacto de la fragmentación antropogénica reciente sobre la diversidad genética y el flujo génico mediado por semillas.

Determinar la diversidad genética y el movimiento de genes a través de polen en poblaciones fragmentadas y árboles aislados.

CAPÍTULO II

SEED MEDIATED CONNECTIVITY AMONG FRAGMENTED POPULATIONS OF
Quercus castanea IN A MEXICAN LANDSCAPE

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**SEED-MEDIATED CONNECTIVITY AMONG FRAGMENTED
 POPULATIONS OF *QUERCUS CASTANEA* (FAGACEAE) IN A
 MEXICAN LANDSCAPE¹**

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- *Premise of study:* Anthropogenic fragmentation is an ongoing process in many forested areas that may create loss of connectivity among tree populations and constitutes a serious threat to ecological and genetic processes. We tested the central hypothesis that seed dispersal mitigates the impact of fragmentation by comparing connectivity and genetic diversity of adult vs. seedling populations in recently fragmented populations of the Mexican red oak *Quercus castanea*.
- *Methods:* Adult individuals, established before fragmentation, and seedlings, established after fragmentation, were sampled at 33 forest fragments of variable size (0.2 to 294 ha) within the Cuitzeo basin, Michoacán state, and genotyped using seven highly polymorphic chloroplast microsatellite markers (cpSSRs). To test whether seed dispersal retains connectivity among fragmented populations, we compared genetic diversity and connectivity networks between adults and progeny and determined the effect of fragment size on these values.
- *Key results:* Seventy haplotypes were identified, 63 in the adults and 60 in the seedlings, with average within-population diversity (h_s) values of 0.624 in the adults and 0.630 in the seedlings. A positive correlation of genetic diversity values with fragment size was found in the seedling populations but not in the adult populations. The network connectivity analysis revealed lower connectivity among seedling populations than among adults. The number of connections (edges) as well as other network properties, such as betweenness centrality, node degree and closeness, were significantly lower in the seedlings network.
- *Conclusions:* Habitat fragmentation in this landscape is disrupting seed-dispersal-mediated genetic connectivity among extant populations.

Key words: chloroplast DNA; Fagaceae; forest fragmentation; genetic connectivity; genetic variation; microsatellites; *Quercus castanea*.

Genetic diversity is a critically important property of populations because it shapes the ability of populations to respond to environmental changes (Templeton et al., 1990; Frankham, 1996; Young et al., 1996; Frankham et al., 2003; Reed and Frankham, 2003). One specific mechanism of maintaining genetic variation is gene flow across a landscape (Sork et al., 1999; Sork and Smouse, 2006). The pattern and extent of pollen and seed exchange determine the connectivity among populations (Templeton et al., 1990; Ennos, 1994; Young et al., 1996; Sork and Smouse, 2006), which can influence the probability of extinction of local

populations (Slatkin, 1985; Ouborg et al., 1999). For plant populations, a key mechanism of maintaining connectivity is the movement of seeds (Taylor et al., 1993; Cordeiro and Howe, 2003; Herrera and García, 2010). At regional scales, seed dispersal allows colonization of new sites and augments existing populations with new individuals and genetic variation. Thus, seed movement, which shapes both the genetic and demographic processes of a metapopulation, can mitigate the effects of landscape transformation or can be impeded by landscape change.

The disruption of natural connectivity in forest landscapes through fragmentation is a major threat for the conservation and maintenance of ecological processes. Fragmentation and, consequently, the isolation of resulting patches may lead to spatially structured habitats in which the overall pattern of gene exchange among populations is reduced (Lowe et al., 2005; Honnay and Jacquemyn, 2007). Population changes associated with habitat degradation can lead to an erosion of genetic variation and to the increase of interpopulation genetic divergence, due to reduced gene flow and elevated inbreeding and random genetic drift (Young et al., 1996; Lowe et al., 2005; Honnay and Jacquemyn, 2007). In wind-pollinated forest trees, gene flow by pollen can effectively connect fragmented populations (Nason and Hamrick, 1997; White et al., 2002; Dick et al., 2003; Fernández and Sork,

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2005; Sork and Smouse, 2006), reducing or preventing a negative impact of even chronic fragmentation on genetic diversity levels in remaining fragments. However, seed dispersal, which acts after pollen dispersal, can reduce the impact of pollen flow in established seedling populations (Grivet et al., 2008; V. Sork et al., unpublished manuscript). The extent to which fragmentation could affect seed dispersal will be influenced by the degree of isolation of remaining patches and its impact on the dispersal vector. Disruption of seed flow is expected to have important demographic consequences because an interruption of recolonization of new sites could increase the extinction risk of the metapopulation on the long term.

A major methodological problem in the study of seed dispersal is the difficulty associated with recovering seeds that have been already dispersed and then determining unambiguously the maternal parent from the nuclear genotype of seedlings (Godoy and Jordano, 2001; Sork and Smouse, 2006). For species with maternally inherited chloroplast DNA (cpDNA), genetic markers have been used effectively in resolving phylogeographical patterns and for inferring historical connectivity among populations at broad geographical scales (Dumolin-Lapegue et al., 1997; Soltis et al., 1997; Schaal et al., 1998; Newton et al., 1999; Grivet et al., 2006, 2008). Unfortunately, for most plant species, the polymorphism in cpDNA markers on a regional scale is not usually sufficient to document patterns of genetic diversity and gene flow through seeds at the landscape level (Banks and Birky, 1985). However, at least in the case of some plant species, cpDNA simple sequence repeat (cpSSR) loci might bear enough within-population variation to make these studies feasible (Pleines et al., 2009). For such species, the use of these markers would be ideal for analyzing consequences of habitat fragmentation on seed-mediated connectivity because haploid cpDNA is expected to be more sensitive to the effects of drift, given its lower effective population size and the usually limited dispersal distances of seeds.

A recent analytical development in the field of landscape genetics has been the application of graph theory to the study of connectivity among populations (Dyer and Nason, 2004; Garroway et al., 2008; Rozenfeld et al., 2008). This method provides a powerful framework to characterize processes that take place in complex interconnected systems, such as gene flow through geographical landscapes (Garroway et al., 2008). This framework offers the possibility of simultaneously visualizing the genetic relationships among all studied populations instead of relying on the usual pairwise differentiation statistics and addressing a variety of questions concerning genetic differentiation, isolation by distance, population assignment, and historical genetic connectivity (Dyer and Nason, 2004). This methodology has been recently used to characterize genetic connectivity in different natural systems (Li et al., 2007; Giordano et al., 2007; Garroway et al., 2008; Rozenfeld et al., 2008; Fortuna et al., 2009).

In this paper, we test the hypothesis that seed dispersal maintains high cpDNA haplotype diversity within populations and seed-mediated connectivity among highly fragmented populations of *Quercus castanea* Née (Fagaceae) within a regional metapopulation in Mexico. Forests in Mexico are rapidly being cleared and fragmented for agriculture, cattle grazing, and urban areas (Arredondo-León et al., 2008). Statistics of current deforestation rates in Mexico show that approximately 167 000 ha of temperate forests are lost per year (Masera et al., 1997), equivalent to an annual deforestation rate of 0.64%. In particular, Michoacán state, where this study was undertaken, has a very

high deforestation rate of about 1.8% per year (Bocco et al., 2001). To determine the impact of recent anthropogenic fragmentation on cpDNA genetic variation and seed-mediated gene flow, we employed a design consisting of sampling in forest fragments both adult individuals, established before the large-scale fragmentation process, and seedling individuals, established after fragmentation. First, we document levels of haplotype diversity and compare them between adults and seedlings and analyze the correlation between genetic diversity and fragment size. Second, we use a general application of graph theory to analyze patterns of population connectivity among adult and seedling populations.

MATERIALS AND METHODS

Study site—This study was conducted in the catchment basin of Lake Cuitzeo, with an area of ~4000 km². It is located in the Trans-Mexican Volcanic Belt in the northern part of Michoacán state and the southern part of Guanajuato state (Fig. 1). The climate is temperate with seasonal (summer) rainfall. The average annual temperature is 17°C, and annual rainfall is about 800 mm (Carlón-Allende et al., 2009). The soils and landforms in most of the basin are derived from volcanic materials (lavas and pyroclasts) (López et al., 2006; Mendoza et al., 2006). The dominant land covers and land uses form a highly fragmented landscape of scrubland, forests (mainly pine, oak, and mixed forests), and agricultural land, combined in a mosaic pattern (López et al., 2006). The basin has a very long history of human occupation encompassing thousands of years with an impact on vegetation (Metcalf et al., 1989), but unprecedented habitat fragmentation has occurred during the last decades. Analyses of land cover and land-use changes indicated that the period 1986–1996 was characterized by high rates of deforestation and forest degradation in the basin (for more details, see Mendoza et al., 2011). As a result of these processes, the once large, continuous oak populations have been reduced to a large number of small patches of variable size due to strong human pressures like growth of urban areas, expansion of the agricultural frontier, and the removal of trees for charcoal production (López et al., 2006).

Study species—*Quercus castanea* Neé belongs to section *Lobatae* (the red oaks). It is a moderately large forest tree 10–18 m tall. It is wind-pollinated and monoecious. Adults produce flowers from March to June and bear mature acorns from October to December. *Quercus castanea* has a broad geographic and altitudinal distribution, with populations in the Sierra Madre Occidental, the Central Plateau, the Trans-Mexican Volcanic Belt, and the Sierra Madre del Sur. The species is found throughout the entire Cuitzeo basin, except in the northeastern portion. In the study region, populations can be found at 2000 to 2800 m a.s.l. The main seed dispersers in the Cuitzeo basin are acorn woodpeckers (*Melanerpes formicivorus*) and golden-fronted woodpeckers (*Melanerpes aurifrons*) (Schondube et al., 2010).

Sampling—On the basis of a digitized vegetation map of the Cuitzeo basin, we identified populations of *Q. castanea* situated at forest fragments of different sizes. In total, 33 fragments were chosen for sampling, ranging in area from 0.2 to 293.9 ha (mean ± SE, 44.4 ± 13.5 ha) (Table 1, Fig. 1). Within sites, 10 adult trees with diameters at breast height (DBH) equal or higher than 100 cm and separated by at least 20 m from each other were sampled from each population by taking a few young, intact leaves and immediately placing them on ice. Even though the relationship between DBH and age is not known for *Q. castanea*, a few stands in the Cuitzeo Basin that according to landowners were cut for the last time in the 1920s currently have *Q. castanea* individuals with maximum DBHs of about 80 cm (L. Herrera-Arroyo, personal observations). Therefore, this species probably grows as slowly as other red oaks (e.g., Loewenstein et al., 2000), and selected individuals may conservatively be 80–100 yr old and thus pre-date the recent large-scale fragmentation. Ten seedlings (<30 cm tall and also separated by at least 20 m) were sampled per population. In the laboratory, samples were stored at –80°C until processed. In total, 650 individuals were analyzed.

