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PERSISTIENDO EN EL LÍMITE

Flujo génico, reproducción y conservación de poblaciones refugio de *Quercus robur* L.

Eva Moracho Martínez

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Directores: Arndt Hampe, Pedro Jordano y Gerardo Moreno

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DEPARTAMENTO DE ECOLOGÍA INTEGRATIVA

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Flujo génico, reproducción y conservación de poblaciones refugio de *Quercus robur* L.

* * *

Memoria presentada por la Licenciada en Biología Eva Moracho Martínez para optar al Título de Doctora en Biología por la Universidad de Sevilla, Sevilla.

Fdo. Eva Moracho Martínez

Sevilla, 2019

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CERTIFICAN:

Que la tesis doctoral titulada **Persistiendo en el límite: flujo génico, reproducción y conservación de poblaciones refugio de *Quercus robur*** L. presentada por la Lcda. Eva Moracho Martínez ha sido realizada bajo nuestra dirección y tutoría, y reúne las condiciones de calidad y rigor científico para que pueda ser presentada y defendida ante la comisión nombrada al efecto.

Y para que así conste y surta los efectos oportunos, firmamos el presente certificado en Sevilla, 20 Marzo de 2019.

Directores

Fdo. Arndt Hampe

Fdo. Gerardo Moreno Marcos

Fdo. Pedro Jordano Barbudo

Tutor

Fdo. Juan Arroyo Marín

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A nuestros preciados bosques

y la gran biodiversidad que sustentan

CONTENIDO

LISTA DE PUBLICACIONES Y MANUSCRITOS EN REVISIÓN INCLUIDOS EN ESTA TESIS

Capítulo 2

Pulido, F., Castagneyrol, B., Rodríguez-Sánchez, F., Moracho, E., Cáceres, J., Pardo, A., Kollmann, J., Valladares, F., Ehrlén, J., Jump, A., Svenning, JC., & Hampe, A. Asymmetry in marginal population performance foreshadows widespread species range shifts. *In review*, Febrero 2019.

* * *

Capítulo 3

Moracho, E., Moreno, G., Jordano, P. and Hampe, A. (2016) Unusually limited pollen dispersal and connectivity of Pedunculate oak (*Quercus robur*) refugial populations at the species' southern range margin. *Molecular Ecology*, 25, 3319-3331.

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Capítulo 4

Moracho, E., Moreno, G., Jordano, P. and Hampe, A. Ecological drivers of individual mating patterns in refugial populations of Pedunculate oak (*Quercus robur*). *In review*, Febrero 2019

* * *

Capítulo 5

Moracho, E., Jordano, P., Hampe, A. (2018) Drivers of tree fecundity in Pedunculate oak (*Quercus robur*) refugial populations at the species' southwestern range margin. *Plant Biology*, 20 (Suppl. 1), 195-202.

RESUMEN

Numerosas especies alrededor del mundo están desplazando su rango de distribución hacia mayores latitudes o altitudes como consecuencia del calentamiento global moderno. Las poblaciones periféricas constituyen piezas clave para predecir la envergadura y velocidad de este proceso. El desplazamiento direccional del rango geográfico se produce cuando las nuevas condiciones ambientales permiten el establecimiento y la expansión de nuevas poblaciones en el llamado margen de avance (leading edge) mientras que causan la extinción de aquellas situadas en el llamado margen de retaguardia (rear edge). Mientras los cambios en la distribución y abundancia de las especies han sido ampliamente documentados, escasean evidencias empíricas sobre la dinámica y el *fitness* de las poblaciones periféricas a gran escala que podrían servir para anticipar futuros cambios en los rangos de las especies (capítulo 1). Más allá de este patrón global, en las poblaciones situadas en el margen de retaguardia, las especies pueden desarrollar estrategias de resiliencia que aumenten su viabilidad en situaciones de estrés ambiental. Además, estas poblaciones persisten frecuentemente en refugios climáticos a escala local (microrefugios) los cuales conservan un clima propicio que se está perdiendo o ya ha desaparecido a escala regional (climas relictos). Las poblaciones relictas así establecidas desde tiempos pasados representan reservorios de diversidad genética, historia evolutiva y potencial adaptativo cuya pérdida sería irremplazable. Tal es el caso de numerosas especies de árboles de climas templados y boreales cuyas poblaciones relictas a lo largo del límite meridional de la distribución han sido capaces de persistir en la Cuenca Mediterránea a través de las grandes oscilaciones climáticas del Cuaternario. Su distribución actual en núcleos pequeños y aislados tiene profundas consecuencias para los procesos demográficos y genéticos que determinan su viabilidad a largo plazo. La falta de conocimiento acerca de los mecanismos que aseguran el buen funcionamiento de estas poblaciones constituye un reto para el conocimiento científico y la conservación de la biodiversidad que ha motivado esta tesis. Usando como organismo de estudio la especie arbórea *Q. robur* en un conjunto de poblaciones relictas, he estudiado el flujo génico (con énfasis en la dispersión de polen) y la reproducción, aspectos clave para la diversidad genética, el potencial de regeneración y en definitiva la viabilidad de las poblaciones a largo plazo. He caracterizado la variabilidad de estos procesos a distintas escalas (desde el

individuo hasta el paisaje) e investigados sus principales determinantes ecológicos y genéticos son investigados.

El marco conceptual en el que se encuadra la tesis es demostrado empíricamente en un estudio a escala global basado en la síntesis de una colección de datos mediante técnicas de meta-análisis (capítulo 2). Los resultados ponen de manifiesto un cambio asimétrico en el *performance* de las poblaciones marginales de acuerdo a las predicciones del cambio climático moderno. Las poblaciones del margen de latitudes bajas muestran un *performance* reducido respecto a otras áreas del rango (centrales y latitudes altas), mientras que aquellas del margen de latitudes altas no sobresalen frente al estimado en áreas centrales pero tampoco quedan atrás (como predice la teoría centro-periferia). Este efecto guarda relación con el diferencial de temperatura entre las zonas de estudio y constituye una tendencia general a través de los reinos y tipos de hábitat. Por tanto, encontramos un impacto del cambio de clima doblemente negativo donde ya se evidencia la vulnerabilidad a la extinción local de las poblaciones del margen de baja latitud, pero además, el retraso de las poblaciones de latitudes altas en su reacción de expansión como respuesta al desplazamiento de las condiciones climáticas.

El estudio de los patrones de flujo génico histórico y contemporáneo en poblaciones relictas de *Q. robur* (capítulo 3) se beneficia del muestreo exhaustivo de todos los adultos presentes en el área de estudio (*n* = 135 en un total de 10 rodales), y las progenies muestreadas sobre 72 árboles madre (*n* = 724). Mediante técnicas de clustering genético y de topología de redes caracterizamos la estructura y el flujo génico histórico, mientras que la dispersión de polen contemporáneo se basa en potentes análisis de paternidad. Este estudio ofrece un resultado sobresaliente para una especie anemófila ya que observamos una excepcional escasez de flujo génico y una limitada conectividad poblacional. La fuerte estructura genética de la población adulta salpicada de eventos esporádicos de dispersión histórica es coherente con el limitado flujo de polen que se detecta entre rodales del mismo valle (2.6%) o desde el valle contiguo (4,4%), lo que indica que la débil conectividad no es un fenómeno a corto plazo. El reducido tamaño y el alto aislamiento de los rodales junto a otros aspectos físicos del paisaje refugial suponen un obstáculo a la dispersión de polen. Como consecuencia, el patrón de conectividad es fuertemente heterogéneo espacialmente, con rodales que funcionan predominantemente como fuente o sumidero de polen y otros aislados prácticamente al flujo génico. A pesar de ser escasa, la inmigración de polen puede llegar a tener un impacto exacerbado en la genética de las poblaciones si operan procesos de purga genética.

A una escala menor, detectamos una gran variabilidad entre individuos en los patrones de cruzamiento que responde mayoritariamente a diferencias en el ambiente local y rasgos intrínsecos del individuo (capítulo 4). La polinización en la mayoría de los individuos ocurre sólo entre individuos del mismo rodal. Una reducida fracción de los árboles es responsable de los infrecuentes eventos de inmigración procedente de la misma especie (7%) o de la especie hermana *Q. pyrenaica* con la que coexiste (0.6%). Los eventos de autofecundación son excepcionalmente frecuentes (6.8%) para una especie predominantemente autoincompatible. Este incremento de la tasa de autofecundación observada en nuestras poblaciones relictas ha sido igualmente descrito en poblaciones marginales de otras especies, lo que parece sugerir algún tipo de ventaja asociada a la adquisición de un sistema de cruzamiento mixto en estas situaciones. Los patrones de dispersión de polen (local, inmigrante y propio) son moldeados por un conjunto de factores que operan a escala fina, relacionados con aspectos del vecindario local (densidad de conespecíficos y grado de parentesco entre ellos). La autofecundación se ve además influida por rasgos intrínsecos del individuo (el tamaño y la producción de polen). En resumen, parece que las poblaciones relictas son propensas a albergar individuos de gran tamaño, grandes productores de polen que favorecen la autofecundación (fenómeno a escala fina) cuando no disponen de polen en abundancia y de calidad, mientras que la existencia de núcleos de población densos en las proximidades facilitaría la dispersión de polen inmigrante (fenómeno a escala de paisaje).

Adicionalmente, profundizamos en los patrones de cruzamiento dentro de los rodales de mayor tamaño (4 réplicas, n = 22-35 individuos) aplicando modelos de cruzamiento espacialmente explícitos que permiten inferir la red de cruzamiento entre todos los individuos del rodal y caracterizar las componentes femenina y masculina del proceso de polinización (capítulo 4). Existe una red de polinización muy conectada en el interior de rodales, donde los árboles de gran tamaño, productores de vastas cantidades de polen y los individuos que ocupan posiciones centrales del rodal juegan un importante rol como conectores. Además se demuestra una gran variabilidad entre individuos en la fecundidad masculina (liberación de polen que llega a producir frutos) que está íntimamente ligado al tamaño y la capacidad productora del árbol.

Como aproximación al potencial de regeneración que presentan las poblaciones relictas de *Q. robur* estudiamos la fecundidad y el éxito reproductivo (capítulo 5) durante 2 años sobre 159 árboles localizados a través de 12 núcleos de población. La variabilidad en la fecundidad femenina (medida como la producción total de semillas viables y su peso), al igual que la masculina, se debe principalmente a diferencias en el tamaño del árbol. Los árboles mas grandes producen una mayor cantidad de flores femeninas (y masculinas) y bellotas de manera casi invariable a través de los años. Además las bellotas producidas son más grandes y pesadas en los árboles de gran tamaño, lo que puede tener particular relevancia en el contexto de refugios climáticos en ambientes mediterráneos debido a las ventajas asociadas a la germinación, crecimiento y supervivencia de las semillas con más reservas. Por otra parte, el éxito reproductivo (fruit set) en estas poblaciones relictas se ve influido por factores actuando a escala de paisaje (tamaño y aislamiento poblacional), lo que evidencia limitación de polen a esta escala, y no a escala local a pesar de los numerosos indicadores ecológicos y genéticos empleados. La aparente ausencia de restricciones genéticas puede explicar la predominante fertilización local observada en poblaciones relictas. Los patrones de dispersión y cruzamiento parecen garantizar la reproducción exitosa de la especie siempre que se alcance un tamaño poblacional mínimo y/o el aislamiento no sea elevado; de otro modo el escaso flujo de polen no logra compensar la limitación de polen existente. No obstante, la variabilidad interanual en el proceso reproductivo es notable de manera que la limitación de polen tendrá efectos más profundos en años de poca productividad, y los episodios donde la regeneración ocurra se limitarán a años muy productivos asociados a condiciones de benevolencia climática.

Las estrategias de conservación en poblaciones relictas deben centrarse en un correcto funcionamiento interno mediante prácticas que aseguren un número y densidad suficiente de individuos reproductivamente muy activos. Especial énfasis debe hacerse en la protección de los árboles de gran tamaño que pueden desempeñar funciones centrales en la conectividad mediada por polen dentro de los rodales y a través del paisaje. Además, poseen múltiples ventajas a nivel de fitness ya que exhiben una mayor éxito reproductivo tanto masculino como femenino, no solo en términos de cantidad sino muy posiblemente en términos de calidad de sus progenies.

CAPÍTULO 1

Introducción

Look deep into nature and you will understand everything better

(A. Einstein 1879-1955)

Few there are [...] who seem to clearly realize how broad a lesson on the life-history of plants is written in the trees that make the great forest regions of the world.

(C. B. Clarke 1894)

LA RESILIENCIA DE LAS POBLACIONES ANTE EL CAMBIO CLIMÁTICO

El cambio climático actual impone alteraciones en el rango de distribución de las especies (IPCC 2014), determinadas en gran medida por la reacción de las poblaciones en los márgenes donde las condiciones ecológicas son próximas a su límite de tolerancia (Parmesan & Yohe 2003). La respuesta local de las poblaciones se define por su capacidad de amortiguar las variaciones ambientales *in situ* a través de reacciones plásticas o adaptación genética, o mediante movimientos geográficos "persiguiendo" las condiciones óptimas (Aitken *et al.* 2008). De este modo, el futuro de cada población dependerá de la interacción de una serie de procesos demográficos y evolutivos determinados por la sensibilidad intrínseca de cada especie (ej. rasgos de la historia de vida, límites fisiológicos, etc.) y el grado de cambio climático al que se enfrenta (conocido como "exposición") (Dawson *et al.* 2011, Moritz & Agudo 2013). En general, se prevé una respuesta asimétrica en los márgenes frente al cambio climático distinguiéndose un límite septentrional con potencial para la expansión (*leading edge*) y un límite meridional vulnerable a la extinción de poblaciones locales (*rear edge*) (Hampe & Petit 2005, Parmesan 2006) (Figura 1.1). Sin embargo, esta tendencia global todavía no ha sido demostrada empíricamente. Además, existe una falta de conocimiento acerca de los procesos biológicos implicados en la respuesta de las poblaciones que limita la capacidad de predecir futuros cambios de rango (Gavin *et al.* 2014).

En el margen de retaguardia (*rear edge*), las dinámicas de resiliencia (i.e. capacidad de mantener poblaciones viables en situaciones de estrés ambiental) adquieren un papel primordial. Una importante consideración aquí son las áreas topográficamente complejas que sirven y han servido como refugio climático a escala local (lo que se denomina microrefugios) al conservar un clima que se está perdiendo o ya ha desaparecido a escala regional (climas relictos *sensu* Dobrowski 2011) (Figura 1.2). Presumiblemente, estos refugios pueden funcionar facilitando la migración (*stepping-stones*), la persistencia a largo plazo (microrefugios) o sosteniendo poblaciones por un tiempo limitado antes de su extinción (*holdouts*) (Hannah *et al.* 2014). Se ha reconocido una gran importancia de las poblaciones en refugios (a las que nos referimos como "poblaciones relictas" en la presente tesis) como reservorios de la diversidad genética, la historia evolutiva y el potencial adaptativo cuya pérdida sería irremplazable (Hampe & Petit 2005, Petit *et al.* 2003) (Figura 1.2). Constituyen, además, valiosos laboratorios naturales para el estudio ecológico y microevolutivo de los procesos que permiten la persistencia de las especies en ambientes abióticos adversos (Woolbright *et al.* 2014). Gran cantidad de estudios previos se han centrado en caracterizar e identificar refugios que permitan predecir con mayor precisión el impacto del cambio climático en la distribución de las especies (Ashcroft *et al*. 2009, Gavin *et al*. 2014, Storlie *et al*. 2014), y en última instancia contribuir a la conservación de la biodiversidad (Keppel *et al.* 2012). Sin embargo, existe un gran desconocimiento sobre el funcionamiento de las poblaciones marginales a pesar de suponer una información clave para predecir su respuesta en el futuro (Thuiller *et al.* 2008).

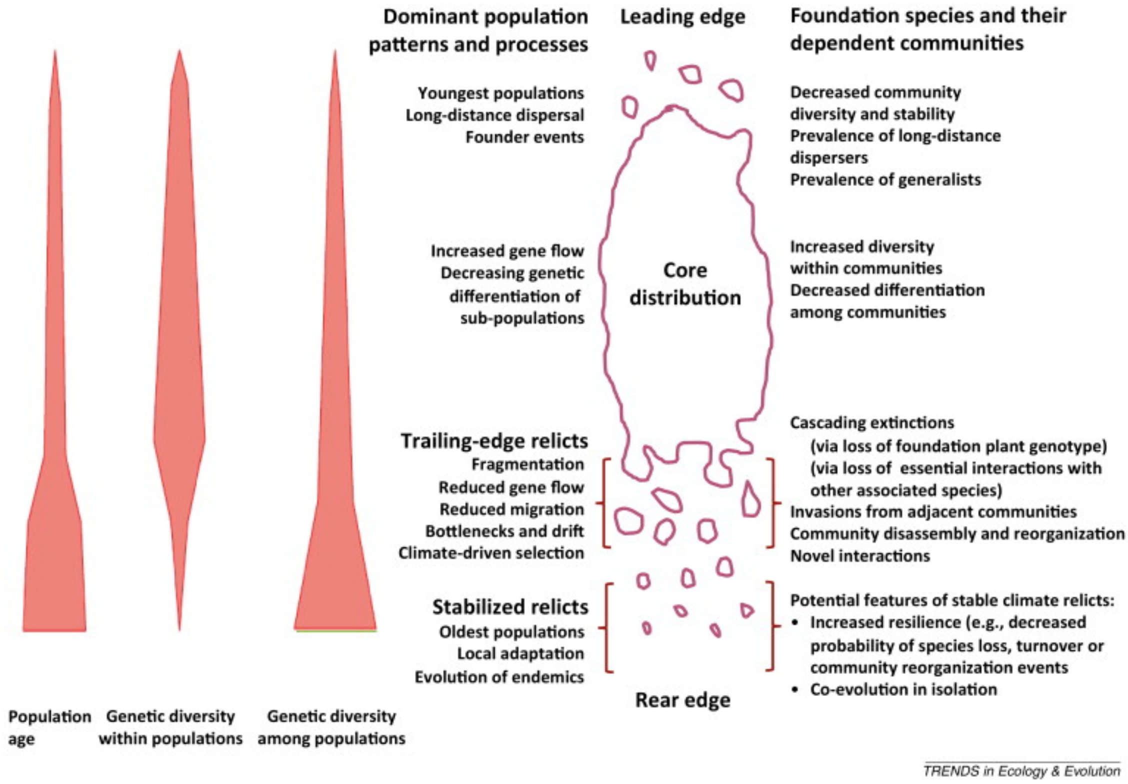

Figura 1.1. Procesos que emergen durante el cambio de rango direccional para una especie hipotética. Las barras rojas en la izquierda son una representación cuantitativa de la edad y la diversidad genética dentro y entre poblaciones. A ambos lados del mapa de distribución se resumen los procesos dominantes más probables a escala de población (izquierda), comunidad y ecosistema (derecha) en respuesta al cambio climático. A lo largo de un gradiente latitudinal se ilustran el denominado “borde rezagado” reciente y los antiguos y estables relictos climáticos. No obstante, la distribución actual de los relictos climáticos de diferentes edades probablemente varían con la topografía y otras características ambientales (Woolbright et al. 2014) Adaptado de Willis & Birks (2006) y Hampe & Petit (2005).

LAS POBLACIONES RELICTAS DE ÁRBOLES COMO MODELO DE RESILIENCIA

El impacto del cambio climático a escala global va a depender en gran medida de la capacidad de respuesta de los árboles y los ecosistemas que sustentan (Ozanne *et al.* 2003, Petit *et al.* 2004, 2005, Aitken *et al.* 2008). Los árboles son organismos de un tamaño y una longevidad excepcional que representan iconos de estabilidad y resiliencia entre los seres vivos (Morris *et al.* 2008). No obstante, sus rangos han sido y continúan siendo extremadamente dinámicos (Petit *et al.* 2008). El registro paleoecológico (la combinación de evidencias fósiles y filogeografía) ha revelado cambios a gran escala en la distribución de especies arbóreas como respuesta a las oscilaciones climáticas de la transición Pleistoceno-Holoceno, pero a su vez sugiere una capacidad robusta (resiliencia) al cambio (Hamrick 2004, Rodríguez-Sánchez *et al.* 2010).

En la actualidad, muchas de las especies de árboles de climas templados y boreales cuentan con poblaciones a lo largo del límite meridional de su distribución que han sido capaces de persistir *in situ* desde el Cuaternario (Bennett *et al.* 1991, Gavin *et al.* 2014), restringidos a islas de hábitat favorable (i.e. microrefugios) más allá de los márgenes del rango de distribución de las especies (Hampe & Jump 2011). Generalmente, configuran una distribución en núcleos pequeños y altamente dispersos a través de un paisaje heterogéneo con profundas consecuencias para los procesos demográficos y genéticos que determinan su viabilidad a largo plazo. La persistencia en estas condiciones depende de estrategias que maximicen el tamaño efectivo poblacional, y minimicen los efectos *Allee* y la depresión por endogamia. De este modo, los procesos de flujo génico y conectividad a distintas escalas adquieren gran relevancia, junto a mecanismos que promuevan una reproducción exitosa (Ghazoul 2005). No debemos olvidar que en estos refugios las limitaciones climáticas se suman a las restricciones que impone el reducido tamaño poblacional y el aislamiento geográfico, determinando en su conjunto el balance de los procesos ecológicos y evolutivos que experimentan las poblaciones y en última instancia su vulnerabilidad a la extinción (Williams *et al.* 2008). Existe una gran necesidad por entender los mecanismos que favorecen la viabilidad a largo plazo de poblaciones relictas, pues supone una valiosa información a ser integrada en los planes de manejo que fomenten su conservación (Morelli *et al.* 2016) y en la predicción de la respuesta que especies similares puedan tener en el futuro.

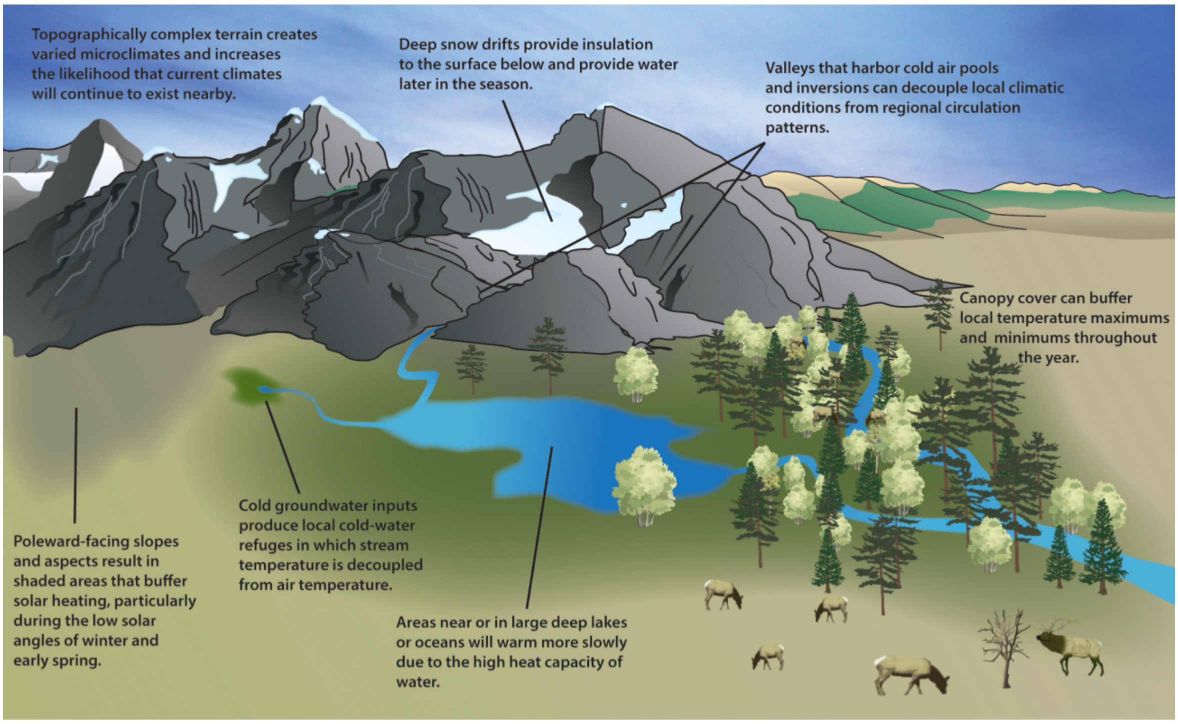

Figura 1.2 Ejemplos de refugios geográficos asociados al ambiente físico cuya probabilidad de verse afectados por el cambio climático es menor (Morelli *et al.* 2016).

DIVERSIDAD Y FLUJO GENÉTICO EN POBLACIONES RELICTAS

Diversos rasgos relacionados con la historia de vida hacen a los árboles organismos particularmente resistentes a la erosión de la diversidad genética poblacional, incluyendo su longevidad, una prodigiosa fecundidad y su capacidad para dispersar polen y semillas a larga distancia (Petit & Hampe 2006). Existen estudios que demuestran la dispersión anemófila de polen en especies forestales a través de grandes distancias (revisión en Kremer *et al.* 2012), algunos de ellos en poblaciones de árboles pequeñas y alejadas de núcleos densos de población (ej. Buschbom *et al.* 2011, Robledo-Arnuncio 2011, Lesser & Jackson 2013). No obstante, en poblaciones relictas las condiciones de aislamiento prolongado y limitado reclutamiento a menudo se imponen al elevado potencial dispersivo propio de las especies arbóreas, dando como resultado diversidades genéticas reducidas dentro de la población (Petit *et al.* 2003), aunque a escala regional podamos observar valores excepcionalmente altos consecuencia de la diferenciación genética entre poblaciones (Petit *et al.* 2003, Hampe & Petit 2005).

Existen evidencias contrastadas, ya descritas por Kramer *et al.* (2008) en "la paradoja de la genética de los bosques fragmentados", que demuestran una gran variabilidad en los patrones de diversidad y flujo génico de las poblaciones. Particularidades de cada fenómeno de fragmentación, ya sea natural o antropogénico, reciente o antiguo, relacionadas con su historia demográfica (e.g. cuellos de botella) y los procesos evolutivos que han experimentado (e.g. selección natural, deriva génica…) son clave para comprender los patrones observados. Así mismo las diferencias en la capacidad dispersiva de las especies atribuible a rasgos de la historia de vida como características del polen o la capacidad productora de propágulos (Duminil et al. 2007) generan patrones diversos. Mas allá de los numerosos factores que generalmente afectan la dispersión efectiva de polen (como el tamaño y la viabilidad del grano de polen (Bohrerova *et al.* 2009, Lindgren *et al.* 1995, Niklas 1985), las dinámicas de los vientos predominantes (Millerón *et al.* 2012, Nathan & Katul 2005) y los tipos de vectores de dispersión); en áreas de refugio el flujo génico a distancia puede verse especialmente limitado por: (i) el grado de aislamiento (distancia entre parejas potenciales dentro y entre núcleos de población), (ii) la ausencia de grandes poblaciones actuando como fuente de propágulos, (iii) las características del paisaje relacionadas con una topografía abrupta, una desarrollada cobertura vegetal o un microclima húmedo, y (iv) diferencias fenológicas (Trakhtenbrot *et al.* 2005, Nathan *et al.* 2008), entre otros.

El flujo génico en poblaciones relictas, aunque previsiblemente sea escaso, puede tener un efecto magnificado sobre la diversidad genética poblacional al producirse una intensa purga genética de individuos consanguíneos durante etapas sucesivas del reclutamiento (Hampe *et al.* 2013). La acción continuada de los procesos de purga en pequeñas poblaciones fragmentadas desde antiguo probablemente hayan minimizado problemas de incompatibilidad y depresión por endogamia asociados al cruzamiento entre vecinos, que amenazan poblaciones de aislamiento reciente. De cualquier modo, la conectividad y un mínimo número de individuos en estas poblaciones de árboles parecen clave para su mantenimiento a largo plazo (Sexton *et al.* 2011), más aún cuando el tamaño efectivo poblacional puede estar muy reducido debido a la gran desigualdad en la fecundidad de árboles individuales (Oddou-Muratorio *et al.* 2005, Moran & Clark 2012). El estudio del flujo génico a distintas escalas en poblaciones relictas es vital ya que constituye un elemento clave para garantizar la diversidad genética, la reproducción y en último término el potencial evolutivo de estas poblaciones.

ÉXITO REPRODUCTIVO EN POBLACIONES RELICTAS

Las poblaciones pequeñas y aisladas son susceptibles de experimentar fenómenos de limitación de polen, ya que cierta dispersión puede ocurrir pero generalmente no en una cantidad o calidad adecuada para una óptima producción de semillas (Knapp *et al.* 2001, Sork *et al.* 2002, Wilcock & Neiland 2002, Jump & Peñuelas 2006). El fallo de la polinización tiene un impacto directo sobre el reclutamiento de plántulas al verse reducida la contribución a la lluvia de semillas (Ashman *et al.* 2004). Estos problemas se agravan en especies que dependen casi completamente del cruzamiento con otros individuos (*outcrossing*) o que presentan tasas de autopolinización mínimas. Las especies de árboles presentan distintos rasgos que ayudan a sobrellevar situaciones donde la limitación de polen tiene lugar, como su gran longevidad y reproducción iterópara (que determinan un mayor solapamiento de generaciones y por tanto del tamaño poblacional efectivo), la sincronización inter-anual de la reproducción e intra-anual de la fenología o incluso la hibridación con especies cercanas (Ghazoul 2005, Petit & Hampe 2006). Algunas teorías que explican la sincronización de una reproducción intermitente, fenómeno conocido como vecería o *masting* (común en especies del género *Quercus*) se apoyan en la limitación de polen para explicar la sincronización de la floración de los individuos de una población (Koenig *et al.* 2015, Pearse *et al.* 2015). La selección tendería a hacer coincidir la floración de los individuos que de otro modo se exponen a una nube de polen poco densa, con consecuencias negativas para su *fitness*. Por otra parte, el flujo génico entre especies interfértiles es un fenómeno ampliamente observado, al que se ha reconocido un papel primordial en procesos evolutivos (p. ej., especiación, dinámicas de sucesión, etc.) (Lepais & Gerber 2011, Petit *et al.* 2004), y se ha propuesto como mecanismo de rescate del efecto Allee que emerge a bajas densidades poblacionales (Chybicki *et al.* 2012). Muchos estudios apoyan la hipótesis del rescate genético en condiciones de limitación de polen, al demostrar el efecto de la abundancia relativa de las especies implicadas sobre la frecuencia y la dirección de la introgresión (Lepais *et al.* 2009, Lagache *et al.* 2013, Chybicki *et al.* 2012).

Otros procesos como el aumento de la auto-fecundación como un fenómeno denso-dependiente, extendido en poblaciones al margen de su distribución (Restoux *et al.* 2008), poblaciones de pequeño tamaño (Robledo-Arnuncio *et al.* 2004) y poblaciones con bajas densidades (Ward *et al.* 2005) tiene consecuencias dudosas sobre el fitness de los individuos. Aunque se reconocen una serie de ventajas evolutivas asociadas a los sistemas de cruzamiento mixtos (p. ej., asegurar la reproducción, mejorar la transmisión de alelos beneficiosos y facilitar la purga de la carga genética) (Knight *et al.* 2005, Cheptou & Schoen 2007), en especies de árboles el fuerte coste de la depresión por consanguinidad hace improbable que semillas autofecundadas se establezcan exitosamente como individuos adultos (Petit & Hampe 2006).

Los mecanismos arriba mencionados junto a la extraordinaria fecundidad, hacen a las especies forestales menos dependientes de la producción anual de semillas, pudiendo soportar grandes variaciones en el éxito reproductivo y el reclutamiento sin que se vea afectada cierta tasa de crecimiento poblacional a largo plazo (Hylander & Ehrlen 2013). En el margen meridional, la adversidad del clima al que se enfrentan las poblaciones sumado a los efectos de la limitación de polen, a menudo limitan la regeneración a unos pocos episodios particularmente húmedos (Hampe & Arroyo 2002). El estudio de la fecundidad y el éxito reproductivo representa un buen indicador del *fitness* de las plantas y ofrece una primera conexión entre la efectividad de la polinización y el potencial de regeneración que poseen las poblaciones.

OPORTUNIDAD QUE OFRECEN LAS POBLACIONES RELICTAS COMO SISTEMA DE ESTUDIO

Comprender la diversidad de los procesos biológicos y los factores que los determinan depende estrechamente de la escala espacial y temporal a la que son estudiados, de manera que emergen preguntas como: ¿son consistentes a lo largo del tiempo los patrones de flujo génico y reproducción? ¿como varían los patrones de dispersión de polen entre individuos (escala local) y entre poblaciones (escala de paisaje)? ¿Cuán importantes son las consecuencias de esta variación a distintas escalas sobre el *fitness* de los individuos y las dinámicas poblacionales?

Las poblaciones de árboles relictas constituyen un sistema natural cuyas peculiares características permiten abordar un estudio integral cubriendo distintas escalas temporales y espaciales. La aproximación en el eje temporal es posible gracias al escenario de aislamiento a largo plazo (*versus* poblaciones de aislamiento reciente) donde los actores son organismos muy longevos que permiten el estudio de su historia pasada al retener la huella de los procesos a los que se han visto sometidos (i.e., selección, flujo génico y deriva génica) (Sork *et al.* 1999). Los patrones históricos de flujo génico y divergencia basados en la estructura genética de los individuos adultos, pueden ser complementados y contrastados con estimas precisas del flujo génico contemporáneo dando robustez a los procesos inferidos. Por otra parte, es posible una aproximación a distintas escalas espaciales dada la configuración de las poblaciones en núcleos dispersos a través del paisaje que permiten establecer varias escalas de estudio: los individuos, los núcleos o rodales de población y el paisaje. En las poblaciones naturales, los individuos se exponen a una gran heterogeneidad de ambientes abióticos y bióticos que afectan desde el modo en que se recibe el polen hasta el éxito reproductivo. Analizar la variación del modo de dispersión, el sistema de cruzamiento o la reproducción a escala de individuos es imprescindible ya que el abordaje a escalas superiores a menudo impide desvelar el mecanismo fundamental tras los patrones encontrados (Robledo-Arnuncio *et al.* 2004, García *et al.* 2005, Oddou-Muratorio *et al.* 2006, Chybicki & Burczyk 2013, Gaüzère *et al.* 2013). Además, la variación de estos procesos entre poblaciones adquiere gran relevancia en escenarios relictos donde la dinámica a escala de paisaje juega un papel clave pudiendo compensar o evitar extinciones locales (Lesser & Jackson 2013). La aproximación a múltiples escalas que ofrecen estas poblaciones es de gran valor para adquirir un profundo conocimiento de los procesos biológicos y los principales factores ecológicos que los determinan.

Adicionalmente, la fisionomía de las poblaciones relictas proporciona dos ventajas experimentales únicas para los estudios de dispersión efectiva de polen (p. ej., Jones 2003, Robledo-Arnuncio 2011). El muestreo completo de todos los individuos en una amplia y aislada área geográfica permite la discriminación total de la gran mayoría de los donantes de polen mediante análisis de paternidad y por consiguiente, el rastreo del movimiento de polen a través del paisaje (Broquet & Petit 2009). Esto permite caracterizar de forma precisa la componente de dispersión de polen a larga distancia a menudo difícilmente descrita (Ashley 2010). Asimismo, disponer de información genética tanto para los árboles adultos como para sus progenies permite realizar una aproximación demo-genética a los factores ecológicos que gobiernan el flujo genético y la reproducción en estas poblaciones. De este modo, podemos testar de forma integrada hipótesis relacionadas con la distribución espacial de los individuos (p. ej., variación en la densidad poblacional, la densidad del vecindario o el aislamiento geográfico), distintos rasgos genéticos (p. ej., la diversidad genética, el nivel de endogamia, y el parentesco entre árboles cercanos), y caracteres intrínsecos a los individuos (p. ej., el tamaño del árbol, la producción de polen, y la fenología).

INFORMACIÓN DE INTERÉS DE LA ESPECIE Y ÁREA DE ESTUDIO

Los robles (género *Quercus*) son especies de árboles dominantes en el hemisferio norte que representan piezas clave para la biodiversidad de los ecosistemas terrestres desde zonas tropicales hasta boreales. Su distribución en Europa ha cambiado repetidamente durante los periodos interglaciales y glaciales. Al final del último máximo glacial, los bosques de robles quedaron restringidos a la Península Ibérica, Italia y los Balcanes, desde donde expandieron alcanzando el amplio rango actual (Petit *et al.* 2002). El roble pendunculado o roble carballo (*Q. robur*), especie modelo de estudio de la presente tesis, se distribuye a través de la Europa templada y su límite sur alcanza la Península Ibérica donde ocupa zonas de influencia oceánica de la región noroccidental (Ducousso & Bordacs 2004). Las poblaciones objeto de estudio forman parte del margen suroeste del rango de distribución de la especie, en la región más occidental del Sistema Central (norte de la provincia de Cáceres, Extremadura). Diversos estudios evidencian la distribución en el pasado del grupo de los robles blancos (entre ellos *Q. robur*) en esta región del centro peninsular durante el Último Máximo Glacial (19-26,5 kyr BP, Clark *et al.* 2009), así como en áreas refugio de los Pirineos y noroeste (Olalde *et al*. 2002, Petit *et al*. 2002). Sin embargo, ni el registro polínico ni los estudios filogeográficos permiten distinguir *Q. robur* de sus congéneres. La similitud existente entre la distribución actual de *Q. robur* y la reconstrucción del pasado inferida mediante modelado (Benito *et al*. 2007) sugiere que las poblaciones bajo estudio han experimentado un largo e intenso aislamiento respecto a las áreas de influencia atlántica, donde la especie fue capaz de hacerse abundante y ampliar su territorio. El clima en este área es localmente templado y húmedo lo que permite mitigar la adversidad del clima mediterráneo que predomina a escala regional. Además, aquí las poblaciones de *Q. robur* generalmente se restringen a zonas próximas a cursos de agua (ver Figura 1.3).

Los robles pedunculados son grandes árboles caducifolios que alcanzan una altura de 30-40 m y pueden vivir más de 800 años (véase Figura 1.4). Esta especie y en general el género *Quercus* se caracteriza por una reproducción sincronizada e intermitente (fenómeno conocido como vecería o *masting*) con años donde se reproducen la gran parte de los individuos y años con una tasa de reproducción mínima. Al igual que sus congéneres, presentan una polinización típicamente anemófila con tasas variables de autofecundación o hibridación con especies hermanas dependiendo del individuo y del contexto (Chybicki *et al.* 2012, Lepais *et al.* 2009, Lagache *et al.* 2013). En el área de estudio *Q. robur* solo coexiste con el roble melojo (*Q. pyrenaica*), siendo posible la hibridación entre ellos al solapar parcialmente sus periodos de floración (ver Figura 1.5). El roble pedunculado alcanza su edad de producir bellotas entorno a los 40 años, y presenta desde entonces una producción alterna entre años. La dispersión de las bellotas está mediada por aves (especialmente el arrendajo, *Garrulus glandarius*), ardillas y otros roedores (den Ouden *et al*. 2005).

CUESTIONES ABORDADAS

Esta tesis ha sido motivada por la necesidad de aportar conocimiento acerca del funcionamiento y perspectivas de las poblaciones de árboles en el margen de retaguardia del rango de distribución de las especies. El objetivo general ha sido evaluar la vulnerabilidad de las poblaciones relictas ante el clima cambiante y profundizar en los mecanismos que les permiten persistir durante largos periodos de tiempo en condiciones próximas a los límites de tolerancia. Los aspectos estudiados en el proyecto constituyen una información fundamental para ayudar a predecir la respuesta de las poblaciones forestales en el futuro (i.e. persistencia, extinción o cambios de rango), ante la amenaza que supone el cambio climático.

