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**Ecological and Genetic Analysis of
Plant-Animal Interactions in
Mediterranean Environments**

**Análisis Ecológico y Genético de
Interacciones Planta-Animal en
Ambientes Mediterráneos**

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Ecological and genetic analysis of plant-animal interactions in Mediterranean environments

Análisis ecológico y genético de
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“What escapes the eye, however, is a much more insidious kind of extinction: the extinction of ecological interactions”

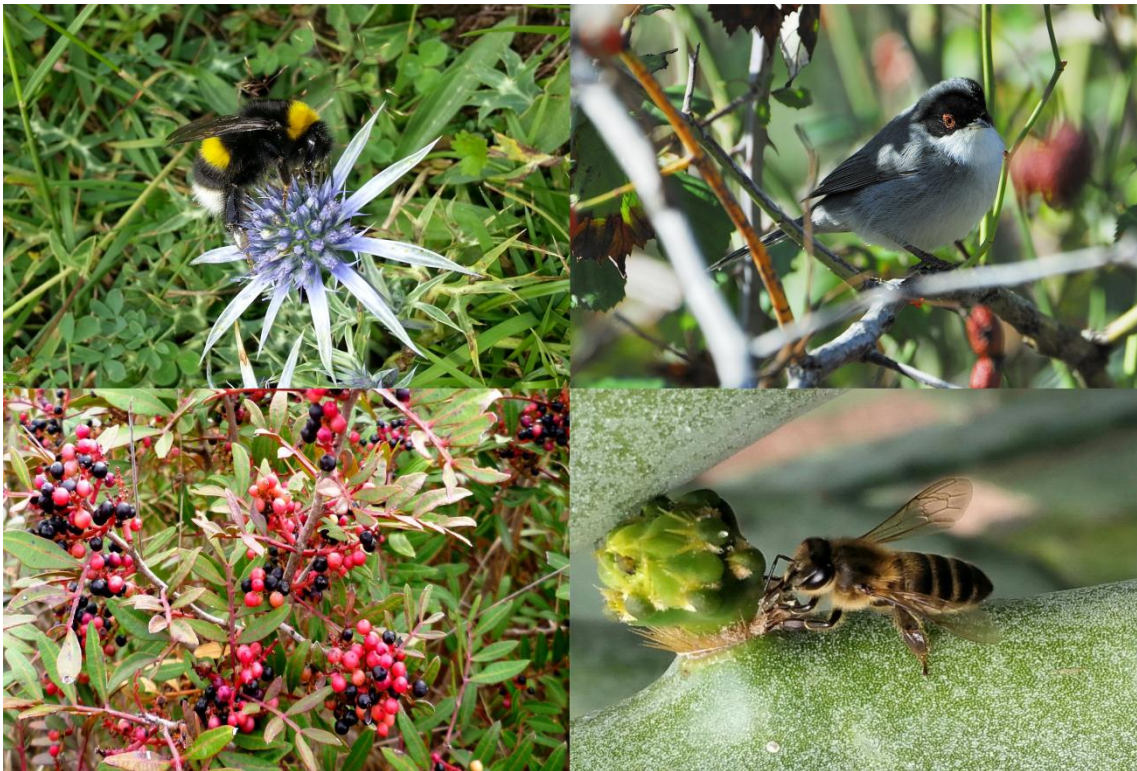
Daniel Janzen (1974)

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SPANISH SUMMARY

(Resumen en español)



1. Mutualismos planta-animal. Importancia y técnicas de estudio

El mutualismo es un proceso ecológico en el que dos especies interactúan entre sí y ambas reciben un beneficio por tal interacción (Bronstein, 2015). Las interacciones mutualistas están extendidas en los ecosistemas naturales (Janzen, 1985), y juegan un papel clave en el mantenimiento de la biodiversidad y de los servicios ecosistémicos en todo el mundo (p. ej. Albrecht et al., 2014). Todos los organismos están directa o indirectamente involucrados en interacciones mutualistas (Thompson, 2009). De hecho, todos los eucariotas podrían considerarse como mutualistas en sí mismos, ya que de acuerdo con la teoría endosimbiótica la célula eucariota es el resultado de múltiples procesos de asociación entre diferentes células procariontas (Margulis, 1967). Se han descrito muchos tipos de mutualismos en la naturaleza: hongos interactuando con las raíces de las plantas para incrementar la movilidad de los nutrientes (micorrizas, Van Der Heijden, Martin, Selosse, & Sanders, 2015); bacterias y nódulos de las raíces de las plantas leguminosas para fijar nitrógeno en el suelo (Kiers, Rousseaus, West, & Denison, 2003; Heath & Tiffin, 2007); líquenes formados por la asociación de hongos y algas (Grube & Wedin, 2016); ácaros y hojas de las plantas (domatía, Romero & Benson, 2005) (Figura 1) y plantas y animales (p. ej. polinización, Willmer, 2011; y dispersión de semillas, Howe & Smallwood, 1982). De entre todas estas interacciones, esta tesis se centra en la polinización y la dispersión de semillas, que son dos tipos de interacciones mutualistas cruciales en el ciclo reproductivo de las plantas que dependen de animales para la polinización de sus flores y/o la dispersión de sus semillas (Jordano, 2000; Ollerton, Winfree, & Tarrant, 2011).

Las interacciones mutualistas han llamado la atención de los científicos durante milenios. Las primeras descripciones de mutualismos datan de la Antigua Grecia. Hay mucha bibliografía de finales del siglo XIX describiendo la historia natural de diferentes mutualismos. Sin embargo, el campo no avanzó conceptualmente hasta la década de 1960, y la mayoría de los estudios fueron realizados a partir de 1980 (Bronstein, 2009; Bascompte & Jordano, 2013). Los estudios de Janzen (1966) sobre la interacción entre las acacias (*Acacia cornigera* (L.) Willd) y las hormigas que las habitan (*Pseudomyrmex ferruginea* F. Smith) son un ejemplo de estos estudios pioneros que proporcionaron unas bases sólidas en el campo

del estudio de los mutualismos. El papel de las hormigas que viven en acacias había sido discutido por muchos científicos desde que Belt describió la interacción en 1874. Decenas de investigadores, incluyendo a Darwin (1877), participaron en ese intenso debate que dio lugar a diferentes hipótesis sobre la interacción acacia-hormiga. Mientras algunos científicos consideraban a las hormigas como protectoras de la planta, otros las veían cómo parásitos. Sin embargo, faltaban estudios experimentales para testar estas teorías que estaban principalmente basadas en observaciones. El debate permaneció abierto hasta los estudios de Janzen (1966). El describió el mutualismo que ocurría entre la acacia y las hormigas tras eliminar las hormigas en algunos árboles y registrar su desarrollo en contraste con el de acacias todavía ocupadas por hormigas. Encontró que los árboles no ocupados sufrían una presión de herbivoría más elevada y una mayor competencia con otras plantas en comparación con las ocupadas, en las cuales las hormigas protegían al árbol de los herbívoros y eliminaban a otras plantas competidoras en los alrededores de la acacia.

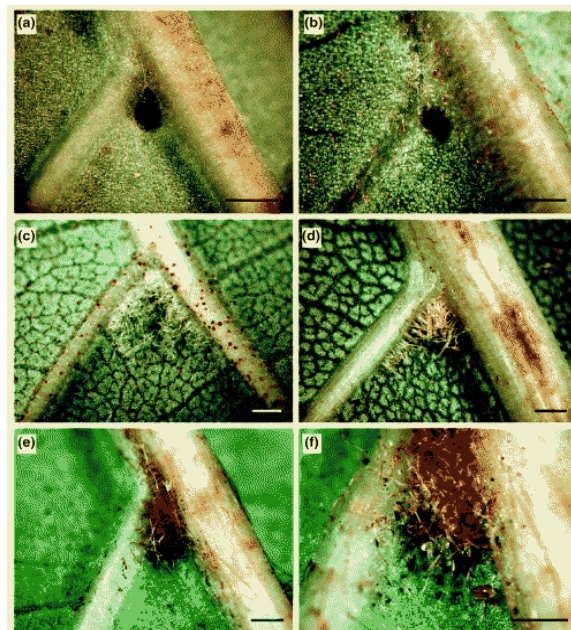


Figura 1. Diferentes ejemplos de domatía entre ácaros y plantas. En estas interacciones las plantas ofrecen refugio a los ácaros, mientras que los ácaros protegen a las plantas de patógenos y herbívoros. Las barras de escala representan 0,5 mm (Figura de Romero & Benson, 2005).