Laboratory analyses—Genomic DNA was isolated from 100 mg of frozen leaf tissue using the Lefort and Douglas (1999) protocol. Seven chloroplast microsatellite loci were screened for polymorphism using previously designed

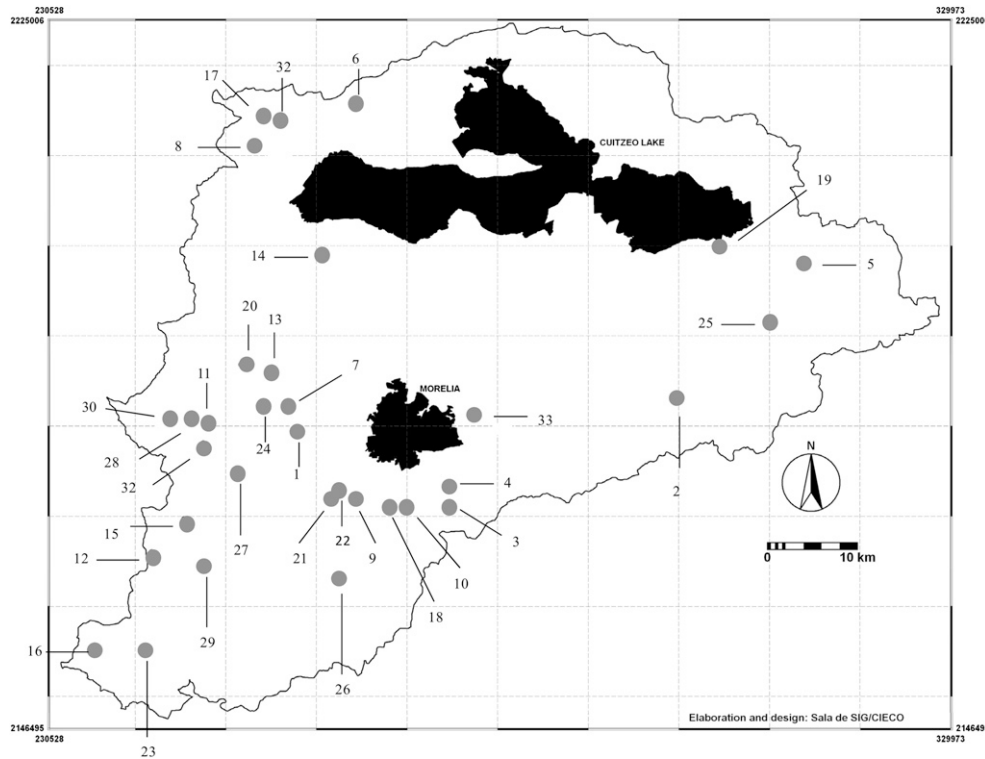


Fig. 1. Map of the geographical distribution of the forest fragments studied. The numbering of populations is as in Table 1.

primers: cmcs6, cmcs7 and cmcs12, developed from *Q. petraea* (Sebastiani et al., 2004), ccmp2 from *Nicotiana tabacum* (Weising and Gardner, 1999), and udt1, udt4 and ucd4, from *Q. robur* and *Q. petraea* (Deguilloux et al., 2003). The multiplex amplifications were performed using the Qiagen (Germantown, Maryland, USA) Multiplex Kit, in 5- μ l reactions as following: 1 \times multiplex PCR Master Mix, 2 μ mol/L of each primer and 5 ng of DNA. Forward primers were fluorescently labeled. The thermal cycling program included an initial denaturation step at 94°C for 4 min; then 25 cycles each at 94°C for 30 s, annealing at 45°C for 30 s, and extension at 72°C for 30 s; and a final extension at 72°C for 10 min. Multiplex products were diluted 1 : 1 in H₂O and run in an ABI PRISM 3100-Avant Sequencer with the GeneScan-500 Liz size standard included (Applied Biosystems, Foster City, California, USA). Fragment analysis and final sizing was performed using Peak Scanner version 1.0 (Applied Biosystems).

Data analyses—Genetic diversity—For each adult and seedling population, we estimated the number of haplotypes and the haplotype diversity (h_s) with the Arlequin 3.0 software (Excoffier et al., 2005). To evaluate whether the number of haplotypes and h_s values differ between adults and seedlings, a nonparametric Wilcoxon test was performed. The relationship between the number of haplotypes and h_s with fragment size was determined with Spearman correlation analyses separately for adults and seedlings. The JMP program (SAS Institute, 2005) was used for these tests.

Connectivity analysis—The connectivity among *Q. castanea* populations was analyzed separately for adults and seedlings using the POPGRAPH software (<http://dyerlab.bio.vcu.edu/software.html>; Rodney Dyer, Virginia Commonwealth University, Richmond, Virginia, USA). This analysis creates a network of population linkages and describes the amount of genetic variation within populations (Dyer and Nason, 2004). In the POPGRAPH framework, the set of nodes represents sampled populations, and the edges represent the multivariate measures of genetic covariance among populations. The difference in node size reflects differences in within-population genetic variability, while the edge length represents the among-population component of genetic variation due to the connecting nodes. Thus, this analysis examines specifically the connections among individual populations that maintain genetic connectivity among all populations (Dyer and Nason, 2004). Additionally, we compared pairwise physical distances among populations with the corresponding pairwise edge lengths with a χ^2 test (Garrick et al., 2009; Sork et al., 2010). Significantly longer spatial

distances than expected from genetic distances indicate long-distance dispersal, while significantly shorter spatial distances than expected suggest the existence of a barrier to dispersal (Garrick et al., 2009; Sork et al., 2010).

We also used POPGRAPH to test for isolation by distance among populations. In this case, instead of a traditional pairwise matrix of genetic differentiation regressed against a matrix of geographical distances among populations, we used a matrix of graph distances. The graph distance between two nodes i and j is the shortest path through the graph connecting them, l_{ij} , which corresponds to the among population component of genetic variance (Dyer and Nason, 2004). We applied a regression of log graph distances on log geographical distances using a Mantel test because of the lack of independence of data.

We further assessed connectivity among populations by calculating three other properties from the networks (Freeman, 1978; Costa et al., 2007): (1) betweenness centrality (i.e., the number of shortest paths from all vertices to all others that pass through a node, which measures the importance of each node to the whole network), (2) node degree (i.e., the number of connections that a node has to other nodes), and (3) closeness (i.e., the number of steps separating a given node from every other node). These analyses were conducted using IGRAPH (Csardi, 2009) for the R statistical software (R Development Core Team, 2009). To test for differences in population connectivity between the seedlings and the adults, we compared the three network properties between both networks using nonparametric Wilcoxon tests. The correlation of the three connectivity metrics with fragment size was determined with Spearman correlation analyses. These tests were conducted with the JMP program (SAS Institute, 2005).

RESULTS

Genetic diversity and structure—The cpDNA diversity of *Quercus castanea* populations in the study area was extremely high. With seven microsatellite loci, a total of 26 alleles were identified in the adult stage and 36 alleles in the seedling stage. These alleles combined into 70 cpDNA haplotypes, from which 53 were shared between the two life stages, while 10 were exclusively observed in the adults and seven in the seedlings. The number of haplotypes observed per population ranged between 1 and 6 (mean \pm SE, 3.6 ± 0.26) in the adults and between 1 and

TABLE 1. Geographical location, size of fragment, and haplotype diversity parameters within populations for adults and seedlings populations of *Quercus castanea*.

Population		Coordinates		Fragment size (ha)	Adults		Seedlings	
No.	Code	W	N		No. haplotypes	h_S (SD)	No. haplotypes	h_S (SD)
1	Con2	257522	2180023	0.2	3	0.600 (0.130)	2	0.355 (0.159)
2	Huer	299347	2182866	0.2	3	0.667 (0.104)	2	0.600 (0.175)
3	JMBO	274205	2172831	0.3	6	0.844 (0.102)	5	0.800 (0.100)
4	JMP	274270	2173596	0.5	4	0.750 (0.139)	2	0.571 (0.119)
5	Zapo	310273	2191569	1.2	3	0.607 (0.164)	4	0.714 (0.180)
6	Tupa	263912	2216049	1.4	2	0.200 (0.154)	4	0.733 (0.119)
7	Taci	251488	2174946	1.5	2	0.355 (0.159)	3	0.555 (0.165)
8	Snic	253482	2211343	1.7	3	0.644 (0.101)	1	0.000 (0.000)
9	Cepa	262484	2173122	1.9	3	0.377 (0.181)	2	0.533 (0.094)
10	Zimp	269279	2171398	2.8	3	0.600 (0.130)	2	0.200 (0.154)
11	Cter	246817	2180842	2.9	6	0.844 (0.102)	6	0.888 (0.075)
12	Font	241795	2166073	3.0	4	0.644 (0.151)	4	0.800 (0.075)
13	Sajo	245963	2180856	3.3	4	0.694 (0.147)	4	0.733 (0.100)
14	Cruc	260320	2199021	3.8	7	0.944 (0.070)	5	0.709 (0.136)
15	Cor2	245388	2169707	4.1	6	0.844 (0.102)	8	0.933 (0.077)
16	Acui	235525	2155684	4.9	1	0.000 (0.000)	1	0.000 (0.000)
17	Sisi	253801	2214497	5.2	3	0.688 (0.103)	4	0.745 (0.097)
18	Zim2	269766	2171013	9.4	2	0.535 (0.123)	4	0.800 (0.088)
19	Coro	304454	2200628	13.8	5	0.818 (0.082)	3	0.667 (0.104)
20	Tiri	251676	2186862	17.8	3	0.533 (0.094)	4	0.644 (0.158)
21	Autp	261538	2171928	24.7	5	0.861 (0.087)	3	0.688 (0.103)
22	Pres	263963	2171983	24.8	1	0.000 (0.000)	2	0.200 (0.154)
23	Cuan	241026	2155451	26.9	2	0.533 (0.094)	3	0.511 (0.164)
24	Cuto	255580	2185831	41.1	6	0.888 (0.091)	6	0.892 (0.111)
25	SJR	314049	2197966	43.7	3	0.733 (0.076)	5	0.709 (0.136)
26	Umec	262840	2163110	56.7	2	0.200 (0.154)	3	0.644 (0.101)
27	Conc	256853	2181972	70.7	3	0.533 (0.094)	3	0.777 (0.090)
28	Rtir	254368	2182345	70.7	4	0.844 (0.079)	6	0.854 (0.080)
29	Corr	247545	2164696	117.9	4	0.644 (0.151)	4	0.822 (0.096)
30	Icua	243253	2181621	123.5	5	0.667 (0.163)	3	0.377 (0.183)
31	Sis2	254061	2214248	227.8	4	0.763 (0.099)	7	0.944 (0.070)
32	Capu	247491	2178122	266.5	4	0.777 (0.900)	6	0.911 (0.062)
33	Punh	277189	2181422	293.9	2	0.500 (0.262)	—	—
				Mean	3.576	0.610	3.844	0.635

8 (mean 3.8 ± 0.30) in the seedlings (Table 1). The mean (SE) h_S value for the adult populations was 0.610 (0.04) and 0.635 (0.04) for the seedling populations. The values of haplotype number and h_S did not differ significantly between adults and seedlings according to the Wilcoxon tests ($P > 0.05$).