El marco conceptual en el que se encuadra la tesis es reforzado con un primer estudio (capítulo 1) basado en un meta-análisis a gran escala con el que se pretende aportar evidencias empíricas que demuestren una diferencia en *performance* de poblaciones localizadas en los márgenes del rango de distribución (i.e., latitudes altas vs. bajas) respecto a poblaciones centrales, de acuerdo a las predicciones del cambio climático actual. Debido al negativo pronóstico que acecha a las poblaciones del margen de retaguardia, el énfasis de los siguientes trabajos se centra en desvelar los mecanismos que han contribuido a garantizar la supervivencia de estas poblaciones a largo plazo haciendo frente a grandes oscilaciones climáticas. Con este objetivo, empleo un conjunto de poblaciones relictas de *Q. robur* en el límite meridional de su rango de distribución para estudiar dos procesos biológicos clave para el funcionamiento de las poblaciones vegetales: (i) la dispersión, especialmente vía polen (capítulos 2 y 3) y (ii) el potencial de regeneración medido como fecundidad (capítulo 4). La variabilidad de estos procesos es caracterizada a distintas escalas (desde el individuo hasta el paisaje) y sus principales determinantes ecológicos y genéticos son investigados. Los patrones de flujo génico histórico y contemporáneo se examinan a escala superior (de paisaje y población) (capítulo 2), para después profundizar en la variabilidad entre individuos en los patrones de cruzamiento y los agentes actuando a escala más local (capítulo 3). Por último, los patrones de fecundidad y sus determinantes son estudiados a distintas escalas evaluando hipótesis relacionadas con la dispersión efectiva de polen (capítulo 4) como una manera indirecta de desvelar situaciones de limitación de polen en nuestras poblaciones relictas.

Las cuestiones específicas que han sido abordadas se exponen a continuación:

¿Existen evidencias empíricas que indiquen un cambio en la *performance* de poblaciones marginales, de acuerdo a lo que predice el cambio climático actual? (Capítulo 1)

La aproximación a esta pregunta de escala global se basa en una rigurosa revisión bibliográfica de cientos de artículos que estudian diferencias en el fitness de poblaciones marginales respecto a poblaciones del rango continuo. Los datos que proporcionan los casos de estudio seleccionados (n = 47) se analizan mediante técnicas de meta-análisis que permiten obtener tendencias generales a partir de los resultados obtenidos en cada estudio particular.

¿Qué patrones de flujo génico histórico y contemporáneo presentan las poblaciones relictas de *Q. robur* y cómo se ven afectados por el escenario relictual? (Capítulo 2)

Este estudio se beneficia del muestreo exhaustivo de todos los adultos presentes en en el área de estudio (n = 135), y las progenies muestreadas sobre 72 árboles madre (n = 724). El modelado del flujo génico histórico se basa en el análisis de la topología de redes poblacionales (Dyer *et al.* 2010), que complementa la estructura genética inferida para el conjunto de núcleos poblacionales (n = 10) mediante técnicas de "clustering" genético. Mientras que el estudio de la dispersión contemporánea de polen a escala de paisaje ha sido abordado a través de tests de paternidad, un método potente y directo para rastrear los movimientos de polen en poblaciones pequeñas y aisladas.

¿Existe variación entre individuos de *Q. robur* en los patrones de cruzamiento y dispersión de polen y qué relación guarda con factores intrínsecos y del ambiente local? (Capítulo 3)

El análisis de paternidad realizado en el capítulo anterior se complementa con modelos de cruzamiento espacialmente explícitos (Klein *et al.* 2008) que profundizan en los patrones de polinización dentro de los núcleos poblacionales. Estos modelos (denominados *mixed-effects mating models,* MEMM) permiten inferir la red de cruzamiento entre los individuos de la población y caracterizar las componentes femenina y masculina del proceso de polinización. Entre las variables que explican la variabilidad entre individuos se incluyen factores ecológicos y genéticos del ambiente próximo e intrínsecos de los individuos.

¿Cómo afectan la configuración de las poblaciones y los rasgos de los árboles individuales a los patrones de fecundidad de *Q. robur* en ambientes relictos? ¿Se evidencian restricciones genéticas en los patrones de cruzamiento? (Capítulo 4)

El estudio de los patrones de fecundidad en poblaciones relictas de *Q. robur* se basa en el seguimiento durante 2 años de la producción de flores y frutos en 159 árboles dispersos a través de 12 núcleos de población. Se testa el efecto que de diversos factores ecológicos y genéticos relacionados con la dispersión efectiva de polen a múltiples escalas sobre los patrones de fecundidad observados.

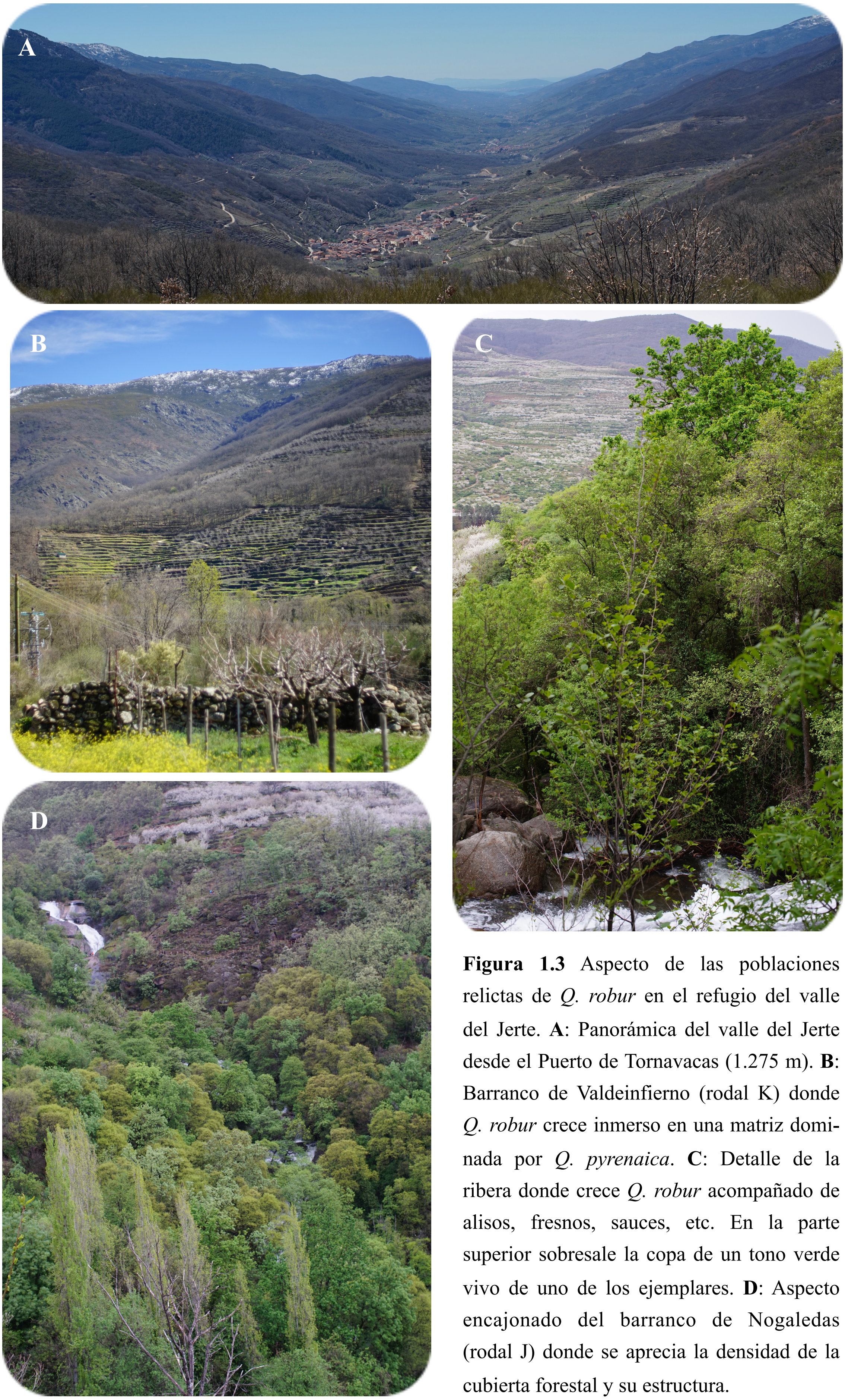

Figura 1.3 Aspecto de las poblaciones relictas de *Q. robur* en el refugio del valle del Jerte. **A**: Panorámica del valle del Jerte desde el Puerto de Tornavacas (1.275 m). **B**: Barranco de Valdeinfierno (rodal K) donde *Q. robur* crece inmerso en una matriz dominada por *Q. pyrenaica*. **C**: Detalle de la ribera donde crece *Q. robur* acompañado de alisos, fresnos, sauces, etc. En la parte superior sobresale la copa de un tono verde vivo de uno de los ejemplares. **D**: Aspecto encajonado del barranco de Nogaledas (rodal J) donde se aprecia la densidad de la cubierta forestal y su estructura.

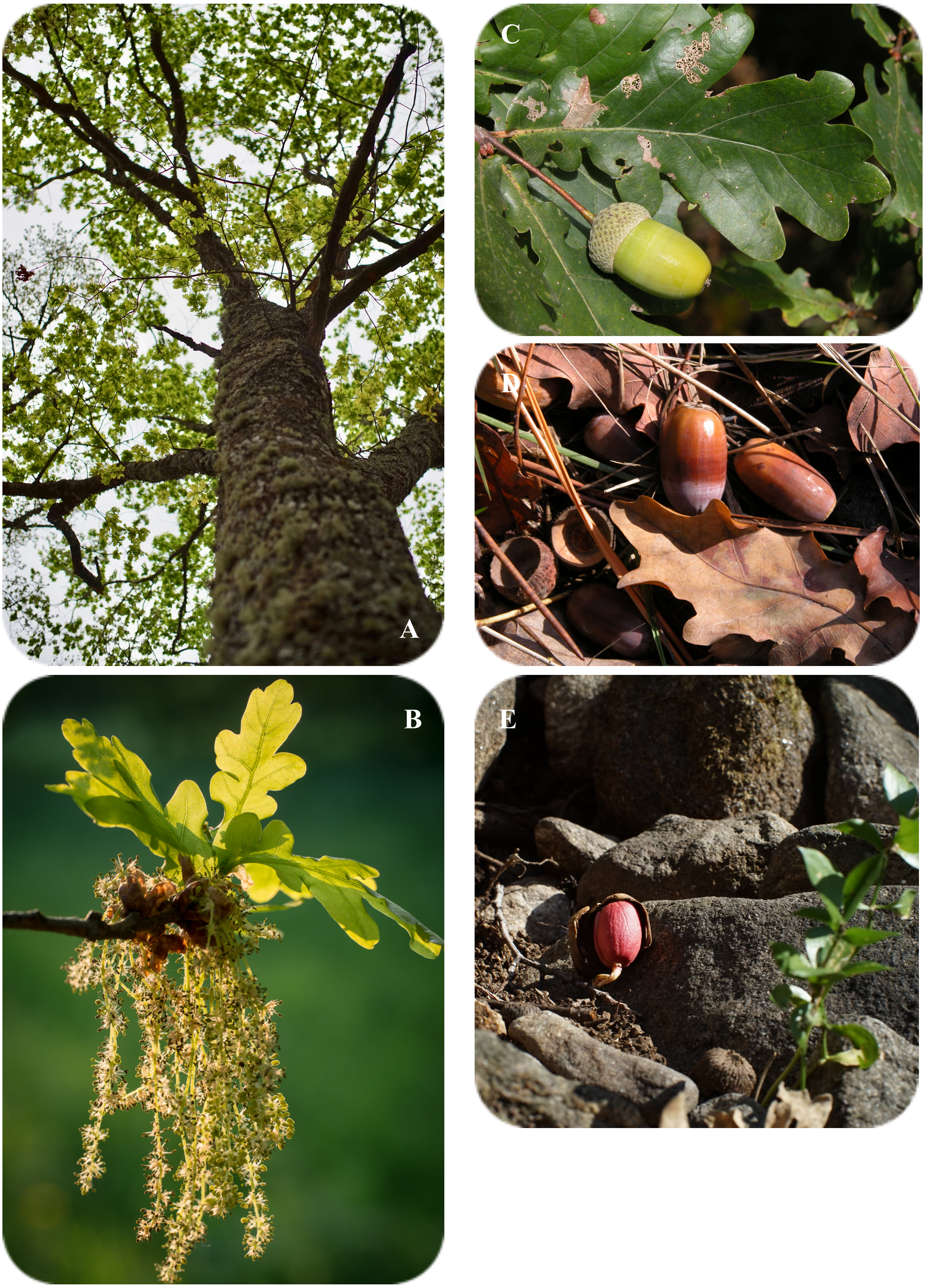

Figura 1.4 **A**: Porte de un individuo de *Q. robur* con un desarrollo foliar incipiente. **B**: Detalle de las flores masculinas maduras en amentos colgantes, verdes-amarillentos, que nacen solitarios o en grupos de ramillas del año anterior. **C**: Detalle de las hojas simples, lobuladas y lampiñas de color verde intenso por el haz, y las bellotas colgantes de un largo pedúnculo (rasgo que motiva su nombre común). **D**: Detalle de las hojas caducas y las bellotas caídas en octubre. **D**: Detalle de una bellota de *Q. robur* germinada en la orilla de un arroyo.

Figura 1.5 A: Expansión foliar en el robledal de Valdeinfierno durante el mes de abril. Destaca la regeneración de *Q. pyrenaica* (roble melojo) en este área donde se ha producido el abandono de los cultivos ante su declaración como espacio natural protegido. **B**: Detalle de la fenología de ambas especies de robles, donde se hace patente la fenología más temprana de *Q. robur* respecto a la de *Q. pyrenaica*.

CAPÍTULO 2

Asymmetry in marginal population performance foreshadows widespread species range shifts

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In review, Febrero 2019.

ABSTRACT

While current climate change is altering the distribution of species worldwide (IPCC 2014), a poor understanding of the mechanisms involved limits our ability to predict future range dynamics. Range shifts are expected to occur when populations at one range margin perform better than those at the other margin (Svenning & Sandel 2013), yet no such global trend has been demonstrated empirically. Here we show that populations at high-latitude range margins generally perform as well as those from the range centre, whereas populations at low-latitude margins perform markedly worse. The trend is moderate but pervasive across plants and animals and terrestrial and marine environments. Such asymmetry in performance between range edges has not been reported before and signals that species are in disequilibrium with current environmental conditions. Our findings are consistent with predicted impacts of a warming climate and imply that the geographic ranges of species are undergoing directional changes. They highlight the pressing need for a more thorough knowledge of population dynamics across species ranges as a mean to forecast climate change impacts on the structure and function of ecosystems across the globe.

Ongoing climate changes are anticipated to result in major impacts on life on Earth (IPCC 2014). As these changes continues, increasing mismatches between current conditions and the climate to which populations are adapted are predicted to create range-wide asymmetry in population growth rates (Svenning & Sandel 2013), with positive rates at expanding and negative rates at contracting species range edges. Such an asymmetry has been hypothesized to be the principal biological mechanism driving large-scale geographical range shifts (Parmesan *et al.* 1999, Sexton *et al.* 2009, Lenoir & Svenning 2015). Yet we do not know how widespread asymmetries are globally. Although population growth rates are difficult to monitor directly, they are the result of demographic processes, such as survival and fecundity, which are possible to observe. Quantifying the global extent of asymmetry in measures of population performance should allow us to assess existing disequilibrium of species ranges with climate and hence the propensity of species to shift their range. Such knowledge is crucial to accurately forecast future climate-driven range shifts (Dullinger *et al.* 2012, Normand *et al.* 2013) and changes in ecosystem functioning, and for informing resource use and conservation planning.

Changes in the performance of marginal populations should represent a much more direct and immediate indicator of species' response to climate warming than the more widely monitored distribution changes (Chen *et al.* 2011). Although the implicit assumption of most studies on range shifts is that climate, either directly or indirectly, is a major determinant of species ranges, range limits can also be triggered by non-climatic constraints, such as habitat availability, dispersal limitation, or, notably, biotic interactions (Louthan *et al.* 2015), and it is a matter of intense debate to which extent range limits reflect different constraints (Hargreaves *et al.* 2014, Lee-Yaw *et al.* 2016, Pironon *et al.* 2017). Even when range limits are directly determined by contemporary climate, its effects on population dynamics might difficult to detect except in meteorologically extreme years. Detailed real-time observations of marginal population dynamics remain rare, especially for contracting range margins (Hill *et al.* 2011, Pearce-Higgins *et al.* 2015). The scant empirical evidence currently prevents wide-ranging comparisons of population dynamics at expanding and retreating range edges.

Here, we use the abundant empirical literature spawned by the so-called centre-periphery (CP) paradigm to examine differences in performance between range centres and high- and low-latitude margins for a wide range of taxa. This paradigm states that the size, density and long-term growth rate of populations tend to decrease from the centre towards the periphery of the range as environmental conditions become increasingly less favourable (Brown 1984, Sagarin *et al.* 2006, Sexton *et al.* 2009) (Fig. 2.1). The CP paradigm has spawned hundreds of empirical studies that have compared numerous indicators of population performance (including measures of individual survival or fecundity, population viability and others) in central and marginal populations. We use a comprehensive sample of published studies to compare measures of population performance in sites located at the centre and at the high-latitude margins (HLM) or low-latitude margins (LLM) of species ranges. We predict that if impacts of ongoing climate change on population performance are widespread, then HLM populations should perform as well as central populations whereas LLM populations should perform worse (Fig. 2.1). To test this prediction, we quantify the empirical support for this hypothesized asymmetry in the performance of HLM and LLM populations compared to central populations, and test if patterns are consistent across taxonomic kingdoms (plants *vs*. animals) and habitats (marine *vs*. terrestrial). We also predict that if climate is a real driver of population performance, then the difference in population performance should increase with the difference in climate between central and marginal populations (Fig. 2.1). To test this

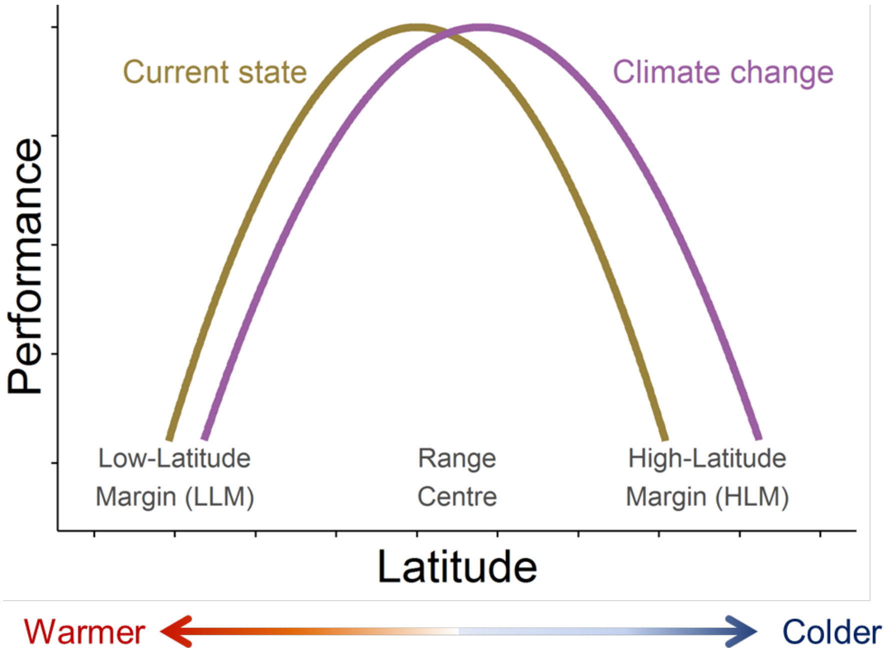

Figure 2.1 The centre-periphery hypothesis postulates that population performance is maximal around the range centre and decreases towards the margins of the distribution range, as environments become less suitable. Under current climate change, the optimal climate zones would displace polewards so that high-latitude populations (HLM) would increase their fitness whereas low-latitude populations (LLM) would experiment a decrease.

prediction, we relate the observed differences in performance between central and peripheral populations with the actual differences in climate.

We searched the scientific literature for peer-reviewed publications published by 23rd May 2017 using keywords related to CP comparisons of population performance, retaining papers that provided data for at least two populations from the range centre and two populations from one latitudinal range margin (HLM or LLM), respectively, each occurring in the species' natural environment. We only considered primary papers reporting demographic performance metrics that could clearly be identified as estimators of individual fecundity, individual survival, or lifetime fitness. We identified a total of 96 CP comparisons involving 623 populations in the 42 papers that fulfilled our criteria (HLM: $n = 58$, LLM: $n = 38$; see Methods and Appendix A). We conducted a mixed-effects meta-analysis on all those studies reporting information that allowed the calculation of effect sizes (*i.e.,* mean, standard deviation and sample size). We used Hedges' d effect sizes for a standardized comparison of performance in central

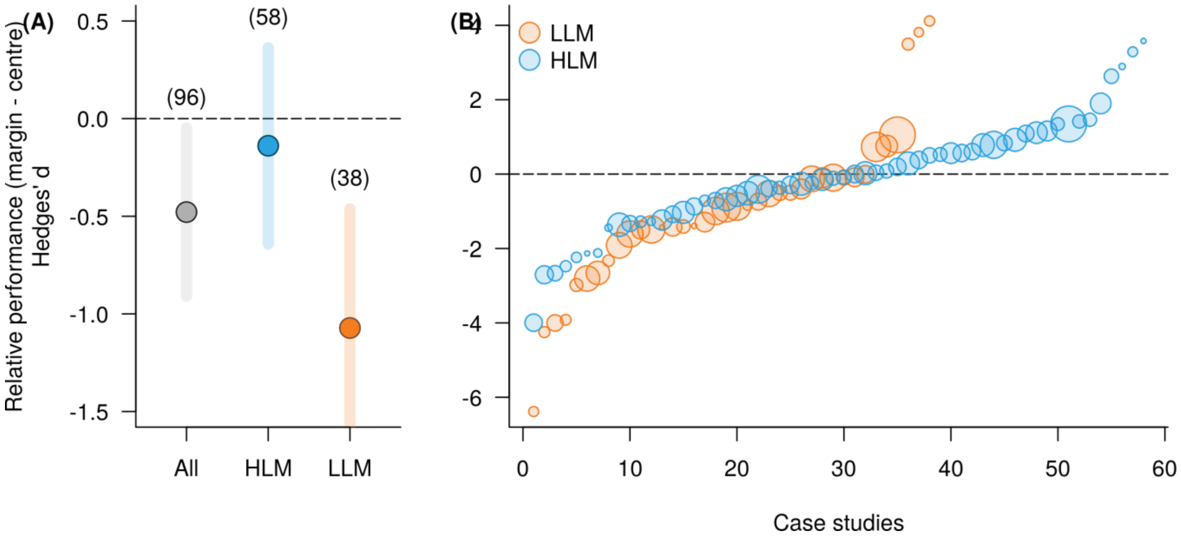

Figure 2.2 Observed differences in performance (Hedges'*d* effect sizes) between marginal (high-latitude, HLM, and low-latitude, LLM) and central populations across all species and studies. **(A)** Grand mean (grey) and margin-specific (blue and orange) combined effect sizes. Error bars represent 95% confidence intervals. Numbers in parentheses correspond to the number of case studies. **(B)** Individual effect sizes for HLM and LLM case studies ranked from the lowest to the highest value. Dot size is proportional to the weight of individual effect sizes in the meta-analysis. Both in **(A)** and **(B)**, positive and negative values indicate higher and lower performances in marginal than in central populations, respectively. Horizontal dashed lines represent the null hypothesis of no difference in the performance of central vs. marginal populations.

Grand mean effect size (± 95% CI) was negative (-0.37; 95% CI: -0.71, -0.04), meaning that marginal populations on average performed worse than central populations. There was a significant amount of total heterogeneity, with 61% of it arising from among-study heterogeneity ($\tau^2 = 1.49$, $I^2 = 0.61$, $Q_E = 289.27$, $P < 0.0001$). Performance declined from the range centre towards the LLM (-1.07; 95% CI: -1.67, -0.47), estimated from the model with Margin as the sole moderator but not towards the HLM (-0.14; 95% CI: -0.64, 0.36) (Fig. 2.2). Thus, HLM populations showed overall similar performance as central populations. Margin type was the most important moderator ($w_H = 0.96$) but the best model only explained 4% of total heterogeneity (HLM-LLM difference: $z = -2.69$, $P = 0.007$). Residual heterogeneity (best model: $\mathrm{Q_E} = 260.63$, $P < 0.0001$) was neither explained by habitat ($w_H = 0.69$, difference between marine and terrestrial habitats in the best model: $z = –1.55$, $P = 0.121$) nor kingdom ($w_H = 0.54$; difference between animals and plants in the best model: $z = 1.33$, $P = 0.184$) (Appendix B: table B1; Appendix C: figure C1).

The differences in performance between marginal and central populations were moderately but significantly related to the difference in their average temperature in the period 1990–2013 (Fig. 2.3; Appendix F: table F1; total deviance explained by an additive mixed model: 24.9%). As expected, performance decreased with increasingly departing temperatures from central populations, while the decline was stronger in LLM than in HLM populations. Thus, HLM populations 5 ºC colder than central populations have similar fitness, whereas 5 °C warmer LLM populations perform worse (Fig. 2.3). These differences in performance were not related to geographical distance between marginal and central populations (Appendix F: figure F1).

Overall, our study revealed that populations from the centre of the range tend to outperform those residing at the LLM but not those at the HLM. Albeit indirectly, this implies that HLM populations tend to outperform LLM populations. Such latitudinal asymmetry is predicted when the environmental conditions relevant for population performance are directionally displaced (Fig. 2.1) (Lenoir & Svenning 2015). Global warming has provoked a rapid large-scale poleward displacement of climatic zones since the 1970s, and the trend is predicted to further accelerate through the coming decades (IPCC 2013). Changes in population performance in response to environmental or climate change are expected to occur with no or only short time lags, and the observed difference is therefore likely to largely result from ongoing climate change (although we cannot exclude effects of changes in factors unrelated with current climate) (Normand *et al.* 2011, Hargreaves *et al.* 2014, Louthan *et al.* 2015). Hence, demographic rates could act as early warning signals of impending range shifts.

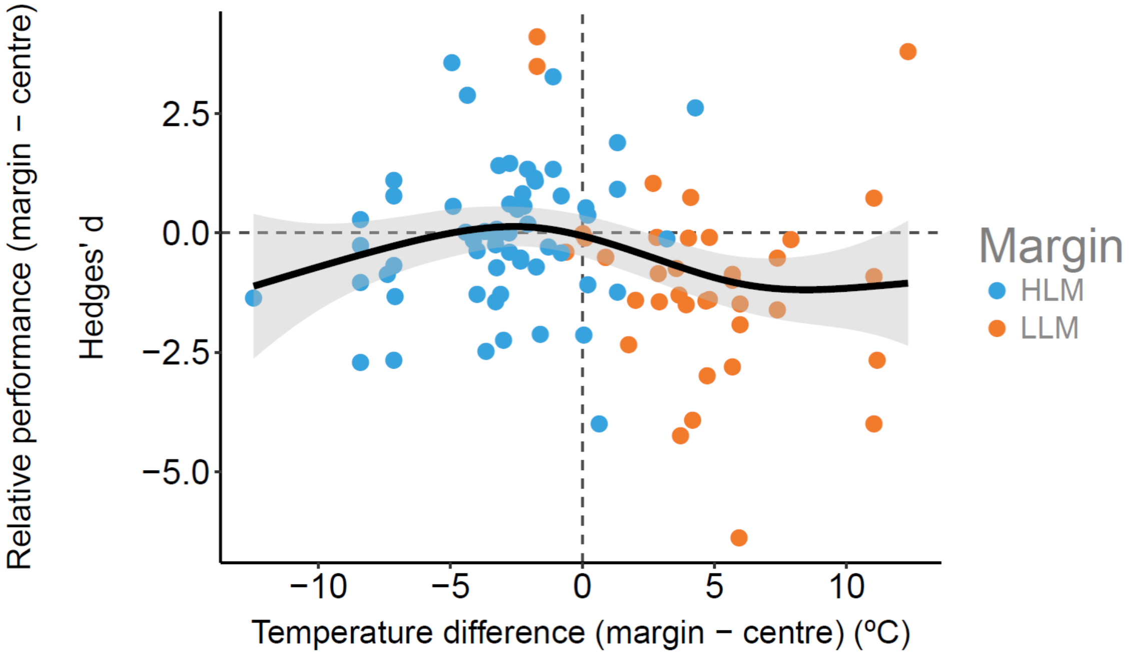

Figure 2.3 Relationship between the observed difference in performance (Hedges' *d*) and the difference in average temperatures between peripheral and central populations for the period 1990-2013 (when most studies were performed). Positive values of Hedges' *d* indicate better performance in the margin compared to central populations, and vice versa. The curve represents the fit of a generalized additive mixed model with temperature and study as predictors. The shaded area represents the 95% confidence interval. HLM = high-latitude margin, LLM = low-latitude margin.

The type of range margin (HLM or LLM) explained only a moderate 4% of the overall variation in the relative performance of marginal populations. This is unsurprising given the great variety of organisms, response variables, and ecological contexts considered in our analysis. In addition, most primary studies reported only short-term data that are likely to stem from meteorologically 'normal' years (whereas range shifts might primarily be catalyzed by extreme years) (Zimmermann et al. 2009). Finally, performance at some specific life stages is not necessarily a reliable predictor of lifetime fitness and population growth rates (Villellas *et al.* 2015, Lee-Yaw *et al.* 2016). Despite these limitations, the type of range margin was the most relevant predictor of performance in marginal populations. Our findings imply that latitudinal asymmetries exist worldwide, for animals as well as plants, and for terrestrial as well as marine species (Appendix A; Appendix C: figure C2). This pervasive nature of the phenomenon is the more striking as climatic constraints and the responses of populations differ greatly between groups of organisms. For instance, plants generally tend to have a greater capacity to buffer climatic stress through phenotypic plasticity and persistent life cycle stages enhancing resilience than animals (Villellas *et al.* 2015), which would allow them to slow population declines and accumulate a higher extinction debt (Jackson & Sax 2009, Jump *et al.* 2009). Moreover, climate is shifting at different pace in marine and terrestrial environments, with median temperatures increasing more than three times faster on land than at sea (Burrows *et al.* 2011). Water temperature and related properties drive population dynamics of marine species, whereas many LLM populations of terrestrial species are primarily constrained by water balance (Hampe & Jump 2011). This difference may also explain why marine ectothermic animals tend to more fully occupy the latitudinal ranges situated within their thermal tolerance limits than terrestrial ectotherms, which are commonly absent in the warmest parts of their potential range (Sunday *et al.* 2012). However, even these important differences between organisms and environments do not blur the effect of the range margin as the most consistent predictor of population performance.

Overall, our results demonstrate that latitudinal asymmetry in marginal population performance is a common phenomenon, indicating that many extant species ranges are not in equilibrium with current climates even though they have to date not experienced perceivable shifts. They thus underpin that differences in population performance can represent a powerful early indicator of impending range shifts (Parmesan *et al.* 1999, Lenoir & Svenning 2015), and that considering empirical fitness trends in marginal populations will substantially increase the realism of population-based approaches to species distribution modelling (Anderson *et al.* 2009, Mair *et al.* 2014). Given that latitudinal range shifts are likely to be ongoing or impending for many species, such improved predictive capacity is needed if we are to forecast their implications for biodiversity and ecosystem function. Disequilibrium dynamics are likely to become increasingly pervasive across the next 50–200 years (Svenning & Sandel 2013), and the present results show that they are already widespread. Given their potentially massive impact of climate and environmental change on the future distribution of species and communities (Dullinger *et al.* 2012, Normand *et al.* 2013) and on ecosystem functions and services upon which we depend as human populations, there is urgent need for an improved understanding of the mechanisms underlying such changes.

METHODS

Data compilation – We searched the Thomson Web of Knowledge® and Scopus until 23rd May 2017 for publications in peer-reviewed international scientific journals using key search terms in the title or the abstract. In addition, we searched Google Scholar using the same terms in the whole text of articles and restricting our selection to the first 200 references. The terms 'centre-periphery', 'central-marginal', 'abundant centre', and ‘latitudinal cline’ were introduced in combination with performance related terms including ‘fecundity’, ‘performance’, ‘survival’, ‘recruitment’ and ‘population growth rate’. We identified additional papers by searching the literature cited sections of these articles.

Selection criteria and data collection – Three filters were applied to the obtained collection of primary papers. First, we only considered studies reporting field data from natural populations (including control populations of transplant experiments if these were measured at their home sites and met all other criteria). Second, we only considered studies with at least two central and two peripheral populations (i.e., true replicates). Third, we only considered papers that provided sufficiently clear criteria for the definition of central and peripheral range parts relative to the global range of the target species. This filtering procedure resulted in a total of 42 retained primary papers with 96 CP comparisons of 44 species including woody plants (17%), herbs (45%), different invertebrates (27%), birds (6%), and reptiles (5%), with 31 (70.5%) being terrestrial and 13 (29.5%) marine organisms (see the list in Appendix A).

We extracted the reported performance metrics from each primary paper and assigned them to one of the following categories: (i) ‘Survival’ (e.g. mortality of individuals or ramets, rates of fruit abortion or germination), (ii) ‘Reproduction’ (e.g. proportion of actively reproducing individuals, seed number, gonadal mass, total seed or egg mass), or (iii) ‘Lifetime fitness’ (different estimates of population growth rate). Moreover, we assigned each case study to one of two major categories of taxonomic status (plants *vs*. animals) and habitat (terrestrial *vs*. marine). Two major kinds of papers provided suitable information: i) explicit CP comparisons of mean performance values from populations classified as central or marginal by the authors, and ii) papers reporting on latitudinal clines. In the first case, we followed the criteria of the original authors for classifying populations as central or marginal. In the second case, we selected the three most central and the three most marginal populations along the gradient (rarely more if several populations were located closely together). We extracted quantitative data for our target metrics either manually from text and tables or from figures with Dagra digitizing software version 2.0.12 (http://www.blueleafsoftware.com/Products/Dagra/). We recorded average values for each individual population (Appendix A) and pooled them subsequently to calculate the average performance, sample size and resulting variance for C, HLM and LLM, respectively.

Effect Sizes – We used Hedges' *d* statistic as our standardised measure of effect size. Hedges' *d* is the most appropriate effect size to compare raw means when both positive and negative values are present in data[71]. Hedges' *d* was calculated as:

$$d = J \frac{\overline{X_{margin}} - \overline{X_{center}}}{s_{pooled}}$$

where

$$\sigma_{pooled} = \sqrt{\frac{\sigma_{center} \times \left(n_{center} - 1\right)^2 + \sigma_{margin} \times \left(n_{margin} - 1\right)^2}{n_{center} + n_{margin} - 2}}$$

and

$$v = \frac{n_{center} + n_{margin}}{n_{center} \times n_{margin}} + \frac{d^2}{2\left(n_{center} + n_{margin}\right)}$$

and $\overline{X}$, n and S^2 the mean, sample size and variance.

Negative values of *d* indicate lower performance in marginal (either HLM or LLM) populations than in central populations (consistent with the CP paradigm), whereas positive values indicate higher performance. The variance of effect sizes was:

$$v = \frac{n_{center} + n_{margin}}{n_{center} \times n_{margin}} + \frac{d^2}{2\left(n_{center} + n_{margin}\right)}$$

Note that *v* contains information about both the sample size and the standard deviation (within d^2) of the original studies; it hence can be used to weight the relative importance of studies within the meta-analysis (see also Fig. 2.2). In some papers, both HLM populations and LLM populations were compared to the same central populations, resulting in an overestimated pooled sample size ($N = n_{center} + n_{margin}$) because, for such primary papers, n_{center} is counted twice. We manually corrected *N* in all such cases before conducting the analysis.

Meta-analytical models – Our dataset had a hierarchical structure as some primary papers contained several case studies. We accounted for this potential non-independence of cases by estimating model variance from multiple sources: (i) among true effect sizes, (ii) among CP comparisons stemming from the same primary papers (by computing the variance-covariance matrix among all effect sizes) and (iii) among groups of moderators. This was done using multi-level error meta-analysis (Koricheva *et al.* 2013) with the *rma.mv* function of the R package *metafor* v. 2.0-0 (Viechtbauer 2010, R Core Team 2016).

Primary paper identity was declared as a random factor and individual CP comparisons were nested as random factor within primary papers. We estimated variance components for primary papers (σ_1^2) and case studies (σ_2^2) together with intra-class correlations (ρ), that is, correlations between true effect sizes from the same study (such that $\rho = \sigma_1^2 / (\sigma_1^2 + \sigma_2^2)$).

We first calculated grand mean effect size as the overall weighted mean across all effect sizes (Borenstein *et al.* 2007). This corresponded to a random-effect meta-analyses, where heterogeneity among true effect sizes (τ^2) is used to weight individual effects sizes (weight $= 1/(v + \tau^2)$), which allows inferences for CP comparisons not included in the analysis. Then, we used multi-level (hierarchical) meta-analyses to test the effect of three moderators: *Margin* (HLM vs. LLM), *Kingdom* (animals vs. plants) and *Habitat* (marine vs. terrestrial).We built a set of the 17 possible models including all possible combinations of simple effects ($n = 7$ models) and two-way interactions among *Margin*, *Kingdom* and *Habitat* ($n = 10$ models). We ranked these 17 models plus the null model (*i.e.*, intercept only) according to their AICc using the R package *glmulti* v. 1.0.7 (Calcagno 2013). For each model, we calculated ΔAICc and AICc weight (w_i). Models within ΔAICc < 2 typically are considered as competing best models, given the model set and the data (Appendix B: table B1). AICc weights represent the probability that a given model is selected as the best model. For each moderator, we then estimated its relative importance (w_H) by summing all w_i of the models including this moderator ($w_H = \Sigma w_i$). w_H can be interpreted as the probability that a given moderator is included in the best model (Appendix C: figure C1). Finally, we estimated model parameters for all competing models with ΔAICc < 2. We report model parameter estimates for the best model and, whenever necessary, for competing models (Appendix B: table B2).

Publication bias – Several assessments of inherent reliability of the meta-analysis, including publication bias, balanced representation of moderators, etc. is presented in Appendix D and E.

Collection of climate data – We gathered the geographical coordinates of all populations included in the meta-analysis from the primary papers (n = 623 populations; see map in Appendix A). For each population, we calculated the average annual temperature between 1990 and 2013 (when most studies were performed) based on monthly temperature data, from CRU TS 3.22 (Harris et al. 2014) for terrestrial species and HadISST 1.1 (Rayner et al. 2003) for marine species. We then aggregated populations to calculate average temperatures for each combination of study, species, performance variable, and region (either central, HLM, or LLM). We could then relate each comparison of performance between a margin (HLM or LLM) and the central range (i.e. Hedges' *d*) with the difference in average temperatures between both regions.

Analysis of relationships between climate and population performance – To assess the relationship between the differences in performance and the differences in climate between marginal and central populations, we used additive mixed models (function *gamm* in the R package *mgcv*, version 1.8-17; Wood 2006) using the temperature differences as predictor, and the study as random effect (to control for lack of independence). We weighted performance effect sizes by their variances so that their influence in model calibration was inversely related to their uncertainty.