Los estudios de interacciones planta-animal generalmente han sido realizados en grupos pequeños de especies (Nilsson, 1988; Johnson & Steiner, 1997). Sin embargo, los mutualismos planta-animal forman redes

complejas de interacciones entre especies. Por tanto, el funcionamiento del sistema solo puede entenderse si se estudian las interacciones al nivel de comunidad (Jordano, Bascompte, & Olesen, 2003). Los parámetros estructurales de estas redes complejas tienen consecuencias importantes para la estabilidad de las comunidades, así que las redes mutualistas han sido consideradas como la arquitectura de la biodiversidad (Bascompte & Jordano, 2007) y comprenderlas es crucial para la conservación de los sistemas ecológicos (p. ej. Rico-Gray & Oliveira, 2007). Aunque este enfoque ya se aplicó en algunos estudios durante la década de 1980 (p. ej. Janzen, 1980; Herrera, 1982; Jordano, 1987), no se desarrolló hasta la aparición de herramientas analíticas adecuadas que pudieran desvelar las propiedades estructurales de estos sistemas complejos de interacciones. En este sentido, el desarrollo de redes bipartidas (aquellas en las cuales las especies están divididas en dos niveles tróficos y que no pueden interactuar con las especies en el mismo nivel, Figura 2) (Jordano et al., 2003) al principio del siglo XXI, ha incrementado la cantidad de estudios realizados en el campo de los mutualismos a nivel de comunidad.

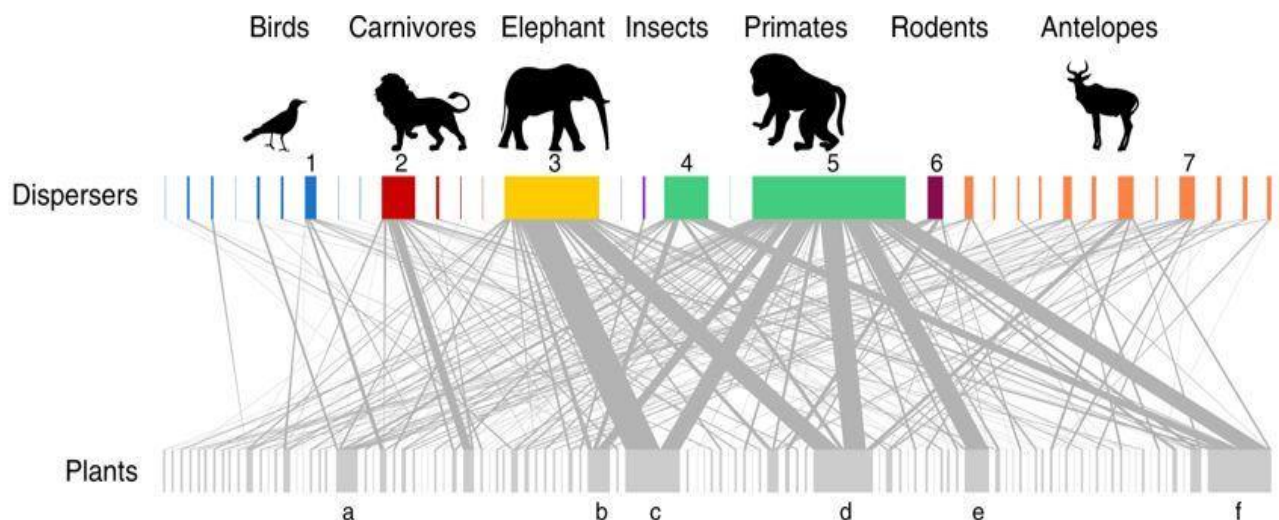


Figura 2. Red bipartida cuantitativa de dispersores de semillas (animales, arriba) y plantas (abajo) en el Gran Rift. Principales dispersores: 1. *Pycnonotus tricolor*, 2. *Civettictis civetta*, 3. *Loxodonta africana*, 4. *Cercopithecus pygerythrus*, 5. *Papio ursinus*, 6. *Hystrix africae australis*, y 7. *Redunca arundinum*. Plantas que fueron más comúnmente dispersadas: a *Centaurea praecox*, b *Grewia inaequilatera*, c *Hyphaene natalensis*, d *Sclerocarya birrea*, e *Tamarindus indica*, y f *Ziziphus mucronata*. Los enlaces representan la interacción entre los taxones animales y vegetales, y su grosor es proporcional al número de interacciones (Figura de Timóteo, Correia, Rodríguez-Echeverría, Freitas, & Heleno, 2018).

La estructura de las redes mutualistas condiciona su resiliencia a perturbaciones antrópicas (Fortuna & Bascompte, 2006). Por tanto, es

fundamental comprender la arquitectura de las redes ecológicas para predecir su robustez a perturbaciones (Montoya, Pimm, & Solé, 2006; Solé & Bascompte, 2006). Las redes mutualistas son anidadas, lo que significa que las especies especialistas interactúan solo con un subconjunto de las especies que interactúan con las generalistas (Bascompte, Jordano, Melián, & Olesen, 2003). Este patrón implica que la estructura de las redes está formada principalmente por un núcleo central de interacciones, y por tanto, algunas especies son más importantes para la estabilidad de las redes que otras (Domínguez-García & Muñoz, 2015). Hay especies que se consideran “piezas clave”, cuya extinción puede desencadenar efectos cascada en los ecosistemas conduciendo a extinciones secundarias. Por lo tanto, algunas especies pueden desaparecer de los ecosistemas naturales con pocas consecuencias, mientras que la extinción de otras puede causar el colapso del ecosistema (Memmot, Waser, & Price, 2004).

La disrupción de mutualismos entre plantas y animales implica la pérdida de especies acompañantes lo que puede afectar negativamente a las redes de interacción y puede derivar en la aparición de “especies mutualistas viudas” (*sensu*, Olesen & Valido, 2004), o en otras palabras, especies que pueden experimentar una reducción de su aptitud ecológica después de que se elimine a su compañero mutualista (p. ej. Given, 1995). Por ejemplo, la extinción de la megafauna en el Pleistoceno desencadenó un efecto cascada de extinción en sus especies mutualistas acompañantes (Galetti et al., 2018). Además, la desaparición de estos animales de gran tamaño implicó la pérdida de servicios de dispersión a larga distancia (Pires, Guimarães, Galetti, & Jordano, 2018). Sin embargo, hay algunos ejemplos de disrupciones de mutualismos planta-animal con consecuencias negativas en épocas mucho más recientes. La extinción del dodo (*Raphus cuculatus* L.) al final del siglo XXVII condujo a la casi extinción del tambalacoque (*Sideroxylon grandiflorum* (A.DC.) Dubard), una planta cuyas semillas necesitaban ser consumidas por el dodo para germinar (Temple, 1977). Del mismo modo, la extinción de los lagartos nativos en Menorca debido a la introducción de mamíferos carnívoros ha causado la extinción virtual de un arbusto endémico (*Daphne rodriguezii* Texidor) (Traveset, 2002). Hay muchos ejemplos de las dramáticas consecuencias de la disrupción de mutualismos en islas. Sin embargo, estos son casos extremos ya que las redes de interacción en las islas son más simples que en el continente debido a que generalmente tienen un menor número de especies. Es común

encontrar que la extinción de una especie implicada en una interacción mutualista puede paliarse mediante su sustitución por otra especie. Por ejemplo, la planta *Cneorum tricoccon* L. es dispersada a día de hoy por los mamíferos carnívoros que causaron la desaparición de sus dispersores primarios (los lagartos nativos) en Mallorca y Menorca (Traveset, 2002). Este proceso se conoce como la capacidad de recableado de las redes mutualistas (Schleuning et al., 2016 y referencias). Sin embargo, las consecuencias de este fenómeno pueden ser impredecibles y no siempre positivas a largo plazo (Traveset, González-Varo, & Valido, 2012).