In the adult populations, the number of haplotypes and h_S values were not correlated with the area of the forest fragments (Fig. 2A, 2C). However, in the seedling populations the correlation between haplotype number and fragment size was positive and significant (Fig. 2B) and marginally significant for h_S and fragment size (Fig. 2D). Furthermore, a positive correlation of the difference in haplotype number between the two life stages (i.e., seedlings – adults) in each fragment, with fragment size (Fig. 3) indicates that on the smaller fragments seedlings have on average less haplotypes than the adults, but in the larger fragments seedlings have on average more haplotypes than the adults.

Connectivity analysis—The POPGRAPHS networks representing the minimal topologies for 33 adult populations and 32 seedling populations are presented in Fig. 4A and 4B, respectively. In total, the adult network had 50 edges, while the seedling network had 38 edges. In the adult network, 10 edges (20%) were significantly longer than expected from genetic distances, and 10 (20%) indicated barriers to dispersal. In the seedling network, four edges (10%) indicated long-distance dispersal, and two edges (5%) indicated barriers (Fig. 5A and 5B). The tests

for isolation by distance performed with POPGRAPHS were nonsignificant for both adults and seedlings. The adult populations with the highest node degree, betweenness and closeness were Sis2[31] and Coro[19], indicating important gene flow across these populations (Table 2; Fig. 4A). In the seedling network, populations Font[12] and Cepa[9] had the highest network connectivity values; although these were lower in comparison with the values in the adult populations. Furthermore, a group of three seedling populations (Zimp[10], Cuan[23], and Acui[16]) appeared disconnected from the rest of the network (Table 2; Fig. 4B). In general, the degree of connectivity among populations was significantly lower in the seedling network than in the adult network, according to the likelihood ratio and Wilcoxon tests for the comparison of closeness and node degree ($\chi^2 = 48$, $df = 1$, $P < 0.0001$ and $\chi^2 = 4.6$, $df = 1$, $P = 0.03$, respectively) but not for betweenness centrality ($\chi^2 = 0.32$, $df = 1$, $P = 0.57$). The three connectivity metrics were not correlated with fragment size in the adults and the seedling networks.

DISCUSSION

Our study shows evidence that fragmentation has affected seed movement of the current generation in comparison to the previous generation. Because *Quercus castanea* populations in the Cuitzeo basin have very high levels of haplotypic diversity

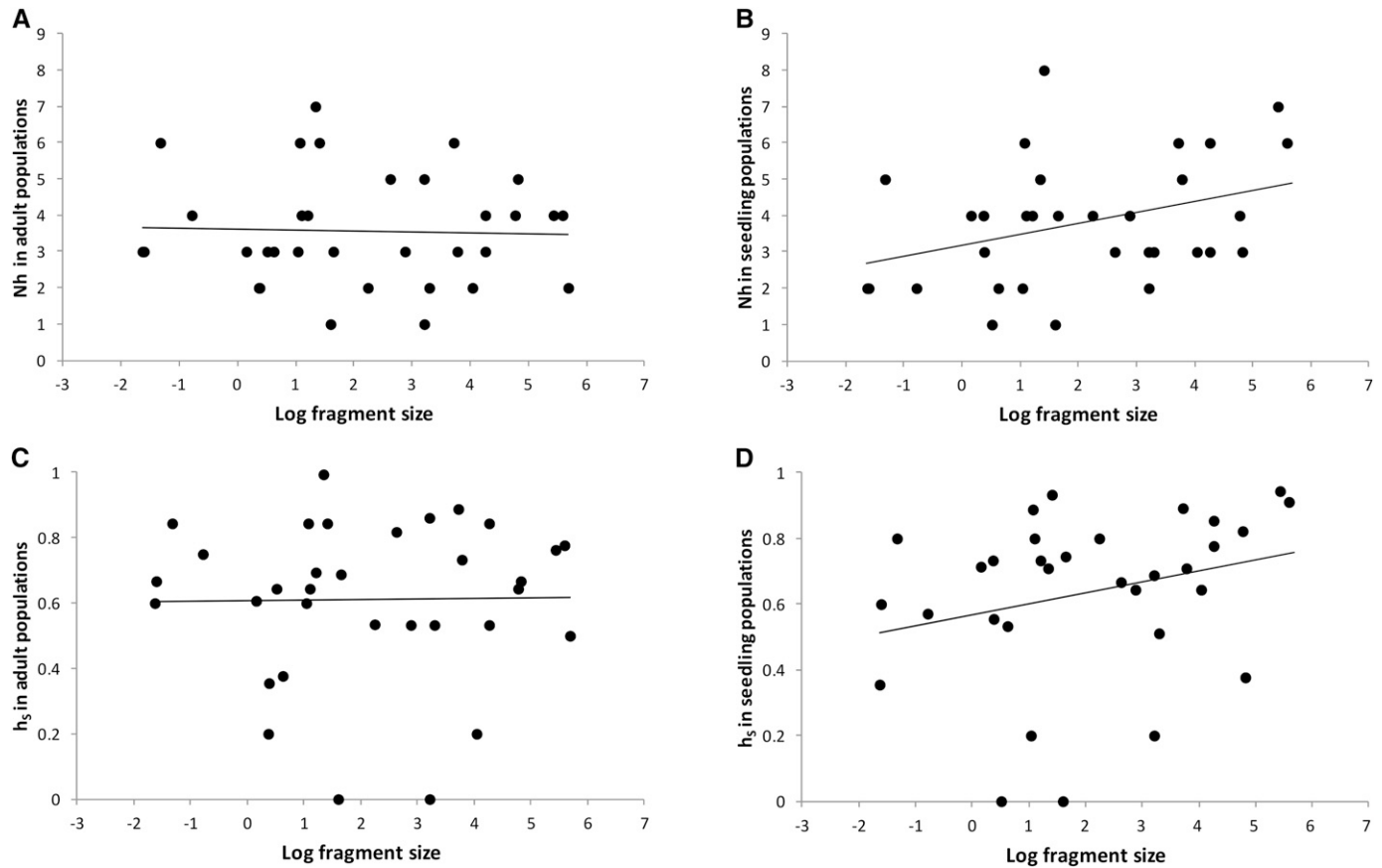


Fig. 2. Relationships between fragment size (hectares, log) and (A) number of haplotypes (N_h) in adult populations (Spearman's $\rho = 0.02$, $P = 0.91$), (B) number of haplotypes (N_h) in seedling populations (Spearman's $\rho = 0.36$, $P = 0.04$), (C) haplotype diversity (h_s) in adult populations (Spearman's $\rho = 0.05$, $P = 0.78$), and (D) haplotype diversity (h_s) in seedling populations (Spearman's $\rho = 0.33$, $P = 0.06$).

at maternally inherited cpSSR loci (70 haplotypes in total) in both the adult and seedling stages, we were able to use these markers to analyze recent seed movement. Such high levels of diversity were also found in a separate study of *Q. castanea* across the complete distribution area of the species (J. M. Peñaloza-Ramírez et al., Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México, unpublished data). These levels of genetic diversity are much higher than those reported in investigations on oak species in previously glaciated areas of North America and Europe (Deguilloux et al., 2004; Magri et al., 2007; Marsico and Hellmann, 2009) and moderately higher than found in oak populations of California, which was not glaciated during the Pleistocene (Grivet et al., 2006). Thus, the very high haplotype diversity values found in *Q. castanea* even at the regional scale of the Cuitzeo Basin (~4000 km²), made it possible to evaluate the effects of recent anthropogenic forest fragmentation on seed-mediated gene flow, through detailed comparisons of genetic diversity and connectivity between adult and seedling samples, as well as evaluating correlations of genetic diversity and connectivity metrics with the size of the fragments.

Effects of fragmentation on genetic diversity—We did not find an overall difference in the number of haplotypes and haplotype diversity (h_s) between the adult and seedling populations. However, these two diversity values had a positive correlation with fragment size in the case of the seedling populations, but

not in the case of the adult populations. Furthermore, the correlation of the difference in the number of haplotypes (i.e., the number of haplotypes in the seedlings minus the number of haplotypes in the adults) in each fragment with fragment size, suggests that the smaller fragments actually tend to have less haplotypes in the seedlings than in the adults, while the reverse is true for the larger fragments. Therefore, the reduction

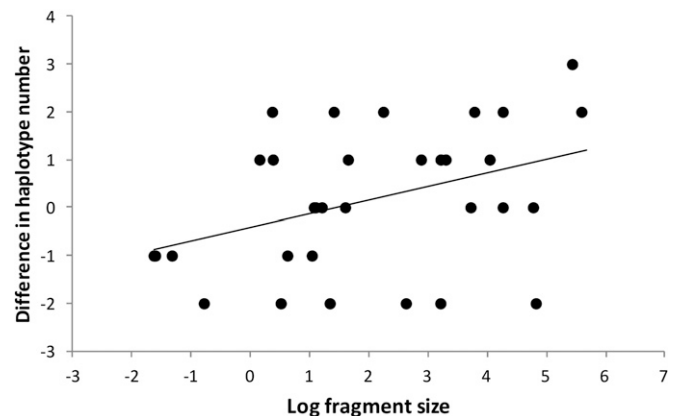


Fig. 3. Relationship between fragment size (hectares, log) and difference in haplotype number (seedlings – adults) (Spearman's $\rho = 0.37$, $P = 0.04$).