ACKNOWLEDGEMENTS

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CAPÍTULO 3

Unusually limited pollen dispersal and connectivity of Pedunculate oak (*Quercus robur*) refugial populations at the species' southern range margin

Moracho, E., Moreno, G., Jordano, P. & Hampe, A. (2016)

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ABSTRACT

Low-latitudinal range margins of temperate and boreal plant species typically consist of scattered populations that persist locally in microrefugia. It remains poorly understood how their refugial habitats affect patterns of gene flow and connectivity, key components for their long-term viability and evolution. We examine landscape-scale patterns of historical and contemporary gene flow in refugial populations of the widespread European forest tree Pedunculate oak (*Quercus robur*) at the species' southwestern range margin. We sampled all adult trees (n = 135) growing in a 20 km long valley and genotyped 724 acorns from 72 mother trees at 17 microsatellite loci. The ten oak stands that we identified were highly differentiated and formed four distinct genetic clusters, despite sporadic historical dispersal being detectable. By far most contemporary pollination occurred within stands, either between local mates (85.6%) or through selfing (6.8%). Pollen exchange between stands (2.6%) was remarkably rare given their relative proximity and was complemented by long-distance pollen immigration (4.4%) and hybridization with the locally abundant *Quercus pyrenaica* (0.6%). The frequency of between-stand mating events decreased with increasing size and spatial isolation of stands. Overall, our results reveal outstandingly little long-distance gene flow for a wind-pollinated tree species. We argue that the distinct landscape characteristics of oaks' refugial habitats, with a combination of a rugged topography, dense vegetation and humid microclimate, are likely to increase plant survival but to hamper effective long-distance pollen dispersal. Moreover, local mating might be favoured by high tree compatibility resulting from genetic purging in these long-term relict populations.

KEYWORDS: genetic differentiation, genetic diversity, landscape scale, marginal populations, paternity analysis, rear edge

Many temperate and boreal plant species have populations along the low-latitudinal limit of their current distribution range that have persisted roughly in situ through the numerous major climate transitions of the Quaternary (Gavin *et al.* 2014). These so-called rear-edge populations are important targets for the preservation of the genetic diversity, phylogenetic history and evolutionary potential of species (Hampe & Petit 2005). They are, moreover, excellent models for studying local adaptation under natural conditions and for understanding the processes that help tree populations to successfully persist in an adverse abiotic environment (Woolbright *et al.* 2014). Rear-edge populations typically survive locally in microrefugia: areas with climatic conditions that have existed for an extended period of time but are currently rare within their surroundings ('relict climates' sensu Dobrowski 2010). Hence, populations are typically small, highly scattered and restricted to particularly favourable habitats within heterogeneous landscapes. It remains poorly understood which ecological mechanisms have enabled them to thrive under the constraints of their climate-driven long-term fragmentation (Hampe & Jump 2011). In particular, we know little about how their particular relictual distribution affects patterns of gene flow and population connectivity, a key component for their genetic diversity, long-term resilience and evolution.

Trees share life history characteristics that render them particularly resistant to the erosion of population genetic diversity, including a long lifespan, a prodigious fecundity and a great propensity for long-distance gene dispersal (Petit & Hampe 2006). A rapidly accumulating body of evidence indicates that pollen- mediated gene flow between tree populations regularly spans various kilometres (reviewed in Kremer *et al.* 2012). In particular, in wind-pollinated species, small and geographically isolated tree populations commonly experience non-negligible amounts of mating events with immigrant pollen arriving over great distances (e.g. Buschbom *et al.* 2011; Robledo-Arnuncio 2011; Lesser & Jackson 2013). This pollen-mediated gene immigration can be further exacerbated through the purging of inbred individuals during the subsequent recruit-ment process (Hampe *et al.* 2013). On the other hand, tree populations also are more dependent on efficient gene exchange than other plants because they are mostly self-incompatible and disproportionately susceptible to inbreeding depression (Petit & Hampe 2006). In addition, the effective population size of trees is often much smaller than apparent in the field because of great inequality in fecundity, with a few individuals contributing very many offspring and the great majority little or nothing (Oddou-Muratorio *et al.* 2005). Hence, a minimum size and connectivity of relict populations seem crucial for their fitness and long-term persistence in a changing environment (Sexton *et al.* 2011).

Relict tree populations at the rear edge of species ranges share many features with other small and isolated tree populations. However, they also assemble some specific characteristics that could render them particularly prone to experiencing lower levels of effective pollen flow. First, they typically grow far away (i.e. tens to hundreds of kilometres) from large pollen sources such as extensive populations belonging to the continuous distribution range. Second, their continued persistence at relatively low population size implies that they probably have undergone extensive genetic purging and may be suffering less from incompatibility and inbreeding depression than more recently isolated populations (such as pioneer stands at the leading edge or those resulting from recent anthropogenic fragmentation). Third, their particular relictual habitats usually are located in topographic settings that help maintain a constant minimum humidity (Hampe & Jump 2011), such as shady valleys, gorges or ravines; these habitats often occur in rugged terrain and contain a dense vegetation that tends to represent an obstacle to long-distance pollen flow (Damschen *et al.* 2014; Shohami & Nathan 2014). Hence, relict populations residing at the rear edge of species ranges could experience less long-distance gene flow than many other tree populations that have experienced fragmentation and isolation as a consequence of human activity (Kramer *et al.* 2008). Yet we still have a limited understanding of how landscape complexity combines with individual tree traits to result in the distinct fecundity patterns of relictual scenarios (Bacles & Jump 2011).

One of the most direct and powerful methods for studying the movement of genes within and among small, isolated populations consists in performing population-specific paternity tests. The precise detection of male parents and their spatial position allows to retrace pollen movements, providing information about the spatial patterns of mating across complex landscapes, and the potential effects of the landscape context on spatial genetic structure and contemporary gene flow (Klein *et al.* 2011). Documenting these patterns across networks of tree stands connected at a landscape scale can unveil demographic and environmental correlates of gene flow patterns (Dyer & Nason 2004; Cheptou & Schoen 2007; DiLeo *et al.* 2014) and ultimately mechanisms of isolation associated to their relictual situation. Finally, complementing these analyses with an in-depth assessment of historical gene flow patterns as reflected by the adult tree populations can reveal how consistent through time and hence biologically relevant observed patterns of pollen flow are.

Here, we use a particularly suited model system to examine landscape-scale patterns of historical and contemporary gene flow and connectivity in relict tree populations residing at the southwestern range limit of the species. This particular setting provides a rare opportunity for comprehensively assessing landscape-scale mating patterns in a major European forest tree. Specifically, we (i) describe the genetic structure of adult trees and patterns of historical gene flow based on an exhaustive sampling of individuals, (ii) test hypotheses

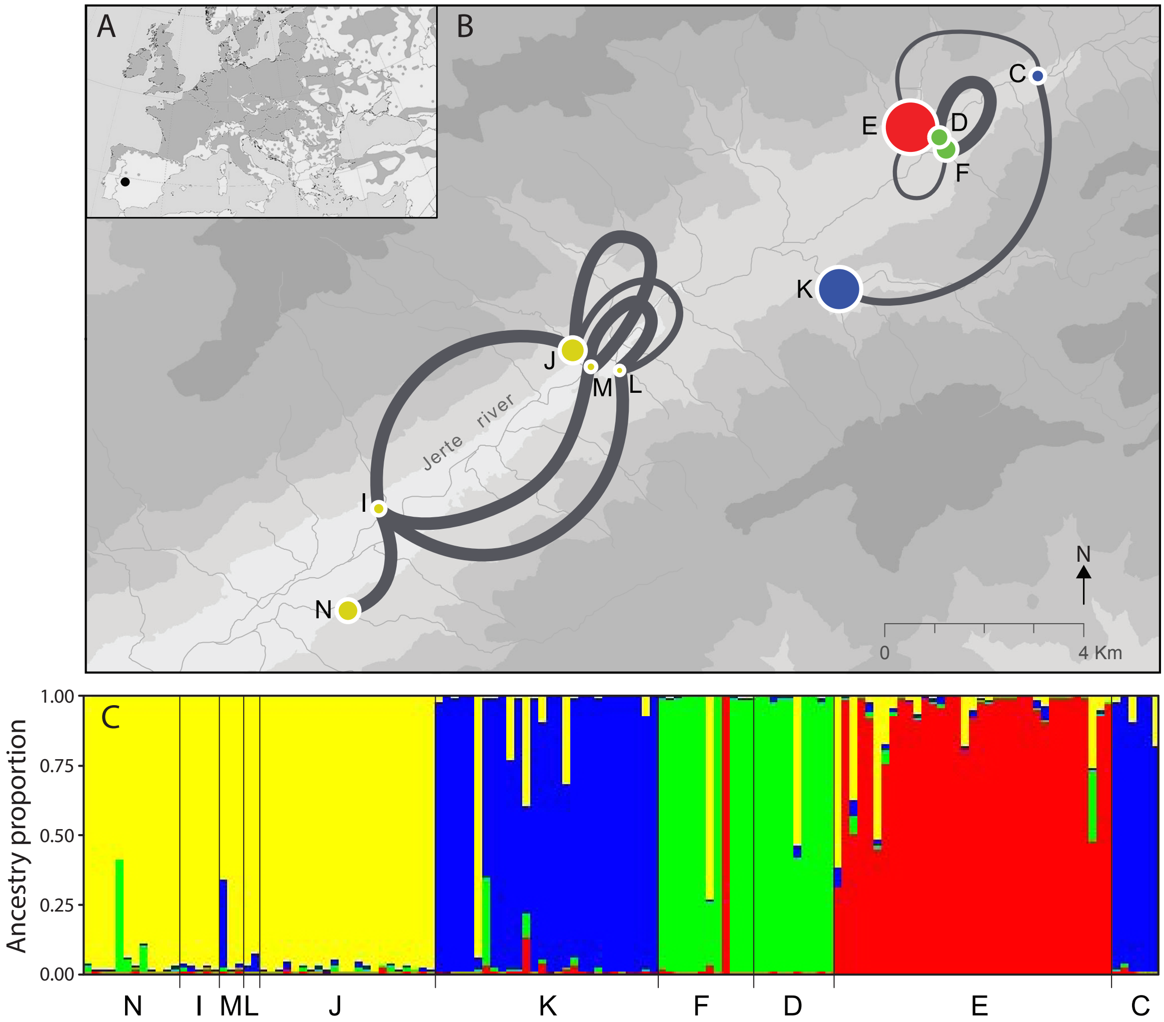

Figure 3.1 (a) Distribution area of *Quercus robur* according to EUFORGEN with a black dot indicating the study area. (b) Map of target stands. Shading indicates altitudinal ranges in 500 m steps from <500 m asl (white) to >2000 m asl (dark grey). Study stands are indicated by circles with circle size indicating the number of adult trees and circle colour the dominant genetic cluster as identified by STRUCTURE. Dark grey lines indicate significant genetic covariance ($0.01 \leq \alpha \leq 0.1$) between stands according to Population Graph analysis and their thickness is proportional to the cGD value, i.e. the genetic similarity of stands. (c) Bar plots showing STRUCTURE ancestry proportions for $K = 4$ clusters. Each individual is represented as a line segment, which is vertically partitioned with different colours representing the individual's estimated proportions of ancestry in each cluster (cluster 1 in yellow; 2 in red; 3 in green and 4 in blue). Letters below the graph refer to stands as shown in plate b.

about contemporary pollen flow limitation within and among stands as well as limited pollen immigration (either through long-distance pollen dispersal or through hybridization with the locally abundant sister species *Quercus pyrenaica*), and (iii) test the effects of stand characteristics (size, geographic isolation and phenology) on patterns of within and among-stand gene exchange.

METHODS

Study species

Pedunculate oak (*Quercus robur* L.) is one of the most prominent and widely distributed European forest tree species. Its southwestern range margin is located in the mountain ranges of central Spain, where scattered populations occur along water courses and in other environments that mitigate the summer drought characteristic of the regional Mediterranean climate.

Quercus robur is an almost exclusively outcrossing, wind-pollinated species. Extensive long-distance pollen flow into highly disjunct stands has been documented in this and other oak species (e.g. Buschbom *et al.* 2011; Hampe *et al.* 2013; Gerber *et al.* 2014). In the study area, it flowers from early to late April and acorns ripen in late September and early October. Hybridization with the sister species Pyrenean oak (*Quercus pyrenaica*) is possible due to partial overlap of flowering periods (Lepais *et al.* 2009). *Quercus robur* shows alternate fruit bearing in the area (G. Moreno, unpublished) as it does in many other parts of its range.

Study area and field sampling

The study was conducted in the Jerte valley (40° 13' N, 5° 44' W; Caceres province), ca. 25 km northeast of the town of Plasencia in western Spain. The valley stretches almost linearly over ca. 30 km descending from an elevation of 1275 m a.s.l. at the header (Fig. 3.1a,b). Its relatively temperate and humid local climate has favoured the development of an extensive fruticulture in the smoother, more accessible slopes while steeper and higher slopes are covered by broadleaved forests dominated by *Q. pyrenaica*. Streamsides and gorges harbour mesic and riparian tree species including *Q. robur, Celtis australis, Castanea sativa, Fraxinus angustifolia* and *Alnus glutinosa* among others.

Our study benefited from a comprehensive survey of adult and close to adult *Q. robur* trees throughout the Jerte valley conducted between 2002 and 2003 (Pulido *et al.* 2007). The species' earlier budburst and later shedding of leaves allows, during certain periods of the year, spotting and distinguishing individuals over hundreds of metres from the far more abundant sister species *Q. pyrenaica*. The survey detected a total of ten small *Q. robur* stands (n = 2–35 adult trees) spread over ca. 20 km of the Jerte valley (Fig. 3.1b). Somewhat larger populations (i.e. with a few hundred trees) are located at ca. 10–15 km, although outside the valley and therefore separated by a mountain chain with an altitudinal differential of up to 1000 m.

We mapped all adult and close to adult *Q. robur* trees known to occur in the Jerte valley (n = 135) and collected several leaves from each tree that were stored in silica gel until genetic analyses. We collected up to 20 acorns (mean: 19.0) in October 2009 from or, if impossible, beneath the canopy of 72 trees from all ten stands (i.e. virtually all individuals reproducing this year in the Jerte valley). Acorns were weighted, frozen and stored until being processed. We extracted the embryo from each acorn and dried it for genotyping. A pilot analysis of acorns from 10 randomly chosen mother trees using genetic diversity accumulation curves of multilocus genotypes indicated that a sample of 8–11 acorns per mother adequately describes the genetic diversity of seed families (see Appendix G: Fig. G1). We hence decided to analyse a minimum of 10 acorns per mother tree, resulting in a total of 724 acorns from 72 seed families. Finally, we characterized the flowering phenology of all adult trees in the Jerte valley on two dates somewhat before and after the peak of the flowering period, respectively (28 March 2015 and 4 April 2015). Four phenological stages of male flowers were distinguished during each survey: (i) swelling buds (score 0); (ii) emerging and immature catkins (score 1); (iii) mature catkins (score 2); and (iv) old, dried catkins (score 3). The phenological stage of the upper and the lower part of each tree was separately recorded and subsequently averaged. A similar survey in the previous year (2014) allowed us to corroborate that the phenological sequence of trees within stands remains reasonably consistent from year to year (Spearman rank correlation, $r_S = 0.38$, $P = 0.004$ (see also Bacilieri *et al.* 1995).

Microsatellite genotyping

All adult trees and acorns were genotyped using the 20 nuclear microsatellite markers described in Guichoux *et al.* (2011), integrated in two multiplex kits of twelve and eight markers each. DNA extraction, PCR amplifications and genotype scoring with an ABI 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) were performed following the protocols described by these authors. Electropherograms were independently scored by two persons to minimize genotyping error. We actually quantified the error rate using 44 randomly chosen acorns as blind samples. Three independent extractions, amplifications and analyses of each blind sample, respectively, resulted in an estimated overall genotyping error rate of 0.85% (n = 880 scorings), thus confirming the high marker quality pointed out by Guichoux *et al.* (2011).

Two loci (S19 and AB) did not fully amplify across all acorns and another one (Qr20) was found to strongly deviate from Hardy–Weinberg equilibrium due to allele dropout. All three loci were discarded, leaving 17 loci for the paternity analyses (while we used all 20 loci for the analyses of adult trees).

Data analysis

Adult genetic diversity and relatedness

The following statistics were calculated overall and for each stand using the GSTUDIO package in R version 3.1.0 (R Development Core Team 2014): mean number of alleles (N_a), effective number of alleles (N_{ae}), observed and expected heterozygosity (H_o and H_e) and fixation index (F_{IS}). We tested the null hypothesis H_0: $F_{IS} = 0$ using 10 000 randomizations of alleles among individuals. The effective number of alleles was estimated according to Nielsen *et al.* (2003) to account for the unequal sample sizes among stands. Gene diversity (H_e) was also corrected for sample size (Nei 1978).

We estimated genetic relatedness (Queller & Goodnight 1989) among all within-stand adult tree pairs with SPAGEDI 1.4 (Hardy & Vekemans 2002). The expected relatedness values (R) are 0.5 among full-sibs, 0.25 among half-sibs and 0.0 for unrelated individuals. To calculate within-stand pairwise relatedness we used the allele frequency of the total, pooled 10 stands in the valley.

Adult genetic differentiation and historical migration

We assessed overall and pairwise genetic differentiation among stands computing F_{ST} (Weir & Cockerham 1984) in SPAGEDI 1.4 (Hardy & Vekemans 2002). F_{ST} was tested against the null expectation of absence of population structure based on 10 000 permutations. We also tested for isolation by distance between stands by means of a Mantel test with 9999 permutations in GENALEX 6.5 (Peakall & Smouse 2012). For this purpose, we calculated the geographic distance among stands (taking their altitude into account).

Bayesian clustering of the genetic data was performed using STRUCTURE v.2.3.4 (Pritchard *et al.* 2000). We ran STRUCTURE with K ranging from 1 to 10, and with 10 runs for each K value. A burn-in period of 50 000 iterations was followed by 200 000 MCMC repetitions assuming allele frequencies to be correlated among populations and an admixture model of population structure. No prior information was used to assist clustering in the first 10 x 10 runs, whereas we included stand identity as prior information in a second series of 10 x 10 runs. Results were highly consistent between both approaches, and we report the results including stand identity. We selected the K value that best described the data from the change in likelihood (delta K) as proposed by Evanno *et al.* (2005) and the highest posterior probability (LnP(D|K)).

We further explored patterns of historical gene exchange between stands using Population Graphs (Dyer & Nason 2004) as implemented in the popgraph library in R version

3.1.0. (R Development Core Team 2014). This graph theoretic approach analyses how genetic variation is distributed across the investigated landscape by plotting historical migration. The advantage of the approach is to explicitly account for differences in genetic covariation associated with both direct and indirect connectivity (gene flow) among populations, making it potentially better suited for use in landscape genetic modelling than more conventional measures of pairwise genetic distance such as F_{ST} and D_{c} (Dyer *et al.* 2010). Within a graph, populations are represented as nodes and the genetic covariation among populations determines the topology. To achieve this, the genetic variance–covariance matrix among populations is inverted and standardized to obtain a partial correlation matrix, with the significance of individual partial correlations determined. If the partial correlation between populations i and j is significantly greater than expected by chance, then an edge is placed between nodes i and j (Dyer & Nason 2004). By setting the significance level from 0.1 to 0.01, we assure robustness of the topology. The Population Graph is constructed by applying this procedure to all possible population pairs, resulting in a graphical model of population genetic structure. The pattern of connections among populations is thus estimated conditional on the entire data set, improving the way genetic covariance is estimated and quantified in landscape models. The shortest path connecting pairs of populations across the entire graph is defined as the conditional graph distance (cGD; Dyer *et al.* 2010). It can be used as a metric of genetic distance that, contrary to traditional F_{ST} approaches, considers the entire population network when comparing pairs of populations (Dyer 2015).

Paternity analysis of acorns

Before starting the paternity assignment, we checked and removed all offspring whose multilocus genotypes did not match their putative mother tree (indicating sampling errors committed in the field). The final progeny array consisted of 684 acorns, with 9.4 ± 1.3 (mean ± SD) acorns per mother tree. We used categorical paternity assignments to identify candidate father trees. This approach was facilitated by the high exclusion power of our molecular markers (0.99995, see Appendix G: Table G1) and the exhaustive sampling of candidate fathers within the Jerte valley. The paternity of each offspring was determined by likelihood ratios with CERVUS 3.0 (Kalinowski *et al.* 2007) assuming the strict confidence criterion (95%) for assignments. Preliminary tests showed that an assumed error rate of 0.5% provided the most reasonable balance between considering genotyping errors and getting a biologically meaningful set of assignments. We performed simulations with the following parameters: number of offspring genotypes = 50 000, number of candidate fathers = 150, proportion of candidate fathers sampled = 0.9, mistyping rate = 0.005 and proportion of loci typed = 0.9936. These parameters correspond to the adult population size of the valley plus an incoming gene flow of 10%, which seems reasonable given our study context (e.g. Gerber *et al.* 2014). Most acorns were assigned a single pollen parent (650 of 684 seeds analysed) and no acorns showed multiple paternity assignments. A posterior check confirmed that all unassigned acorns (n = 34) showed mismatches with the most likely candidate father at a minimum of two loci.

Pollen dispersal

Following paternity assignment, we used three different approaches to characterize pollen dispersal within and among stands. First, we constructed a frequency distribution of observed inter-mate distances from all acorns for which we had identified a single candidate father. This allowed us to infer a global frequency distribution of pollen dispersal distances for the study populations. Second, we quantified the proportions of different types of pollination events by assigning each acorn to one of four classes: (i) selfing: the proportion fertilized by pollen from the same mother tree; (ii) local pollination: the proportion fertilized by any pollen donor candidates from within the oak stand of the mother tree; (iii) between-stand pollination: the proportion fertilized by any pollen donor candidates from stands other than the stand of the mother tree; and (iv) unassigned: the proportion fertilized by pollen from an unsampled *Q. robur* donor (i.e. a long-distance immigration event from outside the Jerte valley) or from a *Q. pyrenaica* donor (i.e. a hybridization event). This classification ignores cryptic gene flow (i.e. local pollinations that cannot be distinguished from immigration events), which we consider unlikely given the high exclusion probability of our markers and the exhaustive sampling of candidate trees. We computed, for each individual mother tree, the proportions of pollination events belonging to each of the four classes. Third, we tested if mating within stands (corresponding to our local pollination class) occurred at random or primarily between nearby trees. For this purpose, we computed and compared, for each stand, the median distance of observed within- stand pollination events and the median distance between all trees.

An additional analysis was performed to infer whether our non-assigned acorns stemmed from long- distance pollen immigration or from hybridization with *Q. pyrenaica*. For this purpose, we conducted an assignment test using STRUCTURE and a data set that included all unassigned acorns, all 135 *Q. robur* trees and a sample of 109 adult *Q. pyrenaica* trees collected next to our focal trees and analysed with the same SSR loci. We ran STRUCTURE with the same configuration as described above and, unsurprisingly, identified $K = 2$ as the by far most likely scenario (Appendix I: Fig. I1).

Stand-level correlates of pollen dispersal

We hypothesized that stand-level mating parameters should be governed by the size, the location and the flowering phenology of stands. We constructed three variables to characterize these components (see Table 3.1): (i) Stand size measured as the number of trees. (ii)

Table 3.1 Stand features, genetic diversity estimates for adult trees (H_E, H_O and F_{IS}), R, adult relatedness and mean rates of pollination types according to paternity analysis (selfing, local pollination, between-stand pollination and unassigned) for each *Quercus robur* stand

Stand	No. of trees	Sample size (trees/acorns)	Altitude	Phenology	Isolation	H_E	H_O	F_{IS}	Selfing	R (CI)	Local pollination	Between-stand pollination	Unassigned	m_{obs}/m_{all}
C	6	5/46	757.9	2.5 (0.11)	0.80	0.54	0.54	−0.01	0.16	0.35 (0.24—0.47)	0.82	0.00	0.03	0.61
D	10	7/66	628.8	1.6 (0.42)	0.61	0.61	0.60	−0.11	0.04	0.27 (0.21—0.33)	0.86	0.10	0.00	0.24
E	35	17/162	742.3	2.0 (0.41)	0.62	0.65	0.65	−0.07	0.03	0.15 (0.13—0.16)	0.86	0.01	0.10	0.34
F	12	7/66	629.9	1.8 (1.10)	0.62	0.64	0.64	−0.03	0.04	0.19 (0.14—0.24)	0.87	0.02	0.07	1.16
I	5	4/41	467.4	2.5 (0.18)	0.85	0.63	0.62	−0.12	0.00	0.28 (0.18—0.38)	0.85	0.00	0.15	0.98
J	22	14/129	622.2	2.4 (0.24)	0.57	0.63	0.63	0.06*	0.08	0.17 (0.15—0.18)	0.89	0.00	0.02	0.62
K	28	11/107	699.4	1.9 (0.44)	0.55	0.61	0.61	0.04	0.13	0.19 (0.18—0.21)	0.86	0.01	0.00	0.27
L	2	1/10	523.1	1.7 (0.18)	0.54	0.75	0.80	0.19*	0.10	−0.05	0.40	0.10	0.40	—
M	3	3/29	484.7	1.7 (0.18)	0.55	0.61	0.58	−0.28	0.10	0.43 (0.36—0.50)	0.69	0.21	0.00	0.12
N	12	3/28	577.7	2.5 (0.14)	1.00	0.72	0.72	−0.05	0.00	0.04 (0.01—0.08)	1.00	0.00	0.00	0.06
Overall	135	73/684		—	—	0.71	0.65	0.08*	0.07	0.17 (0.16—0.18)	0.86	0.03	0.05	—

H_E, expected heterozygosity; H_O, observed heterozygosity; F_{IS}, fixation index; *$P < 0.05$, R, adult relatedness. Phenology indicates the mean phenological state (SD) for each stand from individual tree scorings, where 0, 1, 2 and 3 scores represent swelling buds, emerging and immature catkins, mature catkins and old dried catkins, respectively, at the peak of the flowering period. Isolation is computed as the mean distance to the centroid of every stand in the valley (expressed in 0—1 range). The ratio between the median distance of observed within-stand mating events and the median distance between all trees (m_{obs}/m_{all}) is also presented.

Isolation measured as the mean distance (controlling for altitude) from the focal stand to every other stand within the valley. Note that this measure integrates the 'centrality' of stands within the Jerte valley: those near the valley ends are on average more distant from all others than those situated in the centre. (iii) Flowering phenology measured as the average of the phenological stages (scored between 0 and 3) of all trees in a given stand. This averaging of the individual phenological scores resulted in a numerical value where early-flowering stands score near three and late-flowering stands score near zero. We used generalized linear mixed models (GLMM) with a binomial distribution to test for effects of stand size, isolation and phenology on the proportions of the four pollination types described above (selfing, local pollination, between-stand pollination and unassigned). The proportion of each pollination type on each mother tree was used as response variable, stand size, isolation and phenology as fixed effects and the stand identity of the mother tree as random effect. GLMM analyses were performed using the LME4 package in R version 3.1.0 (R Development Core Team 2014). Previous tests showed that collinearity among predictor variables and correlations among response variables (i.e. the proportions) were moderate (predictor variables: Spearman $r_s \leq 0.63$; response variables: Spearman $r_s \leq 0.54$).

We also tested whether large or dense stands showed a stronger tendency for mating between nearby trees than small or sparse stands. For this purpose, we calculated the ratio between the median distance of observed within-stand mating events and the median distance between all trees (see Table 3.1). This ratio was then regressed against stand size and density.

RESULTS

Adult genetic diversity and relatedness

We detected a relatively high total number of alleles per locus (mean 11.1, range 5–19) and a markedly lower effective number of alleles (mean 4.4, range 1.2–9.1; Appendix G: Table G1). Allelic composition varied greatly among stands and private alleles occurred in all but one (M, Appendix G: Table G2). Observed and expected heterozygosities were overall moderate although again with great variation among stands (Table 3.1). Significant heterozygote deficit was only detected in stands J and L.

Mean relatedness among adult trees in the Jerte valley was 0.167 (CI: 0.159–0.176). Mean values for stands ranged from 0.046 in L to 0.430 (0.36–0.50) in M (Table 3.1). Except for stand L, all stands with <10 trees had R values above 0.25 (the value expected for half-sib pairs). When excluding the outlier stand L (with only two adult trees), R showed a significant tendency to decrease with increasing stand size (linear regression: $F = 8.69$, df $= 7$, $P = 0.02$).

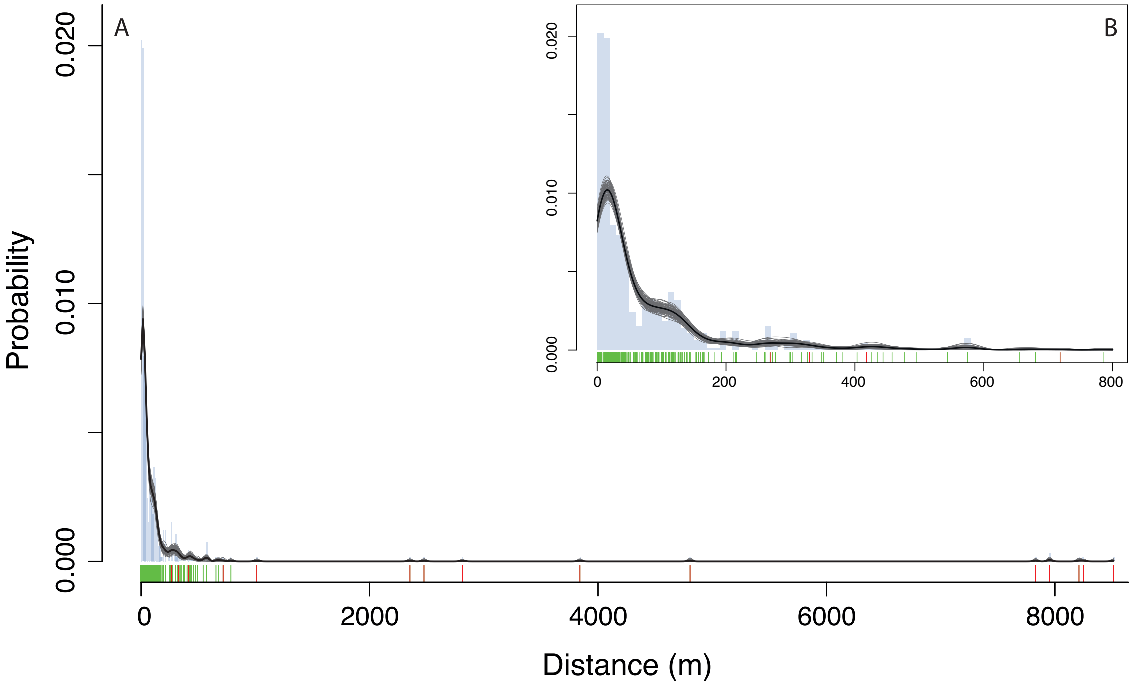

Figure 3.2 (a) Frequency distributions of pollen dispersal distances for *Quercus robur*, estimated via paternity analysis. The blue line indicates the nonparametric smoothing spline fit to the empirical distance distribution together with bootstrapped estimates (n = 100 randomizations). Individual pollination events are indicated by vertical lines under the plot, with light green and red lines indicating within-stand and interstand pollinations, respectively. (b) Zoom spanning 800 m.

Genetic differentiation of and historical gene flow among stands

Overall differentiation among stands was high ($F_{ST} = 0.12$; $P < 0.001$; see also Appendix H: Table H1 for pairwise F_{ST} values). We observed weak signs of isolation by distance (Mantel test: $R^2 = 0.06$; $P = 0.08$). The STRUCTURE analysis indicated the existence of four distinct genetic clusters (Fig. 3.1b, c; see also Appendix H: Fig. H1): Cluster 1 comprised the five stands located in the middle to lower parts of the valley (J, M, L, I and N); cluster 2 dominated stand E; cluster 3 assembled the nearby stands D and F; and cluster 4 included the relatively distant stands C and K. The majority of trees had high probabilities of ancestry in a single cluster. Some individuals were assigned to two different clusters with ancestry probabilities around 50%, indicating that they stemmed from between-cluster pollination events. This phenomenon involved primarily trees located in the stands dominated by clusters 2–4 that were partly assigned to cluster 1. We also observed two trees strongly assigned to one cluster that were situated within populations dominated by another cluster (suggesting that they stemmed from between-cluster acorn dispersal events; see stands F and K in Fig. 3.1c).

The Population Graph analysis produced an invariant network topology (Fig. 3.1b) across the range of a values examined (0.01–0.1). The most salient feature of the network was the disconnection between stands of the upper and the lower valley. The lower valley stands were on average markedly less differentiated among them than the upper stands (average pairwise cGD = 0.75 vs. 1.61; *t* test: $t = 4.86$, df = 10.8, $P < 0.001$; see Appendix H: Table H1). The Population Graph analysis also revealed some links between nearby pairs of stands that belonged to different STRUCTURE clusters (C and E as well as E and D).

Contemporary pollen dispersal

The frequency distribution of mating events and its associated pollen dispersal kernel were robustly estimated based on the high proportion of paternity assignments (95%). The shape of the pollen dispersal curve was leptokurtic (Fig. 3.2), with the median and the 95th percentile pollination distances being 30.5 m and 390.1 m, respectively. A small fraction of mating events (1.8%) spanned distances between 1000 and 8500 m, the maximum pollen dispersal distance observed. Within stands, the median distance between mates was significantly smaller than the median distance among trees (22.6 vs. 60.6 m; paired *t* test: $t = 2.61$, df = 8, $P = 0.03$). This trend was unrelated with the size or density of stands (linear regressions: $F = 0.21$, df = 7, $P = 0.66$; $F = 0.83$, df = 7, $P = 0.39$, respectively).

Observed proportions of different pollination types varied extensively among stands (Table 3.1). By far most pollination events (85.6%) involved fathers from the stand of the mother. This fraction was even higher when adding the percentage of selfing events (6.8%). On the contrary, few events (5.0%) involved fathers located outside the Jerte valley or *Quercus pyrenaica* trees, and even fewer between-stand pollination events (2.6%) were observed. Our STRUCTURE analysis indicated that only four of the acorns without identified fathers (i.e. 0.6% of the overall acorn sample) are likely to stem in first generation from a

Table 3.2 Effect of stand characteristics on the proportion of each pollination type

	Selfing			Local pollination			Between-stand pollination			Unassigned		
	Estimate	SE	*P*	Estimate	SE	*P*	Estimate	SE	*P*	Estimate	SE	*P*
Fixed effects												
Intercept	−0.23	2.11	ns	−1.20	1.28	ns	6.58	0.01	***	−7.81	5.88	ns
Size	−0.02	0.03	ns	0.03	0.02	—	−0.06	0.01	***	−0.09	0.08	ns
Isolation	−0.57	0.43	ns	0.34	0.24	ns	−0.21	0.01	***	−1.11	1.05	ns
Phenology	0.68	1.55	ns	0.11	0.85	ns	−3.90	0.01	***	5.75	4.80	ns
Random effects (SD)												
Stand	0.54			0.23			0.42			1.76		

Results of generalized linear mixed-effects models with binomial errors are shown. $P < 0.1$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

hybridization event with *Q. pyrenaica* (i.e. probability of assignment to cluster '*Q. pyrenaica*' around 0.5, see Appendix I: Table I1).On the other hand, a few of our adult *Quercus robur* trees show signs of being hybrids in first or second generation (see Appendix I: Fig. I1).

We observed a marked directionality of pollen movements between stands (Fig. 3.3): Of the 18 events detected overall, 11 (61%) were from upstream to downstream stands, six (33%) involved stands growing nearby (<1 km) across the valley and only one long-distance pollination event (5%) occurred in upstream direction.

Correlates of variation in pollen dispersal among stands

As revealed by the GLMM model fit, stand characteristics influenced the proportions of between-stand mating events and slightly with local pollinations (see Fig. 3.3), whereas no effect on the proportions of selfed and unassigned acorns was evident (Table 3.2). The frequency of local pollinations tended to increase with stand size, whereas that of between-stand pollinations decreased with increasing stand size and in particular isolation. We also found a marked effect of phenology on the proportion of between-stand pollinations, with late- flowering stands receiving more immigrant pollen than early-flowering stands (Table 3.2).

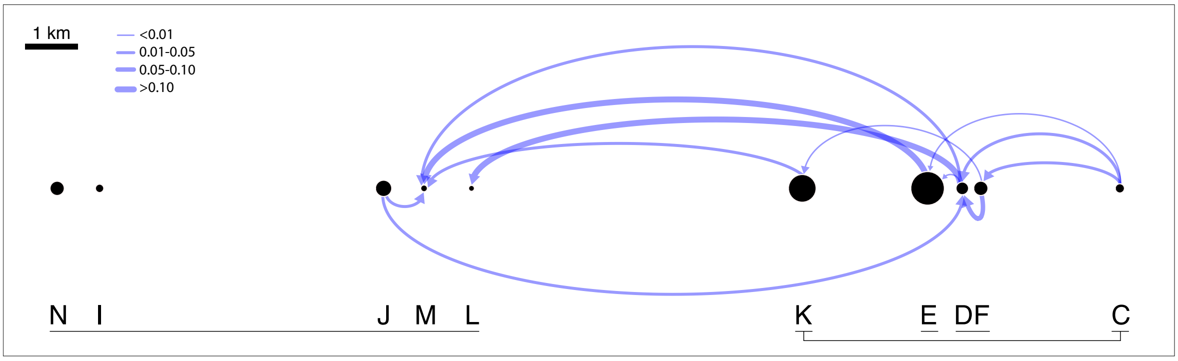

Figure 3.3 Contemporary pollen-mediated connectivity of the investigated *Quercus robur* stands. Stands are drawn as nodes with circle size being proportional to stand size. Arrows represent pollen dispersal probability from source to sink according to the proportion of between-stand pollen dispersion inferred by paternity analysis. Letters beneath circles indicate the identity of stands while horizontal lines below stand codes indicate those stands that belong to the same genetic cluster.

DISCUSSION

Adult population structure and historical gene flow

Our study stands showed a relatively high level of genetic diversity given their geographical isolation and relatively small population size [global $H_E = 0.71$, compared to 0.75–0.85 (Buschbom *et al.* 2011), 0.79 (Hampe *et al.* 2010), 0.79 (Muir & Schlötterer 2005;), or 0.87 (Streiff *et al.* 1998) for SSR-based estimates from other *Quercus robur* populations]. This diversity was strongly structured, indicating that gene flow and population connectivity along the Jerte valley have been weak through an extended period of time. We observed no <4 well-supported genetic clusters and an overall F_{ST} (0.12) that clearly exceeds those of far more geographically separated oak populations from other ecological settings (e.g. Bruschi *et al.* 2003; Muir *et al.* 2004; Craft & Ashley 2007, 2010; Cavender-Bares & Pahlich 2009). Even though the F_{ST} estimate might be somewhat inflated due to the small size of some stands, our study system clearly is at odds with the widespread notion that disjunct populations of wind-pollinated temperate trees tend to experience extensive long-distance gene exchange (Kramer *et al.* 2008; Kremer *et al.* 2012). Stands of the upper and those of the lower valley actually were largely disconnected, although no apparent topographic or other landscape-related barriers to gene flow exist between the upstream and the downstream stands (except for population K, which is located in a lateral valley; see Fig. 3.1b). The observed disconnection should hence largely be driven by the mere spatial distance between the two groups (ca. 8 km). Note also, however, that the STRUCTURE analysis identified a certain number of trees (ca. 10%) that stem from between-cluster pollination or acorn dispersal events.