Las redes mutualistas han mostrado una alta sensibilidad al cambio climático (Tylianakis, Didham, Bascompte, & Wardle, 2008), en especial a la extinción de especies de plantas (Schleuning et al., 2016). Los estudios de simulación llevados a cabo por Memmot et al., (2007) revelaron que entre en 17% y el 50% de las redes mutualistas podrían sufrir una disrupción en los suministros de alimento, lo que se traduciría en extinciones y posibles efectos cascada en los ecosistemas. Además, perturbaciones antrópicas como la pérdida de hábitat y la defaunación amenazan el funcionamiento de los ecosistemas (Dirzo & Miranda, 1990; Fortuna & Bascompte, 2006), conduciendo a la desaparición de las interacciones ecológicas, y por tanto, a la pérdida de los servicios ecosistémicos que proporcionan. Muchos científicos han manifestado la importancia de preservar las interacciones entre especies (Kaiser-Bunbury, Traveset, & Hansen, 2010; Toby-Kiers, Palmer, Ives, Bruno, & Bronstein, 2010; Tylianakis, Laliberté, Nielsen, & Bascompte, 2010). Sin embargo, las estrategias de gestión y conservación se siguen enfocando en las especies, a pesar del hecho de que la evidencia científica ha demostrado que la pérdida de interacciones ecológicas es mucho más preocupante que la pérdida de riqueza de especies en sí misma (Janzen, 1974; Kearns, Inouye, & Waser, 1998; Traveset & Richardson, 2006; Bascompte & Jordano, 2013 y allí referencias incluidas).

Las interacciones ecológicas pueden desaparecer antes de la extinción de las especies. Por ejemplo, la reducción extrema de hábitat reduce el consumo de frutos de *Ziziphus lotus* (L.) Lam. por parte de su principal dispersor, el zorro (*Vulpes vulpes* L.), lo que produce un colapso en la dispersión de semillas de la planta (Cancio et al., 2016). Algunas especies amenazadas de loros son actualmente mucho más abundantes en la

ciudades que en los ecosistemas naturales ya que así pueden escapar de la caza furtiva, sin embargo, esta tendencia implica la pérdida de su funcionalidad en los ecosistemas naturales (p. ej. como dispersores de semillas) (Luna, Romero-Vidal, Hiraldo, & Tella, 2018). Por lo tanto, quizás es el momento de que la interacción ecológica reemplace a la especie como unidad básica en la Biología de la Conservación.

El estudio del funcionamiento de las interacciones mutualistas planta-animal como la dispersión de semillas y la polinización mediante técnicas ecológicas clásicas puede ser bastante complicado (p. ej. estudios observacionales). La construcción de redes mutualistas entre plantas y animales requiere campañas de trabajo de campo intensivas que permitan caracterizar el conjunto de interacciones que se dan en los ecosistemas. Además, abordar estos estudios mediante observaciones directas podría proporcionar resultados sesgados debido a las diferentes tasas de detectabilidad de las especies (Kéry et al., 2010), el comportamiento elusivo de algunas especies (Thompson, 2013), o los cambios de comportamiento en los animales debido a la presencia del observador (Crofoot, Lambert, Kays, & Wikelski, 2010 y referencias). Hay algunos procesos ecológicos ligados a las interacciones planta-animal que no pueden ser abordados eficientemente a través de métodos clásicos debido a su escala temporal y/o espacial. Este es el caso de los eventos de dispersión a larga distancia que son estocásticos y difíciles de rastrear mediante métodos convencionales. Además, las consecuencias de la disrupción de mutualismos pueden mostrar un desfase temporal, o en otras palabras, los impactos negativos de la pérdida de interacciones pueden necesitar tiempo para ser detectados. Este es el caso de las plantas longevas que pierden a sus dispersores o polinizadores, en los que las señales demográficas pueden tardar muchos años en ser detectadas (p. ej. el caso del dodo y el tambalacoque, Temple, 1977). Por lo tanto, la aplicación de técnicas indirectas para el estudio de estas interacciones puede beneficiar a las investigaciones al reducir el esfuerzo de muestreo y proporcionar resultados más precisos. En este sentido, el desarrollo de herramientas moleculares como los loci de microsatélites y su aplicación en estudios ecológicos dió lugar a la Ecología Molecular, lo que hizo posible aumentar nuestro conocimiento sobre las interacciones planta-animal y sus procesos asociados (Monsen-Collar & Dolcemascolo, 2010). Los primeros estudios en el campo de la Ecología Molecular datan de 1980 (p. ej. O'Brien, Wildt,

Goldman, Merrill, & Bush, 1983), sin embargo, el número de estudios en el campo se ha incrementado exponencialmente desde el año 2000 en adelante (Beebee & Rowe, 2004). Si nos centramos en el campo de los mutualismo planta-animal, las técnicas moleculares han ayudado a arrojar luz sobre las distancias de dispersión de polen y semillas (Godoy and Jordano, 2001; García, Arroyo, Gogoy, & Jordano, 2005). Este conocimiento hizo posible desentrañar el papel de cada dispersor en función de las distancias a las que dispersaban las semillas. Jordano et al., (2007) caracterizaron la contribución de mamíferos carnívoros y aves de mediano y pequeño tamaño, a las distancias de dispersión de semillas en *Prunus mahaleb* L. (Figura 3). Encontraron que los mamíferos carnívoros dispersaban semillas mucho más lejos que las aves pequeñas. Estudios más recientes incluso han revelado el papel de las diferentes especies de aves respecto a las distancias de dispersión de semillas y los micrositios de deposición de las mismas mediante la amplificación del ADN aviar en las semillas encontradas en las heces (González-Varo, Arroyo, & Jordano, 2014). Estos hallazgos también hicieron posible obtener redes de dispersión de semillas cuantitativas.

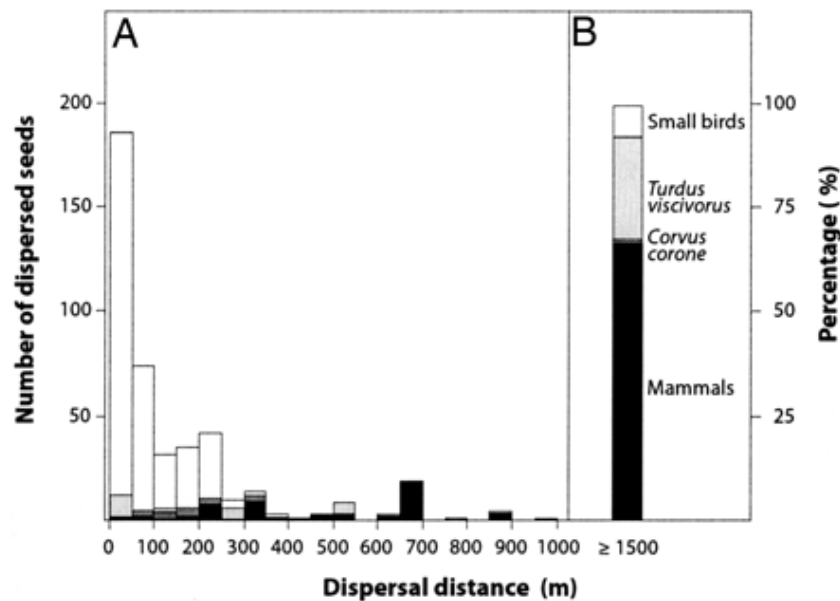


Figura 3. Contribución diferencial de aves pequeñas (blanco), aves de medio tamaño (gris), y mamíferos carnívoros a las distancias de dispersión de semillas de *Prunus mahaleb* (Figura de Jordano et al., 2007).