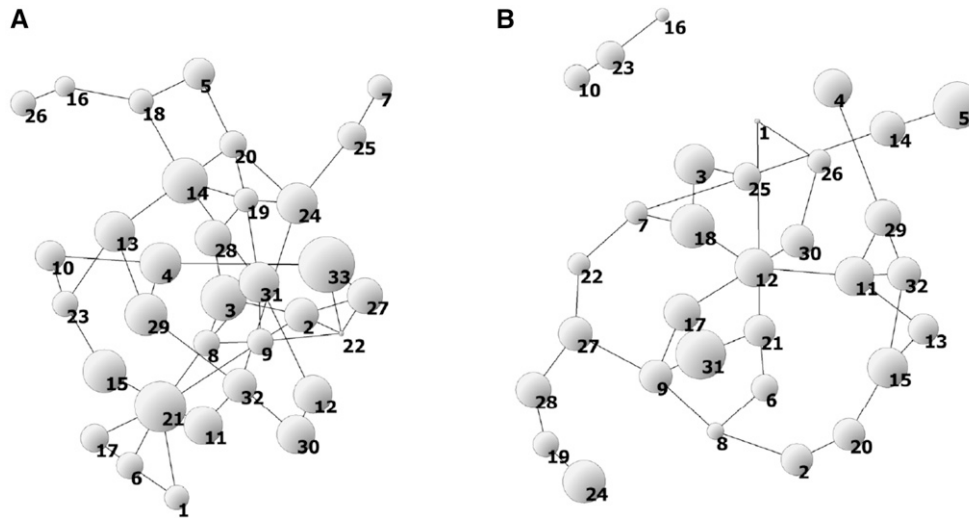


Fig. 4. Network topologies of connectivity relationships among (A) adult populations (before fragmentation) and (B) seedlings populations (after fragmentation) of *Quercus castanea*. Differences in node size and edge length reflect the differences in within and between population variance. Numbering of populations is as in Table 1.

in haplotype number for the seedling populations in the smaller fragments is compensated by an increase in the larger fragments, explaining the absence of an overall difference between adults and seedlings.

These results are what would be predicted under a scenario in which parental trees were established when larger and more continuous forests existed, and individuals that established after fragmentation are the offspring being produced in populations with smaller effective population size and experiencing restricted levels of seed flow, particularly in the smaller fragments. Studies on several tree species in fragmented landscapes have also reported a reduction in genetic diversity levels in recently established individuals in comparison to older individuals, and/or a positive correlation of diversity levels with fragment size (Fernández-M. and Sork, 2005; Jump and Peñuelas, 2006; Aguilar et al., 2008; Ortego et al., 2010; Zhang et al., 2012). However,

most of these studies have been based on nuclear markers and in very few cases cpDNA markers have been used (Petit et al., 2002; Zhang et al., 2012).

Recent reviews have pointed out that the key factors determining the consequences of forest fragmentation on the genetic composition of plant populations are the patterns of genetic variability of the original populations previous to fragmentation, the effective population size within fragments, and the number of generations elapsed in fragmentation conditions (Nason and Hamrick, 1997; Hamrick, 2004; Aguilar et al., 2008). Plant species most likely to be affected by habitat fragmentation are those that are abundant and distributed continuously across a broad geographic range, rather than those that have a natural patchy distribution (Holsinger, 1993). *Quercus castanea* is a species that probably has maintained large and continuous populations during its evolution, associated with a considerable amount of

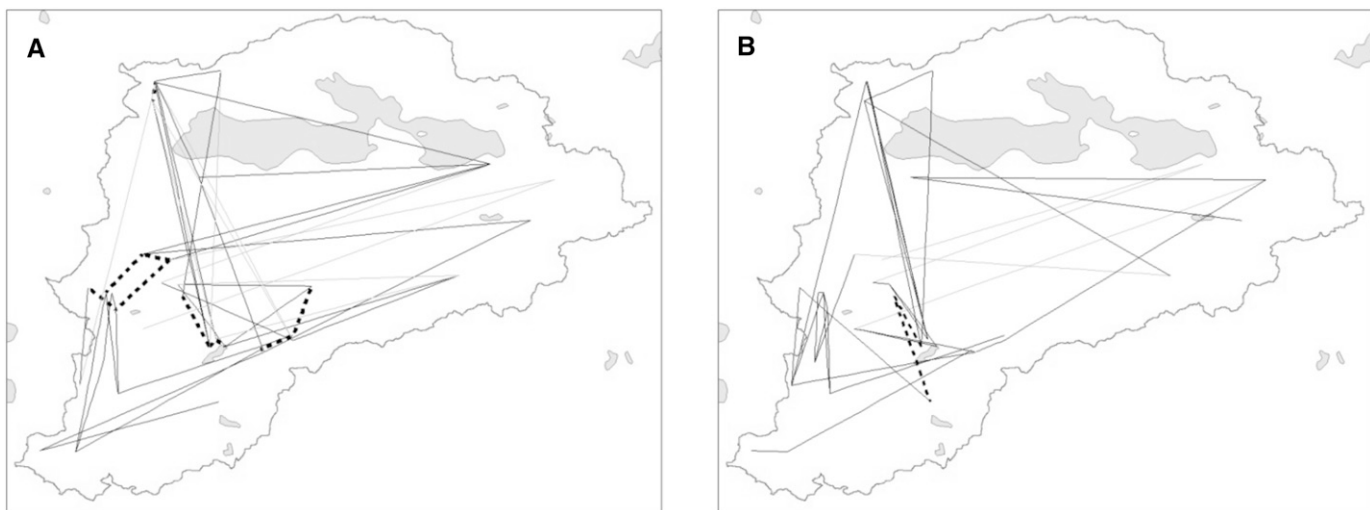


Fig. 5. Map of networks showing sites that are spatially more distant than expected from their genetic differences, indicating long-distance dispersal (thin black lines), and sites spatially closer than expected from genetic distances, indicating barriers (dashed lines) for (A) adults and (B) seedling populations of *Quercus castanea*.

TABLE 2. Betweenness, closeness, and node degree parameters obtained for the network topology of adults and seedlings of *Quercus castanea* populations (see results for details).

Population	Adults			Seedlings			
	No.	Code	Betweenness	Closeness	Node degree	Betweenness	Closeness
1	Con2	0.000	0.218	2	9.833	0.142	2
2	Huer	20.811	0.283	4	27.933	0.148	2
3	JMBO	25.832	0.314	4	21.200	0.149	2
4	JMP	54.337	0.281	3	0.000	0.130	1
5	Zapo	28.500	0.274	2	0.000	0.116	1
6	Tupa	0.500	0.219	3	9.667	0.145	2
7	Taci	0.000	0.206	1	69.467	0.160	3
8	Snic	43.631	0.320	4	56.100	0.159	3
9	Cepa	105.640	0.337	5	111.767	0.171	4
10	Zimp	12.655	0.244	2	0.000	0.033	1
11	Cter	14.052	0.244	2	100.567	0.163	4
12	Font	27.140	0.305	2	167.567	0.176	4
13	Sajo	55.070	0.302	3	11.367	0.148	2
14	Cruc	61.108	0.333	3	27.000	0.129	2
15	Cor2	3.833	0.219	2	23.900	0.142	3
16	Acui	31.000	0.224	2	0.000	0.033	1
17	Sisi	0.000	0.218	2	77.500	0.172	3
18	Zim2	66.608	0.281	3	81.733	0.166	3
19	Coro	121.498	0.372	5	27.000	0.130	2
20	Tiri	86.904	0.344	4	20.667	0.141	2
21	Autp	87.500	0.274	5	24.333	0.150	3
22	Pres	47.000	0.302	4	43.933	0.157	2
23	Cuan	29.781	0.254	3	1.000	0.033	2
24	Cuto	95.436	0.337	4	0.000	0.117	1
25	SJR	31.000	0.258	2	55.000	0.144	3
26	Umec	0.000	0.184	1	15.667	0.144	2
27	Conc	5.879	0.256	3	104.267	0.163	3
28	Rtir	8.713	0.308	2	52.000	0.146	2
29	Corr	41.146	0.291	3	27.000	0.147	3
30	Icua	19.890	0.288	2	30.833	0.157	2
31	Sis2	168.617	0.372	6	31.667	0.162	3
32	Capu	72.248	0.305	4	19.033	0.150	3
33	Punh	47.670	0.281	3	—	—	—
	Mean	42.85	0.28	3.03	39.00	0.14	2.38

haplotype diversity (J. M. Peñaloza-Ramírez et al., Centro de Investigaciones en Ecosistemas, Universidad Nacional Autónoma de México, unpublished data), but the effects of forest fragmentation that has occurred within the last decades are already detectable on haplotype variation of seedlings established in the smaller fragments. Since cpDNA is more sensitive to effects of drift due to its smaller effective population size, it would be interesting to compare these results with diversity values at nuclear markers, to further assess the utility of analyzing cpDNA variation in this type of study.

Effects on population connectivity—The pattern of population connectivity we observed indicates a significant impact of fragmentation. We found a reduction in the overall connectivity among seedling populations in comparison to the adult populations. Overall, we detected a significantly lower number of connections (edges) among populations in the network of seedlings (38) than in the network of adults (50). Since in the POPGRAPH framework the populations connected by edges are those exchanging migrants and therefore exhibiting significant conditional covariance (Dyer and Nason, 2004; Dyer et al., 2010), this result indeed suggests a reduction in seed exchange among populations in the present generation in comparison to the previous generation. Additionally, in the seedling network, most edges

(84%) were not significantly longer or shorter than expected from spatial distances between populations, in contrast with the adult network, where a higher proportion of long-distance gene exchanges and barriers to dispersal were observed. Moreover, closeness and node degree were significantly higher in the adult network (Table 2). However, connectivity metrics were not correlated to fragment size, suggesting that factors like topographic features, degree of isolation, and the movement routes of seed dispersers probably play a role in the pattern of connectivity.

These results suggest that the whole metapopulation system may become more fragile and vulnerable as a result of the reorganization of the overall gene flow pattern as a consequence of fragmentation. From the adult populations to the seedling populations, the network loses connections and gets simpler, and most of the gene flow concentrates along fewer paths. This seed flow disruption may have serious consequences because of the impact on the recolonization of new sites, which will increase the extinction risk of the metapopulation on the long term, even if it is true that pollen flow could provide more extensive gene exchange in this fragmented landscape.

Our interpretation that connectivity is reduced in seedling vs. adult populations relies on certain assumptions. First, from one generation to the next, the effects of mutation and homoplasia should be negligible, which is plausible in the time frame of our comparison. Second, the frequency of haplotypes should not differ between age classes as a result of selective factors, that is, haplotypes should be selectively neutral. Both of these assumptions are strongly supported in the literature. The mutation rate of cpDNA microsatellites has not been established with certainty, but is usually estimated to be in the range of 10^{-5} to 10^{-4} mutations per generation per locus (Navascués et al., 2009; Heuertz et al., 2010). With seven loci, the mutation rate per haplotype would be between 7×10^{-4} and 7×10^{-3} per generation, which is still sufficiently low to be negligible. Even though there is ample evidence that cpDNA is broadly under strong purifying selection (e.g., Muir and Filatov, 2007), it is not likely that size variants at the studied microsatellite loci are affected by selection at the geographic and time scales involved in this study (Wright et al., 2008).