At a finer spatial scale, the genetic differentiation of stands was not homogeneous throughout the valley but much more pronounced in the upper than in the lower part. This difference is remarkable because the two valley parts do not appreciably differ in the spatial distribution of stands, the topography or greater landscape structure. The strong genetic divergence of stands in the upper part of the valley suggests that the species must have persisted more or less independently at different sites in the valley. Such circumstances and the limited size of these stands probably have led to their rather low genetic diversity ($H_E = 0.61$). Note that high population differentiation combined with low within-population diversity is a key characteristic of stable rear-edge populations (Hampe & Petit 2005); thus, our study stands are likely to be real long-term relicts with a history of persistence in situ through many generations.

On the other hand, the relatively weak differentiation of the lower valley stands compared to their upper valley counterparts could reflect that they are remainders of a once larger and more continuous stand that has undergone shrinking and fragmentation in relatively recent time. Such a process would explain that the lower valley stands show higher levels of diversity than their upper valley counterparts (0.67 vs. 0.61; paired *t* test: $t = 2.42$, df = 19, $P = 0.03$), and that most historical long-distance pollination events occurred from lower towards upper parts of the valley (i.e. from cluster 1 towards the other clusters, see Fig. 3.1c). It is well documented that human land use in the Jerte valley steadily increased in intensity through the 20th century (Cruz 1983), although the remotest horticultural areas are now being abandoned and recolonized by scrub and forest (Ezquerra & Gil 2008). A concomitant size reduction and fragmentation of *Q. robur* stands, especially in the more populated and readily transformable lower part of the valley, appears hence realistic.

Contemporary pollen dispersal

By far most mating events involved trees from the same stand (>90% when adding local pollinations and selfing). Larger stands tended to experience somewhat more local pollinations than smaller ones, although the tendency was weak. The dispersal kernel (Fig. 3.2) and the estimated median pollen dispersal distance of only 30 m underpin moreover that mating occurred primarily between neighbouring trees within each stand. We also detected a remarkably high number of selfing events for a strongly outcrossing species such as *Q. robur* (Bacilieri *et al.* 1996). These stemmed mostly from a few heavily selfing trees (i.e. five individuals accounted for more than half of all selfed offspring), suggesting that breakdown of self-incompatibility has occurred several times in our study system (see Hampe *et al.* 2013 for a similar case in a marginal oak population).

Overall, the probability to receive pollen from other stands within the valley decreased with increasing size of stands and especially with their geographical isolation, whereas it increased from early- to late-flowering stands. The two lowermost stands I and N did not participate at all in the observed pollen flow, whereas the central stand D exchanged pollen with no <6 other stands. The small and late-flowering stands L and M served as sinks for 39% of all observed between-stand pollination events, whereas the more isolated, early-flowering stands J and C served exclusively as pollen sources (see Fig. 3.3). Overall, these patterns point to great spatial heterogeneity in the network of pollen gene flow. The detected phenology effect indicates that between-stand mating tends to increase with decreasing pollen abundance, suggesting that competition for access to ovules could to some extent trigger the observed mating patterns (see also Lagache *et al.* 2013).

Interestingly, patterns of contemporary pollen flow showed two major inconsistencies with those of historical gene flow as reflected by the adult trees. First, we detected several instances of ongoing pollen dispersal across the major genetic disconnection between upper valley and lower valley stands (see Figs 3.1b and 3.3). Second, contemporary pollen movements occurred markedly more often downstream than upstream, whereas the STRUCTURE analysis of the adult trees indicated that historical pollen flow has primarily occurred in the opposite direction (from cluster 1 towards the other clusters). The directionality of contemporary pollen flow is in agreement with the dominant wind direction during the flowering season of our study year (*Sistema de Informacion Agroclimatica para el Regadío*, http://eportal.- magrama.gob.es/websiar/Inicio.aspx). We do not know whether our study year was an exception and upstream winds have been historically prevailing in the Jerte valley. But the dominance of upstream pollen dispersal in the past would be in line with our hypothesis that *Q. robur* could have been more abundant in the lower Jerte valley during past decades than it is today.

Pollen inflow from other sources than our target stands (i.e. either *Q. robur* populations outside the Jerte valley or *Quercus pyrenaica*) was also infrequent. Even the smallest and most isolated stands did not show any signs of being particularly susceptible to experiencing long-distance gene inflow or hybridization. Our STRUCTURE analysis indicated that only a tiny fraction of all mating events (0.6%) involves hybridization with *Q. pyrenaica*. Given the great abundance of this species in the Jerte valley, our finding implies that interspecific gene flow played a very minor role. We observed, however, signs of hybridization in some of our adult *Q. robur* trees, which are roughly in line with the hybridization rate of 5.9% that Lepais *et al.* (2009) reported from a mixed oak stand with *Q. robur* and *Q. pyrenaica*. This discrepancy suggests that hybridization might be more frequent in our study system in years of less abundant flowering and concomitant stronger pollen limitation (cf. Lepais *et al.* 2009; Lagache *et al.* 2013).

Mechanisms behind restricted gene flow in relict Quercus robur stands

We observed only marginal effects of isolation by distance in explaining genetic differentiation; thus, other processes must be triggering levels of gene flow in addition to simple geographic distance effects. *Quercus robur* stands in the Jerte valley exchanged little pollen, and the marked genetic structure of the adult population clearly indicates that this weak connectivity is not only a short-term phenomenon. It is particularly remarkable since oaks and other wind-pollinated forest tree species are known for their great ability to disseminate pollen over great distances (Schueler & Schlünzen 2006; Kremer *et al.* 2012). For instance, Hampe *et al.* (2013) observed 6% of pollen inflow into a small, marginal holm oak (*Quercus ilex*) population 30 km ahead of the nearest larger stands. Buschbom *et al.* (2011) reported that no <35% of all mating events involved immigrant pollen in a small *Q. robur* stand whose closest conspecifics grow >80 km away. And Robledo- Arnuncio & Gil (2005) detected 4.3% of pollen immigration in a *Pinus sylvestris* stand 30 km apart from other populations. So why is gene flow so much more restricted in our study system?

At least two mechanisms arising from the relictual character of our stands could help explain the phenomenon. The first is related to their refugial environment: almost all stands grow within dense riparian forests along water courses that are surrounded by rugged terrain (typically gorges). This setting provides the humidity required by *Q. robur* to withstand the dry Mediterranean summers typical of the region. But it also represents a significant obstacle to both the departure and the arrival of oak pollen. The effect of this refugial habitat is probably twofold. First, by creating abundant small-scale turbulences and weakening thermal air uplift, fine-scale topography and the dense canopy reduce the probability of pollen grains to enter free atmosphere layers where directed air flows could transport them over longer distances (cf. Dupont & Brunet 2008; Dobrowski 2010). Second, the humid environment of these riparian forests renders floating pollen likely to be 'captured' by sticky surfaces such as leaves or branches. This phenomenon is known from tropical rain forests and has been used to explain the scarcity of wind-pollinated species in this biome (e.g. Turner 2001). Our study suggests that it could also constrain gene flow when wind-pollinated species are confined to other humid habitats. Relict trees that persist in these refugial microenvironments may hence result environmentally 'trapped' in terms of dispersal limitation and realized gene flow.

The second mechanism combines environmental and genetic components and refers to the observed high rates of mating between neighbours. Our study stands have apparently persisted through extended periods of time in isolation and at small population size under the constraints of their scattered refugial habitat. This situation has provided opportunities for genetic purging, while the great ability of trees to maintain within-population diversity (Petit & Hampe 2006) would have limited negative genetic effects on population regeneration and viability. This suggests that incompatibility and inbreeding depression do not profoundly hinder extensive mating within refugial populations. An abundant local pollen production, such as in the year of our study, can hence easily saturate stigmas and thwart the arrival of foreign pollen. This so-called pollen 'swamping' from nearby trees has been described from closed oak forests (Lagache *et al.* 2013) and should be even much stronger in the highly disjunct stands of the Jerte valley. On the contrary, cases of extensive pollen inflow into geographically isolated populations typically come from genetically strongly impoverished, little stands where adult trees are likely to experience compatibility issues (e.g. Buschbom *et al.* 2011; Hampe *et al.* 2013). Our findings of moderate genetic diversity and relatively low extent of family structures give support to this mechanism as a plausible cause of the extensive within-stand mating in these relict populations. In addition, two lines of evidence indicate that inbreeding depression is not a major issue in our study system: (i) the size of acorns is well known to trigger seedling establishment and is hence used as a proxy for fitness (e.g. Hampe *et al.* 2013); yet the size and the progeny inbreeding of our acorns were uncorrelated (Pearson $r = 0.16$, $t = 1.38$, df = 70, $P = 0.17$); (ii) a concomitant greenhouse experiment with >1500 seedlings revealed no differences in the performance of individuals from small and from large stands (G. Moreno, unpublished). Our peculiar case study and its unusual results hence suggest that compatibility issues and inbreeding depression could be a key driver shaping long-distance dispersal patterns of small populations of wind-pollinated trees (see also Kremer *et al.* 2012).

Insights into organism–environment relationships within microrefugia

The functioning of microrefugia and their role for the survival of species in a rapidly changing climate are the object of rapidly growing attention (e.g. Hampe & Jump 2011; Gavin *et al.* 2014; Woolbright *et al.* 2014). Refugia offer pockets of suitable climate space where climatic conditions in the surroundings do not permit the existence of the species (Dobrowski 2010). Long-term persistence in strong isolation and resulting microevolutionary differentiation has converted many refugial populations in conservation targets of high priority (Hampe & Petit 2005). Their isolation is usually attributed to their highly scattered distribution within a climatically adverse landscape matrix. Our study suggests that not only the climatic conditions around refugia but also those within them can constrain the connectivity of the populations that they harbour. This notion adds an important piece to our understanding of organism–environment relationships and population dynamics at species' low-latitudinal range margins.

ACKNOWLEDGEMENTS

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CAPÍTULO 4

Ecological drivers of individual mating patterns in refugial populations of Pedunculate oak (*Quercus robur*)

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ABSTRACT

The low-latitude range margins of many temperate and boreal plant species consist of scattered populations that persist locally in microrefugia. It remains poorly understood the mechanisms that allow their long-term persistence such as the maintenance of genetic diversity and adaptive potential. We examined fine-scale patterns of mating and pollen dispersal across ten refugial stands of Pedunculate oak (*Quercus robur*) scattered over 20 km along a valley located at the species' southwestern range margin. We genotyped progenies from 72 mother trees and assessed individual variation in mating using categorical paternity assignments, a spatially explicit mating model and mating network analysis. We then examined how this variation was shaped by numerous features of the tree and its ecological neighbourhood. At valley-wide scale, trees showed unusually strong variation in selfing (0-80%, average 6.8%) and immigrant pollinations (0-89%, average 7.3%). Both were most prevalent in large trees surrounded by few and genetically related neighbours. At within-stand scale, extensive among-tree variation existed in all mating parameters, but consistently more accused for the male function than the female one. Large and centrally-located trees assumed a key role for pollen-mediated connectivity. Overall, our results revealed that the character and situation of individual trees within their forest stands clearly surpass the role of the stands themselves in shaping the mating system. The strong dominance of small-scale phenomena for mating implies that even small changes in the structure of extant stands, such as the removal of key pollen donors, could have severe consequences for their conservation.

KEYWORDS: individual variation, ecological and genetic correlates, gene flow, landscape scale, marginal populations, male fecundity, paternity analysis, mating network, rear edge

Many temperate and boreal tree species maintain populations along the low-latitudinal limit of their current distribution range that have persisted roughly *in situ* through the recurrent climate changes of the Quaternary (Gavin *et al.* 2014). These populations are not only important conservation targets (Hampe & Petit 2005; Fady *et al.* 2016) but also excellent models for investigating how species can successfully persist over extended periods in an adverse abiotic environment (Woolbright *et al.* 2014). Today, they typically are restricted to microrefugia: scattered islands of particularly favourable habitat within heterogeneous landscapes (Dobrowski 2010). Refugial populations are most often small and so isolated that regional population dynamics cannot easily compensate local extinction events. A rapidly growing number of studies have assessed relationships of such populations with spatial variation in their abiotic environment (Keppel *et al.* 2012; Hannah *et al.* 2014; Hylander *et al.* 2015). The intrinsic dynamics that have enabled long-term refugial populations to persist locally under the constraints of their climate-driven confinement remain, however, poorly understood (Hampe & Jump 2011). We ignore in particular how patterns of mating and gene flow, individual fecundity, and resulting effective population size can influence key components for long-term population persistence in refugia, such as the maintenance of genetic diversity, adaptive potential, and ultimately evolution.

Trees have a life history that renders them particularly resistant to the erosion of population genetic diversity, including a long lifespan, a potentially prodigious fecundity, and a great propensity for long-distance gene flow (Petit & Hampe 2006). Effective pollen dispersal among tree populations can regularly span various kilometers (Kremer *et al.* 2012). But tree populations are also more dependent on efficient gene exchange than other plants because they are mostly self-incompatible and disproportionately susceptible to inbreeding depression (Petit & Hampe 2006). The demographic bases for the persistence and resilience of tree populations remain poorly understood, mostly because individual variation in mating success, fecundity and gene dispersal remains largely unexplored. Thus, studies addressing population-level variation of mating system rarely account for its variability among individuals within populations, although this variability is often far from negligible (Gaüzère *et al.* 2013; Chybicki & Burczyk 2013). Similarly, great inequality in individual fecundity is a widespread phenomenon in tree populations, resulting in effective population sizes that are far lower than apparent in the field (Moran & Clark 2012; Gerzabek *et al.* 2017). And among-individual variation in mating and its fine-scale ecological triggers can also scale up to exert a significant influence on landscape-level patterns of contemporary gene flow (Dileo *et al.* 2014). Studying mating system, gene flow and their ecological correlates at the scale of individual trees hence can provide critical knowledge for an efficient management and conservation of refugial and other small and dispersed tree populations.

The effectiveness of mating and gene dispersal within refugial tree populations is likely to be determined by the interplay between the intrinsic attributes, spatial distribution, microhabitat characteristics, and ecological neighbourhood of reproductive trees (García *et al.* 2005, Grivet *et al.* 2009; Gaüzère *et al.* 2013). Intrinsic tree attributes can concern the individual's genotype (for instance regarding incompatibility systems; Hampe *et al.* 2013) as well as phenotypic traits. Traits such as plant size, functional gender, flower production and flowering phenology have all been shown to affect mating patterns and reproductive output (García *et al.* 2005; Gaüzère *et al.* 2013; Sánchez-Robles *et al*. 2014; Moracho *et al*. 2018). Furthermore, the abundance, spatial distribution and number of potential mates are commonly major determinants of the mating system because pollen rarely moves at random and the probability of pollen exchange decreases almost invariably with the distance from the pollen donor (e.g. García *et al.* 2005; Chybicki & Burczyk 2013; Lagache *et al.* 2013). Finally, the exposure of a tree's growing site and the density of the surrounding vegetation can also affect pollen arrival (Millerón *et al.* 2012; Gaüzère *et al.* 2013). Refugial populations are often scattered across heterogeneous landscapes in habitats with rugged terrain and a dense vegetation (Hampe & Jump 2011). In such settings, even small differences in the environment that surrounds a reproductive tree may have large consequences for its flower production and exposure to arriving pollen. This variation may further exacerbate the effects of a sparse and highly heterogeneous spatial distribution of individuals, constrained by the availability of suitable habitat islands. Hence, individual variation in mating patterns is expected to be higher in refugial situations than under more homogeneous ecological conditions, and it may have significant implications for gene flow, individual fitness and ultimately the persistence of the entire population. To fully understand the ecological determinants of mating and gene flow in refugial scenarios, one must therefore assess how landscape complexity combines with the attributes of individual trees (Bacles & Jump 2011).

Paternity tests are one of the most direct and powerful methods to investigate fine-scale patterns of mating and gene dispersal within and among small, isolated populations such as those inhabiting microrefugia. The precise detection of male parents and their spatial position allows to retrace pollen movements, providing insights into the potential effects of individual tree traits, the position of mates and the landscape context on spatial genetic structures and contemporary gene flow (Klein *et al.* 2011). Such information can be further expanded using spatially explicit mating models (Burczyk *et al.* 2002; Oddou-Muratorio *et al.* 2005), a paternity based approach that allows to estimate pollen dispersal kernels and infer mating networks for the entire population. Documenting these patterns can unveil demographic and environmental correlates of gene flow patterns (Dyer & Nason 2004; Cheptou & Schoen 2007; Dileo *et al.* 2014) and ultimately mechanisms of individual reproductive success associated to their refugial situation.

In this study, we explore fine-scale mating patterns in a set of long-term refugial forest stands at the southernmost range margin of Pedunculate oak (*Quercus robur*), a major European forest tree. A previous study (Moracho *et al.* 2016) had shown that these stands experience unusually little gene exchange for a wind-pollinated tree species as a result of their spatial isolation, refugial microhabitat and ability to mate locally. Based on paternity analyses of acorn families sampled on 72 mother trees, we here address the following questions: i) How do patterns of mating and pollen dispersal vary among individual trees (both within and across forest stands)? ii) Which are the most relevant intrinsic and extrinsic determinants of individual variation? iii) Are female and male components of the mating system triggered by the same determinants? We first describe individual variation in pollen dispersal within and across forest stands. Then we construct within-stand mating networks for the four largest stands and infer both the maternal and paternal components of mating. Finally, we disentangle the relative importance of intrinsic (tree size and flower production) and extrinsic (neighbourhood features and physical environment) characteristics in generating individual variation in mating and pollen dispersal. Our expectations are that i) individual mating patterns vary greatly because of the heterogeneous refugial landscape context; ii) maternal and paternal mating patterns differ in their main drivers; and iii) the relevance of different drivers of mating and pollen dispersal depends on the scale of observation (landscape, stand or individual). Finally, we discuss the relevance of our findings for the conservation and eventual microevolution of long-term refugial tree populations in a changing environment.

METHODS

Study species

Pedunculate oak (*Quercus robur* L.) is distributed through much of the European continent and into western Siberia (Fig. 4.1). Its southwestern range margin lies in central Spain, where populations are restricted to riparian forests along mountain streams and other environments that mitigate the summer drought typical of this region. The species is strongly outcrossing (Chybicki & Burczyk 2013; Lagache *et al.* 2013, Vranckx *et al.* 2014), although rare individuals with extensive selfing have been described (Chybicki & Burczyk 2013). Like in many other wind-pollinated forest trees (Kremer *et al.* 2012), isolated oak stands commonly experience extensive long-distance pollen flow spanning many kilometers (e.g. Buschbom *et al.* 2011; Hampe *et al.* 2013). However, refugial populations such as the ones investigated here appear to be less susceptible to recurrent pollen immigration (Moracho *et al.* 2016; see also Ouayjan & Hampe 2018).

In our study area, *Q. robur* flowers between March and April, shortly before bud burst. Hybridization with the closely related and locally far more abundant Pyrenean oak (*Q. pyrenaica*) is possible but appears to be rare (<1%, Moracho *et al.* 2016).

Study area and field sampling

The study took place in the Jerte valley (40° 13’ N, 5° 44’ W; Cáceres province), ca. 25 km northeast of the town of Plasencia in western Spain. Descending from an elevation of 1275 m a.s.l. at the header, the valley stretches almost linearly over ca. 30 km (Fig. 4.1). A comprehensive survey of the area concluded with the mapping of 135 adult and close to adult *Q. robur* trees scattered across the valley in ten small forest stands (n = 2-35 trees per stand). The species typically grows along small streams where it coexists with other mesic or riparian tree species such as *Celtis australis*, *Castanea sativa, Fraxinus angustifolius* or *Alnus glutinosa*. Surroundings are covered by broadleaved forests dominated by *Q. pyrenaica* unless these have been cut for horticulture. Somewhat larger *Q. robur* populations (i.e., with a few hundred trees) are located at ca. 10-15 km, although outside the valley and therefore separated by a mountain chain with an altitudinal difference of up to 1000 m.

All 135 identified *Q. robur* trees were sampled for genetic analyses. In October 2009, around 20 acorns were harvested from or, if impossible, beneath the canopy of all actively reproductive trees (n = 72) and stored at -20°C until DNA isolation. We measured the diameter at breast height (dbh) and the canopy projection of each tree in the valley. Male flower production was surveyed during spring 2014 estimating *a visu* the absolute number of male flowers produced by each tree. Estimates were performed independently by two observers and used to assign each tree to one out of eight scores: 1) no flowers, 2) <10,000,

3) 10,000-20,000, 4) 20,000-40,000, 5) 40,000-80,000, 6) 80,000-160,000, 7) 160,000-320,000, 8) >320.000. The scoring was cross-validated by fully counting the total catkin number of 37 trees that covered the while range of flower production observed (see Moracho *et al*. 2018 for further details).

Categorical paternity analysis and pollen dispersal

A detailed description of the genotyping procedure and paternity analysis is provided in Moracho *et al.* (2016). Briefly, we used the 20 microsatellite markers and the laboratory protocol described in Guichoux *et al.* (2011) to characterize the 72 collected acorn families. Three loci (S19, AB and Qr20) had to be excluded due to amplification issues. Repeated analyses of blind samples (n = 44 acorns) with the remaining loci indicated an overall genotyping error rate of 0.85%, confirming the reported high overall quality of the marker set (Guichoux *et al.* 2011).

Before starting the paternity assignment, we checked and removed all offspring whose multilocus genotypes did not match their putative mother tree (indicating sampling errors committed when inaccessibility of tree canopies force collecting seed at the ground). The final sample thus consisted of 688 acorns (9.6 ± 1.3 per mother tree, mean ± SD). A rarefaction analysis indicated that this sample size properly described the genetic diversity of seed families (Moracho *et al*. 2016). Most loci were highly polymorphic, and all individuals (trees and acorns) had unique genotypes. We performed categorical paternity assignment based on maximum-likelihood methods as implemented in CERVUS version 3.0 (Kalinowski *et al.* 2007). Assuming the strict confidence criterion (95%) for assignments, we were able to identify a unique candidate father for 95% of the acorns analyzed. For the remaining 5%, Moracho *et al*. (2016) inferred based on a Bayesian assignment procedure that 4.4% involved *Q. robur* fathers located outside the Jerte valley and 0.6% hybridization events with *Q. pyrenaica.* Here, all non-assigned acorns will be equally treated as immigrants regardless of their putative origin. Based on the described data set, we computed for each individual acorn family the proportions of pollination events resulting from: 1) selfing; 2) local pollination (known father located within the oak stand of the mother tree); or 3) immigration (known or unknown father located outside the stand of the mother tree).

Spatially explicit paternity model

We used a Bayesian model implemented in the program Mixed Effect Mating Model (MEMM; Klein *et al.* 2008) to jointly estimate patterns of within-stand pollen dispersal and heterogeneity in male fecundity. For this purpose, we run independent analyses for the four largest stands (E, DF, K and J; n = 22-35 trees). The model combines the genotypes of sampled acorns, their mothers and putative fathers with the spatial location of all individuals in the stand. Our model considered that each seed i collected on a mother tree j can be sired either, with a probability of i) m, by a tree located outside the focal stand (immigration); ii) s, by the same mother tree (selfing); or iii) $1-m-s$, by another tree within the focal stand. Given that hybridization was marginal in our study system (see Moracho et al. 2016) we do not include it as a form of pollen immigration in the models; thus, all the pollination events involve *Q. robur* individual trees. For modelling the pollen dispersal kernel, i.e. the proportion of pollen released at the source point (0,0) that contributes to the pollen pool at point (x, y), we used the exponential power family. For this family of

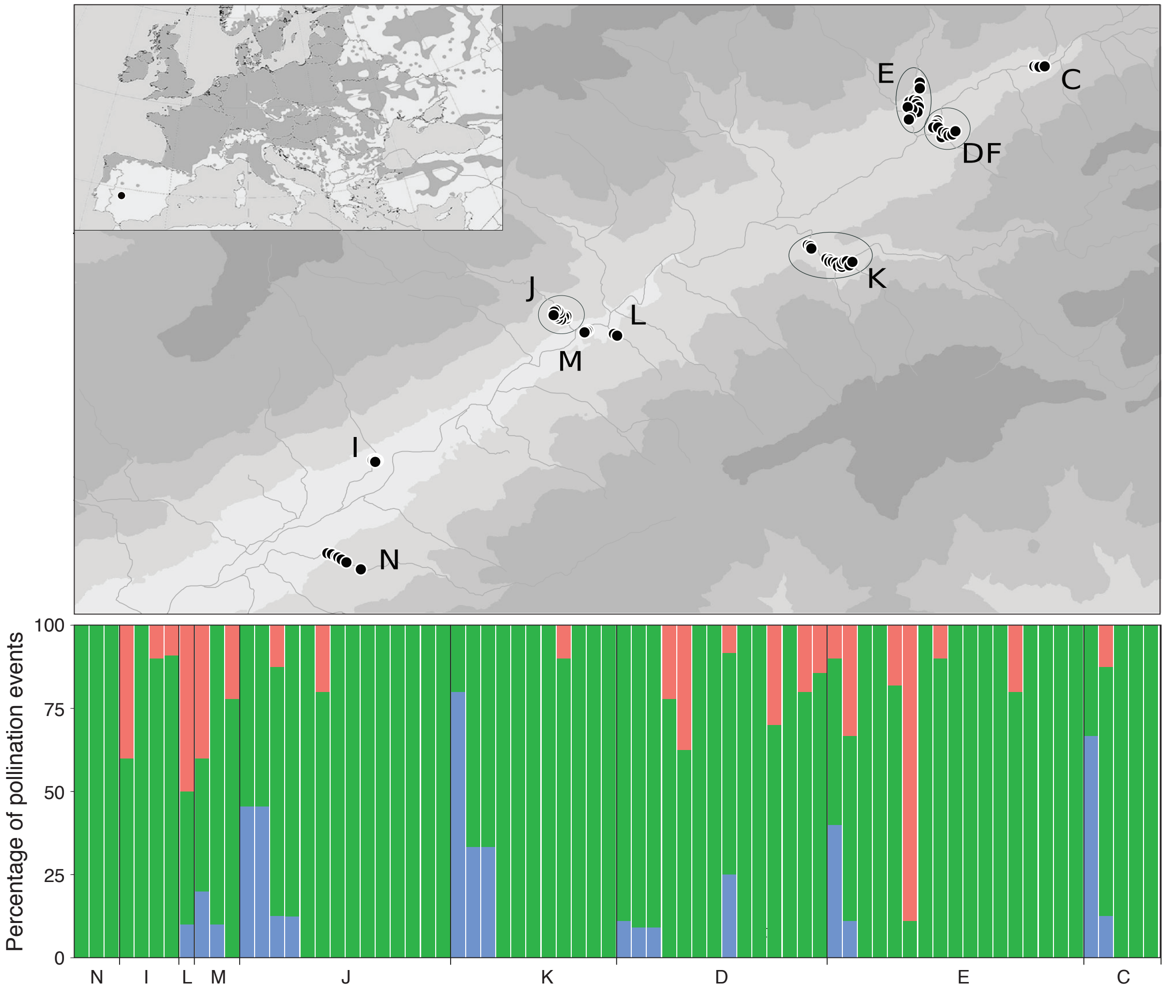

Figure 4.1 (a) Distribution area of *Quercus robur* with a black dot indicating the study area (source: EUFORGEN). (b) Map of the target stands with the four forest stands used for the spatially explicit mating model being indicated by surrounding ellipses. Shading indicates altitudinal ranges in 500 m steps from <500 m asl (white) to >2000 m asl (dark grey). (c) Bar plots showing proportions of pollen dispersal types for seed families. Each mother tree is represented as a line segment that is vertically partitioned with different colours representing the individual's proportions of selfing (blue), outcrossing with individuals from the same forest stand (green) and pollen immigration from outside the stand (red). Letters below the graph refer to the stands as shown in plate b.

functions, two parameters are estimated: the shape parameter (b) and a scale parameter proportional to mean dispersal distance (δ) (see Klein et al. 2008 for details on formulations). Relative male individual fecundities were modelled as random variables that follow a log-normal distribution of mean 1 and variance $\sum 2$. An additional parameter is estimated (d_{obs}/d_{ep}) in relation to the variance of male fecundity $\Sigma 2$, the ratio of the observed (d_{obs}) and the effective density of male reproductive trees (d_{ep}). External allele frequencies were computed from a genotyped sample including all trees growing outside the focal stand in the Jerte valley, plus an additional sample of 252 trees collected in the previously described larger populations located outside the Jerte valley (see Fig. 3.1 in Moracho *et al.* 2018). The initial, minimum and maximum values considered for the Markov chain in this Bayesian approach were, respectively, 10, 0 and 1,000 for δ, 1, 0.1 and 10 for b, 0.5, 0 and 1 for m, 2, 1 and 35 for d_{obs}/d_{ep}, and 0.05, 0 and 0.8 for s.

Using the estimated dispersal kernel and relative individual fecundities together with tree coordinates, we then estimated a global pollination matrix among all trees of each stand. We obtained a symmetric matrix of inferred interactions, where the row vectors represent trees acting as potential fathers and the column vectors represent trees acting as potential mothers (note that each plant of the stand has one row and one column). Each mother column sums to one and each cell estimates the relative fraction of pollen from each father. We binarized the cell values by specifying a threshold MEMM probability value for mating events of $P \leq 0.05$ for the likelihood that two individual trees do not mate (Klein *et al.* 2011). This matrix thus represents all predicted mating events in each stand (i.e., the matrix entries with MEMM values >0.05), where a specific tree can act either as a pollen donor or a pollen receptor.

Finally, we described the predicted pollination pattern within each stand through a set of variables that we calculated for each tree acting either as father or as mother. Thus, we computed for each mother tree: i) the number of pollen donors siring a given mother tree, ii) the mean kinship of pollen donors, iii) the mean genetic distance between pollen donors and the focal mother tree, and iv) the median intermate geographic distance. For father trees, we estimated: i) the number of female mates, ii) the mean kinship of female mates, iii) the mean genetic distance between female mates and the focal father tree, iv) the median intermate geographic distance and v) relative male fecundity. Mean kinship and genetic distance among pairs of mates were estimated in SPAGeDi 1.4 (Hardy & Vekemans 2002). We also calculated a basic measure of network connectivity (degree) for each tree in the stand using the *igraph* package in R version 3.1.0 (R Development Core Team 2016).

Tree-related predictors of mating and pollen dispersal

To assess ecological correlates of individual variation in mating and pollen dispersal, we recorded several intrinsic and extrinsic variables for each of the 72 mother trees as well as for all trees belonging to the four largest stands used for the spatially explicit paternity modelling (n = 107). Our intrinsic correlates were tree size and male flower production. The extrinsic correlates belonged to two groups, one related with the conspecific neighbours (density and genetic relatedness) and the other with the physical environment (topographic position and forest cover) of the focal tree.

Tree size. We constructed a compound measure by mutiplying the dbh and the crown projection of each tree.

Male flower production. Contrary to common expectations, male flowering was not very tightly related with tree size (Pearson $r = 0.65$). Therefore, we directly included our half-quantitative estimates of male flowering (scores 1 to 8) as a surrogate of potential fecundity.

Neighbour density. This variable was estimated as the number of *Q. robur* trees within radius of 25 m. This radius represents the finest spatial scale that could be addressed for all extrinsic predictor variables (see below). In addition, it is close to the median pollen distance in the study area (30.6 m, Moracho *et al*. 2016).

Neighbour genetic relatedness. We computed the mean genetic relatedness of each mother tree with its conspecifics neighbours within the 25 m radius using SPAGeDi 1.4.

Topographic position. The growing site of mother trees was characterized using a topographic position index (TPI) that measures the difference between the elevation of the focal tree and the mean elevation of the surrounding terrain within a radius of 25 m. Positive TPI values indicate a tree that is located on a top while negative TPI values indicate a tree that is growing in a hollow. We used a digital elevation model with a resolution of 5 m (Instituto Geográfico Nacional, distributed by the Centro Nacional de Información Geográfica; http://centrodedescargas.cnig.es) to deduce terrain elevation. The index was computed with the function *tpi* in package spatialEco in R 3.1.0.

Forest cover. The forest cover was estimated in a circular plot of 25 m radius around the mother tree using the Google Earth base map. We derived a semi-quantitative variable with two classes: below and above 50%.

Analysis of individual variation in mating and pollen dispersal

To identify intrinsic and extrinsic ecological determinants of among-tree variation in mating and pollen dispersal, we performed analyses at two complementary levels: across all stands distributed along the Jerte valley (landscape scale) and within stands (local scale).

Valley-wide analysis

We constructed generalized linear mixed models (GLMM) using the proportion of pollen dispersal types (selfing, local pollination and pollen immigration) as binomially distributed response variables. The variables tree size, male flower production, neighbour density, neighbour genetic relatedness, TPI and forest cover were included as fixed effects and the stand of origin as random term. First, we tested the effect of each ecological variable in the saturated model for the different pollen dispersal types. Then, we performed model selection based on the Akaike Information Criterion (AIC). To restrict the total number of models compared, we pooled our explanatory variables in the three groups described above: those intrinsic to the focal tree (I), those related with the conspecific neighbours (N) and those related with the physical environment (E). Then, all possible combinations of these three groups of variables were modelled: I, N, E, I + N, I + E, N + E, I + N + E. We also included a null model without explanatory variables in this procedure. All GLMM analyses were performed using the *lme4* package in R 3.1.0.

Within-stand analysis

This analysis was performed for the four largest stands only to take full advantage of the information obtained through the spatially explicit mating model. We used canonical correlation analysis (CCA) to examine relationships between two multivariate datasets: the set of mating variables obtained from the pollination matrices estimated with MEMM and the set of ecological variables for the individual trees. Two independent CCAs addressed the maternal and the paternal component of mating patterns, respectively. The CCA for the maternal component included the number of pollen donors, the mean kinship of them, the mean genetic distance between pollen donors and the focal mother tree, the median geographic distance between mates, and their network degree centrality. The CCA for the paternal component considered the number of female mates, the median geographical distance between mates, the relative male fecundity and their degree centrality. Both analyses included moreover the previously described ecological variables tree size, male flower production, neighbour density, neighbour genetic relatedness and TPI. We did not use forest cover because of the binary nature of the variable. Instead, we also considered neighbour density within a larger distance (100 m radius) to explore the effect of this variable for more than one spatial scale. Note that neighbour densities at 25 m and at 100 m were only moderately correlated (Pearson r = 0.63). The CCA aims at generating composite variables (canonical variables) that maximize correlations among the multivariate sets of mating and ecological variables. The squared canonical correlation (R^2) corresponds to the percentage of variance in the dependent set explained by the independent set of variables along a given dimension. We assessed whether this relationship is significantly different from zero by means of Bartlett's chi-squared test. We used the cca and yacca packages in R 3.1.0 for this analysis.

RESULTS

Individual variation in mating and pollen dispersal: valley-wide analysis

By far most mating events involved a father tree located within the same forest stand (mean 84.4%, range 0 - 100%; Fig. 4.1). Similar proportions were observed for outcrossing with immigrant pollen (mean 7.6%, range 0 - 89%) and for self-fertilization (mean 6.8%, range 0 – 80%). We detected great variation among individuals. Thus, 19 of the 73 trees analysed experienced at least one selfing event and nine counted with >25% of selfed progenies (Fig. 4.1). A total of 21 trees experienced mating events with pollen from outside the forest stand and six of them displayed pollen immigration rates above 25%. Trees with high rates of selfing or pollen immigration grew across the valley without showing any aggregation in particular stands (Fig. 4.1).

Individual variation in mating and pollen dispersal: within-stand analysis

Table S1 in the Online Appendix provides the average estimates for mating system in each of the four target stands according to MEMM. This analysis produced an adjacency matrix describing within-population patterns of mating, in which each pair of trees was assigned an estimate of the fraction of pollen received by the mother tree from the corresponding father. The spatially explicit representation of this matrix is shown in Fig. 4.2. We generally observed high connectivity among individuals and no major gaps were present among groups of trees except for stand DF. Mating occurred most often among nearby trees. The degree of individual trees within the network was related with their size and

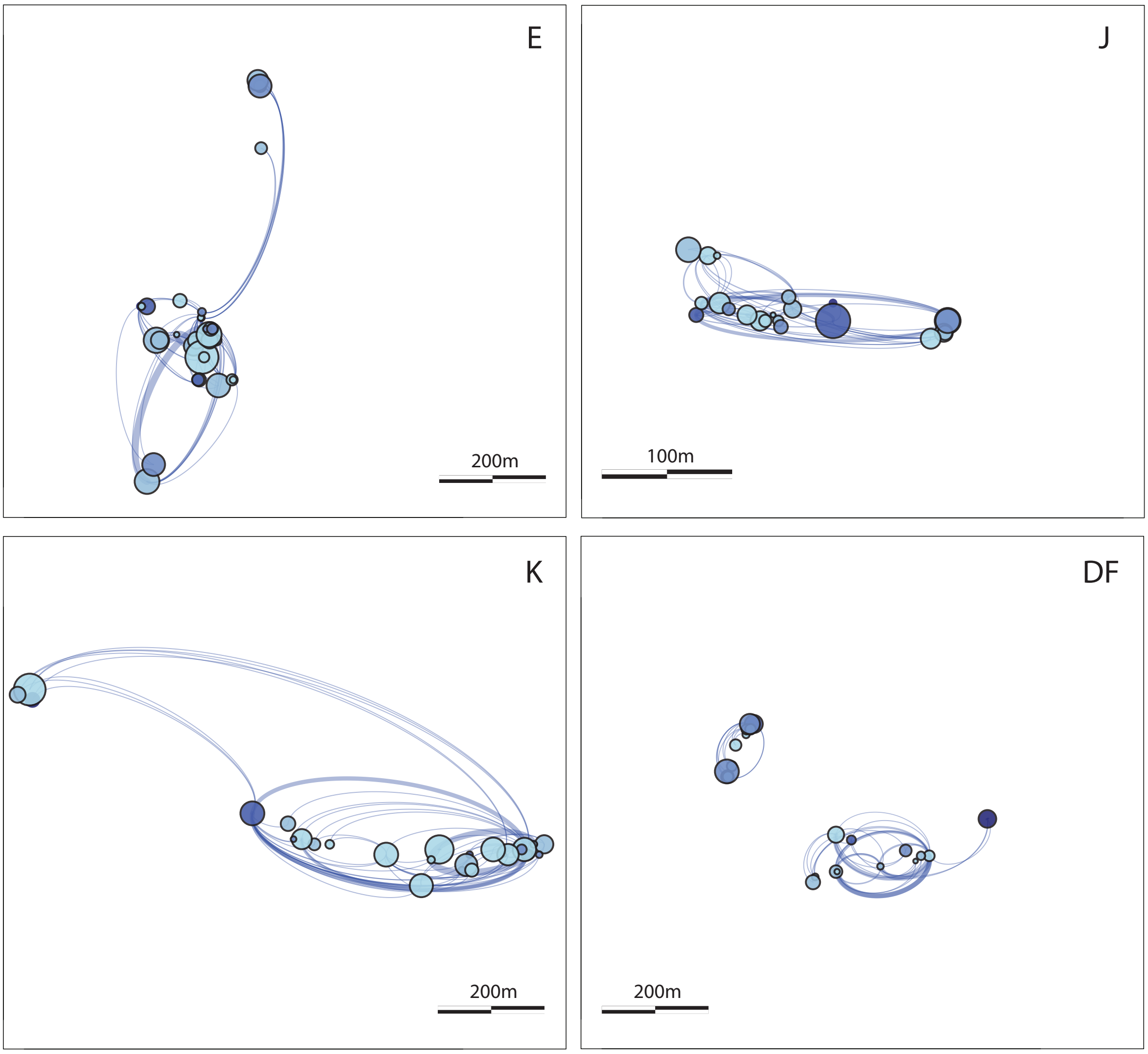

Figure 4.2 Mating networks for the four largest *Quercus robur* forest stands based on the spatially explicit mating model derived with MEMM. Mating events among the mapped individuals of each stand are presented as lines with different colours and thickness according to the probability for a given mother to be sired by a given father. White < 0.05; 0.05 < light blue < 0.1; 0.1 < blue < 0.5; 0.5 < black < 1. Circle size indicates the diameter at breast height (dbh) of the tree and the circle colour represents its degree in the mating network (with darker shading indicating higher degree).

spatial centrality within the stand (GLMM with poisson errors: β= 0.025, SE= 0.002, $P <$ 0.0001; β= -1.280, SE= 0.291, $P < 0.0001$, respectively). Also, the degree was highly correlated to the number of mates for the father perspective (Pearson $r = 0.73$, $t = 10.78$, df = 105, $P < 0.0001$), suggesting that network connectivity is mainly due to the paternal function.All mating parameters inferred from the pollination

matrix showed extensive variation among individuals, both for maternal and paternal progenies (Table 4.1). While the average number of mates was the same for maternal and paternal progenies (4.4), the inequality among pollen donors was much stronger than that among mother trees. Accordingly, MEMM estimated a high variance in male fecundity with most trees contributing little or nothing to effective pollination whilst only 4-18% of the trees in the stand showed fecundities larger than twice the mean value (Fig. S1).