Los estudios moleculares también han permitido a los investigadores detectar eventos de dispersión de semillas a larga distancia (p. ej. Dyer & Nason, 2004), y medir los efectos de la fragmentación en la diversidad

genética de las plantas dispersadas por animales (Martínez-López, De la Rúa, Zapata, & Robledano, 2017; Parejo-Farnés, Robledo-Arnuncio, Albaladejo, Rubio-Pérez, & Aparicio, 2017). Además, estos enfoques metodológicos permiten a los investigadores inferir los efectos de la defaunación en las poblaciones de plantas. Pérez-Mendez et al., (2018) encontraron que la reducción en el tamaño corporal de los lagartos nativos de las Islas Canarias debido a perturbaciones antrópicas tuvo consecuencias genéticas negativas en *Neochamaelea pulverulenta* (Vent.) Erdtman, una planta que dispersaban. Las poblaciones de plantas en las islas con mayor reducción del tamaño de los lagartos mostraron conectividades genéticas más bajas como resultado de la disminución de la distancia de dispersión de semillas vinculada a la reducción de los dispersores animales (Figura 4). Otros estudios también han encontrado que la defaunación de los dispersores de semillas de mayor tamaño tiene consecuencias negativas en los patrones de diversidad genética de las plantas que dispersan (Carvalho, Galetti, Colevatti, & Jordano, 2016).

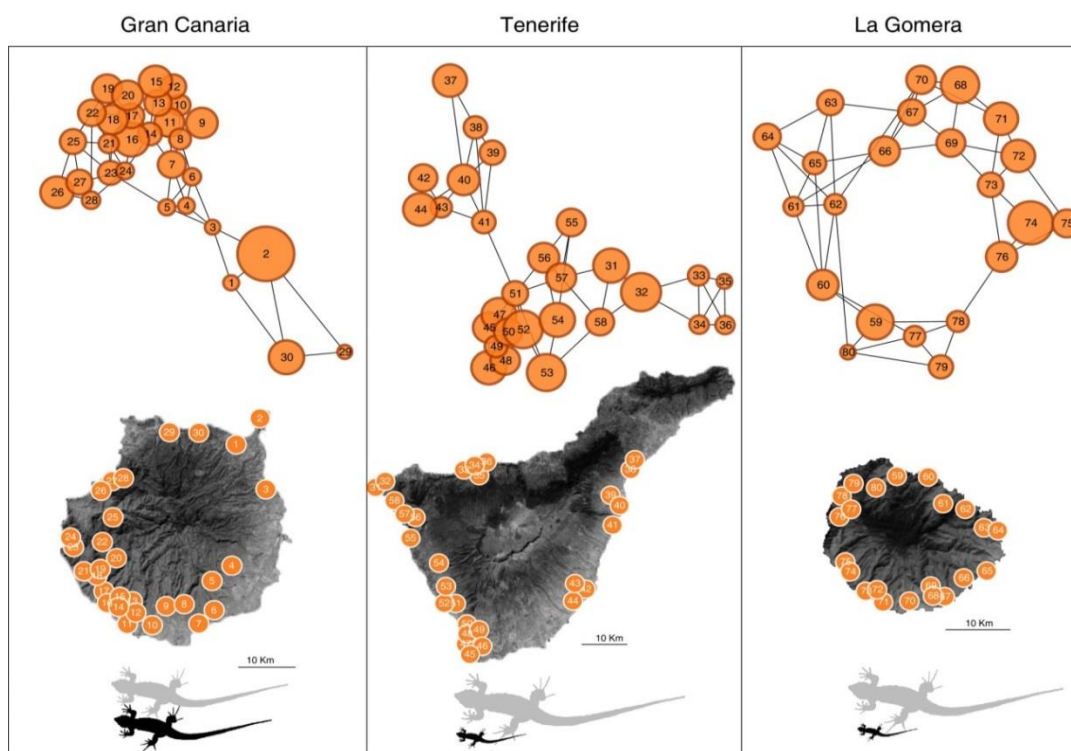


Figura 4. Conectividad genética de *Neochamaelea pulverulenta* en las tres islas estudiadas. Cada nodo representa una población, y el tamaño del nodo es proporcional a la diversidad genética en cada población. La longitud del nodo es proporcional a la distancia genética condicional entre poblaciones. La reducción en el tamaño de los lagartos se muestra abajo (siluetas grises=tamaño de los lagartos en el pasado; siluetas negras=tamaño de los lagartos actualmente) (Figura de Pérez-Méndez et al., 2018).

El estudio de las redes de polinización también ha experimentado un desarrollo notable. Nuevas técnicas como el *metabarcoding* ofrecen la oportunidad de construir redes de polinización cuantitativas de una manera más rápida y detallada que los enfoques palinológicos tradicionales (p. ej. la identificación del polen basada en caracteres morfológicos). En ese sentido Richardson et al., (2015) encontraron que el *metabarcoding* es más sensible que la palinología tradicional para la identificación de plantas polinizadas por la abeja de la miel (19 frente a ocho taxones de plantas identificados, respectivamente). El *metabarcoding* de las cargas de polen de insectos ha demostrado superar a los estudios observacionales tradicionales a la hora de cuantificar las interacciones planta-polinizador en el campo (Pornon et al., 2016). De este modo, la aplicación de estas herramientas moleculares en estudios futuros podría permitir a los investigadores mejorar su conocimiento sobre la biología de la polinización a escalas mayores, tanto en espacio como en tiempo (Bell et al., 2016).

Las herramientas moleculares ofrecen la oportunidad de detectar los impactos de las perturbaciones humanas en la actividad de los polinizadores. Por ejemplo, en tiempos recientes se está prestando mucha atención al impacto de los polinizadores manejados en las poblaciones de los silvestres (ver la revisión de Mallinger, Gaines-Day, & Gratton, 2017, y referencias incluidas). Particularmente la transmisión interespecífica de patógenos ha sido señalada como un factor potencialmente perjudicial que podría provocar una reducción en el *fitness* de los polinizadores. Así, se ha detectado la dispersión de patógenos en las abejas silvestres debido a la introducción de abejas manejadas en ecosistemas naturales (Fürst, McMahon, Osborne, Paxton, & Brown, 2014) gracias a la amplificación por PCR de regiones diana del ADN de patógenos dispersados globalmente o de aquellos considerados como emergentes (Figura 5). Actualmente, la mayor parte del esfuerzo investigador está centrado en los virus de ARN con 11 de los 24 virus conocidos en abejas de la miel ya detectados en especies silvestres (Fürst et al., 2014; Tehel, Brown, & Paxton, 2016). La aplicación de estos enfoques ha permitido incrementar nuestro conocimiento acerca de las dinámicas patógeno-polinizador en ecosistemas naturales lo que facilita el diseño de estrategias de gestión para mitigar estos impactos (Meeus, De Graaf, Jans, & Smagghe, 2010).