Conclusions and implications for conservation—From the conservation standpoint, an important advantage of the network analysis over more traditional approaches is that it can contribute to define the conservation priority of populations, not only depending on genetic diversity levels, but also in terms of their importance as pathways to gene flow across a landscape. For example, in the present study, we identified two populations in the network of adults showing particularly high betweenness, closeness, and node degree (Coro[19] and Sis2[31]), even though these populations did not have the highest number of haplotypes or haplotype diversity values (Tables 1 and 2). Apparently, anthropogenic disturbance has affected gene flow patterns across these populations as evidenced by the much lower connectivity values obtained for the same populations in the seedlings network. Currently, the two more important populations in terms of connectivity seem to be Font[12] and Cepa[9] (Table 2, Fig. 4B). It is interesting to notice that while connectivity values decreased in the majority of the seedling populations in comparison to the adults, these values increased in several populations (for example, population Font[12]). This finding suggests that emphasis should be given to the conservation of fragments with higher probability of retaining connectivity.

A combined approach to conservation planning that considers both population genetic diversity levels and network connectivity

is recommended. Our results illustrate that even if the effects of habitat fragmentation on levels of diversity within populations may affect the smaller fragments initially, the disruption of connectivity among populations suggests that eventually local populations in larger fragments could also be at risk for losing genetic diversity.

In conclusion, this study demonstrates that haplotype diversity and seed dispersal among sites is reduced in fragmented landscapes. The unique characteristic of high chloroplast haplotype diversity in Mexican oak populations has allowed us to isolate the process of seed dispersal to test the impact of forest fragmentation at a regional scale that is not possible in many study systems. We did not assess the extent to which pollen flow would mitigate the loss of nuclear genetic diversity caused by the disruption to seed dispersal, but we assume that wind pollination in oaks should be effective for maintaining genetic connectivity unless sites are extremely isolated. However, lack of seed movement will have demographic consequences in the long run if recolonization of deforested sites is needed in sites that could be restored.

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CAPÍTULO III

GENE FLOW INTERRUPTION IN A RECENTLY HUMAN-MODIFIED
LANDSCAPE: THE VALUE OF ISOLATED TREES FOR THE MAINTENANCE
OF GENETIC DIVERSITY IN *Quercus castanea* NÉE (FAGACEAE).

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Gene flow interruption in a recently human-modified landscape: the value of isolated trees for the maintenance of genetic diversity in *Quercus castanea* Nee (Fagaceae)

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ABSTRACT

Gene flow within and among populations is an important factor in maintaining genetic diversity and preventing population differentiation among them. Nowadays, human land use has led to a large forest conversion creating many fragmented landscapes where remnant trees play an important role in conserving biodiversity. In this study, we analyzed the effects of a recent anthropogenic forest fragmentation on genetic diversity and genetic heterogeneity of pollen pool accepted in *Quercus castanea* trees from fragmented populations and isolated trees in central Mexico. Pollen movement was also evaluated by the analysis of outcrossing rates using seven nuclear microsatellites. We assumed that adult trees are remnants of the populations that existed previous to forest fragmentation and seedlings are the offspring of recent reproductive events occurring after fragmentation. We found high genetic diversity in both adult trees and seedlings in fragmented populations and isolated trees. Adult trees from fragmented populations showed low genetic differentiation ($F_{ST} = 0.018$) although offspring of isolated trees showed a disruption in gene flow ($F_{ST} = 0.247$). Genetic heterogeneity of pollen pool ($\Phi_{FT} = 0.351$) and heterozygous excess ($F_{IS} = -0.120$) indicated that the pollen received by the isolated trees came from no inbreeding sources which gives them a great value on maintaining genetic diversity. Our results provide evidence that gene flow is a sensitive parameter to short-term changes in landscape configuration.

Keywords:

Quercus castanea

Gene flow

Forest fragmentation

Isolated trees

Genetic diversity

Outcrossing rates

1. Introduction

Gene movement within and among populations have an important influence on the patterns of genetic differentiation and on the evolution of local adaptations in plant species (Ennos, 1985; Streiff et al., 1999; Rieseberg and Burke, 2001; Lenormand, 2002). Pollen movement is the most important vector of gene flow in plant species that produce large and relatively immobile seeds (Dow and Ashley, 1998a), and wind pollinated species usually have the greatest potential for pollen movement over long distances (Ennos, 1985). In particular, wind pollinated trees typically show low levels of genetic differentiation among populations, high genetic variation within populations, and high outcrossing rates, which are features associated with high levels of gene flow (Hamrick and Godt, 1996; Dow and Ashley, 1998a). Pollen flow is, therefore, a very important factor in the maintenance of genetic diversity within populations and connectivity among populations in forest trees (Burczyk and Chybicki, 2004; Hamrick, 2004)

However, studies of real-time pollen movement in forest trees have shown that pollen densities decline rapidly from the source and that the majority of the fertilizations of maternal trees are effected by nearby pollen donors (Bacles *et al.*, 2006; Bacles and Ennos 2008), even though some proportion of long distance dispersal also occurs (Bacles and Ennos 2008; Kamm *et al.*, 2009). If a large proportion of pollen movement occurs at a local scale, populations could be genetically subdivided creating local genetic structure (Smouse et al., 2001; Smouse and Sork, 2004). In oaks, recent evidence suggested that most gene flow occurs within moderate distances (Dow and Ashley, 1998b; Streiff et al., 1999) with mating taking place between neighbor trees, including relatives (Smouse et al., 2001; Sork et al., 2002; Valbuena-Carabaña et al., 2005).

Human land use for agricultural and urban development has led to large transformations of natural ecosystems (Ottewell et al., 2009). Considerable extensions of forests around the world have been transformed to agricultural and grassland areas containing a few scattered remnant trees of the original pre-clearance plant communities (Gibbons and Boak, 2002; Manning et al., 2006). This process leads to an increase in geographic isolation and a reduction in connectivity among forest patches, therefore influencing the amount of gene flow between remnant populations (Fahrig, 1997). The increase in genetic isolation and the decline in size may threaten the viability of these populations (Dutech et al., 2005). Therefore, changes associated with habitat degradation could result in an erosion of genetic diversity and in an increase in population genetic divergence (Young et al., 1996). So, the understanding of gene flow at local scales and how landscape changes alter gene movement are very important issues for conservation biology (Fahrig, 1997; Sork et al., 1998; Sork et al., 1999; Smouse et al., 2001).

Some of the consequences of habitat fragmentation and degradation are due to strong impacts on the mating patterns of plants at both population and individual levels. Remnant long-lived trees are usually surrounded by an unfavorable matrix of modified lands, are isolated from other trees, and contribute little to forest regeneration due to reduced pollination and the disruption of natural seed dispersal (Gibbons et al., 2008). Nevertheless, several studies have recorded pollen flow into pasture trees separated by hundreds of meters, indicating that spatially isolated trees may not necessarily be reproductively isolated (Aldrich and Hamrick, 1998; Cascante et al., 2002; Fuchs et al., 2003). However, if recruitment is virtually absent, these isolated individuals will disappear rapidly in agricultural landscapes and may be lost in 90-180 years (Gibbons et al., 2008; Fischer et al., 2010).

Currently, one of the most common features in Mexican forest landscapes is forest fragmentation. Temperate forests, particularly oak forests, confront serious problems of deforestation and fragmentation, with approximately 167 000 ha of temperate forests being lost every year (Masera et al., 1997). These processes are exemplified in the catchment basin of Lake Cuitzeo located in Michoacán state in central México, where an intense land-use change over the last decades has occurred. Large continuous oak forests have been reduced to a large number of small patches of variable size due to strong human pressures like urban growth, expansion of the agricultural frontier, and removal of trees for charcoal production (Bocco et al., 2001; López et al., 2006).

In this study, we were interested in knowing how recent forest fragmentation has altered gene movement in *Quercus castanea*, a long-lived oak species in forest fragments of different sizes as well as isolated individuals in agricultural and pasture lands within the Cuitzeo basin. We analyzed adult trees under the assumption that these represent the surviving genotypes that were established before the fragmentation process, and their respective progenies, which have resulted from recent reproductive events occurring after the fragmentation. We chose *Quercus castanea* as a model system due to its abundance and wide spatial distribution in the study site. The objectives of this work were (i) to assess the genetic diversity and structure of adults and offspring of *Q. castanea* in fragments with two different sizes (< 8 ha and > 12 ha) and for isolated individuals, (ii) to estimate the genetic heterogeneity of pollen pools fertilizing mother trees in three different conditions (trees in isolation, in small fragments and in large fragments), and (iii) to estimate the outcrossing rates of trees under different fragmentation conditions.

2. Materials and methods

2.1. Study system and collecting methods

This study was conducted in the Lake Cuitzeo basin, approximately 4000 km² in area. It is located in central Mexico between the north of Michoacán state and the south of Guanajuato state (Fig. 1). The climate is temperate with seasonal (summer) rainfall. Soils and landforms in most of the basin are derived from volcanic materials (lavas and pyroclasts). The land cover includes temperate forests, scrublands and agricultural lands. A large number of urban and rural settlements from twenty-eight municipalities are included in the study area (López et al., 2006; Mendoza et al., 2006). We have identified 16 oak species in this region, with *Quercus castanea* being the most common species.

Q. castanea (section *Lobatae*) is a moderately large tree reaching up to 18 m in height. This species is widely distributed from northern Mexico to Guatemala and El Salvador, usually found at elevations from 1180 to 2600 m (Bello and Labat, 1987; Valencia, 2004). Flowering occurs in the dry season from March to June and fruiting from October to December. *Q. castanea* is an obligate outcrosser pollinated by wind. Acorns are dispersed by both gravity and bird species such as acorn woodpeckers (*Melanerpes formicivorus*) and golden-fronted woodpeckers (*Melanerpes aurifrons*) (Schondube et al., 2010).

Foliar tissue of 16 mature trees (i. e. > 80 cm in diameter at breast height), separated at least 100 m from each other, were collected in each of four populations, two in fragments of small size (< 8 ha) and two in large fragments (> 12 ha) (Table 1). At these four sites the density of *Q. castanea* trees was estimated with a 100 x 20 m transect. Also, leaves from 16 isolated trees (i.e. with at least 500 m of separation from

the closest conspecific tree) distributed throughout the Cuitzeo basin were also collected. From each of the eighty trees approximately 100 acorns were collected. Acorns were germinated and the first true leaves from ten seedlings per mother tree were harvested and stored at -80°C until DNA extraction.