Ecological correlates of mating and pollen dispersal

We observed a series of consistent relationships between pollination types and the quantified ecological correlates (Table 4.2). The propensity of trees to self was affected by intrinsic as well as extrinsic drivers. Selfing was most common in large trees with abundant male flower production that were surrounded by few, closely related conspecifics and grew in areas with high forest cover. The probability of local mating was likewise linked with intrinsic and extrinsic parameters. Small trees with many, little related neighbours showed the highest rates. Finally, the probability of pollination with immigrant pollen was only triggered by extrinsic variables related with the conspecific neighbourhood. Most immigration occurred on trees with few and closely related neighbours. In summary, model selection showed that neighbourhood-related variables

Table 4.1 Summary of predicted mating system (maternal and paternal component) for the *Quercus robur* trees in the four largest forest stands of the study area (n = 107). PD refers to pollen donors and FM to female mates.

Mating variables	*Mean (SD)*	*Range*
Maternal progenies		
Number of PD	4.3 (1.5)	1 - 8
Genetic distance to PD	22.8 (5.2)	11.3 - 36.3
Kinship of PD	0.11 (0.07)	-0.07 - 0.44
Median distance to PD (m)	100 (87)	6 - 418
Paternal progenies		
Number of FM	4.3 (6.9)	0 - 32
Genetic distance to FM	22.6 (5.5)	12 - 37
Kinship of FM	0.10 (0.06)	-0.04 – 0.26
Median distance to FM (m)	73 (84)	0 - 405
Male fecundity	0.8 (1.8)	0 -13.6
Both		
Degree	4.3 (4.2)	1 - 30

Table 4.2 Parameter estimates (β, standard error and P) for the complete mixed-effect model tested for the proportion of each type of pollen dispersal: selfing, local pollination and pollen immigration. ns, not significant at $P < 0.05$. To test different candidate models, we pooled the explanatory variables in groups of predictors intrinsic to the tree (I), those related with the conspecific neighbours (N) and those related with the physical environment (E). All possible combinations of these 3 groups of variables plus the null model were modelled (see Table S2). ΔAIC, difference between the best and the second model; AICwt, probability that the model will be the best model in the set.

A. Full model

Response variable	β	S.E.	P
Selfing			
Size (I)	0.482	0.193	0.012
Male flower production (I)	1.052	0.332	0.002
Neighbour density (N)	-1.423	0.42	<0.001
Neighbour genetic relatedness (N)	0.532	0.225	0.018
Topographic Position (E)	0.02	0.196	ns
Forest cover (E)	0.785	0.244	0.001
Local pollination			
Size	-0.676	0.205	<0.001
Male flower production	-0.011	0.204	ns
Neighbour density	0.821	0.249	<0.001
Neighbour genetic relatedness	-0.39	0.153	0.011
Topographic Position	-0.115	0.164	ns
Forest cover	-0.043	0.179	ns
Pollen immigration			
Size	0.203	0.277	ns
Male flower production	-0.41	0.265	ns
Neighbour density	-0.758	0.289	0.009
Neighbour genetic relatedness	0.445	0.190	0.019
Topographic Position	0.299	0.245	ns
Forest cover	-0.268	0.255	ns

B. Models with grouped explanatory variables

Response variable	Best model	Δ AIC	AICwt
Selfing	I + N + E	1.33	0.64
Local pollination	I + N	1.94	0.73
Pollen immigration	N	2.19	0.63

consistently explained pollen dispersal patterns as the only category common to all best models (Tables 4.2, S2).

The CCA conducted for the four largest stands likewise revealed several consistent relationships between individual variation in the mating patterns predicted by MEMM and the measured ecological correlates. The first and the first three pairs of canonicalvariates (CV) were significantly correlated for the fathers and the mothers, respectively (Table S3). We only retained the results for the first CV pair for further interpretation, however, because they explained a far greater fraction of the total variance than the following CVs (Table S3).

The best fit model for the mother tree perspective explained 59% of the overall variation (Table S3). The mating canonical variate (Mating) showed a strong positive relation with the density of conspecifics within a 100 m radius and a weaker negative relation with the genetic relatedness and the topographic exposition of the tree (Table 4.3). Its relationship with the mating parameters was characterized by a positive link with the number and the genetic distance to pollen donors, and a negative one with the kinship of mates (Table 4.3). Taken together, the result suggests that the density of conspecific neighbours within 100 m was the most consistent trigger of mother trees' mating system with a positive effect on the number of (male) mates and a negative effect on their kinship.

The best fit model for the father perspective explained 41% of the total variation (Table S3). The mating canonical variable (Mating) was strongly related with tree size and male flower production. This canonical variable defines an overall measure of mating system including all mating parameters, being particularly strong the correlation of the number of female mates, male fecundity and degree. Overall, our results indicate that tree size and flower production were the dominant drivers of male mating system in its different dimensions.

DISCUSSION

A previous study on our refugial oak forest stands (Moracho *et al.* 2016) revealed that they have maintained very little gene exchange - both historically and at present - contradicting the widespread notion that wind-pollinated tree populations tend to regularly experience effective pollen dispersal spanning many kilometers (Kremer *et al.* 2012). The study argued that the low connectivity of the stands would have been caused by their refugial habitats whose topography, humidity and dense vegetation would hamper (especially long-distance) pollen movements. Additionally, an efficient genetic purge of recruiting oaks, maintained over many generations, would allow the majority of pollinations to occur among nearby, little genetically-related trees. Here, we advance on the ecological causal mechanisms underlying the limited pollen dispersal in our refugial stands by providing detailed insights into individual-level variation of mating and pollen dispersal. More generally, this study is one of the first to decompose individual-level mating patterns of forest trees from a local (within-stand) to a landscape (valley-wide) scale (but see also Gaüzère *et al.* 2013). Our results show that different maternal and paternal components of the mating system respond to varying drivers depending on the scale of observation. Thus, they illustrate the great complexity of individual-level mating patterns that underlie the more commonly quantified population-level mating systems (Friedman & Barrett 2009; see also Chybicki & Burczyk 2013; Gaüzère *et al.* 2013).

Table 4.3 Structural correlations of CCA analysis involving mating variables and ecological correlates of individual *Quercus robur* trees, calculated once from a maternal and once from a paternal perspective. Results from the first canonical variate pair are presented with the correlation of each original variable with its corresponding canonical variate (Ecological or Mating) and the original mating variables with the Ecological one. Bold numbers indicate correlation values >0.40.

Original variables	Canonical variables	
Mother tree perspective		
Ecological variables	*Ecological*	-
DBH	0.12	-
Male flower production	0.31	-
Neighbour density $_{25\ \mathrm{m}}$	0.18	-
Neighbour density $_{100\ \mathrm{m}}$	**0.77**	-
Neighbour genetic relatedness	-0.39	-
Topographic Position	**-0.44**	-
Mating variables	*Mating*	*Ecological*
Number of pollen donors	**0.73**	**0.56**
Genetic distance to pollen donors	**0.44**	0.34
Kinship of pollen donors	**-0.78**	**-0.60**
Median intermate distance	0.06	0.05
Degree	0.19	0.15
Father tree perspective		
Ecological variables	*Ecological*	-
DBH	**0.89**	-
Male flower production	**0.85**	-
Neighbour density $_{25\ \mathrm{m}}$	0	-
Neighbour density $_{100\ \mathrm{m}}$	0.05	-
Neighbour genetic relatedness	-0.07	-
Topographic Position	-0.32	-
Mating variables	*Mating*	*Ecological*
Number of female mates	**0.92**	**0.59**
Median intermate distance	**0.5**	0.32
Male fecundity	**0.73**	**0.47**
Degree	**0.69**	**0.44**

Among-individual variation in mating: from landscape to local scale

Valley-wide analysis

In accordance with our expectation, trees displayed extensive variation in their proportions of selfing, local pollination and pollen immigration. Moracho *et al*. (2016) had not detected any stand effect on levels of selfing or local pollination, whereas pollen immigration had been triggered to some extent by the size and geographical isolation of stands. Neither selfing nor pollen immigration showed, however, a noteworthy aggregation in particular stands (Fig. 4.1). Selfing was overall frequent (6.8%) compared to levels reported from other oak populations (Nakanishi *et al*. 2005: 0.1%; Pluess *et al*. 2009: 3.5%; Chybicki & Burczyk 2013: 0.5%; Gerber *et al*. 2014: 2.5%), even small and isolated ones (Ortego *et al*. 2013: 1.4%; Vranckx *et al*. 2014: 0.7%). A closer inspection revealed, however, that 54 trees experienced no selfing at all whereas the remaining 19 trees had up to 80% selfed seeds, and only five individuals produced more than half of all selfed offspring. Such an inequality exceeds by far those observed in other oak populations (maximum individual selfing estimates: *Q. robur*: 2.6%, *Q. petraea*: 7.4% [Chybicki & Burczyk 2013]; *Q. lobata*: 9.8% [Pluess *et al*. 2009]; *Q. salicina*: 0.7% [Nakanishi *et al*. 2005]). One could hence speculate whether long-term refugial populations might be disproportionately prone to sustaining individuals capable of selfing (see also Moracho *et al*. 2016). Relictual settings characterized by low density, low pollen competition, and extreme variability of selfing levels, may result in low effective population size and high variation in individual tree contributions to progeny. Interestingly, Gaüzère *et al*. (2013) also reported a high overall frequency and extensive individual variation in selfing (23 of 60 trees affected with maximum values up to 48%) from a southern marginal population of beech (*Fagus sylvatica*). Yet Ouayjan & Hampe (2018) observed no selfing at all in another long-term refugial population of the same species.

On the other hand, pollen migration showed levels of among-individual variation that were curiously similar to selfing (mean: 7.6%, maximum: 89%, distribution: 21 trees with and 52 trees without events), although they concerned a different set of trees (Fig. 4.1). We are not aware of similar studies assessing individual variation in pollen immigration across disjunct broadleaf forest stands (for studies on conifers see e.g. Robledo-Arnuncio & Gil 2005; O'Connell *et al*. 2007; Lesser & Jackson 2013), but the distribution we observed appears to be unusually unbalanced in comparison with other studies on tree stands within continuous populations (e.g. Chybicki & Burczyk 2013; Gaüzère *et al*. 2013; Lagache *et al*. 2013; Gerber *et al*. 2014), single isolated stands (Buschbom *et al*. 2011; Hampe *et al*. 2013), or single isolated trees (Ortego *et al*. 2011). Note, however, that direct comparisons of migration rates and their individual variation are quite challenging because of the idiosyncrasy of case studies.

Within-stand analysis

We also detected extensive among-tree variation in all mating parameters estimated for the four largest stands (Table 4.1). Interestingly, this variation was consistently higher for the male mating components than for the female components. Thus, female and male trees shared the same average number of mates (4.4) yet the variance was much larger for the male component. This trend went along with a highly unbalanced distribution of male fecundities, with a few trees dominating the effective pollen pool and numerous trees contributing nothing at all (see Fig. S1). Pronounced inequality in male fecundity is a widespread phenomenon in forest trees (e.g. Klein *et al*. 2008; Chybicki & Burczyk 2013; Gerber *et al*. 2014; Ouayjan & Hampe 2018). The resulting reduction in effective population size should however be of special concern for small and isolated tree populations such as those found in long-term refugia.

The only mating parameter involving both sexes, the degree within mating networks, showed intermediate levels of individual variation. The connectivity of within-stand mating networks was relatively high (average 4.3 mates) and the tendency to form local mating clusters not particularly pronounced (Fig. 4.2).

Intrinsic and extrinsic determinants of individual mating patterns

Valley-wide analysis

We found substantial evidence indicating that among-tree variation in the frequency of selfing, local pollination and pollen immigration was driven by a combination of intrinsic and extrinsic determinants related with fine-scale pollen availability. Local pollinations were most prevalent in small trees surrounded by many, genetically little related neighbours. On the contrary, both selfing and pollen immigration occurred most commonly in trees that were surrounded by few and genetically related neighbours. Selfing was moreover most frequent in large trees that produced themselves many male flowers. All these trends suggest that both selfing and pollen immigration are most likely to occur in those trees that are not in reach of an abundant and genetically diverse local pollen cloud. High male flower abundance on the tree itself (i.e. a fine-scale phenomenon) would then favour selfing while the existence of large stands in the vicinity (i.e., a landscape-scale phenomenon) would favour pollen immigration. The observed trend fully corresponds to expectations for a mating system driven by pollen competition, where chance pollination events start to predominate over deterministic competition under low pollen load on stigmas (El-Kassaby & Ritland 1992). Oaks, like many other plants, are known to experience pollen limitation (Koenig & Ashley 2003; Pesendorfer *et al*. 2016). Independent field data actually indicate that the fruit set of our oak stands underlies pollen limitation at the landscape (but not at the local) scale (Moracho *et al*. 2018). Several other studies have shown that even small and fine-scale differences in pollen availability can have an important impact on a tree's mating system. Thus, Lagache *et al*. (2013) demonstrated its effects on hybridization in white oaks (see also Chybicki & Burczyk 2013). Quite in line with our results, Gaüzère *et al*. (2013) observed highest levels of pollen immigration in large mother trees located in low-density patches of *Fagus sylvatica*. On the contrary, they also found that selfing was lowest (and not highest) in large trees and not influenced by the density of conspecific neighbours. This counterintuitive result could arise from an overarching effect of the vegetation surrounding trees that acts as a barrier to pollen flow (Gaüzère *et al*. 2013; see also Millerón *et al*. 2012). We detected a similar vegetation effect; yet in our case it did not counteract but paralleled the described effect of tree size (Table 4.2).

Within-stand analysis

All four stands were characterized by a high connectivity of individuals, in line with the absence of pollen limitation within stands (Moracho *et al*. 2018). However, the amount of connections established by individual trees varied among them being related with their size and spatial location within the stand, suggesting that big and centrally-located trees assumed a main role in the pollen-mediated connectivity. The CCA on female trees revealed that the density of conspecifics within 100 m - but not within 25 m - was the most consistent trigger of the mating system, with a positive effect on the number of (male) mates and a negative effect on their kinship. This relationship is likely to directly influence the reproductive success of mother trees, because their fruit set tends to increase with their number of mates (Moracho *et al*. 2018). The greater neighourhood appeared to be more influential than the direct neighbourhood in the largest stands, whereas we did not detect such an effect in the valley-wide analysis (results not shown) probably as a simple sampling effect: No or very few individuals grow at greater distances in many of the smaller oak stands. The observed inconsistency hence suggests that the importance of the small-scale neighbourhood for individual mating patterns might arise from the small size of the stands, rather than from biological features inherent to the studied species (see Lagache *et al*. 2014). On the contrary, the CCA on male trees revealed that their mating system was primarily determined by their size and flower production. This dominance of intrinsic tree features contradicts our prediction that the heterogeneous refugial environment should exert a significant impact on pollen mobilization and resulting mating success (Moracho et al. 2016). It also is at odds with observations of Chybicki & Burczyk (2013) and Lagache *et al*. (2014) that, in mixed *Q. robur*/*Q. petraea* forest stands, male fecundity decreased systematically from the higher towards the lower parts of the study plots, suggesting a major influence of environmental triggers. This could be a particular effect in relict populations where the outstanding presence of huge old trees, producing a vast amount of pollen relative to medium and small sized one, would have a preponderant effect on determining effective pollination over the environment-related issues.

Oak reproduction and conservation in long-term refugia

Our detailed analysis showed that the intrinsic character and the immediate conspecific environment of individual trees are the dominant drivers of the mating system – in other words: the situation of trees within their forest stands overwhelms the situation of the stands themselves. The only exception was (unsurprisingly) the rate of pollen immigration into stands determined by both local and landscape drivers, but even here the effect became only relevant when both local or self pollen were little abundant. We have to admit that our study was carried out in a year of an abundant fruit crop and that the observed trends could well differ in years with less abundant fructification (Nakanishi *et al*. 2005; Pesendorfer *et al*. 2016). However, the great importance of the local environment for mating and reproductive success is in line with what would be expected for long-term refugial populations (Hampe & Jump 2011). And in any case, high productive years are likely the most determinant for the population dynamics.

In this sense, the observed strong dominance of local pollination and absence of local-scale pollen limitation might even be taken as a reassuring phenomenon as it indicates that our study populations could be less susceptible to negative genetic effects of small population size than many more recently fragmented tree populations (Jump & Peñuelas 2006; Aguilar *et al*. 2008). The mechanism of being fertilized by abundant, local but genetically distant pollen, and otherwise by immigrant or even self pollen may help explain the successful long-term persistence of sparse refugial tree populations in an adverse larger environment. As a matter of fact, field and experimental data provide no evidence for noteworthy inbreeding depression in our study system (Moracho et al. 2016). However, the strong dominance of small-scale phenomena for the mating system, and probably plant reproduction in general, also implies that even small changes in the structure of the extant stands – for instance the removal of a few large trees that act as central and highly fecund pollen donors – could have severe consequences. Thus, our study implies (in line with the field evidence from Moracho *et al.* 2018) that management and conservation activities should pay particular attention to securing the inner functioning of local stands (e.g. through securing a sufficient number and density of actively reproducing individuals, and promoting the survival of old large individuals, which besides have the potential to favor connectivity among stands). Moracho *et al.* (2016) concluded that not only the climatic conditions around refugia but also the environment within them can constrain the connectivity of the populations that they harbour. Here, we can complete the conclusion by adding that even optimal climatic conditions within refugia are not sufficient to secure the performance and hence persistence of refugial populations. Hence, climate-change adapted conservation strategies (e.g. Hannah *et al.* 2014) will not succeed when neglecting the key importance of species ecology.

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CAPÍTULO 5

Drivers of tree fecundity in Pedunculate oak (*Quercus robur*) refugial populations at the species' southwestern range margin

Moracho, E., Jordano, P. & Hampe, A. (2018)

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ABSTRACT

Rationale: The current low-latitudinal range margins of many extra-tropical plant species consist of small and scattered populations that persist locally in microrefugia. It remains poorly understood how their refugial distribution affects mating patterns and reproductive success. Here we examine flower and acorn production and their determinants in refugial populations of the widespread European forest tree Pedunculate oak (*Quercus robur*).

Methods: We monitored male flower, female flower and acorn production in 159 adult trees from 12 oak stands over two years. We related these and derived parameters with a series of ecological and genetic predictor variables extrinsic (stand size, density and isolation as well as elevation, topography and forest cover) or intrinsic (size, phenology and several genotypic measures) to the target tree.

Key results: Tree fertility was unrelated with extrinsic factors but determined by tree size, although we detected size-independent variation in reproductive investment. Female flower number accurately predicted acorn crop size. Fruit set differed between years evidencing the existence of pollen limitation at the landscape but not at the local scale. Fruit set also tended to increase with the number of mates of the target tree. We detected no other evidences for genetic constraints on mating.

Main conclusions: Reproduction was triggered by a combination of small-scale and landscape-scale drivers. Although short-distance mating prevailed, limited pollen flow did not appear to significantly constrain reproductive success. The high intrinsic ability of populations to maintain their reproductive capacity may help explain their successful long-term persistence in an adverse broader environment.

KEYWORDS: climate relict, crop size, ecological and genetic correlates, fruit set, gene flow, marginal populations, pollen limitation

The current low-latitudinal range limits of many temperate and boreal tree species consist of populations that have persisted roughly in place through the multiple glacial-interglacial cycles of the Quaternary (Gavin *et al.* 2014). Today, these populations typically are restricted to scattered islands of favourable habitat within heterogeneous landscapes, termed microrefugia (Dobrowski 2010). Long-term refugial populations are most often small and so isolated that local extinction events cannot easily be buffered by regional metapopulation dynamics. Their performance and viability thus depends heavily on inherent population characteristics (e.g. effective size, genetic diversity) as well as on constraints imposed by the surrounding landscape (e.g. triggering their spatial distribution or gene flow; Sexton *et al*. 2009; Hampe & Jump 2011; Levin 2011). Long-term refugial populations are important conservation targets and excellent models for investigating how species can successfully persist over extended periods close to their environmental tolerance limit (Hampe & Petit 2005; Levin 2011; Woolbright *et al.* 2014). While a rapidly growing number of studies have assessed their relationships with spatial variation in current climate (Keppel *et al.* 2012; Hylander *et al.* 2015), the intrinsic dynamics that have enabled them to persist locally under the constraints of their climate-driven confinement remain poorly understood . Thus, we still have a limited understanding of how patterns of mating and gene flow can influence key components for long-term population persistence in refugia such as effective population size, genetic diversity and adaptive potential. And we ignore how landscape complexity combines with individual tree traits to result in the distinct fecundity patterns of relictual scenarios (Bacles & Jump 2011, Hampe & Jump 2011).

Long-term refugial tree populations share many features with younger small and isolated populations, such as pioneer stands at the leading range margin or those resulting from anthropogenic fragmentation (Kramer *et al.* 2008; Bacles & Jump 2011; Hampe *et al.* 2013). But they also share some specific characteristics that could render them particularly prone to experiencing reduced levels of effective mating and pollen flow and hence to depending on a favourable fine-scale mating environment for successful reproduction. First, they typically grow many kilometres away from large pollen sources such as extensive populations in the core distribution range. Moreover, their particular refugial habitats usually are located in topographic settings that help maintain a certain minimum humidity (Hampe & Jump 2011), such as shady valleys, gorges or ravines; these habitats often occur in rugged terrain and contain a dense vegetation that tends to represent an obstacle to long-distance pollen flow (Damschen *et al.* 2014; Shohami & Nathan 2014). Finally, their continued persistence at relatively low population size implies that refugial populations probably have undergone genetic bottlenecks and extensive drift. These processes may imply a significant loss of alleles at self-incompatibility (S) loci (Levin 2011) as well as increased postzygotic inbreeding depression (especially relevant for tree species; Petit & Hampe 2006). Hence, long-term refugial tree populations could experience stronger pollen limitation and less long-distance gene flow than other tree populations that have experienced fragmentation and isolation more recently as a consequence of human activity (Kramer *et al.* 2008; Bacles & Jump 2011). A detailed knowledge of the ecological drivers that influence tree fecundity, mating success and ultimately fitness therefore can provide key information upon the environmental constraints and conservation challenges that such populations face in their current refugial context.

Here we perform a detailed analysis of the patterns as well as the ecological and genetic correlates of tree fecundity and reproductive success in a set of 12 refugial stands of Pedunculate oak (*Quercus robur*) situated at the southwestern periphery of its range (Fig. 5.1). Pedunculate oak is one of the major European forest tree species and a model organism for tree genetic and molecular ecological studies (e.g. *Petit et al.* 2002, 2004; Plomion *et al.* 2015). A recent molecular study of our target stands (Moracho *et al.* 2016) showed that they are remarkably distinct, indicating that they have experienced little effective gene exchange over an extended period of time. Contemporary pollen flow between stands was likewise little frequent while hybridization with the locally abundant sister species Pyrenean oak (*Q. pyrenaica*) was rare (Moracho *et al.* 2016). In accordance, the stands' genetic diversity was relatively low. The present study combines field and molecular data in order to identify intrinsic (tree size, phenology, and genotypic measures) and extrinsic (stand size and isolation, neighbourhood density, elevation, topography, and forest cover) determinants of tree mating and reproductive success in our study system. For this aim, we assess patterns of flowering and acorn production at both the stand and the individual tree level. Moreover, we investigate the phenotypes of acorn families from our target trees to assess eventual effects of the small-scale pollination environment (or 'pollen cloud') on offspring fitness. Our analysis addresses the following research questions: 1) What are the principal determinants of acorn production, fruit set, functional gender, and acorn size (a proxy for offspring fitness) in our study system? 2) At what spatial scale do these determinants act? 3) What is the relative importance of intrinsic *versus* extrinsic determinants? The ultimate goal of this study, with its tight integration of ecological and genetic perspectives, is to broaden our understanding of the ecological and evolutionary determinants of tree reproductive success and resulting population viability in the particular context of long-term refugia.

METHODS

Study system

The southwestern range limit of *Q. robur* is located in the mountain ranges of central Spain. A comprehensive survey across the north of the autonomous region Extremadura (W Spain, ca. 20,000 km^2) identified a total of 18 refugial *Q. robur* forest stands (Pulido *et al*. 2007). Large and continuous populations of the species occur in northern and northwestern Spain. The present study was conducted across 12 of the detected stands, including six from the ten investigated by Moracho *et al*. (2016). They occur in locally humid environments where the summer dry regional Mediterranean climate is mitigated, most typically along streams where *Q. robur* coexists with other mesic tree species such as *Celtis australis*, *Castanea sativa* and *Alnus glutinosa* among others. The landscape surrounding this habitat is largely covered by broadleaved forests dominated by *Q. pyrenaica* where it has not been transformed for horticulture or livestock exploitations. The investigated *Q. robur* stands vary considerably in size (*n*= 3-340 adult trees) and the level of geographical isolation (Fig. 5.1).

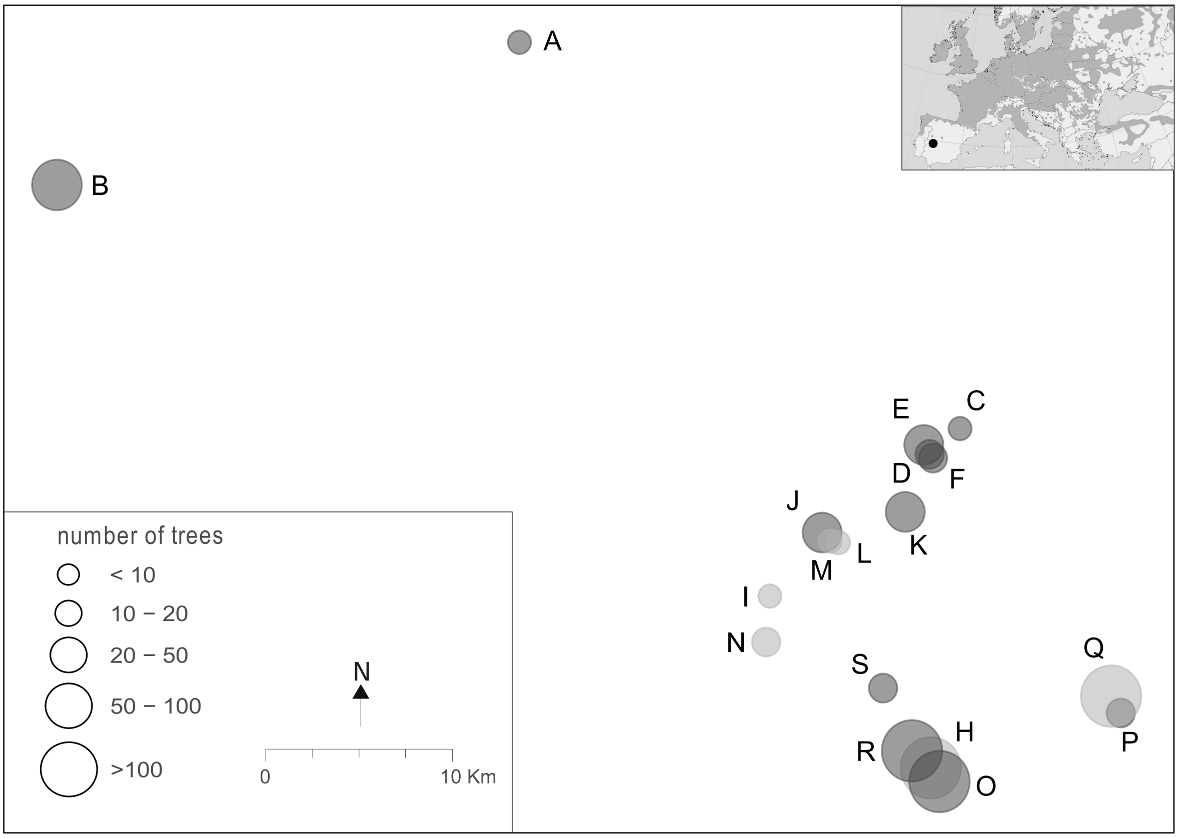

Figure 5.1 (a) Distribution area of *Quercus robur* according to EUFORGEN with a black dot indicating the study area. (b) Map of the investigated refugial stands, represented by circles with circle size indicating the number of adult trees.

Quercus robur is a strongly outcrossing, wind-pollinated species with interannual variation in seed production that can range from alternating to classical masting. In the study area, the species flowers between March and early April, shortly before bud burst, and acorns ripen in late September and early October. Oak populations exhibit a great ability for long-distance pollen flow (e.g. Craft & Ashley 2007; Buschbom *et al.* 2011; Hampe *et al.* 2013). However, different lines of evidence also indicate that pollen limitation could be a widespread phenomenon in oak populations (Knapp *et al.* 2001; Koenig *et al.* 2012; Lagache *et al.* 2013; Pearse *et al.* 2015).

Field sampling

Female flower production: We selected a total of 159 adult trees from across the 12 stands for our field survey of flower and acorn production (Fig. 5.1). This sample included 65% of all the adult trees growing in small stands (i.e., with up to 35 individuals) and a minimum of 20 trees in each of the larger stands. Counts were performed in the first week of November 2012 and 2013, respectively, when virtually all female flower structures had fallen to the ground but remained mostly intact. The number of female flowers produced by a given target tree was estimated by counting the number of aborted and of fully developed acorn cupules in 0.5 x 0.5 m quadrats randomly placed beneath the tree canopy. The number of quadrats sampled was roughly proportional to the total crown projection of the target tree (range: 2-8 quadrats per tree) and their summed surface spanned at least 15% of the projection area. Total flower production per tree was then computed by extrapolating the number of cupules counted in quadrats to the full crown projection. We inferred the overall production of female flowers as the sum of aborted and of fully developed acorn cupules, while fruit set was estimated as the proportion of the overall female flower production that arrived to produce fully developed acorns. Note that our fruit set estimates could be somewhat inflated due to the difficulty in accurately detecting the earliest flower abortions (even though we spent a great effort in identifying them, a task that was facilitated by the conspicuous peduncules of *Q. robur*).

Male flower production: In early April 2013 (that is, near the peak flowering period), we counted the number of catkins on two randomly selected branches from each of the 159 trees also used for the female flower survey. Counts were performed using binoculars and extrapolated to the total crown surface. The validity of this sampling procedure had previously been tested and corroborated by directly counting the total number of catkins on 37 trees that covered the entire range of flower production in the area. Besides, we collected 10 catkins from 12 trees in order to determine the average number of male flowers per catkin (18.5±3.5, mean ±SD) and computed the number of male flowers per tree as the product of the number of catkins and the average number of flowers per catkin.

Floral sex ratio: We used the 2013 field data on female and male flower production to derive the functional gender (G_i) of individual trees. This was done through the function $G_i = g_i/(g_i + a_i \times E)$, where g_i is the number of female flowers produced by the *i* tree, a_i is the number of male flowers, and $E = \sum g_i / \sum a_i$. E is an equivalence factor that equates the probability of androecial units and gynoecial units contributing genes to the next generation (Lloyd 1979, 1980). By this procedure, G_i characterizes the functional "femaleness" of an individual plant.

Acorn mass: We haphazardly collected 11 full-sized, mature acorns from each tree in 2013. Their fresh mass was determined by weighing them to the nearest 0.1 mg.

Tree size and flowering phenology: We measured the diameter at breast height (DBH) of all the trees sampled and characterized their flowering phenology on two dates somewhat before and after the peak of the flowering period 2013 (5 and 23 April, respectively). Four phenological stages of male flowers were distinguished during each survey: (i) swelling buds (score 0); (ii) emerging and immature catkins (score 1); (iii) mature catkins (score 2); and (iv) old, dried catkins (score 3). The phenological stage of the upper and the lower part of each tree was recorded separately and averaged afterwards. Repeating the survey in the following year (2014) allowed us to corroborate that the phenological ranking of trees within stands remains reasonably consistent between years (Spearman rank correlation, $r_s = 0.38$, $P = 0.004$; see also Bacilieri *et al.* 1995).

Ecological and genetic correlates of flowering and acorn production

For each of our 12 target stands, we obtained the following variables: (i) size, calculated as the number of adult trees belonging to the stand and (ii) geographical isolation, measured as the average distance to the other 18 stands. Within stands, we measured the following variables for each target tree: (i) elevation, according to a Digital Elevation Model with a resolution of 5 m (Instituto Geográfico Nacional, distributed by Centro Nacional de Información Geográfica CNIG; http://centrodedescargas.cnig.es); (ii) topographic index (TPI), computed as the difference between the elevation of a focal tree and the mean value of the surrounding landscape (25 m radius) as implemented in package spatialEco function *tpi* (R Development Core Team 2014). This measure describes whether a tree is located on a top (TPI > 0) or in a hollow (TPI < 0); (iii) percentage of forest cover within a 25 m radius around each focal tree (with the two classes <50% and >50 %); and (iv) population density, measured as the number of conspecific trees within the same radius.

All target trees had been genotyped using 20 nuclear microsatellite markers and the procedures are described in detail in Moracho *et al.* (2016). Based on the trees' multilocus genotypes, we computed the mean genetic relatedness of each target tree with its conspecific neighbours growing within a 25 m radius using the coefficient of Queller & Goodnight (1989). In addition, we used the individual genotypes of the acorn families from 47 trees reported in Moracho *et al.* (2016) to calculate, for each mother tree's progeny, the following variables: (i) gene diversity (H_E), (ii) mean kinship (computed using the coancestry coefficient of Loiselle *et al.* 1995), (iii) inbreeding coefficient (F_{IS}), and (iv) number of pollen donors (computed as described in Moracho *et al.* 2016). These variables can be considered to characterize the 'pollen cloud' of individual mother trees. All genetic parameters were obtained with SPAGeDi 1.4 (Hardy & Vekemans 2002) except for the number of pollen donors that was based on paternity analyses conducted in CERVUS 3.0 (Kalinowski *et al.* 2007).

Statistical analyses

Flowering and acorn production

We first tested for correlations between our different response variables (numbers of male and of female flowers, acorn number, fruit set, acorn mass, floral sex ratio). Then we estimated variance components of the female fecundity parameters (female flower number, fruit set and acorn mass) considering four levels: among stands, among individuals within stands, within individuals, and between years. Finally, we performed linear mixed models considering a hierarchical nested structure of random effects to assess the statistical support of our variance components analysis. No fixed terms where included in these models. These analyses were performed in R v.3.0.2 (R Development Core Team 2014) using the packages 'ape' and 'lme', respectively.

Ecological and genetic correlates of flowering and acorn production

We attempted to identify both extrinsic and intrinsic determinants of among-tree variation in the three response variables female flower number, fruit set and acorn mass. Two analyses focused on extrinsic determinants. The first model examined among-stand variation considering stand size and geographical isolation as predictor variables. The second model examined within-stand variation with elevation, topographic index, forest cover, and population density as predictors. Two further analyses focused on trees' intrinsic determinants (again for the three response variables female flower number, fruit set and acorn mass). The first model included DBH, flowering phenology, and mean genetic relatedness with conspecific neighbours as predictor variables. The second model used only the subset of trees for which we had analysed acorn progenies and included the (progeny-based) variables gene diversity (H_E), mean progeny kinship, inbreeding coefficient, and number of pollen donors.

Finally, we constructed two more models with the floral sex ratio as response variable. The first model considered the extrinsic variables elevation, topographic index, forest cover, and population density as predictors, while the second model included the intrinsic variables DBH, phenology and relatedness.

We employed general linear mixed models (GLMMs) in a maximum likelihood framework (Burnham & Andersen 2002) using the lme function (package 'nlme') to specify Gaussian errors and the glmer function (package 'lme4') to specify Poisson errors when estimating models with the variable female flower number. We considered the stand as a random factor and also the individual tree when fecundity data were available for two years (e.g. for female flower number; see Table 5.1). All predictor variables of the six models were considered as fixed factors.

RESULTS

Patterns of flowering and acorn production

Average values for flowering and acorn production are summarized in Table 5.1. Male flower number was positively related with both female flower number (Spearman r_s = 0.68, n = 156, P < 0.0001) and acorn number (r_s = 0.64, n = 155, P < 0.0001). Even after accounting for tree size, male and female flower number retained a clear positive relationship (Spearman r_s = 0.62, n = 156, P < 0.001). Female flower number closely matched acorn production in both years (2012: r_s = 0.92, n = 157, P < 0.001; 2013: r_s = 0.93, n = 157, P < 0.001) and floral sex ratio (measured as 'femaleness': r_s = 0.37, n = 156, P < 0.001) showed a similar albeit weaker trend. Finally, fruit set was unrelated with either fe-

Table 5.1 Summary statistics for the surveyed fecundity parameters of individual *Quercus robur* trees. Integers are rounded to the nearest hundred.

	2012	2013
Male flower number	-	3,387,400 ± 3,106,100
Female flower number	17,000 ± 23,900	10,100 ± 15,700
Floral sex ratio	-	0.455 ± 0.232
Acorn number	7,400 ± 11,600	3,700 ± 7,500
Fruit set (%)	42.6 ± 18.9	33.5 ± 15.6
Acorn mass (g)	5.9 ± 1.7	-

male flower number (r_s = -0.02, n = 157, $P > 0.05$), male flower number (r_s = 0.13, n = 154, $P > 0.05$) or floral sex ratio (r_s = 0.03, n = 154, $P > 0.05$).

Despite marked between-year variation in the number of female flowers, the fertility ranking of individual trees in 2012 and 2013 remained highly consistent (Spearman r_s = 0.84, n = 156, $P < 0.001$). Accordingly, the variance component analysis revealed that the main source of overall variation in female flower number were differences among trees (CV = 0.59; see Fig. 5.2). Fruit set also varied substantially between the two years of study (CV = 0.52), being higher in the year of more abundant female flower production (2012). The ranking of individual trees regarding fruit set was rather consistent between years (Pearson r = 0.60, n = 155, $P < 0.001$) albeit less than female flower number. Acorn weight varied primarily among trees (Fig. 5.2). Among-stand variation was low (although statistically significant) for all fecundity parameters (Fig. 5.2). AIC-based tests indicated that including all these sources of variation as random effects in the models of ecological and genetic correlates significantly improved their fit with respect to the null model.