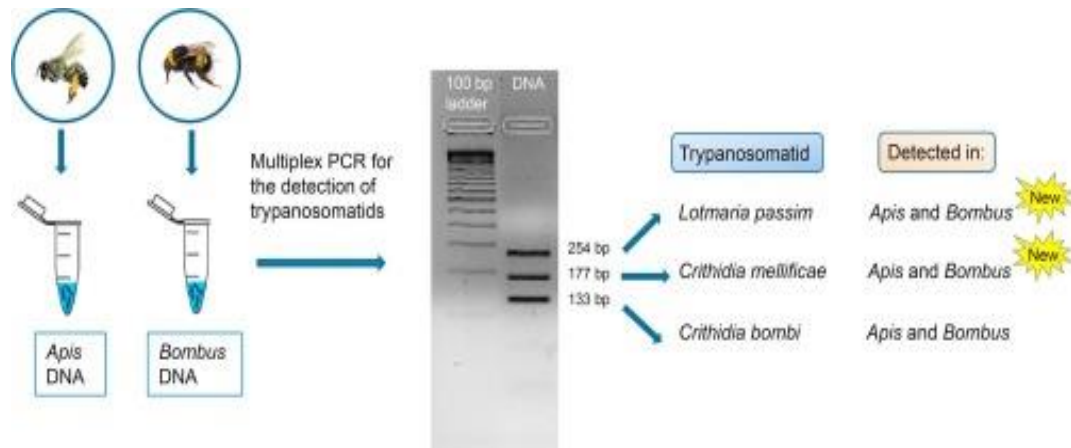


Figure 5. Ejemplo de una PCR múltiple para detectar patógenos en polinizadores manejados y silvestres (Figura de Bartolomé et al., 2018).

2. Estructura de la Tesis

La presente tesis está compuesta de cuatro capítulos en los que se estudian los mutualismos planta-animal desde diferentes enfoques y a diferentes escalas geográficas. Se combinan herramientas ecológicas y genéticas para dar respuesta a las preguntas propuestas. Profundizaremos en estos cuatro capítulos en las próximas secciones de este resumen, pero los temas tratados pueden resumirse brevemente como:

- i) los patrones de estructura genética de un arbusto de fruto carnoso a lo largo de todo su rango de distribución en la Cuenca Mediterránea, y el papel de los eventos de dispersión de semillas a larga distancia en estas tendencias genéticas;
- ii) los efectos de la fragmentación extrema en la diversidad genética de poblaciones remanentes de un arbusto de fruto carnoso dispersado por aves;
- iii) el desarrollo de marcos metodológicos para evaluar la contribución de los dispersores de semillas a la restauración de campos abandonados
- iv) el impacto de las abejas manejadas (la abeja de la miel) en las redes de polinización bajo un grado variable de uso del suelo y de intensidad apícola.

3. Resumen capítulo I. La dispersión de semillas a larga distancia explica la estructura genética poblacional de un arbusto de fruto carnoso que se distribuye en la Cuenca Mediterránea

La dispersión a larga distancia es un proceso fundamental para las plantas ya que promueve el flujo génico entre poblaciones distantes y determina el rango de distribución de las especies. La mayoría de especies de plantas de fruto carnoso dependen de vertebrados frugívoros para dispersar sus semillas a través del paisaje. Los eventos de dispersión a larga distancia son raros y difíciles de registrar, pero unos pocos estudios ecológicos han mostrado que las aves movilizan una cantidad considerable de semillas a través de barreras geográficas como por ejemplo estrechos entre continentes. Si este flujo de propágulos es constante, la distribución espacial de la variación genética y las distancias genéticas entre poblaciones de plantas dispersadas por animales debería explicarse mejor por los movimientos de los frugívoros migradores que por patrones de aislamiento por distancia (IBD por sus siglas en inglés). Aquí, analizamos la diversidad y estructura genética de *Pistacia lentiscus*, un arbusto de fruto carnoso ampliamente distribuido en la Cuenca Mediterránea, para dilucidar si el mar Mediterráneo actúa como barrera geográfica, o si por el contrario, las aves frugívoras migradoras promueven el flujo génico entre poblaciones localizadas a ambos lados del mar. Nuestros resultados sugieren una alta conectividad genética entre poblaciones en diferentes continentes, y una estructura genética este-oeste. Estos hallazgos concuerdan con las rutas conocidas de aves migradoras que conectan los continentes europeo y africano de norte a sur. Nuestro estudio encontró que los movimientos de los frugívoros migradores son coincidentes con la estructura genética espacial de una planta dispersada por animales. Por lo tanto, las perturbaciones antrópicas y/o cambios climáticos que podrían interrumpir la migración de las aves frugívoras tendrían consecuencias genéticas negativas para las especies de plantas de las que se alimentan.

4. Resumen capítulo II. Consecuencias ecológicas y genéticas de la fragmentación en un bosque urbano mediterráneo semiárido

La urbanización amenaza la biodiversidad en todo el mundo y se espera que aumente en el futuro. Esto implica la intensificación de la fragmentación y el aumento de los remanentes de bosques urbanos. En

consecuencia, es importante comprender el funcionamiento de estos parches para la preservación de la biodiversidad local. Las interacciones mutualistas, como la dispersión de semillas, tienen un papel clave en la dinámica de las poblaciones de plantas y podrían ayudar a mitigar los efectos negativos de la fragmentación en las poblaciones silvestres de plantas. En este contexto, utilizamos al lentisco (*Pistacia lentiscus*), un arbusto mediterráneo común, como modelo para estudiar la interacción entre la dispersión de semillas por aves y la diversidad genética local de plantas en un área forestal semiárida altamente fragmentada en el sureste ibérico (Murcia, España). Los resultados revelan una alta diversidad genética y una pobre diferenciación poblacional de los arbustos de *P. lentiscus* en los parches forestales del área, pese a una aparente pobre contribución de las aves a la dispersión de semillas de lentisco. Sin embargo, no se puede descartar la existencia de impactos latentes, ya que al menos uno de los parches muestra algunos síntomas de endogamia.

5. Resumen capítulo III. El barcoding como técnica auxiliar para descubrir los mecanismos de dispersión de semillas en entornos exigentes, y para ayudar a restaurarlos

Las áreas mediterráneas semiáridas están sufriendo importantes cambios en el uso del suelo. En particular, grandes extensiones de campos agrícolas están experimentando un proceso creciente de abandono lo que representa un reto para la restauración. La sequía y la escasez de microhábitats adecuados hace difícil para las plantas colonizar estos ambientes. Un incremento en la dispersión de semillas mediada por vectores animales podría acelerar la sucesión secundaria, pero es imprescindible tener un conocimiento preciso de los agentes implicados para poder aprovechar estas interacciones para la restauración de hábitats. De otra manera, los esfuerzos para fomentar estos procesos de interés pueden estar mal dirigidos.

En el presente estudio proponemos y testamos un marco metodológico basado en la combinación de herramientas ecológicas (censos de aves y fototrampeo) y genéticas (*barcoding*), para evaluar la dispersión de semillas de arbustos de fruto carnoso llevada a cabo por las aves hacia los campos abandonados en una de las áreas más áridas del sureste de España

(precipitación anual < 250 mm). Instalamos atractores artificiales de aves (perchas y puntos de agua) en dos áreas abandonadas con diferente edad de abandono con el fin de fomentar la restauración natural. Además, también proporcionamos microhábitats (rocas), y prevenimos la herbivoría (mediante el vallado) para mejorar el reclutamiento de plantas. Los resultados obtenidos indicaron grandes diferencias en la caracterización de las interacciones de dispersión según las técnicas de estudio empleadas. Mientras que los censos de aves solo permitieron detectar la presencia de los frugívoros, el fototrampeo y el *barcoding* hicieron posible caracterizar las respuestas de las aves a los atractores instalados. Las estructuras de restauración empleadas fueron efectivas para incrementar la llegada de semillas a las áreas de estudio aunque no se observó el establecimiento de plántulas.

Nuestro estudio señala la importancia de combinar diferentes técnicas, incluyendo enfoques moleculares, para caracterizar y evaluar el funcionamiento de las aves dispersoras de semillas. El agua fue sin duda un atractor efectivo que incrementó la llegada de semillas a las parcelas de estudio. Sin embargo, nuestro marco de restauración no mejoró el reclutamiento de plantas en los campos abandonados. Por tanto, el próximo paso lógico hacía la restauración de los campos mediterráneos abandonados será optimizar las condiciones de las restauraciones para mejorar la germinación y el establecimiento de plántulas.