2.2. Microsatellite markers and genotyping

Genomic DNA was isolated from 100 mg of frozen leaf tissue using the protocol of Lefort and Douglas (1999). Seven nuclear microsatellite markers designed for *Q. rubra* (Aldrich et al., 2002) were chosen on the basis of reproducibility and polymorphism assessed in preliminary trials: *quruGA-2F05*, *quru-GA-0I01*, *quru-GA-1F07*, *quru-GA-0A01*, *quru-GA-1F02*, *quru-GA-0M07*, and *quru-GA-0C19*.

Multiplex amplifications were performed using the QIAGEN Multiplex Kit, (QIAGEN) in 5 µl reactions as follows: 1x multiplex PCR master mix, 2 µM of each primer and 5 ng of DNA. Forward primers were fluorescently labeled. The thermal cycling program included an initial denaturalization step at 94°C for 4 min, then 25 cycles each at 94°C for 30 s, annealing at 45°C for 30 s, and extension at 72°C for 30 s. A final extension step at 72°C for 10 min was used. Multiplex products were diluted 1:1 in H₂O and run in an ABI PRISM 3100-Avant Sequencer (Applied Biosystems). Fragment analysis and final sizing was performed using Peak Scanner version 1.0 (Applied Biosystems).

2.3. Statistical data analysis

2.3.1. Genetic diversity and structure analysis

Genetic diversity at the seven nuclear microsatellite loci of adult trees and their offspring was quantified in terms of allelic richness (A), private allelic richness (A_p),

observed heterozygosity (H_o), expected heterozygosity (H_E), and fixation index (F) using the GenAlex software (Peakall and Smouse, 2006). The allelic richness of a sample is affected by sample size with large samples having more alleles than small ones (Kalinowski, 2004, 2005). We applied the rarefaction method to estimate allelic richness and private allelic richness to compensate for differences in sample sizes between fragmented populations and isolated trees, using the HP Rare software (Kalinowski, 2005).

The structure of genetic variation was assessed by an analysis of molecular variance (AMOVA) through the evaluation of the statistics F_{ST} , F_{IT} and F_{IS} , in adults and seedlings from fragmented populations and isolated trees separately, using the software ARLEQUIN 3.0 (Excoffier et al., 2005)

2.3.2. *TwoGener analysis*

The analysis of genetic structure of pollen pools was conducted using the TwoGener approach (Smouse et al., 2001). This method allows estimating the heterogeneity of the pollen cloud of a particular mother tree compared to the global pollen pool of the population. The TwoGener method is based on the analysis of the genetic distances among male gametes. This analysis produces the statistic Φ_{FT} , which is the fraction of the total genetic variation of male gametes that is attributable to maternal differences in their sampled pollen pools. Φ statistics are analogous to Wright's (1969) F_{ST} statistic but with females replacing populations and male gametes replacing diploid individuals. The statistic Φ_{FT} determines if different mother trees have been pollinated by different father trees, taking values from 0 to 1; values close to 0 indicate highly outcrossed populations (i.e., mother trees receive pollen from many trees), and values close to 1 denote highly selfing or inbreeding populations (i.e., fewer pollen donors). The

significance of the Φ_{FT} , was tested by 1000 randomizations. TwoGener analysis was performed using the GeneAlex 6 software (Peakall and Smouse, 2006).

Based on pollen pool structure (Φ_{FT}), we calculated the effective number of pollen donors contributing to the average female using the relationship $N_{ep} = 1 / (2 \Phi_{FT})$ (Smouse et al., 2001), and the average distance of effective pollen dispersal following $\delta = \sigma \sqrt{(\pi / 2)}$, where σ is the adult stem density per hectare. The effective pollination neighbourhood was estimated using $A_{ep} = 4\pi\sigma^2$ (Austerlitz and Smouse, 2001). These analyses were carried out with the GenAlex software (Peakall and Smouse, 2006).

2.3.3 Mating system

The multilocus outcrossing rate (t_m), single-locus outcrossing rate (t_s), outcrossing rates among relatives ($t_m - t_s$), multilocus paternity correlation (r_p), and the single-locus inbreeding coefficient of maternal parents (F) were estimated for each population in fragments and isolated trees. We used the maximum likelihood procedures of Ritland and Jain (1981) as implemented in the multilocus mating system program MLTR (Ritland, 2002). Standard errors for t_m , t_s , and r_p were estimated from 1000 bootstraps replicates with resampling among families. The multilocus paternity correlation was used to calculate effective number of pollen donors (N_{ep}).

3. Results

3.1. Genetic diversity and structure of adult and offspring populations

All microsatellite loci were highly polymorphic in the four populations analyzed and in isolated trees. Levels of genetic diversity measured by allelic richness, private allelic richness, and expected and observed heterozygosity were similar in adults and offspring in small and large fragments and in isolated trees (Table 2). However, the

fixation index (F) showed a significant excess of homozygotes in the offspring of mother trees from fragmented populations, whereas the offspring from isolated trees showed a significant excess of heterozygotes (Table 2).

The analysis of genetic structure among the four populations situated in forest fragments showed no significant genetic differentiation among populations for adult trees ($F_{ST} = 0.018$), but the inbreeding parameter reached a highly significant value ($F_{IS} = 0.110$). Offspring from these trees showed a low but significant population genetic differentiation ($F_{ST} = 0.038$), and the inbreeding parameter was also significant ($F_{IS} = 0.104$) (Table 3). The inbreeding parameter for adults of isolated trees showed a slight but non significant excess of heterozygosity ($F_{IS} = -0.019$). Offspring from these trees showed a strong and highly significant genetic structuring ($F_{ST} = 0.247$) and the inbreeding parameters reached a value of $F_{IS} = -0.120$, which was not significant (Table 3).

3.2. Genetic heterogeneity among pollen pools

The estimated values of Φ_{FT} were between 0.212 and 0.351; with isolated trees having the highest values (Table 4). The effective number of pollen donors (N_{ep}) was very similar in the four populations from forest fragments (between 2.142 and 2.358), but was considerably lower in isolated trees ($N_{ep} = 1.424$). The average distances of pollination were very short in the four populations (δ from 6.67 to 10.78 m), and the effective neighborhood pollination area (A_{ep}) had values from 300 to 899.1 m² (Table 4).

3.4 Mating system

The four populations and isolated trees were highly outcrossed with values of t_m not significantly different from 1 (from 0.993 to 1) (Table 5). The estimates of biparental inbreeding were significantly greater than zero for the four populations and the isolated trees (t_m-t_s : 0.088 to 0.272) (Table 5). The inbreeding coefficient for mother trees was significantly positive for three populations and for the isolated trees and not significantly different from zero in one population (F : -0.01 to 0.123) (Table 5). Multilocus paternity correlation coefficients were low (r_p : 0.102-0.278) but significantly different from zero (Table 5), which indicates that most seeds within families had different fathers, even though the effective number of pollen donors (N_{ep}) was lower for isolated trees.

4. Discussion

4.1. Genetic diversity and structure

It is commonly assumed that habitat fragmentation negatively affects genetic diversity and that it may also increase among-population genetic differentiation within species, due to gene flow reduction, and increased random genetic drift and inbreeding (Young et al., 1996; Sork et al., 1999; Lowe et al., 2005). However, the responses of plant populations to habitat fragmentation may vary depending on life-history traits such as longevity, generation time (Young et al., 1996), systems of sexual and asexual reproduction (Honnay and Bossuyt, 2005; Honnay and Jacquemyn, 2007), ploidy level (Moody et al. 1993) and breeding systems (Loveless and Hamrick, 1984). For this reason and for some species, fragmentation events may even increase gene flow among remnant populations, breaking down local genetic structure (Hall et al., 1994; Hall et al., 1996; Dick et al., 2003).

The immediate effects of fragmentation on the genetic composition of plant populations depend on two factors: the effective population size within fragments and the patterns of genetic variability of the original populations previous to fragmentation (Nason and Hamrick, 1997; Hamrick, 2004). Some studies have indicated that genetic diversity is lost slowly after habitat fragmentation (Lowe et al., 2005; Honnay and Jacquemyn, 2007). Our results showed that *Quercus castanea* adults, both in populations situated in forest fragments, and isolated trees maintain high and similar genetic diversity levels. However, the comparison of diversity values among the progenies from the different groups showed that the offspring of isolated trees had lower genetic diversity values.

The fixation index (F) was markedly different between the progenies of trees located in forest fragments (positive and significant) and isolated trees (negative and significant). As in other studies the negative inbreeding coefficients of offspring indicated that habitat fragmentation processes did not necessarily result in inbred seed at scattered trees level (Hobee *et al.*, 2007; Kamm *et al.*, 2009)

Quercus castanea has high levels of genetic diversity within and between populations and isolated trees that could mitigate the effects of forest fragmentation as has been shown in other tree species (Dick, 2001; White et al., 2002). This great genetic diversity is comparable with other oak species (Valbuena-Carabaña et al., 2005; Craft and Ashley, 2007; Pakkad et al., 2008) and suggests that these populations have maintained great capacity to move their genes through pollen and seeds (Hamrick and Godt, 1996).

Scattered isolated trees represent potential bridges for gene flow because their reproductive potential remains high. This gene flow contributes to maintain the genetic connectivity among remnant populations (Cascante et al., 2002; Manning et al., 2006).

These isolated trees play many key ecological roles such as effective sources of seeds for regeneration (Dorrough and Moxham, 2005), and the maintenance of habitats for wildlife in fragmented landscapes (Fischer and Lindenmayer, 2002; Manning et al., 2006; Manning et al., 2009).

Spatial isolation between populations and individuals caused by habitat fragmentation is expected to reduce gene flow. This reduction of migrant genes combined with genetic drift and allelic loss will increase genetic differentiation among remnant populations. In *Q. castanea*, we found a significant reduction of gene flow in the progenies of fragmented populations and isolated trees, progeny populations were more genetically structured than adult populations. The physical structure of surrounding habitat influences the process of pollen movement altering the population genetic structure (Dyer and Sork, 2001). These physical structures of the surrounding habitat of populations of *Q. castanea* previous (i.e., continuous forest) and post fragmentation events (i.e., isolated fragment remnants and isolated trees) have influenced the gene movement among these populations restricting or facilitating pollen movement.

The analyzed trees from fragmented populations represent individuals of the continuous forests prior to habitat fragmentation. The low genetic differentiation between adult trees from fragmented populations ($F_{ST}= 0.018$), suggest homogeneous gene flow throughout the landscape which avoided the genetic structuring of these continuous populations. However, the disruption of this continuous forest led to the formation and isolation of many patchy populations, which disrupt the gene flow patterns and consequently led to genetic differentiation of offspring in fragmented populations ($F_{ST}= 0.038$). Isolated trees made more markedly this genetic structuring in offspring from these trees ($F_{ST}= 0.247$); both set of individuals, offspring from

fragmented populations and isolated trees were established post fragmentation, so this physical modifications alter ecological processes such as pollen and seed movements, modifying the genetic configuration of the landscape. Therefore, gene flow between *Q. castanea* populations appears to be much more vulnerable to fragmentation than genetic diversity, which creates significant differences in allele frequency distribution among offspring populations respect to adults.