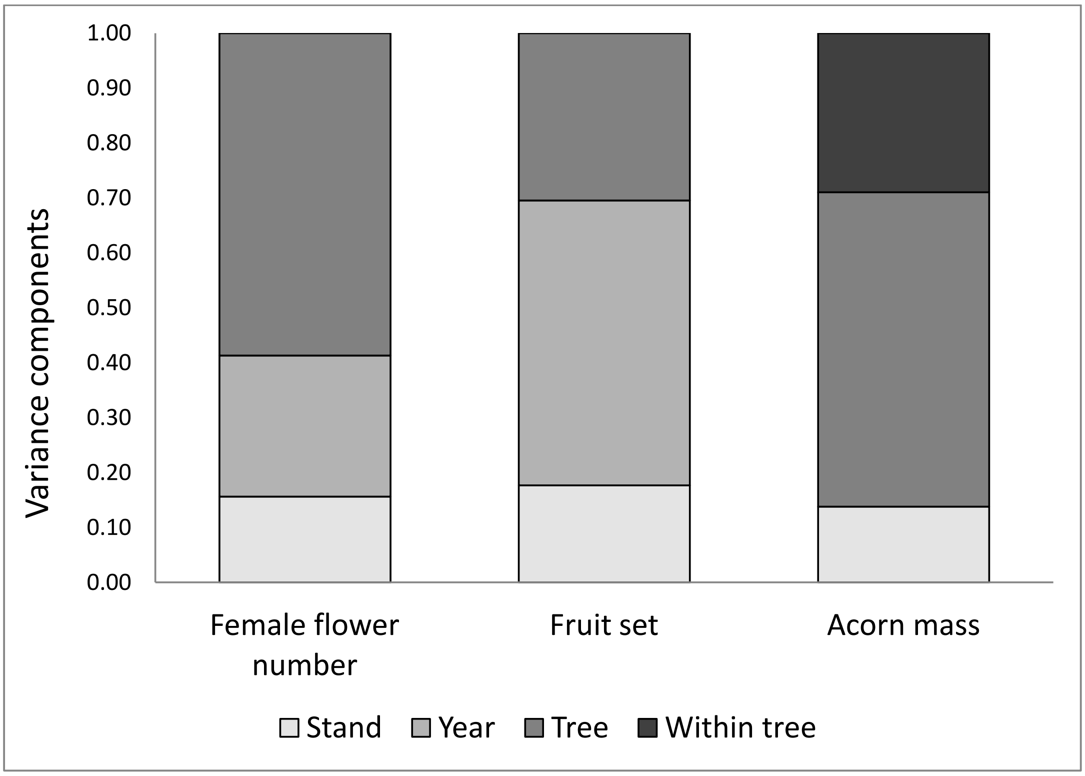

Figure 5.2 Distribution of overall variation in three measures of *Quercus robur* tree fecundity and offspring fitness in refugial forest stands. Four hierarchical sources of variance are considered (within-tree < tree < year < stand). Note that between-year variation among individual trees was not available for acorn mass.

Table 5.2 Effects of different grouped predictor variables ('extrinsic' versus 'intrinsic', each at two different scales) on female flower number, fruit set and acorn mass. The SD of random effects (year/stand) and the sample size are shown for each model. Models for female flower number and fruit set are based on 2 years of sampling (and hence the twofold number of observations); the fact that trees were repeatedly censused was taken into account by including their identity as random effect in the model.

	female flower number			fruit set			acorn mass		
	β	SE	*P*	β	SE	*P*	β	SE	*P*
Extrinsic among-stand									
Stand size	0.32	0.18	–	0.06	0.00	**	−0.45	0.35	ns
Isolation	0.01	0.18	ns	−0.05	0.07	*	−0.05	0.34	ns
Random effects	1.30/0.39			0.10/0.03			–/0.83		
Sample size (n)	318			318			159		
Extrinsic within-stand									
Elevation	−0.29	0.19	ns	−0.01	0.02	ns	0.23	0.26	ns
Topographic index	0.01	0.12	ns	0.00	0.01	ns	−0.06	0.15	ns
Forest cover	−0.39	0.13	**	0.00	0.01	ns	−0.26	0.15	
Population density	−0.10	0.13	ns	0.01	0.01	ns	0.12	0.16	ns
Random effects	1.27/0.49			0.10/0.08			–/0.74		
Sample size (n)	220			220			110		
Intrinsic tree-based									
DBH	1.00	0.13	***	−0.01	0.02	ns	0.62	0.18	***
Phenology	0.11	0.14	ns	0.01	0.02	ns	0.29	0.20	ns
Relatedness	−0.08	0.11	ns	0.01	0.01	ns	0.02	0.16	ns
Random effects	1.03/0.59			0.08/0.06			–/0.30		
Sample size (n)	220			220			110		
Intrinsic progeny-based									
Kinship of progeny	0.12	0.22	ns	0.02	0.02	ns	−0.51	0.50	ns
Pollen donors	0.06	0.22	ns	0.04	0.02		−0.33	0.52	ns
Inbreeding	0.10	0.20	ns	−0.02	0.02	ns	0.50	0.48	ns
Random effects (SD)	1.17/0.62			0.01/0.04			–/1.06		
Sample size (n)	94			94			47		

Results of GLMM are shown: – $P < 0.1$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

Determinants of flowering and acorn production

Extrinsic determinants

Stand characteristics influenced the fruit set (Table 5.2), which increased with greater stand size and decreased with stronger isolation. Stand size also had a marginal effect on female flower number ($P = 0.07$), with trees in larger stands producing more flowers. Acorn mass was unaffected by stand identity.

Within stands, forest cover was the only relevant variable in predicting female flower number, with more fecund trees occurring in less forested vicinities. We detected no effects on fruit set, while a tendency of larger acorns to occur in less forested sites was only marginally significant ($P = 0.1$). Finally, floral sex ratio variation was affected by the elevation of trees' growing sites, although the slope of the relationship was virtually horizontal (slope = 0.001; Table 5.3).

Intrinsic determinants

Unsurprisingly, female flower number was strongly predicted by the DBH of target trees (Table 5.2). The DBH was moderately but significantly related with female flower number ($r_{s\ 2012} = 0.35$, $r_{s\ 2013} = 0.34$), male flower number ($r_s = 0.32$) and acorn number ($r_{s\ 2012} = 0.31$, $r_{s\ 2013} = 0.26$; $P < 0.01$ for all variables). Acorn mass also increased with the DBH, whereas fruit set was unaffected by any of the tested predictor variables (Table 5.2). Our analysis of the trees with available progeny data revealed instead that fruit set tended to be predicted by the number of pollen donors ($P = 0.06$; Table 5.2). On the contrary, neither female flower number nor acorn mass showed any relationship with the genetic properties of the progenies. Finally, the floral sex ratio was marginally affected by trees' flowering phenology ($P = 0.08$; Table 5.3).

Table 5.3 Effect of different predictor variables on floral sex ratio (measured as 'femaleness'). Stand is included as a random factor and sample size is n = 110.

	β	SE	*P*
Extrinsic			
Elevation	0.001	0.000	**
Topographic index	−0.001	0.009	ns
Forest cover	−0.063	0.040	ns
Population density	−0.003	0.006	ns
Random effect (SD)	0.092		
Intrinsic			
DBH	0.000	0.001	ns
Phenology	−0.068	0.039	–
Relatedness	0.101	0.080	ns
Random effect (SD)	0.135		

DISCUSSION

Patterns of flowering and acorn production

We observed ample variation in flower and acorn production as well as in acorn mass. The principal source of variation were differences among individual trees, whereas differences among stands were throughout very minor. Both flower and acorn numbers also differed markedly between the two years of study. These patterns of variation largely correspond to those reported from various other oak species in similar environments (e.g. Sork *et al.* 1993; Pérez-Ramos *et al.* 2015; Pesendorfer *et al.* 2016). At the level of individual trees, the initial number of female flowers was a powerful predictor of the resulting acorn crop (r_S = 0.92-0.93), implying that fruit set in a given year was remarkably consistent among trees (Fig. 5.2; see also Feret *et al.* 1982; Sork *et al.* 1993). At the level of the entire population, however, fruit set was about a third higher in the year with a higher initial abundance of female flowers (2012), indicating considerably more frequent pollination and resulting in a twofold acorn crop. Although based on only two years of data, our observation is fully in line with reports indicating that the mating environment largely triggers interannual variation in oak acorn crops ("wind pollination hypothesis": Koenig *et al.* 1994; see also García-Mozo *et al.* 2007; Koenig *et al.* 2015; Pearse *et al.* 2015; Pesendorfer *et al.* 2016). Meteorological conditions may have further supported the observed trend (Bell & Clark 2016) as the accumulated precipitation during the acorn maturation period was three times higher in 2012 than in 2013 (Agencia Estatal de Meteorología; www.aemet.es). Finally, the strong effect of female flower number on acorn crop size probably triggered also the observed (weaker) relationship between floral sex ratio and acorn production.

Determinants of flowering and acorn production

Unsurprisingly, the production of flowers and acorns was consistently related with tree size (estimated via the DBH). This relationship remained virtually unaltered by the observed between-year variation in flowering and fruiting, suggesting that trees of different sizes tended to behave similarly across years (Sork *et al.* 1993; but see Hirayama *et al.* 2008). We also observed larger female flower numbers in less forested sites; however, this trend is most probably just a secondary effect that arises from a tendency of larger trees to grow in open sites (E. Moracho, unpubl. data).

Male and female flower number retained a remarkably strong positive relationship (r_S = 0.62) even after accounting for tree size, indicating that some trees invested systematically more in reproductive structures than others. This phenomenon has repeatedly been shown in oaks and has been related with small-scale variation in growing site productivity (Knops

& Koenig 2012; Pérez-Ramos *et al.* 2014) or microclimate (Koenig *et al.* 2015). It is in line with the notion that, for long-lived organisms, a plastic resource tracking response to environmental fluctuations may be more adaptive than directly linking life-history traits through trade-offs such as well-defined functional genders (Knops & Koenig 2012; see also Petit & Hampe 2006). Fruit set outstood from the other tree fecundity measures as the only parameter that was primarily determined by stand-level characteristics. Both the size and the geographical isolation of stands affected fruit set in a way that points to the existence of pollen limitation at a landscape scale (cf Fig. 5.1). Fruit set hence appears to underlie the same constraints as previously shown for the rate of between-stand pollen flow (Moracho *et al*. 2016). On the contrary, we found very little evidence for pollen limitation at finer spatial scales despite using several ecological and genetic indicators (population density, tree phenology, genetic relatedness, number of mates, progeny kinship and inbreeding). The only eventual and very indirect indication consists in the fact that fruit set showed a marginally significant trend ($P = 0.06$) of being higher in those trees that received pollen from a greater pool of mates. Our inability to detect local-scale pollen limitation is at odds with several other studies in oaks (Knapp *et al.* 2001; Koenig *et al.* 2012; Lagache *et al.* 2013; Pearse *et al.* 2015) that have led to increasing awareness of the phenomenon (Koenig & Ashley 2003; Friedman & Barrett 2009). The absence of local-scale pollen limitation in our system could also explain why we failed to detect any influence of phenology on fruit set, whereas Koenig *et al.* (2012) reported clear evidence for stabilizing selection on flowering time that arises from pollen limitation of very early or late flowering individuals.

Acorn size also was positively related with tree size, a phenomenon that has previously been reported in detail by Koenig *et al.* (2009). This relationship could be of particular relevance in our refugial context because larger acorns are known to produce more performant offspring (Gómez 2004; Sage *et al.* 2011), an important fitness advantage in Mediterranean-type environments where early germination and rapid growth can greatly enhance seedling survival to summer drought. Hence large trees would have a double fitness advantage in our context, given that their reproductive success exceeds that of smaller conspecifics both in terms of offspring quantity and quality.

Consequences for tree reproductive success in a refugial context

Overall, the reproductive success of our refugial oak stands appears to be triggered by a combination of local-scale and landscape-scale drivers. Flower and acorn production depend basically on individual tree size, whereas mating success in terms of fruit set is primarily determined by the size and geographical isolation of the stand. The fact that pollen limitation became evident at the landscape-scale but not at the local stand scale allows two inferences: (i) most pollen dispersal appears to occur over relatively short distances (Moracho *et al.* 2016; see also Knapp *et al.* 2001; Sork *et al.* 2002) and (ii) the current population structure with adult trees growing at relatively high density within their refugial habitats provides however sufficient opportunities for effective mating. If these inferences are correct, then our study implies that conservation measures should pay particular attention to securing the inner functioning of local stands (e.g., through securing a sufficient number and density of actively reproducing individuals). Moracho *et al.* (2016) showed that, at least in years of abundant acorn crops, by far most mating events occur within local stands. This finding, together with the remarkably strong genetic subdivision of stands, indicate that the fecundity of our long-term refugial populations suffers unusually little from incompatibility and inbreeding, otherwise a widespread phenomenon in small and isolated tree populations (Petit & Hampe 2006). This intrinsic ability to maintain their reproductive capacity may help explain the successful long-term persistence of refugial tree populations in an adverse environment (Hampe & Jump 2011).

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CAPÍTULO 6

Discusión general

Las poblaciones periféricas constituyen piezas clave para predecir el impacto del cambio climático sobre el rango de distribución de las especies (Sexton *et al.* 2009). Como consecuencia del desajuste en las condiciones climáticas, se predice que ocurran diferencias en las tasas de crecimiento poblacional con valores positivos en los bordes en expansión (latitudes y elevaciones altas) y negativos en los bordes de retaguardia (latitudes y elevaciones bajas). El estudio abordado bajo esta hipótesis (Capítulo 2) se basa en analizar el *performance* poblacional en sus diversas componentes (fecundidad, supervivencia y crecimiento poblacional), pues ofrecen un valioso indicador temprano del efecto del cambio del clima sobre las poblaciones. Los resultados ponen de manifiesto **un comportamiento asimétrico de las poblaciones en ambos márgenes de la distribución de las especies de acuerdo a las expectativas del cambio climático** (Capítulo 2: figura 2.2). Las poblaciones del margen a latitudes bajas muestran un *performance* reducido respecto a otras áreas del rango (centrales y latitudes altas), mientras que a latitudes altas no sobresale frente al estimado en áreas centrales pero tampoco queda atrás (como predice la teoría centro-periferia). Este efecto guarda relación con la intensidad de cambio de temperatura en las zonas de estudio (Capítulo 2: figura 2.3), a pesar de que las condiciones ambientales relevantes para las distintas especies pueden ser muy variables. Por tanto, encontramos un impacto del cambio de clima doblemente negativo donde ya se evidencia la vulnerabilidad a la extinción local de las poblaciones del margen de baja latitud, pero además, el retraso de las poblaciones de latitudes altas en su reacción de expansión como respuesta al desplazamiento de las condiciones climáticas. Esta asimetría latitudinal parece una tendencia general a través de los reinos y tipos de hábitat, a pesar de las particularidades biológicas de la diversidad de seres vivos incluidos en el estudio (Apéndice C: figura C2). La notable representación de especies vegetales leñosas (17%) en la base de datos recopilada sugiere que el efecto se hace extensivo a organismos que son iconos de resiliencia entre los seres vivos por su gran capacidad para amortiguar el estrés climático mediante plasticidad fenotípica y su largo ciclo de vida (Morris *et al.* 2008, Villellas *et al.* 2015).

Las evidencias empíricas señalan la gran vulnerabilidad a la extinción local de poblaciones del margen de retaguardia; no obstante, la respuesta de las poblaciones que conforman este margen variará según las diferentes historias ecológicas y evolutivas que hayan experimentado (Capítulo 1: figura 1.1). Aquí destacan los relictos climáticos estables (*stable rear-edge relicts*) (Hampe & Petit 2005) - poblaciones fragmentadas "dejadas atrás" cuando los rangos de las especies cambiaron en respuesta a cambios climáticos del pasado – como modelos de persistencia a largo plazo soportando grandes variaciones ambientales.

La falta de conocimiento acerca de los mecanismos que contribuyen a la alta resiliencia de estas poblaciones relictas ha motivado esta tesis. Usando como organismo modelo la especie arbórea *Q. robur* en un conjunto de poblaciones relictas, he estudiado el flujo génico (con énfasis en la dispersión de polen) y la reproducción, aspectos clave para la diversidad genética, el potencial de regeneración y en definitiva la viabilidad de las poblaciones a largo plazo. Concretamente, (i) he analizado los patrones de flujo génico y fecundidad a escala de paisaje (Capítulos 3 y 5), (ii) para después profundizar en la variabilidad existente entre individuos y los determinantes ecológicos a escala local (Capítulos 4 y 5). A continuación, se expone una discusión general de las principales contribuciones.

EL FLUJO GÉNICO Y CONECTIVIDAD EN POBLACIONES RELICTAS: LA INFLUENCIA DEL ESCENARIO

El estudio de los patrones de flujo génico histórico y contemporáneo en poblaciones relictas de *Q. robur* ofrece un resultado sobresaliente: **la excepcional escasez de flujo génico para un árbol de polinización anemófila** (Capítulo 3: figura 3.1 y 3.2). Encontramos una fuerte diferenciación genética de la población adulta salpicada de eventos esporádicos de dispersión histórica. Estos patrones son coherentes con el limitado flujo de polen existente entre los rodales del valle (2.6%) o desde el valle contiguo (4,4%) lo que indica que la débil conectividad no es un fenómeno a corto plazo. Igualmente resalta el escaso flujo de polen procedente de la otra especie de *Quercus* con la que coexiste (0.6%), mientras domina la polinización a corta distancia entre individuos del mismo núcleo poblacional (85.6%, distancia mediana de dispersión = 30m).

Muchos estudios demuestran que la dispersión de polen en poblaciones de árboles a menudo cubre varios kilómetros (revisión en Kremer *et al.* 2012). Incluso en poblaciones pequeñas y aisladas de robles y otras especies se han documentado tasas de inmigración notables, lo que pone de manifiesto su aislamiento geográfico pero no genético (p. ej., Buschbom *et al.* 2011, Hampe *et al.* 2013, Lesser & Jackson 2013, Robledo-Arnuncio & Gil 2005; Robledo-Arnuncio 2011; Schueler & Schluünzen 2006). Las estimas de flujo genético en mi sistema de estudio claramente contradicen estos patrones. ¿Porqué el flujo génico y la conectividad pueden ser mucho más limitados en áreas de refugio climático?

Los patrones de dispersión de polen observados son fácilmente interpretables como un proceso dependiente de la densidad de polen (Holsinger 1991). Diversos factores (ecológicos, demográficos y físicos) en ambientes relictos (ver Capítulo 3: tabla 3.2) determinan la abundancia relativa de polen local *vs.* inmigrante que marca la competencia por alcanzar los estigmas. La baja frecuencia de dispersión entre los rodales parece guardar relación con dos fenómenos: (i) se produce polen local en cantidad suficiente y (ii) se moviliza una pequeña fracción de polen inmigrante debido al pequeño tamaño de los rodales y su aislamiento geográfico. **El tamaño del rodal afecta a los patrones de dispersión de polen inmigrante**. Por una parte, los núcleos poblacionales de mayor tamaño producen polen local en alta densidad que compite favorablemente con el polen inmigrante. Esta hipótesis se ve reforzada con la moderada diversidad genética y el parentesco relativamente bajo entre los individuos del mismo núcleo (Capítulo 3: tabla 3.1), de manera que aspectos genéticos de incompatibilidad y depresión por endogamia no parecen obstaculizar el cruzamiento entre vecinos (Capítulo 3: tabla 3.1; ver página 53) . Por otra parte, en núcleos de población pequeños donde escasea el polen local aumenta la probabilidad de fertilización con polen inmigrante. No obstante, la frecuencia de estos eventos es baja y se detectan evidencias de limitación de polen en estos núcleos, particularmente si su aislamiento es elevado (ver *fruit set* en Capítulo 5: tabla 5.2). **El aislamiento geográfico supone una barrera adicional al flujo de polen** en nuestro sistema de estudio (Capítulo 3: figura 3.2, tabla 3.2), donde además, otros factores físicos característicos de los refugios climáticos (como una marcada topografía, un microclima húmedo y una densa cobertura forestal) pueden dificultar más tanto la salida como la llegada del polen inmigrante (Di-Giovanni & Kevan 1991). Por último, variaciones fenológicas entre núcleos poblacionales también afecta la composición de la nube polínica en el momento de la fertilización. La probabilidad de recibir polen inmigrante aumenta en los rodales de floración tardía respecto a los de floración temprana (Capítulo 3: tabla 3.2) (ver también Lagache *et al.* 2013). Este efecto parece lógico en el sistema de estudio donde la fenología de la floración es extremadamente corta (E. Moracho, obs. pers.) y se caracteriza por una distribución asimétrica de individuos en el máximo de floración, donde al principio de la estación la mayoría de los individuos están en flor pero existe una cola de individuos rezagados de floración tardía. Dado que las flores femeninas de *Q. robur* están receptivas después de la liberación de polen (protandria), en los rodales más tardíos estarán receptivas cuando haya menos polen disponible y se relaje la competencia, permitiendo la fertilización con polen inmigrante (ver caso contrario de protoginia en Gaüzère *et al.* 2013).

En resumen, existe un débil patrón de conectividad en poblaciones relictas de *Q. robur* influido por factores a escala de paisaje que es fuertemente heterogéneo espacialmente, con rodales funcionando predominantemente como fuente o sumidero de polen y otros aislados prácticamente al flujo génico (Capítulo 3: figura 3.3). Por ejemplo, los núcleos pequeños y de floración tardía (ver L y M) son sumideros de notables cantidades de polen, mientras que las mayores poblaciones del valle contiguo representan una fuente de polen no despreciable (4,4%), que supera a la fracción de polen intercambiado entre los rodales del valle. Como consecuencia, la red de flujo de polen es vulnerable a cambios en la densidad y configuración espacial de los núcleos de población.

LA IMPORTANCIA DE LA VARIABILIDAD A ESCALA FINA EN LOS PATRONES DE POLINIZACIÓN Y FECUNDIDAD

Los patrones de flujo génico entre núcleos poblacionales del valle tienen una clara influencia del escenario relicto (Capítulo 3). Adicionalmente, factores operando a menor escala adquieren relevancia para explicar los patrones de cruzamiento dentro de los rodales (Chybicki & Burczyk 2013, Gaüzère *et al.* 2013, Lagache *et al.* 2013). Los estudios abordados a esta escala desvelan una **gran variabilidad entre individuos en la dispersión de polen, el sistema de cruzamiento y la fecundidad (tanto masculina como femenina) que responde mayoritariamente a diferencias en el ambiente local y rasgos intrínsecos del individuo.** A continuación discutimos los patrones observados y los factores ecológicos y genéticos que los determinan.

La polinización en la mayoría de los individuos ocurre solo entre individuos del mismo rodal (Capítulo 4: figura 1), mientras que los eventos de inmigración (7.6%, sumando todas las fuentes) y la notable autofecundación (6.8%) se atribuyen a una reducida fracción de individuos. Los valores de autofecundación de algunos individuos (hasta el 80% de la progenie) están muy por encima de los registrados en poblaciones pequeñas de robles y relativamente aisladas (Ortego *et al.* 2013, Vranckx *et al.* 2014), sin embargo estudios en poblaciones marginales de otras especies si demuestran tasas similares (Gaüzère *et al.* 2013: 6% en *Fagus sylvatica;* Robledo-Arnuncio & Gil 2005: 25% en *Pinus sylvestris*). Esto sugiere que en condiciones marginales, puede haber una tendencia a superar las barreras de la autoincompatibilidad para así adquirir las ventajas que proporciona un sistema de cruzamiento mixto.

Varios factores que actúan a escala fina parecen moldear la variabilidad individual en los patrones de dispersión de polen al influir en la disponibilidad de polen a escala local (ver Figura 6.1). Cuando el polen producido localmente es escaso o genéticamente similar (dicho de otro modo, en presencia de un bajo número de vecinos o emparentados entre si) se favorece la inmigración de polen y la autofecundación. Este efecto corresponde con lo esperado bajo un modelo de competencia polínica, donde los eventos de polinización al azar comienzan a predominar sobre una competición determinista cuando la carga de polen es baja (El-Kassaby & Ritland 1992). Además, en árboles de gran tamaño productores de vastas cantidades de polen la autofecundación es especialmente frecuente. En resumen,

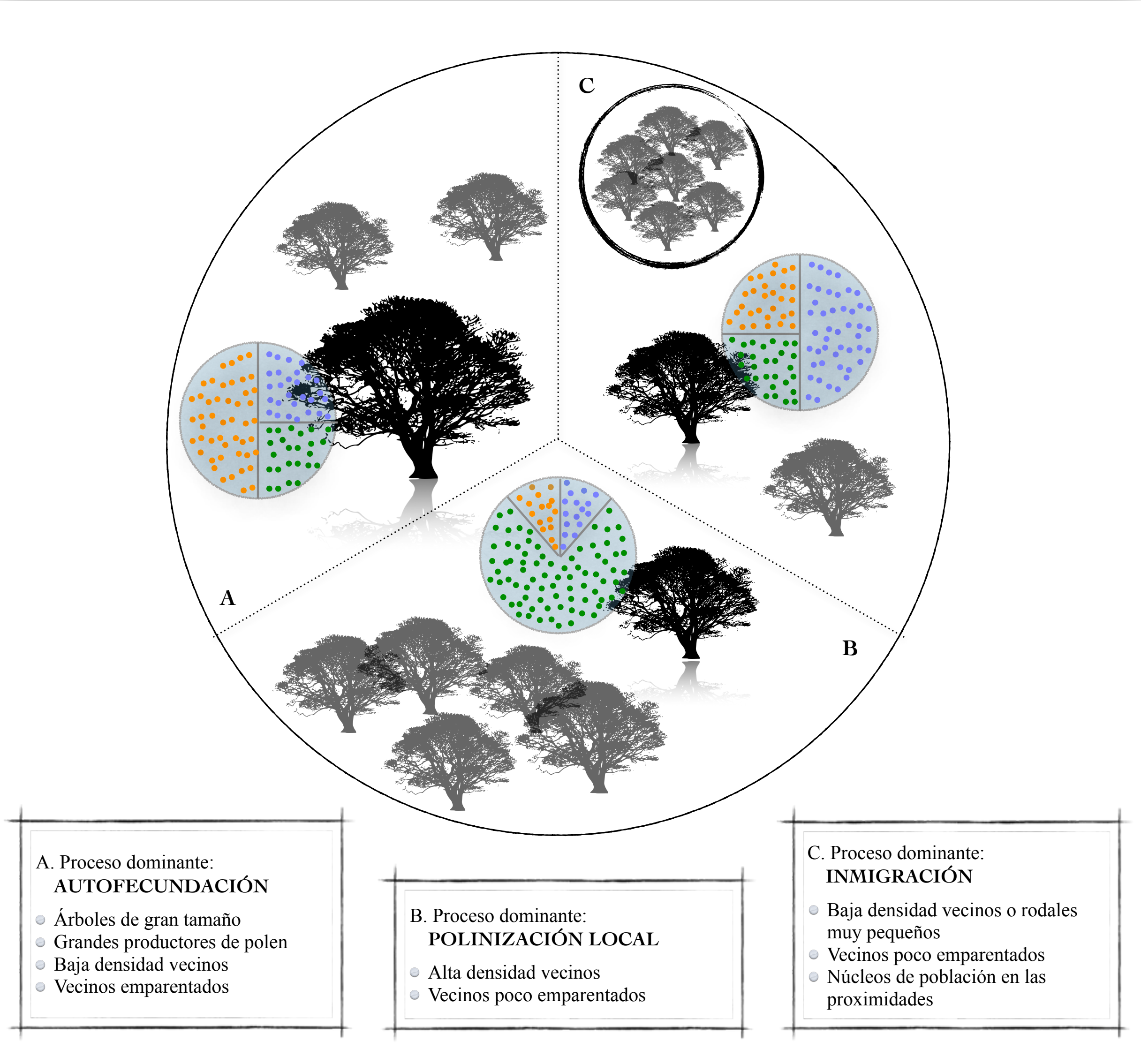


Figura 6.1 Resumen gráfico de los resultados de los modelos que explican los patrones de dispersión de polen en poblaciones relictas de *Q. robur* a escala local (Capítulo 4) y de paisaje (Capítulo 3). Domina la **polinización local** siempre que los árboles dispongan de un vecindario denso y poco emparentado. La **autofecundación** se ve favorecida en árboles grandes productores de polen con una baja densidad de conspecíficos emparentados a su alrededor (A). En un ambiente similar pero con núcleos de población actuando como fuentes de polen en las proximidades la frecuencia de **inmigración** aumenta.

parece que las poblaciones relictas son propensas a albergar individuos longevos grandes productores de polen que favorecen la autofecundación (fenómeno a escala fina) cuando no disponen de polen en abundancia y de calidad, mientras que la existencia de núcleos de población densos en las proximidades facilitaría la dispersión de polen inmigrante (fenómeno a escala de paisaje).

El análisis de los patrones de cruzamiento dentro de los rodales de mayor tamaño (4 réplicas, *n* = 22-35 individuos) revela dos resultados interesantes: (i) Existe **una alta conectividad vía polen en el interior de rodales** contraria a la observada a escala de paisaje (Capítulo 4: figura 2). El rol de conectores dentro de la red de polinización es asumido por árboles que ocupan posiciones centrales del parche y principalmente, por individuos de gran tamaño, productores de vastas cantidades de polen que son muy efectivos en su función paternal (donando polen) (ver Capítulo 4: fig. 4.2). Éstos, además son una fuente de polen importante para favorecer la dispersión de polen a larga distancia y la conectividad a través del paisaje (Figura 6.2 y Apéndice M). (ii) Existe **una gran variabilidad en los patrones de cruzamientos para ambas funciones sexuales (la donación y la recepción de polen), especialmente notable para la componente masculina** (Capítulo 4: tabla 1). El gran desequilibrio encontrado en la fecundidad masculina (liberación de polen que llega a producir frutos) ha sido ampliamente demostrado en poblaciones de especies forestales (p. ej., Chybicki & Burczyk 2013, Gerber *et al.* 2014, Klein *et al.* 2008). Encontramos que la fecundidad, y en general, los patrones de cruzamiento masculino guardan relación con rasgos intrínsecos de los árboles como su tamaño y la producción de polen (Capítulo 4: tabla 3), y no con aspectos del ambiente físico como han demostrado otros estudios (Chybicki & Burczyk 2013, Lagache *et al.* 2014). Los enormes árboles viejos en poblaciones relictas adquieren un papel preponderante para los patrones de polinización (Petit & Hampe 2006), cuyo efecto se sobrepone a aspectos relacionados con el ambiente refugial (ver la distribución del tamaño de los árboles en el sistema de estudio, Figura 6.1).

Por otra parte, el estudio de fecundidad en poblaciones relictas de *Q. robur* (Capítulo 5) revela que **la fecundidad femenina (i.e. la producción de semillas viables y su peso), al igual que la masculina, debe su principal fuente de variación a diferencias en el tamaño del árbol** (Capítulo 5: figura 5.2 y tabla 5.2). Los árboles mas grandes producen una mayor cantidad de flores tanto masculinas como femeninas de manera casi invariable a través de los años (Sork *et al.* 1993; pero ver Hirayama *et al.* 2008). No obstante, al eliminar el efecto del tamaño, encontramos que algunos árboles invierten sistemáticamente más en su función reproductora que otros. Este fenómeno puede adquirir un carácter adaptativo si constituye una respuesta plástica frente a las fluctuaciones ambientales (Knops & Koe-

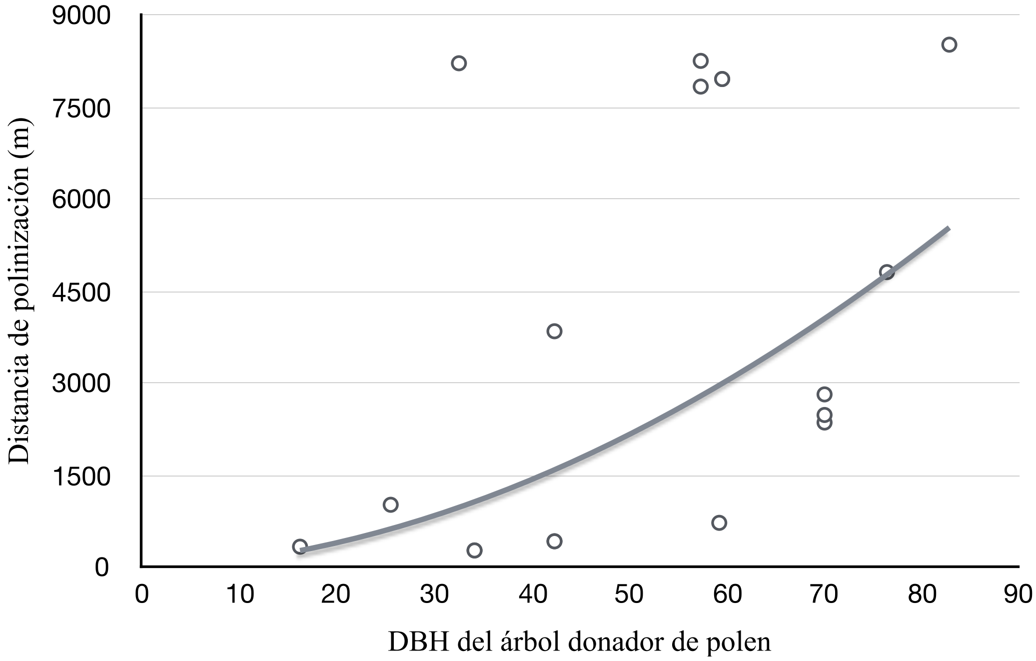


Figura 6.2 Relación existente entre el tamaño del árbol donador de polen (medido como DBH) y la distancia de dispersión efectiva, exclusivamente para los eventos de flujo polínico entre núcleos de población del valle del Jerte. El DBH promedio (SD) de los árboles responsables de estos eventos es 51,7 (18,8). Se representa la línea de tendencia con mayor ajuste ($R^2 = 0,35$; $y = 1,499 x^{1,86}$).

nig 2012, Pérez-Ramos *et al.* 2014, Koenig *et al.* 2015). Además, encontramos una mayor producción de bellotas más grandes y pesadas en árboles de gran tamaño (ver Koenig *et al.* 2009). Este efecto puede tener particular relevancia en el contexto de refugios climáticos, dada la ventaja para el desarrollo que supone disponer de una mayor cantidad de reservas (Gómez 2004, Sage *et al.* 2011), especialmente en ambientes mediterráneos donde la germinación temprana y el rápido crecimiento aumenta la probabilidad de supervivencia de las plántulas durante la sequía estival. En resumen, los árboles grandes tienen una múltiple ventaja a nivel de fitness: su éxito reproductivo supera al de conespecíficos de menor tamaño tanto en su función masculina y femenina, y muy posiblemente en términos de calidad de sus progenies.

El éxito reproductivo (*fruit set*) es la única medida de fecundidad influida por el escenario relicto (Capítulo 5: tabla 5.2), lo que relacionamos con el escaso flujo de polen a este nivel. El efecto del tamaño del rodal y el grado de aislamiento sobre el *fruit set* pone de manifiesto la limitación de polen a escala de paisaje, mientras que a escala fina las evidencias que encontramos son mínimas a diferencia de otros estudios (Knapp *et al.* 2001, Koenig *et al.* 2012, Lagache *et al.* 2013, Pearse *et al.* 2015), a pesar de haber usado nume-

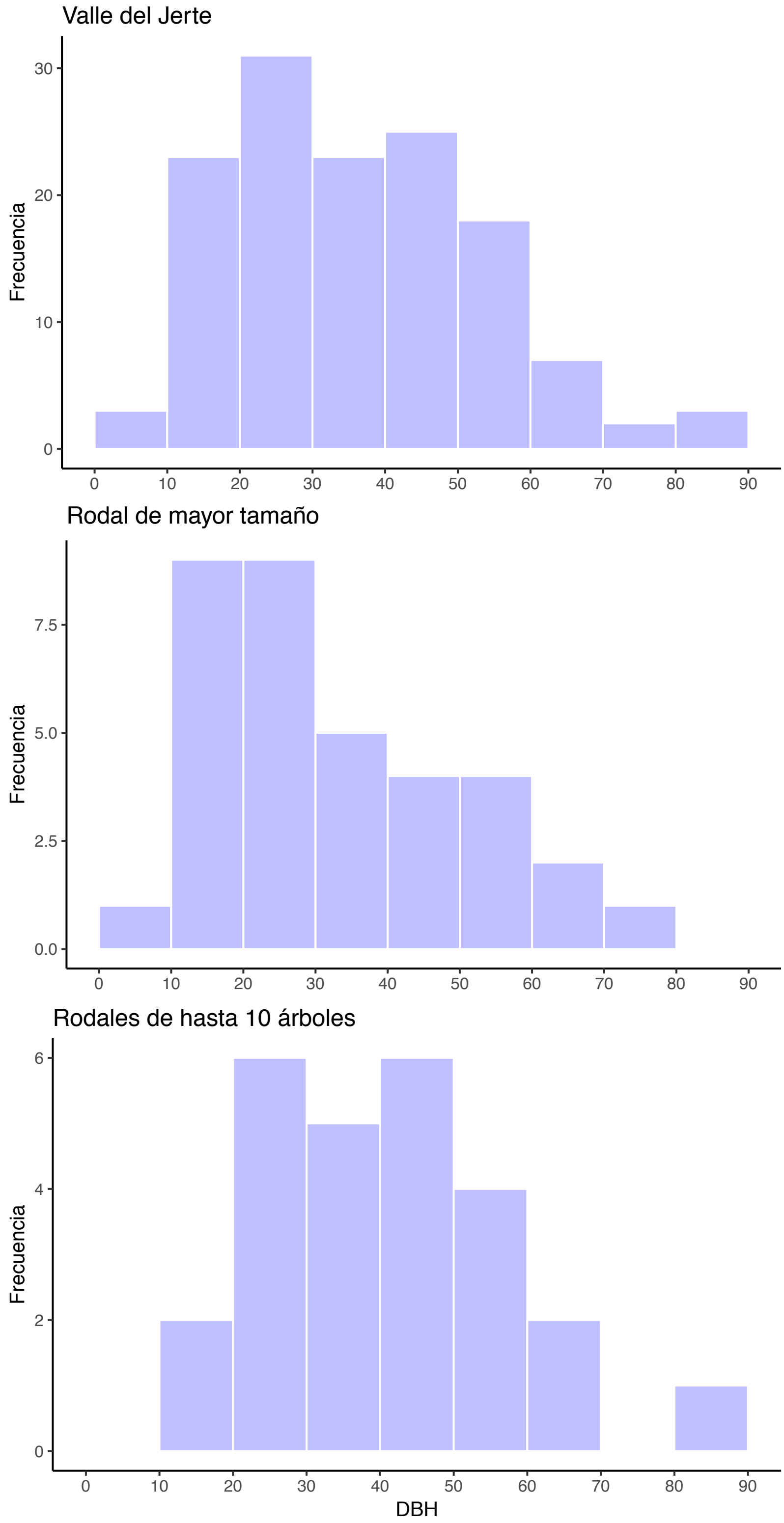


Figura 6.3 Distribución de frecuencias de DBH para el conjunto de árboles distribuidos a lo largo del Valle del Jerte, en el rodal de mayor tamaño (E con 35 individuos), y en el conjunto de rodales más pequeños (compuestos de hasta 10 árboles). La estructura de edades en las poblaciones relictas de *Q. robur* revela la abundancia de árboles de gran tamaño, especialmente en rodales pequeños donde persisten árboles muy longevos. Las poblaciones mayores presentan indicios de cierto reclutamiento a largo plazo con una mayor proporción de individuos jóvenes.

rosos indicadores tanto ecológicos como genéticos. La ausencia de restricciones genéticas que afecten el sistema de cruzamiento en nuestras poblaciones relictas puede explicar que la mayor parte de la dispersión de polen ocurra a cortas distancias entre conjuntos de árboles en suficiente densidad, como para que existan oportunidades para un polinización y reproducción efectiva.