6. Resumen capítulo IV. Efectos opuestos de la apicultura y el uso de la tierra en las redes de plantas y polinizadores, y la prevalencia de patógenos en un ecosistema mediterráneo semiárido

El conocimiento sobre las redes de interacción mutualista entre polinizadores y plantas es crucial para establecer medidas de protección en el actual escenario de declive de insectos polinizadores. Los cambios en el uso del suelo y la transmisión interespecífica de patógenos destacan entre los diferentes factores relacionados con tales disminuciones. Sin embargo, la mayoría de los estudios realizados hasta el momento se han centrado en taxones específicos y rara vez se han dirigido a analizar las comunidades en su conjunto, especialmente en ecosistemas semiáridos menos estudiados.

En este trabajo se investigaron las redes de interacción planta-polinizador (Apoidea) y patógeno-polinizador en el sureste de la península Ibérica durante el final del invierno y la primavera de 2017. Se seleccionaron nueve áreas bajo un grado variable de uso del suelo y de intensidad apícola. Estudiamos los efectos de estas dos variables en la estructura de las redes de polinización, y también el efecto de la abeja melífera en la dispersión de patógenos (*Nosema apis* y *Nosema ceranae*) en las comunidades de abejas silvestres.

La diversidad de las intracciones en las redes planta-polinizador fue mayor al final de la primavera, mientras que la conectividad ponderada decreció ligeramente. El uso del suelo tuvo un efecto débil en las redes planta-polinizador; sin embargo, estos impactos fueron generalmente positivos en el caso de la agricultura, y principalmente negativos en el caso de los matorrales. La densidad de abejas de la miel decreció a lo largo del periodo de estudio, y por tanto también la prevalencia de *N. ceranae*. En contraste, la densidad de abejas silvestres no varió en el tiempo, pero la prevalencia de patógenos se incrementó durante la primavera en los últimos muestreos del estudio.

Nuestros resultados corroboran el impacto de la abeja de la miel en las abejas silvestres también en ambientes semiáridos, al promover la dispersión de patógenos en el paisaje incluso cuando la densidad del hospedador (la abeja de la miel) decreció.

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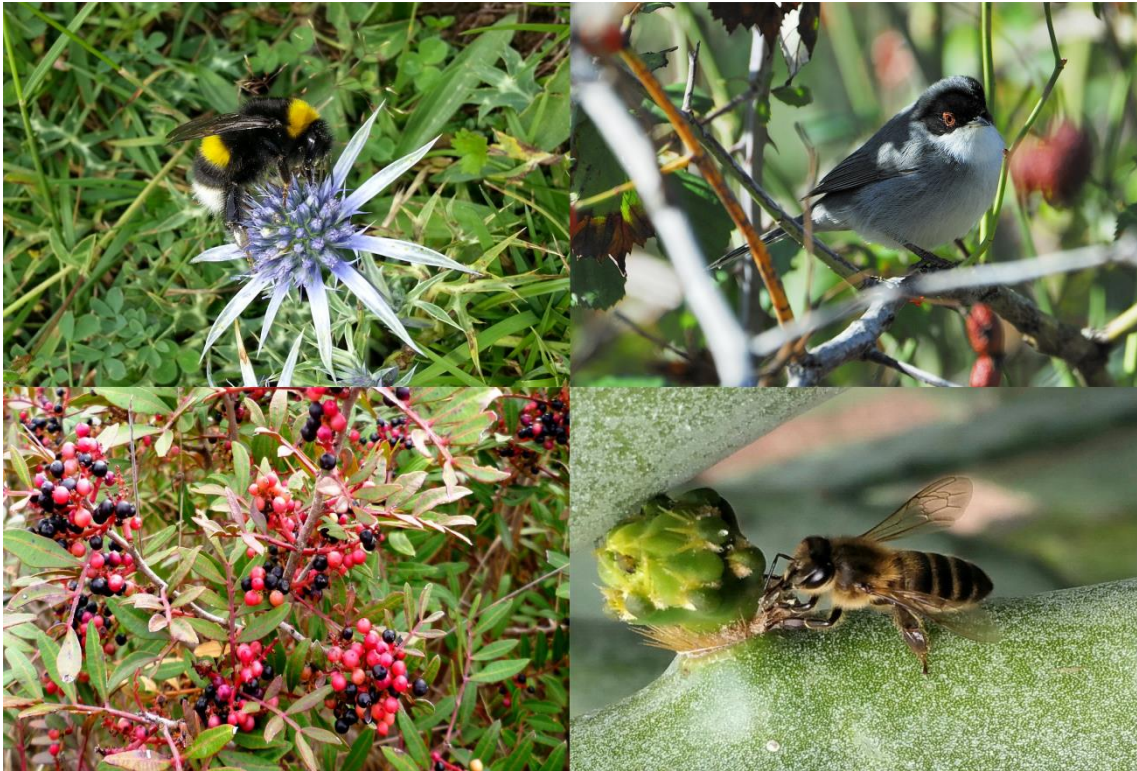
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GENERAL INTRODUCTION



1. Plant-animal mutualisms. Importance and research techniques

Mutualism is an ecological process by which two species interact with each other, and both partners receive a positive trade off from such interplay (Bronstein, 2015). Mutualistic interactions are widespread in natural ecosystems (Janzen, 1985), and play a key role in the maintenance of biodiversity and ecosystem services worldwide (e.g. Albrecht et al., 2014). All organisms are directly or indirectly involved in mutualistic interactions (Thompson, 2009). In fact, all eukaryotes can be considered as mutualistic since according to the endosymbiotic theory, the eukaryotic cell is the result of multiple processes of association among different prokaryotic cells (Margulis, 1967). Many kinds of mutualisms have been described to occur in nature between different taxa: fungi interacting with plant roots to increase nutrients mobility (i.e. mycorrhiza, Van Der Heijden, Martin, Selosse, & Sanders, 2015); bacteria and legume root nodules to fix nitrogen in soils (Kiers, Rousseaus, West, & Denison, 2003; Heath & Tiffin, 2007); lichen-forming fungi and algae (Grube & Wedin, 2016); mites and plant leaves (i.e. leaf domatia, Romero & Benson, 2005) (Figure 1); and plants and animals (e.g. pollination, Willmer, 2011; seed dispersal, Howe & Smallwood, 1982). Among all these interactions, this thesis focuses on two crucial mutualistic interactions in the reproductive biology of many plants that rely on animals either for the pollination of their flowers and/or the dispersal of their seeds (Jordano, 2000; Ollerton, Winfree, & Tarrant, 2011).

Mutualistic interactions have caught the attention of scientists for millennia. The first observational descriptions of mutualisms date back from the ancient Greece. There is a lot of literature from the end of the 19th century describing the natural history of different mutualisms. However, the field did not conceptually advance until the decade of 1960s, with the vast majority of investigations being conducted from 1980s onwards (Bronstein, 2009; Bascompte & Jordano, 2013). Studies of Janzen (1967) about the interaction between acacias (*Acacia cornigera* (L.) Willd) and ants inhabiting them (*Pseudomyrmex ferruginea* F. Smith) are an example of these pioneer studies that gave a solid foundation to the studies about mutualisms. The role of ants inhabiting acacia trees had been discussed by many scientists since Belt described the interaction in 1874. Tens of researchers, including Darwin (1877), participated in such intense debate

which gave rise to different hypotheses about the acacia-ant interaction. While some scientists considered ants as protectors of the plant, others saw them as parasites. However, there was a lack of experimental studies to test those theories that were mainly based on observations. The debate remained open until the studies of Janzen (1967). He described the acacia-ant mutualism by removing ants from some trees and recording their development compared to acacia trees that still had ants left in them. He found that trees without ants suffered higher pressure from herbivores and stronger competition with other plants in comparison to those with ants that protected the trees from herbivores and eliminated other plant competitors in the surroundings of the acacia.