Inbreeding values (F_{IS}) showed different responses in fragmented populations respect to isolated trees. Both, adults and offspring from fragmented populations exhibit a significant excess of homozygotes ($F_{IS} = 0.110$ and 0.104 , respectively). This heterozygous deficiency can be explained by inbreeding, assortative mating, and selection against heterozygous (Stoeckel et al., 2006). Then, although adult trees were surrounded by many trees previous to habitat fragmentation, the probability to get pollen from different individuals was high. However, it seems that the pollen received came from related individuals, which caused the resulting inbred trees.

Adults and offspring from isolated trees also exhibited an excess of heterozygous ($F_{IS} = -0.019$ and -0.120), but values were not significantly different of zero. These results can be explained as a consequence of reproductive small size populations and mating between few individuals (Pudovkin et al., 1996). Reproductive population size of isolated trees is very small and in most of the cases, they are spatially isolated from each other. Crosses occur between unrelated trees so, although the adults are isolated, their progeny are not necessary inbred. Thus, isolated trees can act as important stepping-stones for gene flow between forest fragments providing connectivity and enhancing population variability (White et al., 1999; Dick, 2001; White et al., 2002). These isolated oak trees play a key role in gene movement as important bridges among fragmented landscapes and catching pollen from unrelated

individuals diminishing the inbreed probability. Furthermore, these trees can be managed as potential sources for oak forest regeneration.

4.2. Genetic heterogeneity of pollen pool

Based on the Φ_{FT} estimated values, we can infer that the composition of pollen pool that fertilizes mother trees in both, fragmented populations and isolated trees were significantly different. Apparently the fragment size did not influence on the actual pollen flow since Φ_{FT} and N_{ep} values from fragmented populations were very similar in different size fragments. However, isolated trees showed a major disruption to pollen flow among their individuals; these isolated trees are getting the greatest value on Φ_{ft} parameter.

The higher Φ_{FT} values observed in isolated trees than in fragmented populations indicate that isolated trees are receiving pollen from a few donors ($N_{ep} = 1.424$) while fragmented populations receive pollen from more pollen sources ($N_{ep} = 2.142$ to 2.358). Trees from fragmented populations are immersed in a large number of possible pollen donors while isolated trees have restricted pollen sources due the absence of neighbor trees. The smaller effective number of pollen donors (N_{ep}) of isolated trees confirm this situation of pollen restriction. However, although isolated trees received pollen from fewer donors, that pollen comes from unrelated trees promoting a greater genetic diversity. Also, high values of Φ_{FT} indicate that effective pollination occurs very localized, which is confirmed by the small average distance of pollen dispersion (δ), and the effective neighborhood pollination area (A_{ep}).

4.3. Mating system

MLTR analysis revealed that all the studied populations, including isolated trees, were highly outcrossers (t_m over than 0.98) but with small values of correlation of paternity (r_p). However, it was possible to observe significant effects of biparental inbreeding (t_m - t_s : 0.088 to 0.272), which indicates the occurrence of high proportion of mating among relatives. This proportion was higher than expected, compared with levels found in oak fragmented populations (*Q. semiserrata* t_m - t_s : 0.013) (Pakkad *et al.*, 2008).

One consequence of habitat fragmentation on mating systems is that they can be disrupted thereby resulting an increase on inbreeding and population differentiation (Pakkad *et al.*, 2008), such results are being observed on fragmented populations and isolated trees of *Q. castanea*, because there are an increase on inbreeding levels as well on genetic differentiation, then we observe that although is a relatively young fragmented system, some effects are already evident.

5. Conclusion

Forest fragmentation affects the intensity of gene flow and consequently the genetic diversity and population differentiation in the short-term. Results obtained with seven nuclear microsatellite markers provide evidence that recently fragmented populations of *Q. castanea* maintain high levels of genetic diversity but this habitat fragmentation has induced moderate impacts on these populations. These impacts are more evident on gene flow parameters, specially from isolated trees, however, these impacts are not totally negative; these trees are receiving pollen from lesser donors, but these donors are unrelated with them so, this condition increases the possibility for heterozygosity, which gives these isolated trees an important genetic value within this landscape.

Isolated trees play a major role in the maintenance of genetic diversity and connectivity through pollen movement. Because most individuals are old trees, they

represent the original vegetation prior to forest fragmentation providing important opportunities for conservation of original gene pools and the genetic sources for future landscape restoration.

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Table 1. Description of *Quercus castanea* populations.

	Abbreviation	Coordinates utm		Altitude	fragment size
		N	W		
Fragmented populations:					
Jesus Monte Bosque	JMB	274205	2172831	2136	Small (0.3 ha)
Jesus Monte Pueblo	JMP	274270	2173596	2118	Small (0.5 ha)
Correo	Corr	247545	2164696	2313	Large (117.9 ha)
Umecuario	Ume	262840	2163110	2189	Large (56.7 ha)
<hr/>					
16 isolated trees	Throughout the basin				

Table 2. Mean values of genetic variation of adults and offspring from populations in small and large forest fragments and isolated trees of *Quercus castanea* using seven nuclear microsatellite loci.

Pop	Adults						Offspring					
	n	A	A_p	H_o	H_e	F	n	A	A_p	H_o	H_e	F
JMB	16	9.31	0.2	0.78	0.77	-0.02	156	9.86	0.43	0.74	0.79	0.08
JMP	16	8.43	0.01	0.81	0.82	0.00	156	9.42	0.18	0.73	0.84	0.13
Corr	16	8.86	0.3	0.74	0.81	0.09	153	9.71	0.46	0.68	0.84	0.18
Ume	16	9.43	0.23	0.75	0.80	0.06	153	9.63	0.33	0.77	0.83	0.08
Average	16	9.01	0.19	0.77	0.80	0.03	154.5	9.65	0.35	0.73	0.82	0.12
		(0.22)	(0.06)	(0.03)	(0.02)	(0.03)		(0.09)	(0.06)	(0.03)	(0.02)	(0.03)
Isolated trees												
Average	16	9.86	0.11	0.80	0.82	0.02	154	8.97	0.09	0.69	0.57	-0.22
		(0.74)	(0.06)	(0.04)	(0.04)	(0.03)		(0.94)	(0.04)	(0.02)	(0.01)	(0.04)

n= simple size, A= Allelic richness, A_p = Private allelic richness, H_o = Observed heterozygosity, H_e = Expected heterozygosity, F= Fixation index. Parenthesis indicates standard error.

Tabla 3. F -statistics for adults and offspring from four populations in forest fragments and 16 isolated trees and their offspring of *Quercus castanea*, based on seven nuclear microsatellite loci.

		F_{IS}	F_{IT}	F_{ST}
Fragmented populations	Adults	0.110*	0.127**	0.018
	Offspring	0.104**	0.138**	0.038**
Isolated trees	Adults	-0.019		
	Offspring	-0.120	0.157**	0.247**

* $P < 0.05$; ** $P < 0.01$.

Table 4. Results of pollen structure calculated for four populations in small and large forest fragments and isolated trees of *Q. castanea*.

Population	Φ_{ft}	N_{ep}	δ	A_{ep}
JMB	0.233 *	2.142	6.67 m	300 m ²
JMP	0.212 *	2.358	7.32 m	400 m ²
Corr	0.215 *	2.326	10.78 m	899.1 m ²
Ume	0.232 *	2.155	7.19 m	400 m ²
Isolated trees	0.351*	1,424		

Φ_{ft} = genetic heterogeneity of pollen pool, N_{ep} = effective number of pollen donors, δ = average distances of pollination, A_{ep} = effective neighborhood pollination area. * $P < 0.001$.

Table 5. Mating system parameters obtained in four populations and 16 isolated trees of *Q. castanea* in Cuitzeo's Basin.

Pop	t_m		t_m-t_s		F		r_p		N_{ep}
JMB	0.988	(0.07)	0.088	(0.07)	-0.005	(0.04)	0.149	(0.03)	6.71
JMP	0.998	(0.10)	0.206	(0.10)	0.073	(0.04)	0.143	(0.03)	6.99
Corr	0.993	(0.10)	0.272	(0.09)	0.123	(0.04)	0.278	(0.06)	3.60
Ume	1	(0.0)	0.121	(0.03)	0.11	(0.06)	0.103	(0.03)	9.71
Average	0.995		0.392		0.075		0.168		6.75
Isolated trees	0.987	(0.07)	0.228	(0.07)	0.068	(0.04)	0.245	(0.03)	4.08

t_m = outcrossing multilocus rates, t_m-t_s = biparental inbreeding, F = inbreeding of mother generation, r_p = multilocus correlation paternity coefficient, N_{ep} = effective number of pollen donors. Parenthesis indicates standard deviation.

DISCUSIÓN GENERAL

La destrucción antropogénica de los ecosistemas hoy día es uno de los factores de presión más importantes sobre la biodiversidad. En México, la deforestación es un problema que se ha presentado desde tiempos precolombinos, sin embargo, durante las últimas décadas estos procesos se han incrementado sustancialmente (Aguilar *et al.*, 2010).

Siendo México un país megadiverso, se requiere conocer de manera detallada todos estos procesos de deterioro del ambiente para poder generar propuestas que permitan revertir o remediar el problema de pérdida de biodiversidad.

Fragmentación del hábitat y diversidad genética

Nuestros resultados indican que las poblaciones analizadas de *Quercus castanea*, a pesar de formar parte de un sistema altamente fragmentado (más de 1200 fragmentos de diferente tamaño), mantienen valores de diversidad genética de cloroplasto y nuclear (herencia materna y biparental, respectivamente) considerablemente altos. Así mismo, esta diversidad genética se encuentra ampliamente representada tanto en las poblaciones de adultos, como en las poblaciones de plántulas.

Estos valores de diversidad pueden ser explicados por diferentes razones. Se ha sugerido que algunas especies que tienen una gran capacidad para mover sus genes son capaces de mantener altos niveles de diversidad genética (Hamrick y Godt, 1996).

Quercus castanea es una especie polinizada por el viento, y cuyas bellotas son dispersadas por la gravedad y algunas aves y pequeños mamíferos. Es probable que dichos agentes de dispersión históricamente hayan sido muy eficientes, de manera que las poblaciones en la actualidad mantienen niveles de diversidad genética bastante altos.

Estos altos niveles de diversidad genética también pueden ser atribuidos a la amplia distribución geográfica de la especie (Hamrick y Godt, 1996), y a la permanencia histórica de las actuales poblaciones con grandes tamaños poblacionales (Hewitt, 1996; Rowe *et al.*, 2004; Peñaloza-Ramírez *et al.*, datos no publicados).