¿QUE MECANISMOS FACILITAN LA PERSISTENCIA EN POBLACIONES RELICTAS DE ÁRBOLES?

El flujo génico representa el foco principal de esta tesis ya que constituye un elemento clave para la conectividad y diversidad genética de las poblaciones, el éxito reproductivo, el potencial adaptativo en ambientes cambiantes y en definitiva la persistencia a largo plazo (Sexton *et al.* 2011). Sorprendentemente, los núcleos poblacionales de *Q. robur* en el valle del Jerte intercambian poco polen, y la marcada estructura genética de la población adulta sugiere que la débil conectividad no es solo un fenómeno a corto plazo (véase también Ouayjan & Hampe 2018). El enclave y aislamiento de las poblaciones en refugios junto a la baja densidad poblacional suponen un obstáculo a la dispersión de polen. A pesar de ello, la predominante fertilización entre individuos del mismo rodal garantiza una reproducción exitosa siempre que se alcance un cierto tamaño poblacional, de otro modo el escaso flujo de polen no compensa la limitación de polen existente. El mecanismo de ser fertilizado por polen local abundante y poco emparentado, y en su ausencia, por polen propio o inmigrante parece contribuir al éxito reproductivo de estas poblaciones (Figura 6.1). Además la escasa inmigración de polen puede llegar a tener un impacto exacerbado en la genética de las poblaciones si operan procesos de purga genética (Hampe *et al.* 2013), y la salvaguarda de la autofecundación permitiría la reproducción en circunstancias extremas, aunque la viabilidad de los descendientes procedentes queda por confirmar. Por último, el flujo génico interespecífico no parece tener un papel notable en los patrones de dispersión de *Q. robur*, a pesar de que a baja densidad poblacional o en situaciones de limitación de polen cabría esperar una mayor frecuencia (Lagache *et al.* 2013, Lepais *et al.* 2009). A menudo, el flujo génico entre especies de robles en simpatría es escaso tanto en poblaciones centrales como del margen de la distribución (Chybicki *et at.* 2012, Curtu *et al.* 2007, Lepais *et al.* 2009). Parece que principalmente barreras sexuales prezigóticas incluyendo el desacople fenológico de la floración, la competencia del polen y las interacciones polen-pistilo, limitan el cruzamiento interespecífico entre estas 2 especies. No obstante, aunque la tasa anual de hibridación sea baja, no es desdeñable el rol que puede desempeñar en la adaptación local de las poblaciones. De hecho, la frecuencia de individuos con apariencia híbrida es notable en algunas poblaciones del área de estudio donde la regeneración es patente, lo que podría sugerir un establecimiento ventajoso de los híbridos (E. Moracho, obs. pers).

Las poblaciones relictas de *Q. robur* parecen poco afectadas por los procesos genéticos que amenazan otras poblaciones de pequeño tamaño (Aguilar *et al.* 2008, Jump & Peñuelas 2006). Los siguientes evidencias apoyan esta afirmación: (i) no se demuestra una depresión por endogamia notable en base a datos de campo y experimentales (ver Capítulo 3: página 53); (ii) el grado de parentesco es moderado-bajo (Capítulo 3: tabla 3.1) resultado de la acción de mecanismos prezigóticos (se evita la fertilización con genotipos emparentados) y postzigóticos (por procesos de purga genética); y (iii) las poblaciones preservan en su conjunto una diversidad genética relativamente elevada, aunque inferior a la encontrada en el rango continuo de la distribución de la especie (Hampe *et al.* 2010, Muir & Schloetterer, Streiff *et al.* 1998) o en poblaciones aisladas con altas tasas de flujo génico (Buschbom *et al.* 2011). Dentro de los rodales encontramos valores de diversidad menores y combinaciones de alelos únicas (Capítulo 3: tabla 1) como es de esperar bajo condiciones de aislamiento continuado y tamaño reducido de las poblaciones. Esto junto a la elevada diversidad genética regional, la fuerte diferenciación y las inferencias de la distribución en el pasado (Benito et al. 2007), sugieren que nuestras poblaciones son realmente relictos con una historia de persistencia a través de muchas generaciones (Hampe & Petit 2005) (ver Capítulo 1: figura 1.1).

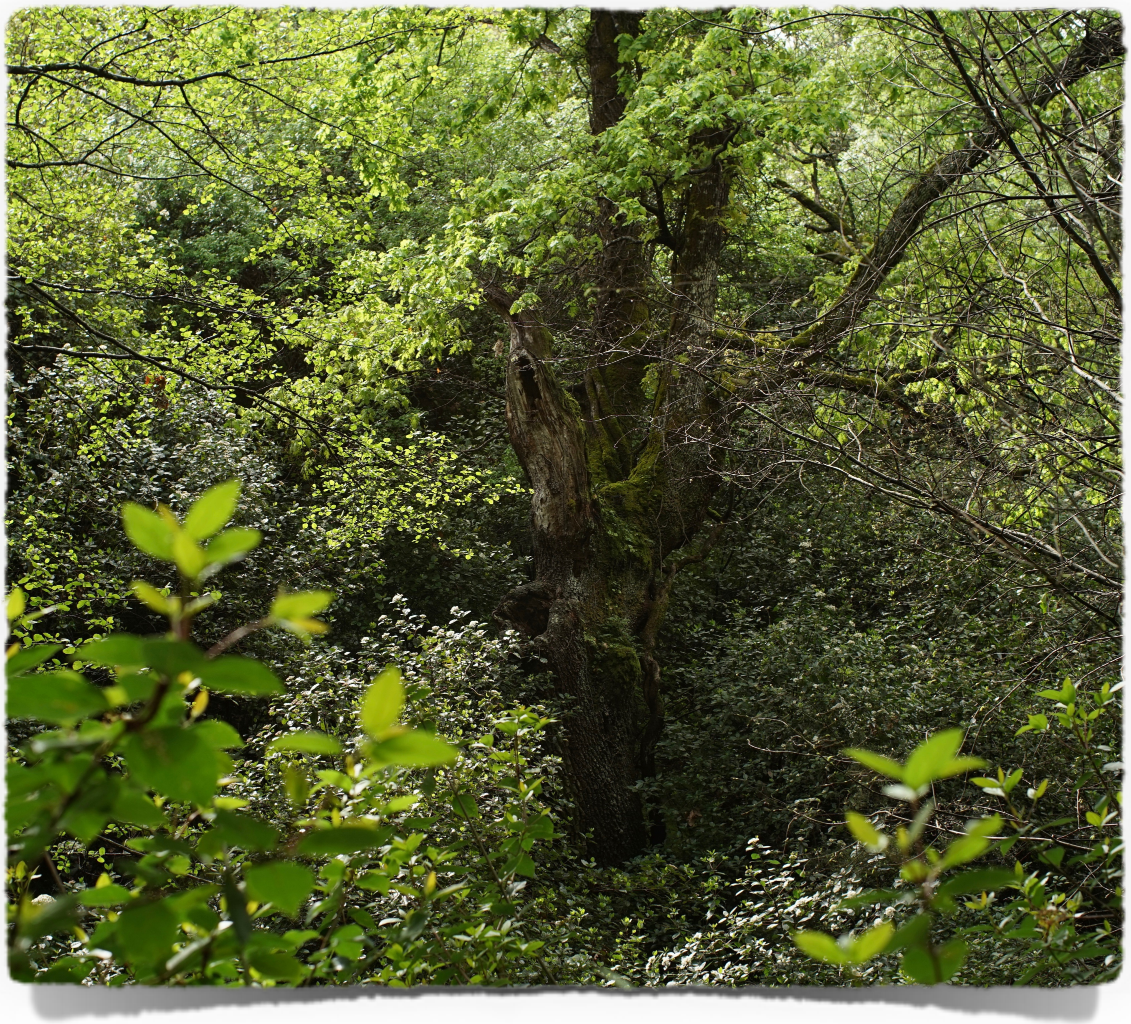
En contraposición, los rasgos de las poblaciones de fragmentación reciente o núcleos de nueva colonización determinan unos patrones de flujo génico sustancialmente distintos. En el margen en expansión, las poblaciones muestran una dependencia especialmente alta por el flujo génico al ser comúnmente originadas por efectos fundadores (Pujol & Pannell 2008, Lesser & Jackson 2013) hasta que alcanzan un cierto tamaño poblacional. Por su parte, las poblaciones fragmentadas por causas antrópicas son susceptibles a procesos de incompatibilidad y depresión por endogamia al cruzarse con árboles vecinos, ya que representan los parches resultantes de procesos de cuello de botella no sometidos a purga por selección. Por tanto, dependen de una alta tasa de flujo génico que a menudo se ve favorecida por la proximidad a núcleos de población mayores y por la apertura del hábitat degradado que facilita la dispersión por el viento entre parches dispersos (Kramer *et al.* 2008).

La estructura de edades en base al dbh de los árboles en el valle del Jerte demuestra la gran frecuencia de árboles de medio y gran tamaño, especialmente sesgada en poblaciones pequeñas (Figura 6.3). En núcleos de mayor tamaño se observa una mayor proporción de árboles más pequeños lo que sugiere que aquí prospera cierto reclutamiento a lo largo del tiempo, así mismo la densidad de plántulas es notablemente mayor (E. Moracho, pers. obs). Los resultados obtenidos sugieren que las poblaciones de *Q. robur* de mayor tamaño o menos aisladas mantienen potencial de regeneración en lo que se refiere a su fecundidad y éxito reproductivo (*fruit set*). Existe una variabilidad interanual no desdeñable (p. ej., Pérez-Ramos *et al.* 2015 Pesendorfer *et al.* 2016, Sork *et al.* 1993) con una producción de semillas mayor en años con altas frecuencias de polinización (capítulo 5), mientras que la limitación de polen tendrá efectos más profundos en años de poca productividad. Numerosos estudios indican que el ambiente de polinización está entre los principales determinantes de la variación interanual en la producción de semillas en robles ('hipótesis de la polinización por el viento': Koenig *et al.* 1994; ver también García-Mozo *et al.* 2007, Koenig *et al.* 2015, Pearse *et al.* 2015, Pesendorfer *et al.* 2016). De todos modos, los episodios de mayor éxito reproductivo y reclutamiento en poblaciones relictas es probable que se limiten a años de benevolencia climática debido a la gran influencia de las condiciones meteorológicas a lo largo del proceso reproductivo, desde la producción de flores y el éxito de la polinización (Bell & Clark 2016, Koenig *et al.* 1996) hasta la maduración correcta del fruto (Espelta *et al.* 2008, Pérez-Ramos *et al.* 2010).

Las evidencias presentadas en esta tesis y los mecanismos discutidos ayudan a explicar la alta resiliencia de las poblaciones relictas de árboles. No podemos olvidar que hablamos de persistencia referida a una escala de tiempo y bajo unas condiciones ambientales determinadas (Hanna *et al.* 2014) y que por ello, mantener la capacidad adaptativa es necesario para asegurar la persistencia a largo plazo. Sería interesante continuar la investigación en esta línea para averiguar si las poblaciones relictas de *Q. robur* pueden experimentar procesos rápidos de adaptación local que les permitan afrontar las condiciones ambientales cada vez más adversas en que crecen, a pesar de sus reducidos niveles de flujo génico.

IMPLICACIONES PARA LA CONSERVACIÓN DE POBLACIONES RELICTAS

La gran relevancia de los fenómenos a escala local para los sistemas de cruzamiento y probablemente para la reproducción de plantas en general, así como la desigualdad existente entre individuos en la fecundidad femenina y sobretodo masculina, implica que incluso pequeños cambios en la estructura de los núcleos de población (como la eliminación de unos pocos árboles de gran tamaño con una función central como donantes de polen y altamente fecundos), pueden tener consecuencias muy severas en poblaciones relictas. Por ello, el manejo y las estrategias de conservación en estos sistemas deben centrarse en asegurar el funcionamiento interno de los propios rodales mediante prácticas que aseguren un número y densidad suficiente de individuos reproductivamente muy activos. Así mismo, es fundamental garantizar la supervivencia de individuos grandes y longevos, que además poseen potencialidad para favorecer la conectividad entre rodales.



CONCLUSIONES

1 El cambio climático está produciendo desplazamientos de la distribución de las especies en todo mundo. Existen evidencias empíricas que demuestran la existencia de asimetrías en el *performance* de las poblaciones en los márgenes comparado con las poblaciones centrales, como un fenómeno global través de los reinos y tipos de hábitat, que refleja su desequilibrio en las condiciones climáticas cambiantes. El impacto de la alteración del clima es doblemente negativo, en el margen de baja latitud las poblaciones muestran un performance reducido, lo que confirma su vulnerabilidad a la extinción local; y sin embargo en el margen opuesto no encontramos un performance sobresaliente lo que sugiere un retraso en la respuesta de las poblaciones en el rastreo de las condiciones óptimas.

2 El flujo génico y la conectividad en poblaciones pequeñas y aisladas constituye una componente clave para la diversidad genética y su potencial adaptativo. Sorprendentemente, el conjunto de poblaciones relictas de la especie anemófila *Q. robur*, intercambian muy poco polen (a lo largo de 20 km de valle), y la marcada estructura genética de la población adulta sugiere que la débil conectividad no es solo un fenómeno a corto plazo. Aspectos físicos del enclave en este refugio climático, junto a la baja densidad y aislamiento poblacional parecen suponer un obstáculo a la dispersión de polen a distancia. No obstante, el éxito reproductivo no se ve comprometido gracias a una predominante fertilización entre individuos del mismo rodal siempre y cuando exista un mínimo tamaño poblacional y otras fuentes de polen en las proximidades.

3 Las tasas de auto-fecundación (notablemente altas) e inmigración (excepcionalmente bajas) encontradas se atribuyen a una pequeña fracción de árboles, en coherencia, factores operando principalmente a escala local determinan la variabilidad existente entre individuos. En conjunto, los patrones de dispersión de polen corresponden a un modelo de competencia polínica en el que predomina la fertilización entre individuos del vecindario cuando su densidad garantiza una producción alta de polen de calidad (competencia determinista), y en su ausencia se favorece la autofecundación especialmente en organismos grandes productores de polen, y la inmigración cuando existen núcleos de población densos en las proximidades (procesos al azar).

4 A pesar del limitado flujo de polen a escala de paisaje, el conjunto de poblaciones relictas de *Q. robur* conservan una diversidad genética relativamente elevada aunque más baja en rodales particulares. No se detecta un grado de parentesco alto entre individuos próximos ni la existencia de restricciones genéticas al cruzamiento con vecinos lo que debe facilitar la predominante polinización a cortas distancias. Parece que la acción de una intensa purga genética evita en poblaciones relictas los problemas de consanguinidad y depresión por endogamia que experimentan otras poblaciones de fragmentación reciente.

5 La dispersión interespecífica (o hibridación) contribuye a muy baja frecuencia en los patrones de flujo génico en poblaciones relictas. No obstante, no descartamos una mayor frecuencia de introgresión de *Q. pyrenaica* en años menos productivos donde la limitación de polen se acentúe. Además, su rol en fases sucesivas del reclutamiento está aún por determinar.

6 Las poblaciones de *Q. robur*, especialmente las de mayor tamaño y menor aislamiento, mantienen un notable potencial de regeneración, en lo que se refiere a su fecundidad y éxito reproductivo, que guarda relación con la alta frecuencia de polinización. La fecundidad masculina (cantidad de polen que genera semillas viables) y la femenina (producción de bellotas) es máxima en los individuos más grandes y longevos que desempeñan un papel primordial para la conectividad y la contribución de propágulos en las sucesivas generaciones.

7 La conservación de poblaciones relictas pasa por asegurar el funcionamiento interno de los núcleos de población mediante prácticas que aseguren un número y densidad suficiente de individuos reproductivamente muy activos. Además de garantizar la supervivencia de individuos grandes y longevos con una fecundidad extraordinaria tanto de polen como de frutos, que adquieren un papel central para las dinámicas de las poblaciones relictas.

APÉNDICES

CAPÍTULO 2

Asymmetry in marginal population performance foreshadows widespread species range shifts

APÉNDICE A

LISTADO Y DISTRIBUCIÓN DE LOS CASOS DE ESTUDIO

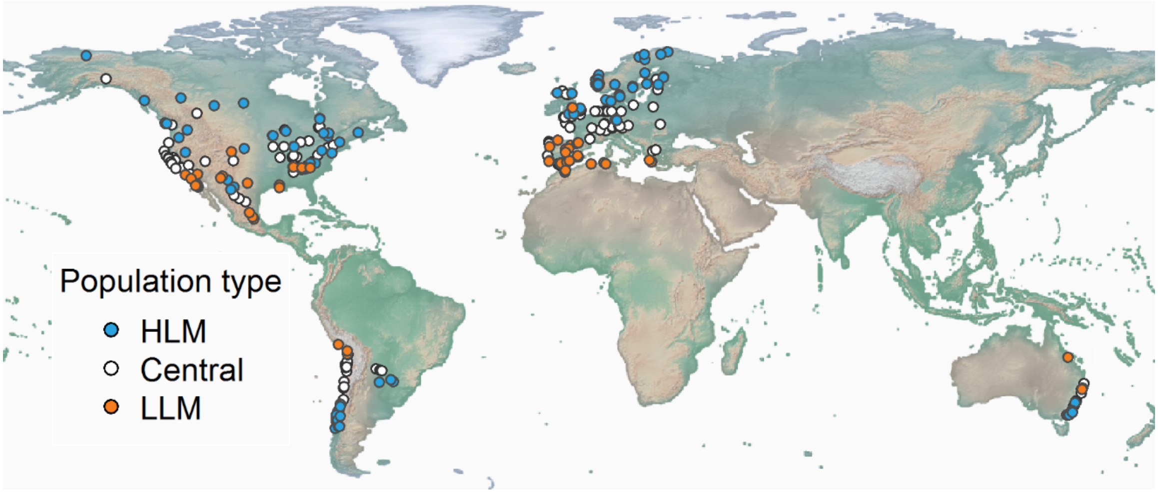


Figure A1. Map of the 623 populations included in this study, classified as 'High-Latitude Margin' (HLM), 'Central' populations, or 'Low-Latitude Margin' (LLM). Note that HLM populations for some organisms can be at lower latitudes than LLM populations of other species (and *vice versa*).

The following list includes the total of 42 retained papers with CP comparisons:

1	Aikens, M. L. & Roach, D. A. Population dynamics in central and edge populations of a narrowly endemic plant. *Ecology* **95**, 1850-1860 (2014).
2	Angilletta Jr, M. J., Niewiarowski, P. H., Dunham, A. E., Leaché, A. D., & Porter, W. P. Bergmann´s clines in ectotherms: illustrating a life-history perspective with sceloporine lizards. *Am. Nat*. **164**, E168-E183 (2004).
3	Araújo, R.M., Serrao, E.A. & Sousa-Pinto I. & Aberg, P. Spatial and temporal dynamics of fucoid populations (*Ascophyllum nodosum* and *Fucus serratus*): a comparison between central and range edge populations. *PLoS ONE* **9**, e92177 (2014).
4	Brante, A., Cifuentes, S., Pörtner, H. O., Arntz, W., & Fernández, M. Latitudinal comparisons of reproductive traits in five brachyuran species along the Chilean coast. *Rev. Chil. Hist. Nat*. **77**, 15-27 (2004).
5	Cardoso, R.S. & Defeo, O. Geographical patterns in reproductive biology of the Pan-American sandy beach isopod *Excirolana braziliensis*. *Mar. Biol*. **143**, 573-581 (2003).
6	Carey, P. D., Watkinson, A.R. & Gerard, F.F.O. The determinante of the distribution and abundance of the winter annual grass *Vulpia ciliata* ssp. *ambigua*. *J. Ecol*. **83**, 177-187 (1995).

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14 **50 m** Graves, G. Geographic clines of age ratios of black-throated blue warblers (*Dendroica caerulescens*). *Ecology* **78**, 2524-2531 (1997).

15 Hidas, E. Z., Russell, K. G., Ayre, D. J., & Minchinton, T. E. Abundance of *Tesseropora rosea* at the margins of its biogeographic range is closely linked to recruitment, but not fecundity. *Mar. Ecol. Prog. Ser.* **483**, 199-208 (2013).

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APÉNDICE B

RESUMEN DE LOS MODELOS Y SELECCIÓN DE LOS MEJORES MODELOS

Table B1. Summary of models with ΔAICc, w_i and heterogeneity. Model selection procedure retained five models within two units of ΔAICc of the best model (bold characters). All included margin type as a moderator and the null model (i.e., intercept only) was excluded. Only models with ΔAICc < 10 are presented. Pseudo R^2 was calculated as 1 – LLR where LLR is the ratio between the log-likelihood of model *i* and the log-likelihood of the null model.

Model	**AICc**	**w_i**	**delta**	**Q_M**	**$P(Q_M)$**	**Q_E**	**$P(Q_E)$**	**Pseudo-R^2**
Habitat + Margin	381.39	0.17	**0.00**	9.70	0.008	413.28	< 0.001	0.04
Margin	381.47	0.17	**0.08**	7.32	0.007	431.87	< 0.001	0.03
Habitat + Margin + Kingdom	382.03	0.13	**0.64**	11.57	0.009	396.76	< 0.001	0.05
Habitat + Margin + Kingdom + Kingdom × Margin	382.55	0.10	**1.16**	13.69	0.008	394.03	< 0.001	0.06
Habitat + Margin + Margin × Habitat	383.12	0.07	**1.74**	10.30	0.016	413.2	< 0.001	0.05
Margin + Kingdom + Kingdom × Margin	383.45	0.06	2.06	10.21	0.017	425.68	< 0.001	0.05
Margin + Kingdom	383.56	0.06	2.18	7.44	0.024	430.11	< 0.001	0.04
Habitat + Margin + Kingdom + Margin × Habitat	383.57	0.06	2.19	12.40	0.015	396.62	< 0.001	0.06
Habitat + Margin + Kingdom + Kingdom × Habitat	384.36	0.04	2.97	11.57	0.021	396.2	< 0.001	0.06
Habitat + Margin + Kingdom + Margin × Habitat + Kingdom × Margin	384.83	0.03	3.44	13.77	0.017	393.8	< 0.001	0.07
Habitat + Margin + Kingdom + Kingdom × Habitat + Kingdom × Margin	384.92	0.03	3.53	13.70	0.018	393.2	< 0.001	0.07
Habitat	385.92	0.02	4.53	2.59	0.108	427.56	< 0.001	0.02
Habitat + Margin + Kingdom + Margin × Habitat + Kingdom × Habitat	385.94	0.02	4.55	12.41	0.03	395.96	< 0.001	0.07
Null	386.17	0.02	4.79	-	-	449.01	< 0.001	0.00
Habitat + Kingdom	386.68	0.01	5.30	4.51	0.105	412.59	< 0.001	0.03
Habitat + Margin + Kingdom + Margin × Habitat + Kingdom × Habitat + Kingdom × Margin	387.26	0.01	5.88	13.77	0.032	393.06	< 0.001	0.08
Kingdom	388.29	0.01	6.90	0.07	0.798	448.06	< 0.001	0.01
Habitat + Kingdom + Kingdom × Habitat	388.84	0.00	7.45	4.60	0.204	412.12	< 0.001	0.04

Table B2. Summary of the five models retained in the set of best models (*i.e.*, with AICc < 2, Appendix G.). Margin explained a significant amount of heterogeneity in each of the five competing best models whereas neither Kingdom nor Habitat explained a significant amount of heterogeneity in any of the five models retained in the set of best models. Q_M and associated *P-values* represent the test associated with each moderator, separately. Pseudo R^2 were calculated as 1 – LLR, where LLR is the ratio between the log-likelihood of model *i* and the log-likelihood of the null model.

Model	**Moderators**	**Q_M (*P*-value)**	**Pseudo-R^2**
Model 1	Margin	7.21 (**0.007**)	0.040
	Habitat	2.25 (0.134)	
Model 2	Margin	7.23 (**0.007**)	0.026
Model 3	Margin	7.40 (**0.007**)	0.053
	Habitat	3.72 (0.054)	
	Kingdom	0.14 (0.705)	
Model 4	Margin	7.65 (**0.006**)	0.065
	Kingdom	3.1 (0.78)	
	Habitat	0.14 (0.705)	
	Margin × Kingdom	1.83 (0.176)	
Model 5	Margin	4.23 (**0.040**)	0.049
	Habitat	2.74 (0.098)	
	Margin × Habitat	0.55 (0.458)	

APÉNDICE C

EFECTO DE LOS MODERADORES Y SUS INTERACCIONES

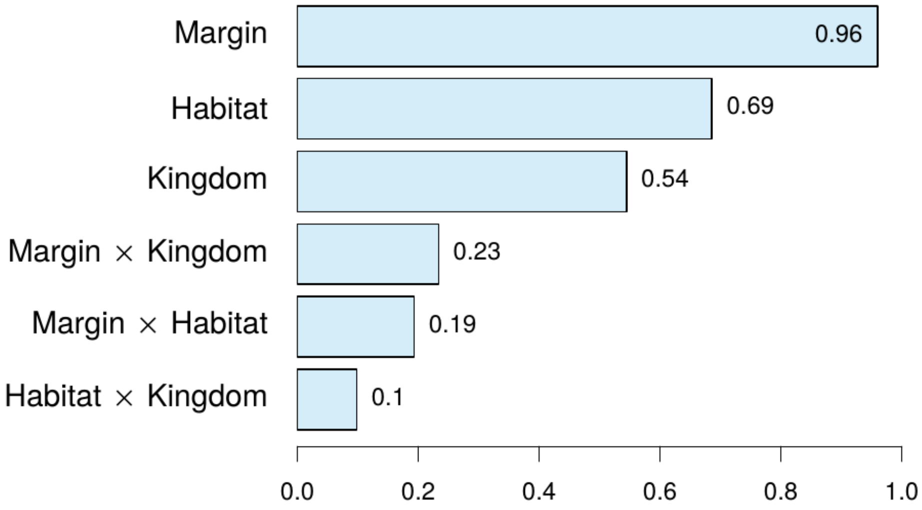


Figure C1. Sum of weights of moderators quantifying the relative importance of individual moderators and their interactions. Values are interpreted as the probability that a given variable is retained in the best model. Among tested moderators, margin type was the most important predictor (wH, margin = 0.96), whereas Habitat (wH,habitat = 0.69) and Kingdom (wH, kingdom = 0.54) received only marginal support, and interactions (Margin × Habitat and Margin × Kingdom) were even less relevant. Fully in line with this result, neither Kingdom nor Habitat explained a significant amount of heterogeneity in any of the five models retained in the set of best models (Appendix H).

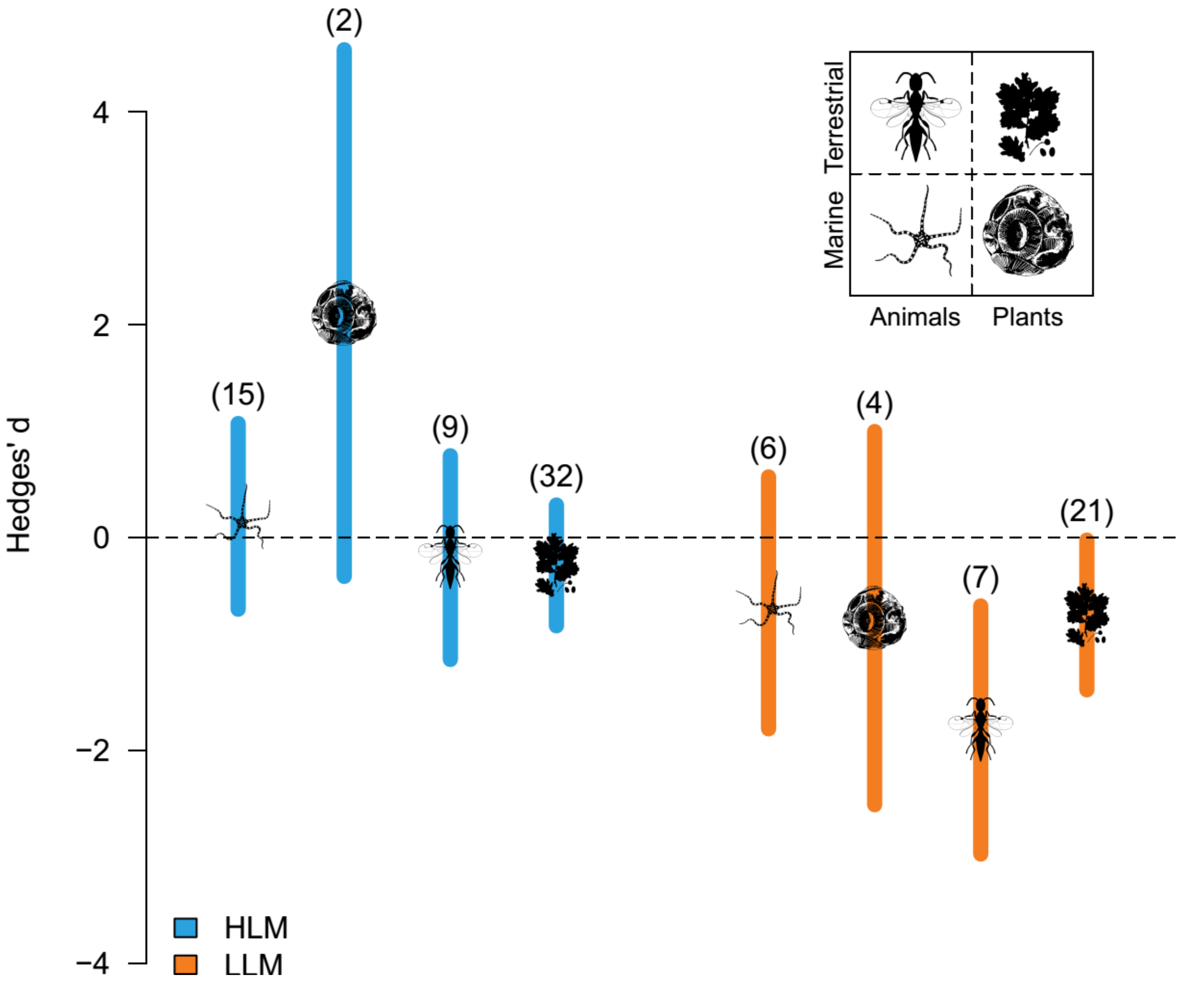


Figure C2. Asymmetry in population performance at High Latitude Margins (HLM) and Low Latitude Margins (LLM) for each Kingdom and Habitat. Symbols representing Habitats and Kingdoms are centered on the mean estimate. Vertical bars represent 95% CI estimated from the multi-level meta-analysis. Negative and positive values indicate lower and higher performance of marginal populations as compared to central populations, respectively. Numbers within parentheses indicate the number of case studies for each category.

APÉNDICE D

META-ANÁLISIS ACUMULATIVO

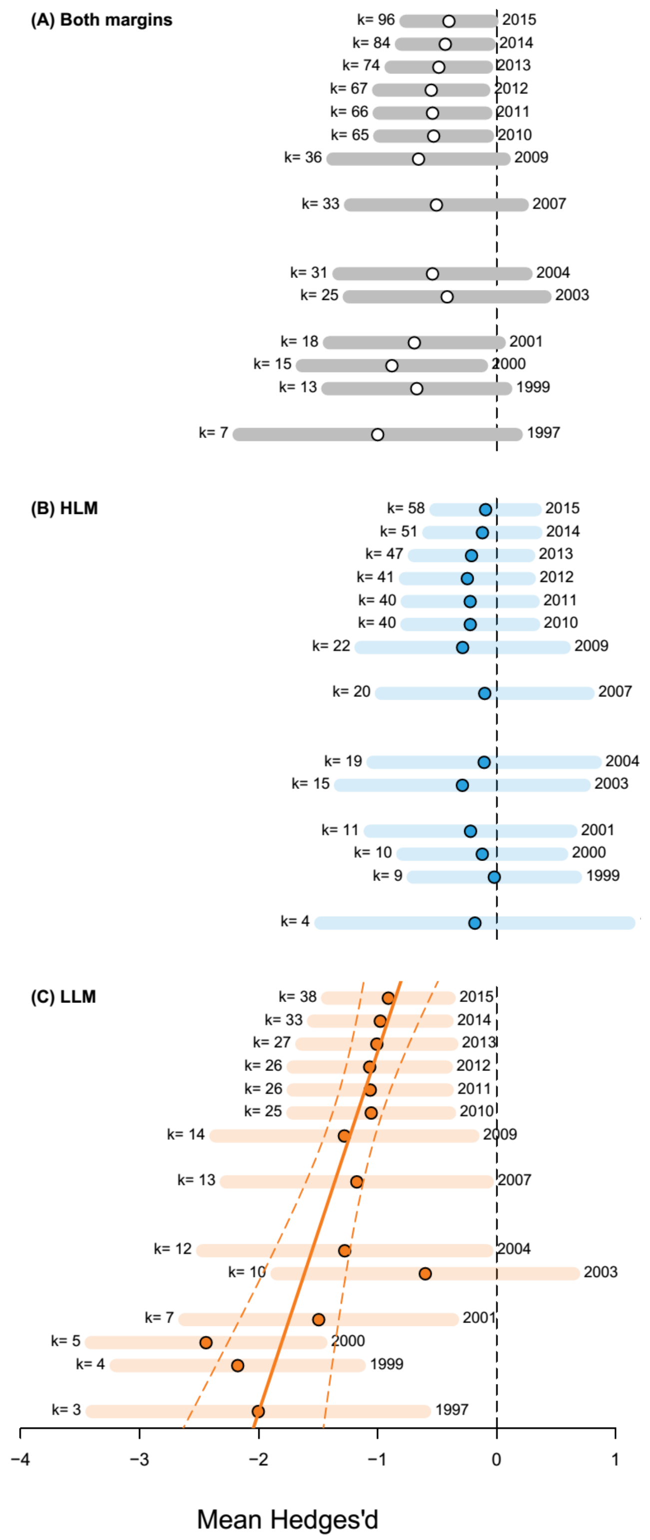


Figure D1. Cumulative meta-analysis. Grand mean effect sizes (dots), 95% CI (bars) and sample sizes (*k*) are shown for each year, including all previous years. Plate (A) depicts the global data set, plates (B) and (C) the datasets for HLM and LLM populations, respectively. Only significant relationships between publication year and effect sizes are shown by a regression line (continuous) and its 95% CI (dotted).

APÉNDICE E

ANÁLISIS DE SENSIBILIDAD

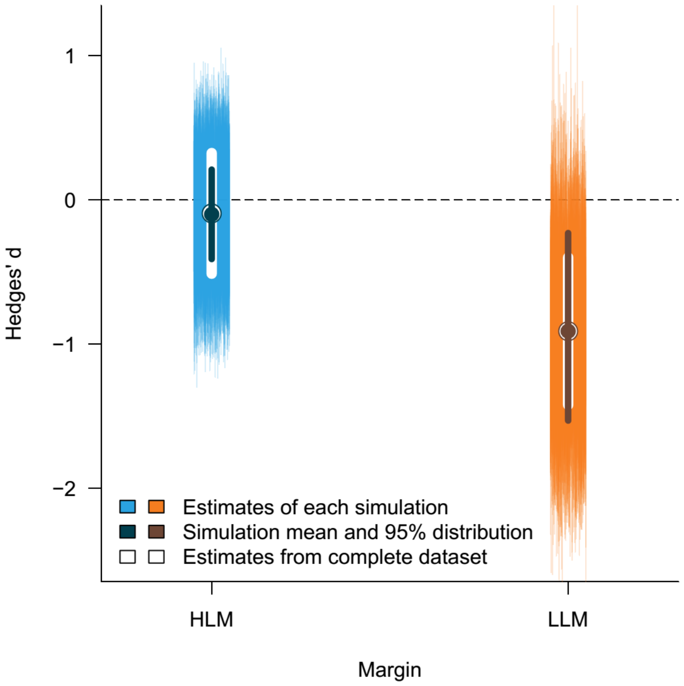


Figure E1. Sensitivity analysis. Thin coloured bars represent the 95% CI of effect sizes estimated for HLM and LLM from the 1000 '*i*' models ran with a random sample of one case per primary paper. Dark dots and error bars represent the corresponding mean and 95% distribution of mean effect sizes. Predictions based on the complete dataset (i.e., those reported in the main text) are shown in white for comparison. The match between the results of the main analysis and sensitivity analysis confirm the robustness of our conclusions about asymmetry in marginal population performance.

APÉNDICE F

EFECTO DE LAS VARIACIONES CLIMÁTICAS Y LA DISTANCIA GEOGRÁFICA

Table F1. Results of the additive mixed model relating relative performance of marginal populations (Hedges' *d*) to the difference in average climate between marginal and central populations. We used temperature difference as predictor, and the study as random effect (see Methods on climate analysis).

	Estimates
(Intercept)	-0.32 (0.16)*
EDF: s(Tmean.dif)	3.16 (3.87)*
EDF: s(study.id)	4.59 (41.00)
Deviance explained	0.25
R^2	0.18
GCV score	1.88
Num. obs.	96
Num. smooth terms	2

*$p < 0.05$

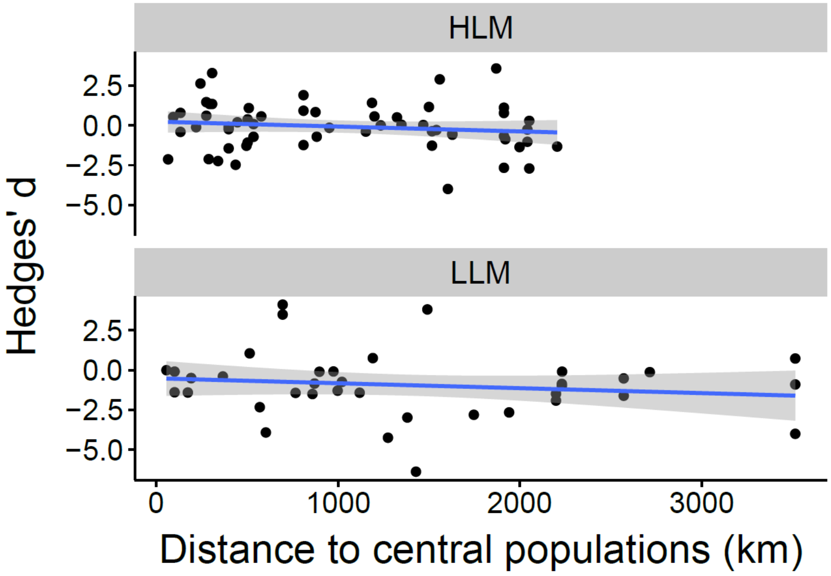


Figure F1. Relative performance of marginal vs central populations (Hedges' *d*) in relation to the geographic distance between them. The latter was calculated as the distance between the centroids of marginal (HLM or LLM) and central populations in each case. We found no evidence for a distance effect on explaining differences in relative population performance, as we found for climate (Fig. 3)

CAPÍTULO 3

Unusually limited pollen dispersal and connectivity of Pedunculate oak (*Quercus robur*) refugial populations at the species' southern range margin

APÉNDICE G

DETALLES DE LA DIVERSIDAD GENÉTICA A NIVEL DE MARCADORES, ÁRBOLES ADULTOS Y PROGENIES

Table G1. Nuclear microsatellite marker diversity across all ten *Quercus robur* stands (*n* = 135 trees).