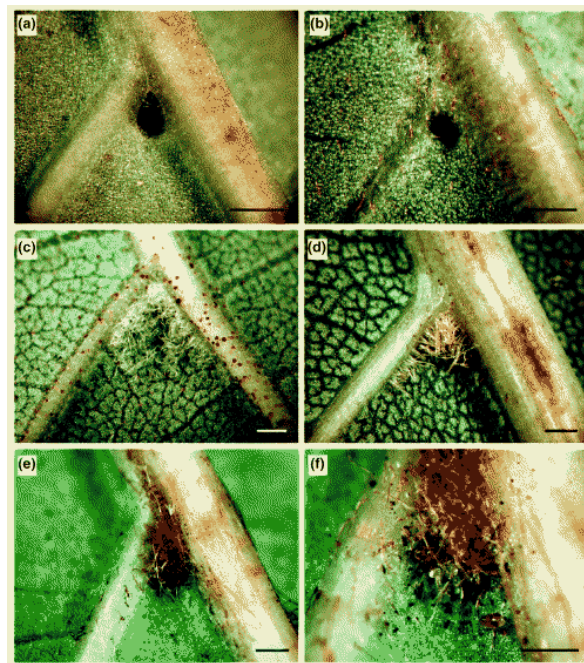


Figure 1. Different examples of leaf domatia between mites and plants. In these interactions plants house mites whereas mites protect plants against pathogens and herbivores. Scale bars represent 0.5 mm (Figure from Romero & Benson, 2005).

Studies of plant-animal interactions have generally been conducted in small groups of species (Nilsson, 1988; Johnson & Steiner, 1997). However, plant-animal mutualisms form complex networks of interactions among species. Thus, system functioning can only be understood if the study of the interactions is addressed at the community level (Jordano, Bascompte, & Olesen, 2003). The structural parameters of such complex networks have important consequences for the stability of the communities, so that mutualistic networks have been regarded as the architecture of biodiversity

(Bascompte & Jordano, 2007) and their understanding is crucial for the conservation of ecological systems (e.g. Rico-Gray & Oliveira, 2007). Although this approach was already applied in some studies during the decade of the 1980s (e.g. Janzen, 1980; Herrera, 1982; Jordano, 1987), the issue remained unclear until the development of suitable analytical tools that could unravel the structural properties of such complex systems of interactions. In that sense, the development of bipartite networks (i.e. those in which species are divided in two trophic levels and they can not interact with species in the same level. Figure 2) (Jordano et al., 2003) at the beginning of the 21st century has increased the body of research conducted in the field of mutualisms at community level.

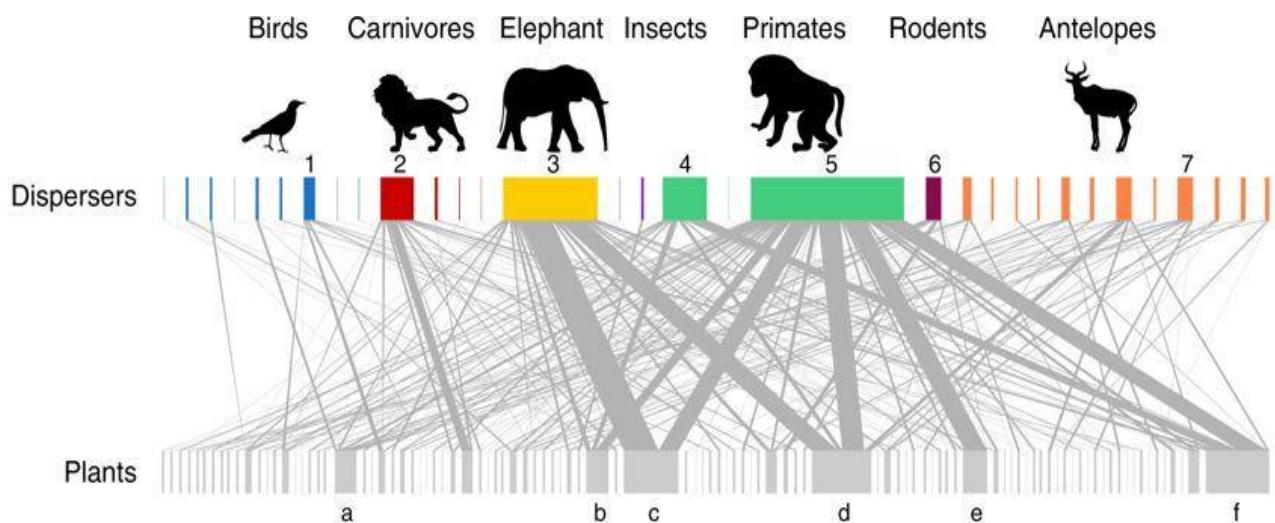


Figure 2. Quantitative bipartite network between seed dispersers (animals, above) and plants (below) in the Great Rift. Main seed dispersers: 1. *Pycnonotus tricolor*, 2. *Civettictis civetta*, 3. *Loxodonta africana*, 4. *Cercopithecus pygerythrus*, 5. *Papio ursinus*, 6. *Hystrix africaeausstralis*, and 7. *Redunca arundinum*. Most commonly dispersed plants: a *Centaurea praecox*, b *Grewia inaequilatera*, c *Hyphaene natalensis*, d *Sclerocarya birrea*, e *Tamarindus indica*, and f *Ziziphus mucronata*. Links represent the interaction between animal and plant taxa and their width is proportional to the number of interactions (Figure from Timóteo, Correia, Rodríguez-Echeverría, Freitas, & Heleno, 2018).

The structure of mutualistic networks conditions their resilience to anthropogenic disturbances (Fortuna & Bascompte, 2006). Thus, it is crucial to understand the architecture of ecological networks in order to predict their robustness to disturbances (Montoya, Pimm, & Solé, 2006; Solé & Bascompte, 2006). Mutualistic networks are nested which means that specialist species interact only with a subset of the species interacting with generalist ones (Bascompte, Jordano, Melián, & Olesen, 2003). This

network pattern involves that the structure of networks are mainly formed by a central core of interactions, and hence, some species are more important for the stability of the network than others (Domínguez-García & Muñoz, 2015). There are some species considered as “keystone”, whose extinction can trigger cascading effects in ecosystems leading to secondary species extinctions. Therefore, some species can disappear from natural ecosystems with few consequences, while the extinction of others can result in the collapse of the ecosystem (Memmot, Waser, & Price, 2004).

The disruption of plant-animal mutualisms entails the loss of partners which negatively impact interaction networks and can result in the appearance of “mutualistic widows” (*sensu*, Olesen & Valido, 2004), or in other words, species that may experience reduced ecological fitness after their mutualistic partner is removed (e.g. Given, 1995). For instance, the extinction of megafauna in the Pleistocene triggered a cascade effect of extinctions in some highly specialized mutualistic partners (Galetti et al., 2018). Furthermore, the disappearance of such large-body size animals involved the loss of long-distance seed dispersal services (Pires, Guimarães, Galetti, & Jordano, 2018). However, there are also examples of plant-animal mutualism disruptions with negative consequences in much more recent times. The extinction of the dodo (*Raphus cuculatus* L.) by the end of the 17th century led to the near extinction of the tambalacoque (*Sideroxylon grandiflorum* (A.DC.) Dubard), a plant whose seeds needed to be consumed by the dodo in order to germinate (Temple, 1977). Similarly, the extinction of native lizards in Minorca due to the introduction of carnivorous mammals has led to the virtual extinction of an endemic shrub (*Daphne rodriguezii* Texidor) (Traveset, 2002). There are many examples of dramatic consequences due to mutualism disruption in islands. However, these are extreme cases since the complex of interactions in those ecosystems are simpler than in mainland as they generally have a small number of species. It is common to find that the extinction of one mutualistic partner can be relieved by its substitution with a new one. For example, the plant *Cneorum tricoccon* L. is nowadays dispersed by the carnivorous mammals which caused the extinction of their primary dispersers (native lizards) in Majorca and Minorca (Traveset, 2002). This is known as the rewiring capability of mutualistic networks (Schleuning et al., 2016 and references therein). Nevertheless, the consequences of such

process can be unpredictable and not always positive in the long term (Traveset, González-Varo, & Valido, 2012).