Grandes niveles de diversidad genética pueden ser capaces de diluir o amortiguar los efectos de la fragmentación del hábitat sobre la diversidad genética. También es posible que no haya transcurrido el tiempo suficiente posterior a la fragmentación del hábitat para colapsar tales niveles de diversidad (Aguilar *et al.*, 2008; Bacles y Jump, 2011). Muchas especies de árboles, particularmente aquellas que son polinizadas por el viento han mostrado ser muy resistentes a los efectos de la fragmentación del hábitat (Jump y Peñuelas, 2006)

Considerando que *Q. castanea* puede ser una especie longeva (hasta 400 años), (Kaul, 1985), y que el proceso de fragmentación del hábitat que se analiza es muy incipiente (más o menos 100 años), es probable que no hayan transcurrido las suficientes generaciones posteriores a la fragmentación que permitan visualizar de manera significativa los efectos que la teoría predice.

Uno de los métodos comúnmente utilizados para identificar las consecuencias de la fragmentación del hábitat es a través de el estudio de las relaciones entre la diversidad genética y el tamaño del hábitat o bien el tamaño poblacional (Zhang *et al.*, 2012).

Nuestro estudio demostró que aunque los niveles de diversidad genética permanecen altos en ambas cohortes, hay una correlación negativa y significativa del tamaño de fragmento sobre la diversidad en las generaciones posteriores a la fragmentación.

Las plántulas que se encuentran en fragmentos de tamaño pequeño, tienen una menor diversidad que aquellas que se encuentran en fragmentos grandes. Así mismo, las

plántulas de fragmentos pequeños, tienen menor diversidad en comparación con los adultos de los mismos fragmentos.

Como señalan Honnay y Jacquemyn (2006), la diversidad genética está directa y positivamente relacionada con el tamaño poblacional por lo que si este proceso de fragmentación continúa o se intensifica, esta incipiente pérdida de diversidad genética que experimentan las poblaciones evaluadas, podría conducir a una importante pérdida de la diversidad en las nuevas generaciones que pudieran establecerse en dichos fragmentos. Esto en conjunción con otros procesos como deriva génica y endogamia, las poblaciones de *Q. castanea* establecidas en fragmentos de tamaño pequeño, podrían poner en riesgo su permanencia y continuidad (Ellstrand y Elam, 1993; Honnay y Jacquemyn, 2006).

Conectividad genética poblacional.

Muchos hábitats fragmentados han perdido la capacidad para soportar poblaciones de plantas lo suficientemente grandes para mantener un equilibrio mutación-deriva y las poblaciones que ocupan estos fragmentos están tan aisladas que el flujo génico es insuficiente para permitir la reposición de alelos perdidos (Honnay y Jacquemyn, 2006).

Dentro de las predicciones más importantes de los modelos teóricos sobre la fragmentación del hábitat está que la fragmentación puede conducir a la disminución del intercambio de genes entre poblaciones (Young *et al.*, 1996; Lowe *et al.*, 2005; Bacles y Jump, 2011).

En nuestro sistema de estudio, esta predicción toma sentido al encontrar que existe un efecto negativo y significativo de la fragmentación del hábitat sobre el

movimiento de genes vía semillas y polen, demostrando que el flujo génico en *Quercus castanea* es un parámetro muy sensible a los efectos de la fragmentación del hábitat.

La conectividad de las poblaciones a través de semillas, nos mostró una significativa disminución en los parámetros del número de conexiones ("node degree") y cercanía ("closeness"), en las poblaciones de plántulas con respecto a las poblaciones de adultos. Esto indica que el movimiento de genes a través de semillas está siendo interrumpido en las cohortes de plántulas, las cuales se establecen posteriores a la fragmentación. Estas diferencias podrían volverse más severas si se eliminan algunas de ellas por un proceso natural de selección, y solamente se establezcan como árboles adultos algunas otras (Hamrick y Godt, 1996), lo cual traerá consigo una pérdida de diversidad genética y con ello una mayor disminución en el flujo génico.

También se encontró evidencia de interrupción del proceso de dispersión de semillas a larga distancia en poblaciones de plántulas. Este hecho tiene importantes implicaciones en los procesos demográficos poblacionales, ya que el reclutamiento de nuevos individuos a través de semillas es un factor importante para el mantenimiento de la diversidad genética en poblaciones de plantas, puesto que este proceso mueve ambos genomas, el materno y el paterno, y es esencial para la persistencia a largo plazo de las mismas (Bruna y Kress, 2002). La modificación de procesos post-polinización, como la dispersión de semillas puede llevar a la reducción en la abundancia de plántulas (Cunningham, 2000), y en consecuencia a la inestabilidad demográfica poblacional.

En general, se espera que los árboles que son polinizados por el viento tengan un menor riesgo a los efectos genéticos por limitación del polen en paisajes fragmentados. Sin embargo, se ha demostrado que la disponibilidad de polen puede disminuir en paisajes fragmentados, provocando con ello la pérdida de la conectividad entre individuos del mismo fragmento incluso (Knapp *et al.*, 2001; Sork *et al.*, 2002).

Nuestros resultados mostraron que el movimiento de polen también experimentó importantes cambios, inducidos por la fragmentación del hábitat. La estructura genética de las poblaciones de progenies presentó un importante incremento, lo que indica que hay una interrupción al flujo de genes, comparado con las poblaciones de adultos que mantuvieron un flujo génico homogéneo lo que les permitió mantener una muy baja y no significativa estructura genética de las mismas.

Este efecto se presenta mucho más acentuado en las progenies de los árboles aislados, ya que éstas, al recibir una menor cantidad de polen de otros individuos o poblaciones la estructura genética moldeada es mucho más fuerte, lo que representa un severo problema para la permanencia y viabilidad de las futuras generaciones (Finger *et al.*, 2012).

Aún cuando los árboles aislados son los elementos del paisaje que menor flujo de genes observan, y que además los genes que reciben provienen de un menor número de padres, representan elementos muy importantes dentro de la conectividad del paisaje ya que el polen que están capturando proviene de árboles menos emparentados, lo cual supone un vecindario de polinización más amplio, resultando en progenies menos endogámicas, las cuales representan una importante fuente de semillas para el restablecimiento de la vegetación (Manning *et al.*, 2006; Ottewell *et al.*, 2009). De esta manera, los árboles aislados están representando un papel muy importante en la conectividad de los diferentes fragmentos, como sucede en otros sistemas fragmentados (Lander *et al.*, 2010).

Muchos de los programas de conservación han centrado su atención en las grandes poblaciones que mantienen altos niveles de diversidad genética y han ignorado las contribuciones potenciales que pueden tener las poblaciones pequeñas y mas aún los árboles aislados. Sin embargo, algunas investigaciones (Hoebee *et al.*, 2007; Hanson *et*

al., 2008;) incluyendo la investigación de la presente tesis, han demostrado la importancia de éstos en la conectividad genética en paisajes fragmentados.

CONCLUSIONES

Los análisis genéticos poblacionales utilizan marcadores moleculares capaces de capturar gran parte de la variación natural de las poblaciones de manera que permitan reconstruir la estructura de su diversidad genética y los patrones de flujo génico dentro y entre sus poblaciones. Los marcadores moleculares utilizados en esta investigación fueron capaces de capturar una gran parte de esta variación genética tanto nuclear como de cloroplasto, lo cual nos permitió evaluar los patrones de pérdida de diversidad genética y flujo de genes en un paisaje con fragmentación de origen antropogénico, aún cuando se trata de un proceso relativamente reciente, en términos de procesos ecológicos.

Los análisis comparativos realizados con los marcadores de microsatélites de cloroplasto y núcleo demostraron ser particularmente útiles para esta evaluación, ya que permitieron contestar las preguntas planteadas al inicio de la investigación:

(i) ¿se ha reducido la conectividad vía semillas entre poblaciones de *Quercus castanea* en la cuenca de Cuitzeo, Michoacán como resultado de la fragmentación del hábitat?;

(ii) ¿puede la diversidad genética y el flujo génico a través de polen ser afectados por procesos contemporáneos de fragmentación de hábitat? y

(iii) ¿cuál es el papel de los árboles aislados en este proceso de fragmentación del hábitat?

La alta diversidad genética encontrada tanto en cloroplasto como en núcleo, así como la distribución geográfica de esta diversidad en los diferentes fragmentos permitió reconocer los efectos de procesos antropogénicos contemporáneos de fragmentación del hábitat sobre la diversidad y el flujo de genes.

Al proveer información complementaria acerca de la estructura genética, diversidad y diferenciación así como el flujo de genes dentro y entre poblaciones nos

permitieron conocer y entender los procesos pre- y post-fragmentación a nivel de paisaje en una especie forestal, lo cual puede facilitar la toma de decisiones para su manejo.

Respecto a las hipótesis planteadas, solo se acepta la primera: las poblaciones actuales de *Quercus castanea* históricamente han mantenido conectividad genética a través de semillas. Las otras dos hipótesis planteadas originalmente se rechazan: los procesos actuales de fragmentación de hábitat, no afectan la diversidad genética de cloroplasto, y respecto al movimiento de genes a través de polen, se espera encontrar diferencias en los patrones de apareamiento en función del tamaño del fragmento. Es decir, el número de padres que polinicen a los árboles madre en los fragmentos de menor tamaño será diferente al número de padres que polinizan a los árboles madre en los fragmentos de mayor tamaño.

Los resultados de los siete marcadores de microsatélites de cloroplasto y siete marcadores de microsatélites nucleares utilizados en este estudio son consistentes y sugieren algunas poblaciones muy importantes para el mantenimiento de la conectividad genética y en consecuencia, para la retención de la diversidad en las próximas generaciones.

Se encontraron dos poblaciones que mantienen una alta conectividad en adultos y dos para plántulas, indicando que esta conectividad está siendo modificada en un lapso de tiempo muy corto (menos de 100 años), por lo que es importante propiciar la conservación principalmente de estas poblaciones, mismas que están actuando como puentes en el mantenimiento de la diversidad genética.

Además, es necesario no perder de vista a los árboles aislados y sus progenies, ya que al servir como puentes en la captura de polen, forman importantes estructuras

clave en el paisaje, por lo que pueden utilizarse como estrategia para facilitar el mantenimiento de la diversidad y conectividad genética

La dispersión de semillas de los árboles aislados también es un factor que debe ser tomado en consideración, ya que al estar los árboles aislados inmersos en una matriz de vegetación modificada resulta difícil la dispersión de semillas y en consecuencia, el establecimiento de plántulas por lo que es necesario, tomar en consideración este aspecto para formular estrategias de conservación.

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