Locus	***Na***	***Nae***	H_O	H_E	***PIC***	***NE-1P***	***NE-2P***	***NE-PP***	***NE-I***	***NE-SI***	***HW***	***F* (Null)**
FamQr7	15	9.1	0.79	0.89	0.88	0.37	0.23	0.08	0.02	0.31	ND	0.058
FamMsQ13	11	3.4	0.69	0.71	0.69	0.67	0.48	0.26	0.11	0.42	NS	0.020
VicQr112	5	1.2	0.13	0.14	0.13	0.99	0.93	0.87	0.75	0.87	ND	0.069
VicQr20	17	5.7	0.70	0.83	0.81	0.50	0.33	0.14	0.05	0.35	*	0.086
PetQp15	7	3.3	0.66	0.70	0.65	0.71	0.54	0.35	0.14	0.44	NS	0.028
PetQp110	12	2.7	0.59	0.63	0.60	0.76	0.57	0.36	0.17	0.48	NS	0.035
NedQr96	15	2.5	0.56	0.60	0.58	0.78	0.59	0.37	0.18	0.50	NS	0.021
NedQr11	16	5.2	0.72	0.81	0.78	0.55	0.37	0.18	0.06	0.36	NS	0.053
FamD20	9	2.8	0.60	0.64	0.58	0.78	0.62	0.44	0.19	0.48	NS	0.031
FamA11	9	3.0	0.56	0.67	0.64	0.72	0.54	0.33	0.14	0.45	NS	0.093
FamS19	16	7.5	0.80	0.87	0.85	0.42	0.26	0.10	0.03	0.33	NS	0.043
VicF	11	7.7	0.83	0.87	0.85	0.43	0.27	0.11	0.03	0.33	NS	0.023
VicD31	9	3.1	0.62	0.68	0.62	0.75	0.59	0.42	0.17	0.45	NS	0.052
VicG	6	2.1	0.49	0.52	0.48	0.85	0.69	0.51	0.27	0.56	NS	0.025
PetC	12	4.1	0.72	0.76	0.72	0.63	0.45	0.26	0.10	0.40	NS	0.025
PetA15	5	3.0	0.69	0.66	0.61	0.76	0.59	0.41	0.17	0.46	NS	0.024
PetAO	11	5.6	0.689	0.82	0.80	0.52	0.35	0.17	0.06	0.35	NS	0.085
NedAK	10	5.4	0.72	0.82	0.79	0.54	0.37	0.18	0.06	0.36	NS	0.066
NedAB	19	7.3	0.77	0.86	0.85	0.42	0.27	0.10	0.03	0.33	NS	0.058
NedA3	7	3.0	0.65	0.67	0.62	0.75	0.57	0.39	0.16	0.46	NS	0.011
Mean	*11.1*	*4.4*	*0.65*	*0.71*	*0.68*	*0.64*	*0.48*	*0.30*	*0.14*	*0.43*		*0.043*

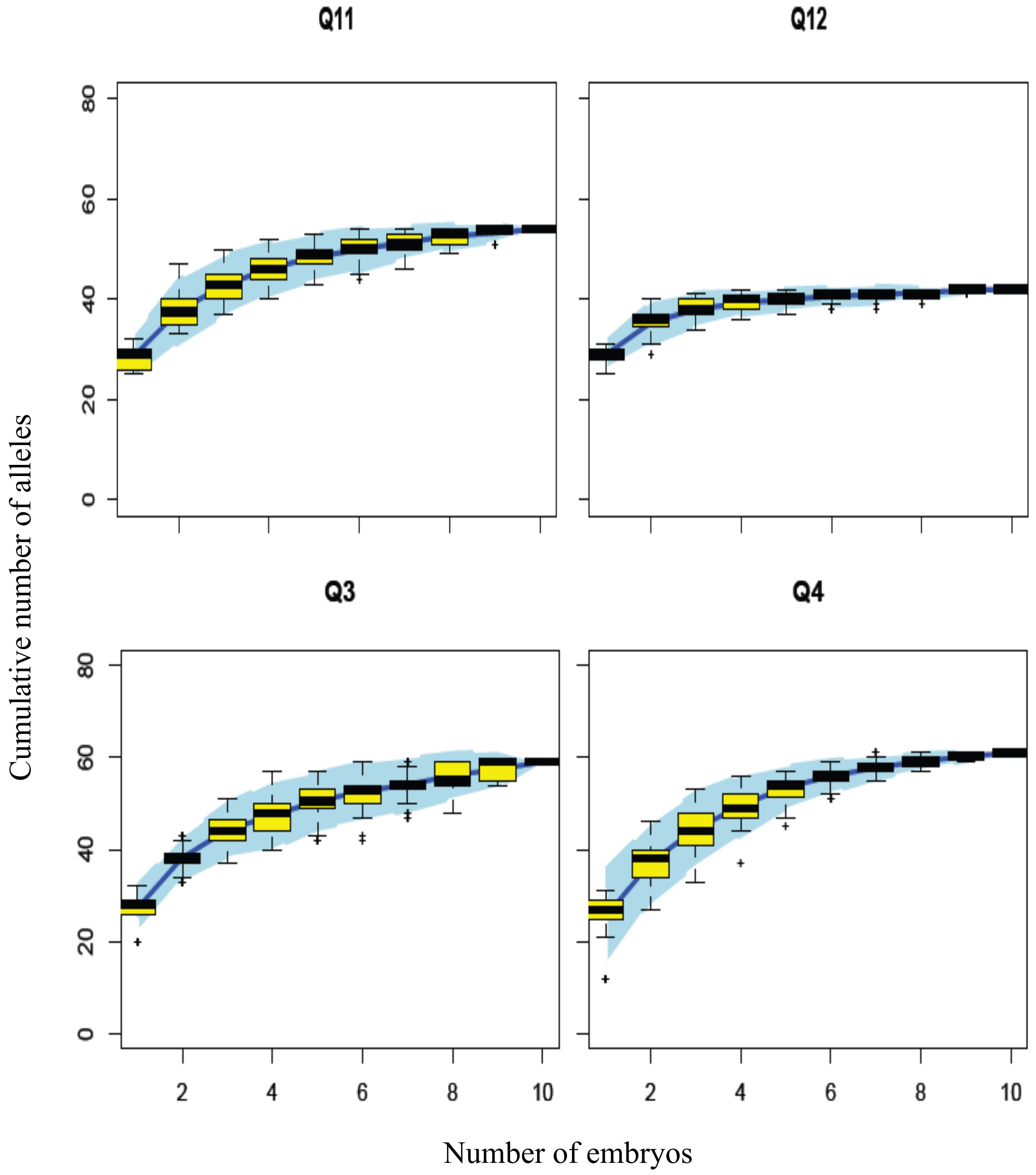
*N*a: Number of alleles per locus. *N*ae: Effective number of alleles (Nielsen *et al*. 2003). H_O: Observed heterozygosity. H_E: Expected heterozygosity or gene diversity corrected for sample size (Nei 1978). *PIC*: Polymorphic information content. *NE-1P, NE-2P, NE-PP, NE-I, NE-SI*: Combined non-exclusion probability of first parent, second parent, parent pair, identity and sib identity, respectively. *HW*: Hardy-Weinberg equilibrium test. *F*(Null): Null allele frequency estimate.

Table G2. Genetic diversity of adult trees for each stand.

	N	*N*a	*N*ae	*AR*	*PrAll*	H_E	H_o	F_{Is}
C	6	2.85	2.41	2.11	0.15	0.54	0.54	-0.01
D	10	4.15	**3.29**	2.39	0.10	0.61	0.60	-0.11
E	35	6.45	3.44	2.51	0.75	0.65	0.65	-0.07
F	12	5.10	3.64	2.50	0.15	0.64	0.64	-0.03
I	5	3.65	3.00	2.41	0.20	0.63	0.62	-0.12
J	22	5.50	3.24	2.43	0.35	0.63	0.63	0.06*
K	28	5.80	3.16	2.40	0.40	0.61	0.61	0.04
L	2	2.80	3.08	2.80	0.10	0.75	0.80	0.19*
M	3	2.60	2.67	2.27	0.00	0.61	0.58	-0.28
N	12	6.00	4.71	2.75	0.75	0.72	0.72	-0.05
All	**135**	**11.10**	**4.38**	**2.72**	**-**	**0.71**	**0.65**	**0.08***

N: Total number of trees censused in the stand. *N*a: Mean number of alleles per locus. *N*ae: Mean effective number of alleles. *AR*: Allelic richness (expected number of alleles among 4 gene copies). *PrAll*: Private alleles. H_E: gene diversity corrected for sample size (Nei 1978). H_O: Observed heterozygosity. F_{IS}: Individual inbreeding coefficient (*: significant differences from zero using 10000 randomizations of alleles among individuals).

Figure G1. Genetic diversity accumulation curves for sampled acorn progenies of some illustrative mother trees (Q11, Q12, Q3 y Q4). The number of embryos at which the number of alleles stabilizes (ca. 10) represents a sufficiently large sample size for adequately characterizing the genetic diversity of acorn families.



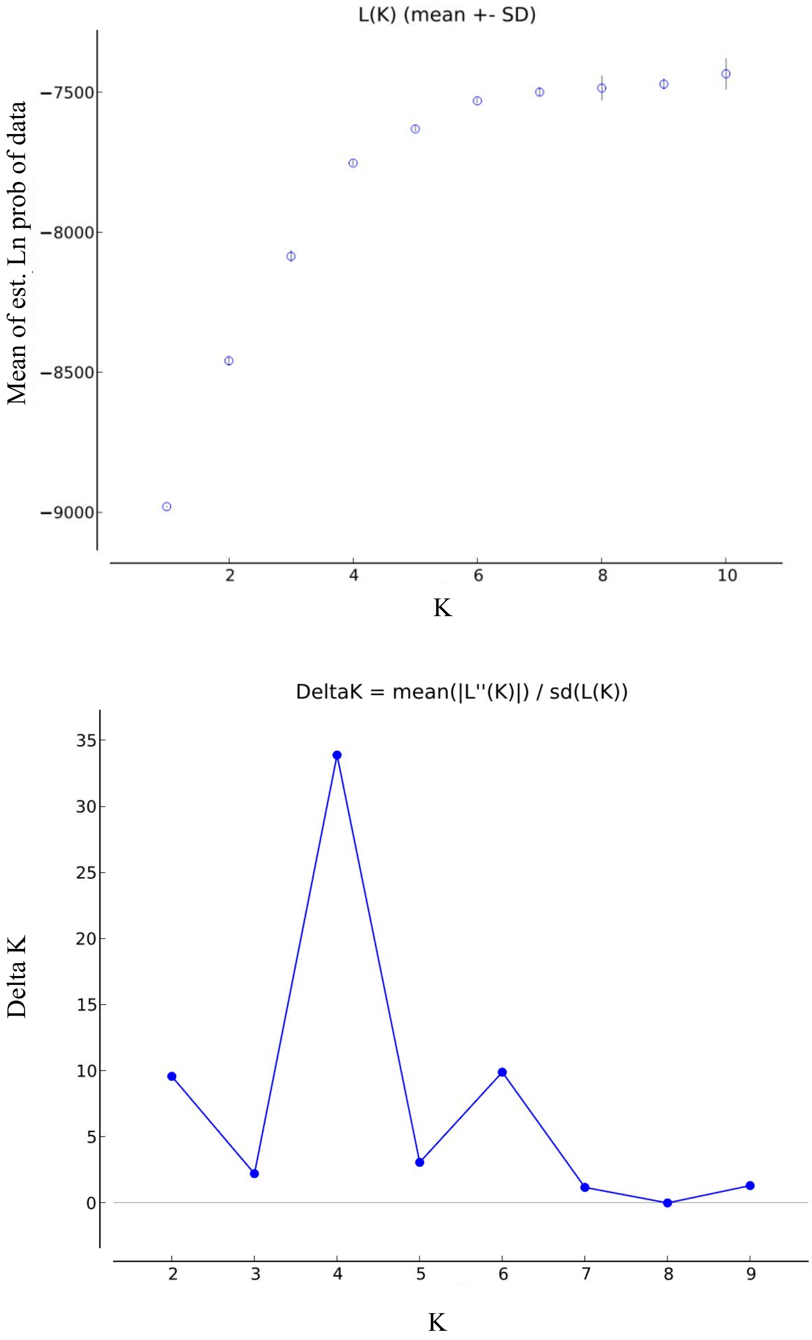
APÉNDICE H

DIFERENCIACIÓN ENTRE NÚCLEOS POBLACIONALES Y ESTRUCTURA GENÉTICA DE LOS ADULTOS

Table H1. Pairwise differentiation (F_{ST}, below diagonal) and pairwise conditional genetic distance (cGD, upper diagonal) among stands. F_{ST} values different from zero according to permutation tests are shown in bold (when $P < 0.01$) or bold italics (when $P < 0.05$).

	C	D	E	F	I	J	K	L	M	N
C		1.590	1.721	1.663	1.471	1.996	0.762	1.536	1.661	1.050
D	**0.180**		2.061	0.560	1.835	2.027	1.978	1.933	1.989	1.270
E	**0.149**	**0.140**		1.684	1.589	2.010	2.027	1.668	1.704	1.584
F	**0.161**	0.005	**0.103**		1.661	1.867	2.074	1.779	1.821	1.219
I	**0.194**	**0.196**	**0.126**	**0.150**		0.742	1.400	0.580	0.557	0.700
J	**0.168**	**0.135**	**0.110**	**0.121**	**0.103**		1.882	0.815	0.655	1.097
K	**0.114**	**0.138**	**0.115**	**0.138**	**0.128**	**0.098**		1.432	1.529	1.143
L	***0.200***	***0.198***	**0.154**	***0.160***	***0.129***	**0.120**	**0.139**		0.589	0.857
M	***0.235***	**0.218**	**0.156**	**0.175**	***0.186***	**0.108**	**0.130**	0.097		0.901
N	**0.135**	**0.093**	**0.093**	**0.087**	**0.069**	**0.073**	**0.079**	***0.086***	**0.123**	

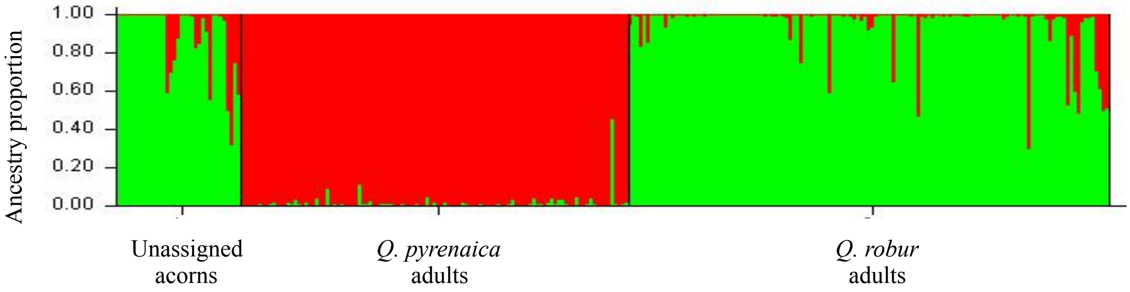
Figure H1. Posterior probability and change in likelihood used for detecting the K value that best described the adult genotypes. Plot of mean likelihood L(K) and variance per K value from STRUCTURE on a dataset containing 135 adult *Quercus robur* trees genotyped at 20 polymorphic microsatellite loci (upper panel). Change in likelihood (delta K) plot as proposed by Evanno *et al.* (2005) points to *k* = 4 as the best number of groups fitting the data (lower panel). Results generated by STRUCTURE have been collated and summarized using the program STRUCTURE HARVESTED.



APÉNDICE I

ANÁLISIS DE LOS EVENTOS DE HIBRIDACIÓN

Figure I1. Bar plots of STRUCTURE ancestry proportions for K = 2 clusters including unassigned acorns (*n* = 35, individuals indicated as group 1 below the plot), adult *Q. pyrenaica* trees (*n* = 109, group 2) and adult *Q. robur* trees (*n* = 135, group 3). Each individual is represented as a line segment, which is vertically partitioned with different colours representing the individual's assignment to one of the two clusters (green or red). In the figure below, change in likelihood (delta K) is presented, which point to K = 2 as the best value describing the data.



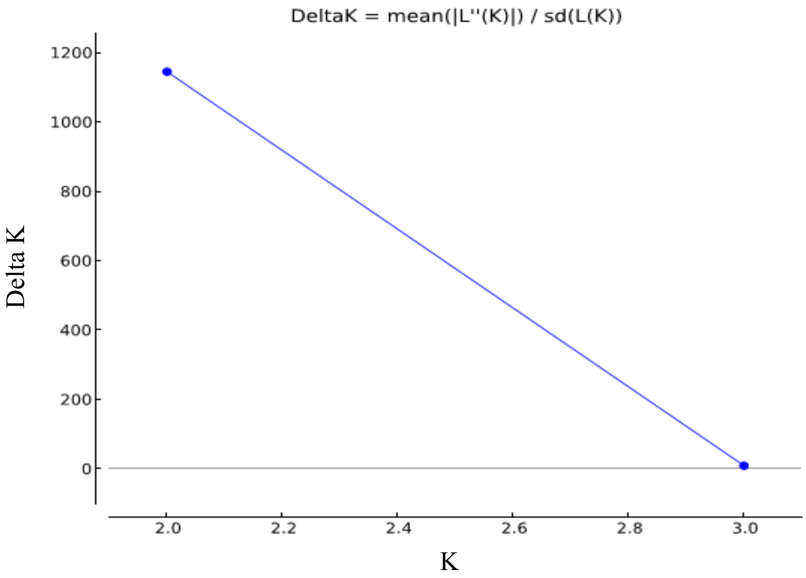


Table I1. Probability of non-assigned acorns to stem in first generation from a hybridization event with *Q. pyrenaica*. Assignment probability of acorns to clusters "*Q. robur*" and *Q. pyrenaica*" was computed in STRUCTURE. From 35 acorns without identified fathers, only 4 are likely to be first generation hybrids (i.e., probability of assignment to cluster "*Q. pyrenaica*" 0.4 < value < 0.6).

Acorn ID	**Qrob_cluster**	**Qpyr_cluster**	**Resulting assignment**
Unass_1	0.996	0.004	Qrob
Unass_2	0.995	0.005	Qrob
Unass_3	0.995	0.005	Qrob
Unass_4	0.995	0.005	Qrob
Unass_5	0.996	0.004	Qrob
Unass_6	0.995	0.005	Qrob
Unass_7	0.995	0.005	Qrob
Unass_8	0.995	0.005	Qrob
Unass_9	0.996	0.004	Qrob
Unass_10	0.995	0.005	Qrob
Unass_11	0.994	0.006	Qrob
Unass_12	0.996	0.004	Qrob
Unass_13	0.994	0.006	Qrob
Unass_14	0.997	0.003	Qrob
Unass_15	0.588	0.412	Hybrid
Unass_16	0.696	0.304	Qrob
Unass_17	0.758	0.242	Qrob
Unass_18	0.872	0.128	Qrob
Unass_19	0.994	0.006	Qrob
Unass_20	0.995	0.005	Qrob
Unass_21	0.994	0.006	Qrob
Unass_22	0.986	0.014	Qrob
Unass_23	0.825	0.175	Qrob
Unass_24	0.846	0.154	Qrob
Unass_25	0.981	0.019	Qrob
Unass_26	0.913	0.087	Qrob
Unass_27	0.557	0.443	Hybrid
Unass_28	0.996	0.004	Qrob
Unass_29	0.996	0.004	Qrob
Unass_30	0.992	0.008	Qrob
Unass_31	0.967	0.033	Qrob
Unass_32	0.499	0.501	Hybrid
Unass_33	0.320	0.680	Hybrid
Unass_34	0.743	0.257	Qrob

CAPÍTULO 4

Mating patterns and their ecological drivers in long-term refugial populations of Pedunculate oak (*Quercus robur*)

APÉNDICE J

ESTIMAS BASADAS EN LOS MODELOS DE CRUZAMIENTO (MEMM)

Table J1. Summary descriptors of mating patterns in the four largest forest stands according to MEMM simulations: rates of immigration (m) and selfing (s), median dispersal distance (δ) and shape parameter (b), as well as the ratio of observed (d_{obs}) and effective (d_{ep}) densities of male reproductive trees. The 95% confidence intervals [shown in brackets] were obtained by computing the 2.5% and 97.5% quantiles from 50,000 retained values of the parameter in the MCMC simulation. Forest stands showing significant differences (i.e., non-overlapping CI) are identified by different superscript letters.

	E	**J**	**K**	**DF**
m	0.21 [0.15, 0.27]^{a}	0.19 [0.13, 0.26]^{a}	0.075 [0.03, 0.13]^{b}	0.15 [0.10, 0.22]^{ab}
s	0.018 [0.004, 0.048]^{a}	0.090 [0.051, 0.150]^{b}	0.099 [0.051, 0.170]^{b}	0.011 [0.001, 0.041]^{a}
δ	668 [286, 982]^{a}	570 [172, 975]^{a}	595 [258, 974]^{a}	142 [95, 503]^{a}
b	0.34 [0.24, 0.52]^{a}	0.35 [0.23, 0.68]^{a}	0.36 [0.21, 0.65]^{a}	0.58 [0.26, 0.92]^{a}
d_{obs}/d_{ep}	16 [4.5, 33]^{a}	8.6 [2.6, 32]^{a}	23 [7, 34]^{a}	22 [6.9, 34]^{a}

The selfing rates were s_{J} = 9%, s_{K} = 9.9%, s_{DF} = 1.1%, s_{E} = 1.8% and significantly differed among stands with J and K having higher rates than DF and E, according to the CI. Similarly, the average pollen migration rates (m_{K} = 7%, m_{DF} = 15%, m_{J} = 19% and m_{E} = 21%) were significantly different between stands K and E, and between stands K and J.

The average pollen dispersal distance, δ, ranged from 138 m (stand DF) to 672 m (stand E). MEMM yielded longer dispersal distance estimates than previous empirically-estimated frequency distributions of pollen dispersal distances obtained by CERVUS in the study area (ranged from 59.6 to 185.4 in stand J and DF, respectively). This is expected given that MEMM estimates include all trees in each population and the effect of cryptic gene flow, contrary to CERVUS estimates. The shape parameter, b, of the pollen dispersal kernel ($b<1$; range: 0.33 - 0.57) indicated a non-negligible amount of long distance dispersal events. Stand DF tended to exhibit a higher b ($b_{DF} = 0.57$) and a lower δ ($\delta_{DF} = 138$ m) than the other three populations, but pollen dispersal parameters did not significantly differ between the stands (the credibility intervals, CI, overlapped).

There is a extremely high variance of individual male fecundity. This resulted in highly skewed distributions of relative fecundity in all populations (Fig. L1).

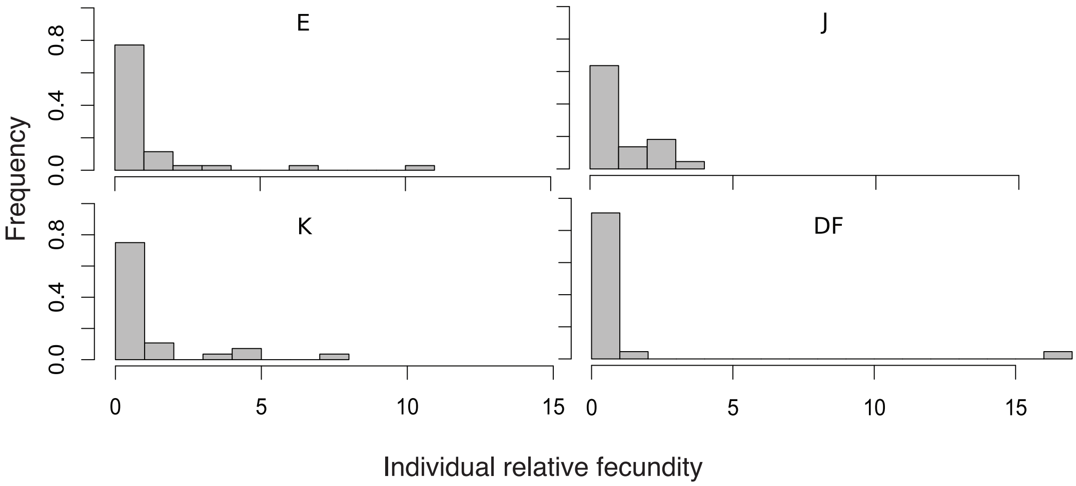


Figure J1. Individual variation on male fecundity for each stand

APÉNDICE K

RESUMEN DE LOS MODELOS DE DISPERSIÓN DE POLEN EMPÍRICOS

Table K1. Ranking of candidate mating models of empirically-estimated pollen dispersal types based on the Akaike information criterion (AIC). To configure the candidate models, we pooled the explanatory variables in groups of predictors intrinsic to the tree (I), those related with the conspecific neighbors (N) and those related with the physical environment (E). Then, all possible combinations of these 3 groups of variables were modeled (including the null model): I, N, E, I + N, I + E, N + E, I + N + E. Δ AIC, difference between the best and the second model. AICwt, probability that the model will be the best model in the set.

Response variable	Candidate model	AIC	Δ AIC	AICwt
Selfing	I + N + E	152.46	0.00	0.64
	I + N	153.79	1.33	0.33
	I	159.22	6.76	0.02
	I + E	163.00	10.54	0
	N	209.16	56.70	0
	N + E	209.95	57.50	0
	null	232.55	80.10	0
	E	236.24	83.78	0
Local pollination	I + N	248.83	0.00	0.73
	I + N + E	250.77	1.94	0.27
	I	269.70	20.88	0
	I + E	272.69	23.86	0
	N	278.16	29.34	0
	N + E	281.75	32.93	0
	null	325.42	76.59	0
	E	325.89	77.07	0
Pollen immigration	N	172.96	0.00	0.63
	N + E	175.15	2.19	0.21
	I + N	176.29	3.33	0.12
	I + N + E	178.46	5.50	0.04
	I	194.51	21.55	0
	I + E	195.56	22.60	0
	E	195.76	22.80	0
	null	197.60	24.64	0

APÉNDICE L

ANÁLISIS DE CORRESPONDENCIA CANÓNICA BASADOS EN LOS MODELOS DE CRUZAMIENTO (MEMM)

Table L1. Test of canonical dimensions. Two independent CCA were applied to mating variables from a mother and father perspective. Four and five dimensions respectively, were inferred following the number of mating variables in the analysis. CC, canonical correlations; R^2, squared canonical correlations; CR, canonical redundancies.

Perspective	Dimension	CC	R^2	CR	Bartlett's test (ρ^2)	χ^2	df	*P*
Mother	1	0.77	0.59	0.16	0.59	170.76	30	<0.001
	2	0.62	0.39	0.06	0.39	93.85	20	<0.001
	3	0.58	0.34	0.07	0.34	51.67	12	<0.001
	4	0.40	0.16	0.04	0.16	15.79	6	0.015
	5	0.90	0.01	0.00	0.01	0.70	2	0.704
Father	1	0.64	0.41	0.22	0.41	69.64	24	<0.001
	2	0.43	0.19	0.03	0.19	22.38	15	0.098
	3	0.17	0.03	0.00	0.03	3.83	8	0.872

CAPÍTULO 6

Discusión general

APÉNDICE M

DETALLES DE LA DISPERSIÓN DE POLEN ENTRE RODALES DE *Q. robur* EN EL VALLE DEL JERTE

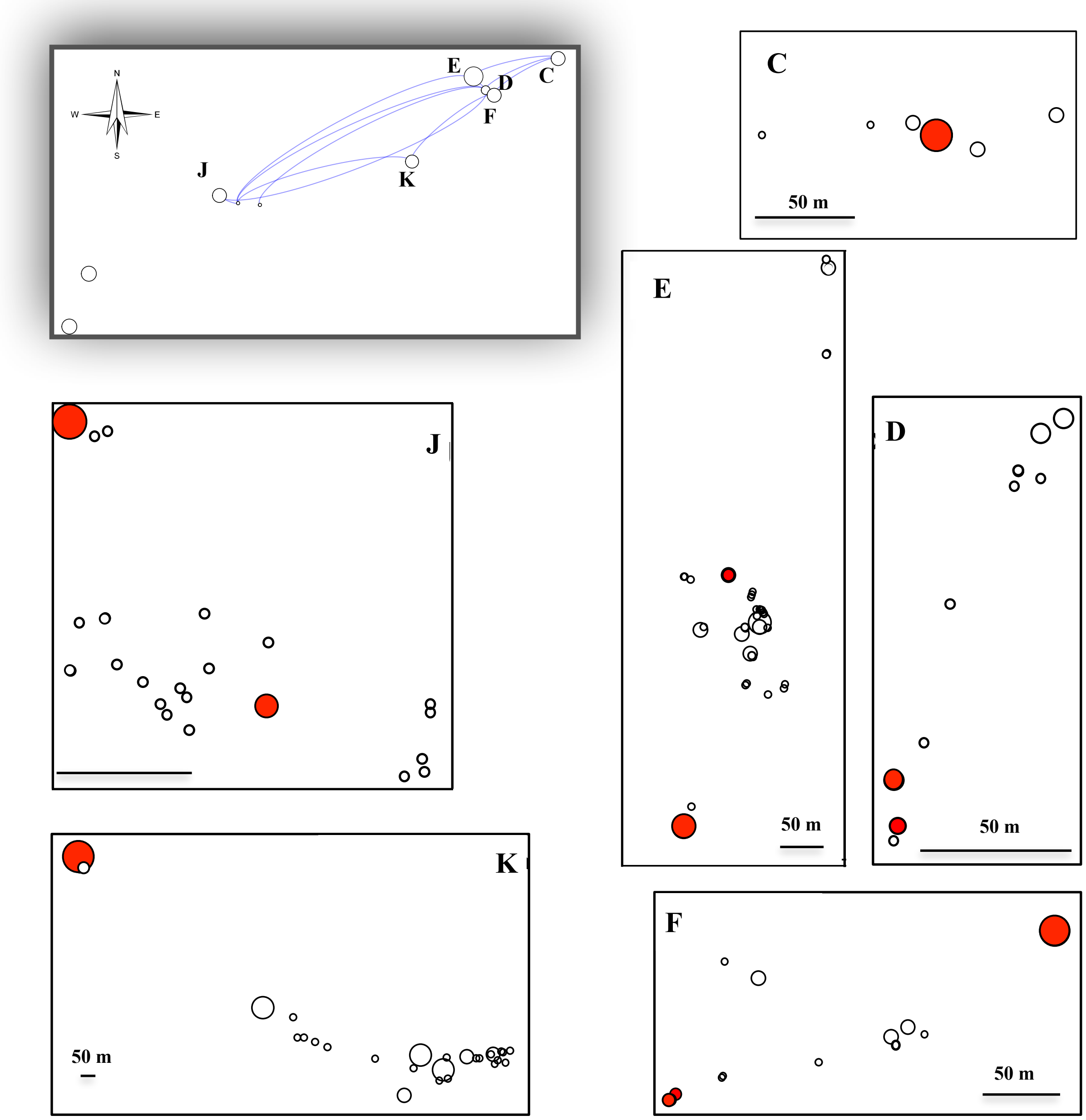


Figura M1. Mapa de la distribución de los rodales de *Q. robur* en el valle del Jerte (esquina superior izquierda) y detalle de la distribución de los individuos en aquellos rodales que participan en la conectividad a través del paisaje. El tamaño de los puntos es proporcional al DBH de los individuos. En rojo se representan los árboles padre responsables de la dispersión de polen efectiva entre núcleos de población. Se observa el gran tamaño de muchos de los árboles implicados en la conectividad de la población.

AGRADECIMIENTOS

Embarcarme en este proyecto de investigación ha sido una oportunidad única desde el punto de vista personal y con certeza lo será en el ámbito profesional. La tesis ha sido un camino de perseverancia, como una expedición a una alta montaña llena de retos y satisfacciones y con una gran recompensa, el desarrollo de una mentalidad crítica y constructiva con la que poder promover la conservación de la naturaleza. Todo lo aprendido a lo largo de este camino se lo debo a grandes personas con las que he tenido la suerte de cruzarme, así como a instituciones que lo han financiado.

Mis directores representan los pilares de este proyecto, dándome apoyo incondicional y motivación en cada momento. Además de grandes científicos, son grandes personas y maestros en varios sentidos. Con ellos he aprendido lo que es la ciencia y cómo a través de ella podemos contribuir a una conservación más eficaz de nuestra preciada y amenazada biodiversidad. Guiada por su experiencia y su saber, lo que era un esbozo inicial de proyecto ha llegado a ser la tesis que aquí presento con satisfacción. Han sido el acompañamiento que nunca falta pero que incentiva la autonomía y la seguridad en cada paso dado. Me siento afortunada de haber aprendido a hacer ciencia de nivel de la mano de estos señores. En particular, agradezco a Arndt su pedagogía (basada en la discusión y en el por qué de las cosas), su claridad y sus críticas siempre constructivas que han hecho posible una inmejorable tutela a distancia. Darme oportunidades desde el inicio para crecer como investigadora, haciéndome participe en colaboraciones internacionales y diversas actividades científicas. Su optimismo y las valoraciones siempre positivas de mi trabajo han hecho que ame esta profesión y culmine la tesis con entusiasmo. Gracias por esta bonita idea de proyecto y transmitirme tu gran conocimiento acerca de la ecología de bosques que forma parte de las grandes motivaciones de mi vida. Agradezco a Pedro, haber compartido su pasión y saber por cada proceso biológico y cada organismo que habita la Tierra. Por ser el ejemplo de que la curiosidad no se pierde sino que se alimenta con la sapiencia y debe estar en todo lo que hacemos. Por cada conversación entre risas y sin prisas en su apretada agenda, y una atención inmediata cada vez que la he requerido, siempre apreciando mis avances y llenándome de motivación. Por dar el toque maestro y aportar una visión holística a los trabajos, desbordando creatividad y expresión artística. A Gerardo, le agradezco su cálida acogida en tierras extremeñas, que compartiera conmigo sus rincones naturales y el disfrute de estudiarlos. Con él he aprendido ecología desde lo más profundo y bello de los bosques, con las botas puestas. Sin intimidarse ante los muestreos más impracticables, hemos alcanzado hojas, bellotas y troncos con maniobras habilidosas en barrancos y riachuelos. A estas inolvidables experiencias debo la querencia por esa tierra.

Al equipo extremeño de la UEX le agradezco poner a mi disposición todos los recursos e instalaciones y facilitarme siempre mis desplazamientos a su entorno. Quiero destacar la enorme ayuda de Juan Carlos Benito (Espi) como técnico de campo, especialista en todo tipo de muestreos arriesgados, que ha mantenido su buen talante a través de intensos y largos días de campo. No han faltado buenos momentos entre descenso de barrancos, rap y sandía pocha. Agradezco a Adahara esa gran generosidad y conversación calmada cada vez que me acogía en su casa. Y por último, a Fernando Pulido le debo haberme incluido en el grupo de colaboración "los marginales" del que salió el Capítulo 2 de la tesis. Ha sido un placer compartir momentos de discusión y trabajo con ese carácter afable, llano y divertido que le caracteriza (véase "el caso de la serpiente submarina"). Gracias a esta colaboración, mi formación se benefició de las discusiones y momentos de trabajo que compartí con grandes científicos como Fernando Valladares, Alistair Jump, Johannes Kollmann, Johan Ehrlén y Arndt Hampe.

La tesis tiene la esencia de la gran calidad personal y profesional de mi grupo de trabajo en la Estación Biológica de Doñana (EBD), el *Chirigotive Ecology Group*. De la mano de Pedro y Arndt, Alfredo Valido, Candelaria Rodríguez, Néstor Pérez, Rocío Rodríguez, Francisco Rodríguez, Juan Miguel Arroyo, Juan Pedro González, Marcial Escudero y Cristina García, he disfrutado de largas e intensas discusiones intelectuales cada *Lab meeting*, que me han enriquecido en temas diversos, me han enseñado a expresar ideas con claridad, y han desarrollado mi espíritu crítico. Gracias por vuestra ayuda, consejos y cariño. A destacar, la colaboración de la técnico del proyecto Cristina Rigueiro, que ha sido fundamental para conseguir el genotipado de amplias muestras en un tiempo récord. Además, le debo esa paciencia y empeño durante las clases particulares que recibí sobre técnicas de procesado genético siempre con una sonrisa. Mención especial merece Manolo Carrión (el Lolo), que más allá de ser un gran técnico y naturalista, ha sido La persona con el don de hacer felices a todos los que nos cruzamos en su camino. Gracias por todos los momentos contigo y enseñarme que el trabajo (y la vida) debe estar repleta de carcajadas, disfraces y color cada día. En particular, agradezco todo lo que he aprendido con Rocío como compañera estudiosa de relictos que me ha allanado el camino a través de la genética de poblaciones, y con Paco siempre dispuesto a compartir su gran experiencia en estadística de un modo didáctico y ameno. A Nestor, su tiempo de discusión y sus opiniones tan acertadas. Y en especial a Cande, mi gran compañera a lo largo de toda la tesis, le agradezco su energía positiva, su escucha y conversación calmada, todas sus enseñanzas personales como profesionales (desde cómo hacer una buena gráfica hasta cómo compaginar la maternidad y la investigación). Su amistad junto a la de Patricia Martínez han supuesto un apoyo fundamental y necesario para mi.

El soporte que ha supuesto la EBD como centro de investigación ha sido inmejorable, por la gran cantidad de científicos de nivel que mantiene, la calidad de sus instalaciones y sus recursos. A destacar, el aprendizaje brindado por el Laboratorio de Sistemas de Información Geográfica y Teledetección (LAST) de la mano de David Aragonés en el procesado de datos espaciales y elaboración de mapas, así como el personal y el buen funcionamiento del Laboratorio de Ecología Molecular (LEM).

Mi familia representa la guinda del pastel de todas las grandes personas que han contribuido a que este proyecto salga a delante. Pablo Villalva, Noa y Mar han sido una fuente de alegría y motivación cada día, vital para no perder sino aumentar el entusiasmo por conservar la naturaleza y querer hacer un mundo mejor. El incondicional apoyo de Pablo ha sido indispensable desde un punto de vista emocional así como intelectual y técnico. Su interés constante por temas dispares y sus ideas creativas fluyendo a borbotones han impregnado toda la tesis desde intensos debates sobre ecología y evolución, diseños de muestreo, presentaciones y manuscritos. Agradezco que haya sido el fotógrafo de la tesis y que sus dotes artísticas hayan embellecido todos los trabajos. Así como toda la ayuda y tiempo voluntario que ha dedicado en multitud de muestreos de campo, enseñándome a tomar y recuperar datos espaciales, siendo pieza clave para contrastar opiniones y agilizando los largos e intensos días de muestreo. Compartir mi vida con un conservacionista acérrimo como es él, me enriquece cada día. Mis niñas, Noa y Mar, han sido la revolución que ha marcado los últimos 5 años. A ellas les debo mi gran felicidad y su contribución a mi desarrollo personal que han trascendido también a nivel profesional. La madurez adquirida con ellas me ha ayudado a sintetizar e integrar conceptos, y a ver el proyecto y sus diferentes cuestiones desde un prisma más elevado. Me han convertido en una persona eficaz capaz de hacer de los tiempos más cortos momentos de alta eficiencia. Con ellas he sentido que trabajar en investigación es un placer. A mis raíces, mi madre, mi padre y mis hermanos, les agradezco lo que soy, el respeto al medio ambiente que me han inculcado y su apoyo siempre.

Por último, el proyecto nunca podría haberse realizado sin mi tutor, Juan Arroyo, que ha facilitado todos los trámites a través del programa de doctorado de Universidad de Sevilla, y por supuesto, sin el respaldo económico que diversos diversos organismos e instituciones han aportado. La idea original desarrollada en el denominado proyecto PERSLIM (CGL2010-18381) ha sido financiada por el Ministerio de Economía y Competitividad, el

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