Mutualistic networks have shown to be quite sensitive to global change (Tylianakis, Didham, Bascompte, & Wardle, 2008), particularly to the extinction of plant species (Schleuning et al., 2016). The simulation studies carried out by Memmot et al., (2007) revealed that between 17% and 50% of them could suffer disruption in food supplies which would turn into extinctions and possible cascade effects in ecosystems. Furthermore, anthropogenic disturbances such as habitat loss and defaunation threaten ecosystem functioning (Dirzo & Miranda, 1990; Fortuna & Bascompte, 2006), by leading to the disappearance of ecological interactions among species, and hence, to the loss of the ecosystem services they provide. Much research has claimed the importance of preserving species interactions (Kaiser-Bunbury, Traveset, & Hansen, 2010; Toby-Kiers, Palmer, Ives, Bruno, & Bronstein, 2010; Tylianakis, Laliberté, Nielsen, & Bascompte, 2010). However, management strategies remain anchored in species-based conservation strategies, in spite of the fact that scientific evidence has proved that the loss of ecological interactions is much more worrying than the loss of species richness itself (Janzen, 1974; Kearns, Inouye, & Waser, 1998; Traveset & Richardson, 2006; Bascompte & Jordano, 2013 and references therein). Ecological interactions can disappear before species extinction. For instance, the extreme habitat loss reduces the consumption of *Ziziphus lotus* (L.) Lam. fruits by their main seed dispersers (foxes, *Vulpes vulpes* L.), which results in seed dispersal collapse of this plant (Cancio et al., 2016). On the other hand, some threatened species of parrots are nowadays much more abundant in cities than in natural ecosystems since they can escape from poaching, however, this trend involves the loss of their functionality in natural ecosystems (e.g. as seed dispersers) (Luna, Romero-Vidal, Hiraldo, & Tella, 2018). Therefore, it is time for the ecological interactions to replace the species as the basic unit in Conservation Biology.

The study of the functioning of plant-animal mutualistic interactions such as seed dispersal and pollination can be difficult to achieve through classical ecological techniques (e.g. observational studies). The construction of mutualistic networks between plants and their animal partners requires intensive field work campaigns in order to properly

characterize the set of interactions occurring in a given ecosystem. Furthermore, addressing these studies through direct observations could provide skewed results due to the different detectability rate of species (Kéry et al., 2010), the elusive behaviour of some species (Thompson, 2013), and/or behavioural changes in animals due to the observer presence (Crofoot, Lambert, Kays, & Wikelski, 2010 and references therein).

There are some ecological processes linked to plant-animal interactions that can not be efficiently addressed through classical methods due to their spatial and/or temporal scale. This is the case of long-distance dispersal events which are stochastic and difficult to track through conventional methods. Moreover, consequences of mutualisms disruption can show a time lag, in other words, negative impacts of the interaction loss can take time to be noticeable. This is the case of long-lived plants that lose their seed dispersers or pollinators, in which demographic signals can take many years before being detected (e.g. the dodo and the tambalacoque, Temple, 1977). Therefore, the application of indirect techniques to monitor these interactions can benefit scientific studies both by reducing sampling effort and by providing more accurate results. In that sense, the development of molecular tools (e.g. microsatellites) and their application in ecological studies giving rise to the Molecular Ecology, make possible to increase our knowledge about plant-animal interactions and their associated processes (Monsen-Collar & Dolcemascolo, 2010).

The first studies in the field of Molecular Ecology date back from the 1980s (e.g. O'Brien, Wildt, Goldman, Merrill, & Bush, 1983), however, the number of studies in the field has increased exponentially from 2000 onwards (Beebe & Rowe, 2004). Focusing on the field of plant-animal mutualisms, molecular techniques have helped to shed light on seed and pollen dispersal distances (Godoy and Jordano, 2001; García, Arroyo, Gogoy, & Jordano, 2005). This knowledge enabled to disentangle the role of each disperser taxa according to seed dispersal distances. Jordano et al., (2007) characterized the contribution of carnivorous mammals, medium size and small birds to seed dispersal distances of *Prunus mahaleb* L. (Figure 3). They found that carnivorous mammals dispersed seeds much farther than small birds. Recent studies have even unraveled the role of different bird species regarding seed dispersal distances and microsite deposition of dispersed seeds through the amplification of avian DNA in

droppings found with seeds (González-Varo, Arroyo, & Jordano, 2014). These findings also make more feasible to obtain complete quantitative seed dispersal networks.

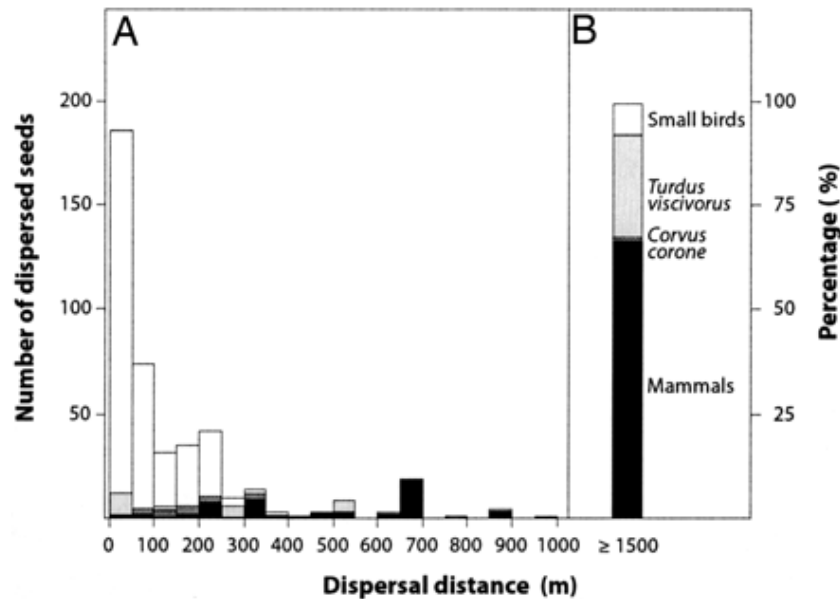


Figure 3. Differential contribution of small (white), medium size birds (grey), and carnivorous mammals to seed dispersal distances in *Prunus mahaleb* (Figure from Jordano et al., 2007).

Molecular studies have also allowed researchers to detect long-distance dispersal events (e.g. Dyer & Nason, 2004) and to measure the effects of fragmentation on the genetic diversity of animal-dispersed plants (Martínez-López, De la Rúa, Zapata, & Robledano, 2017; Parejo-Farnés, Robledo-Arnuncio, Albaladejo, Rubio-Pérez, & Aparicio, 2017). Moreover, these methodological approaches allow researchers to infer the consequences of defaunation on plant populations. Pérez-Mendez et al., (2018) found that lizard's body size reduction in the Canary Islands due to anthropogenic disturbances led to negative genetic consequences in *Neochamaelea pulverulenta* (Vent.) Erdtman, a plant they disperse. Plant populations in those islands with greater reduction in lizard's body size showed lower genetic connectivity as a result of decreased dispersal distance linked to the downsizing of animal dispersers (Figure 4). Other studies have also found that the defaunation of larger seed dispersers negatively impacts the genetic diversity patterns of the dispersed plants (Carvalho, Galetti, Colevatti, & Jordano, 2016).

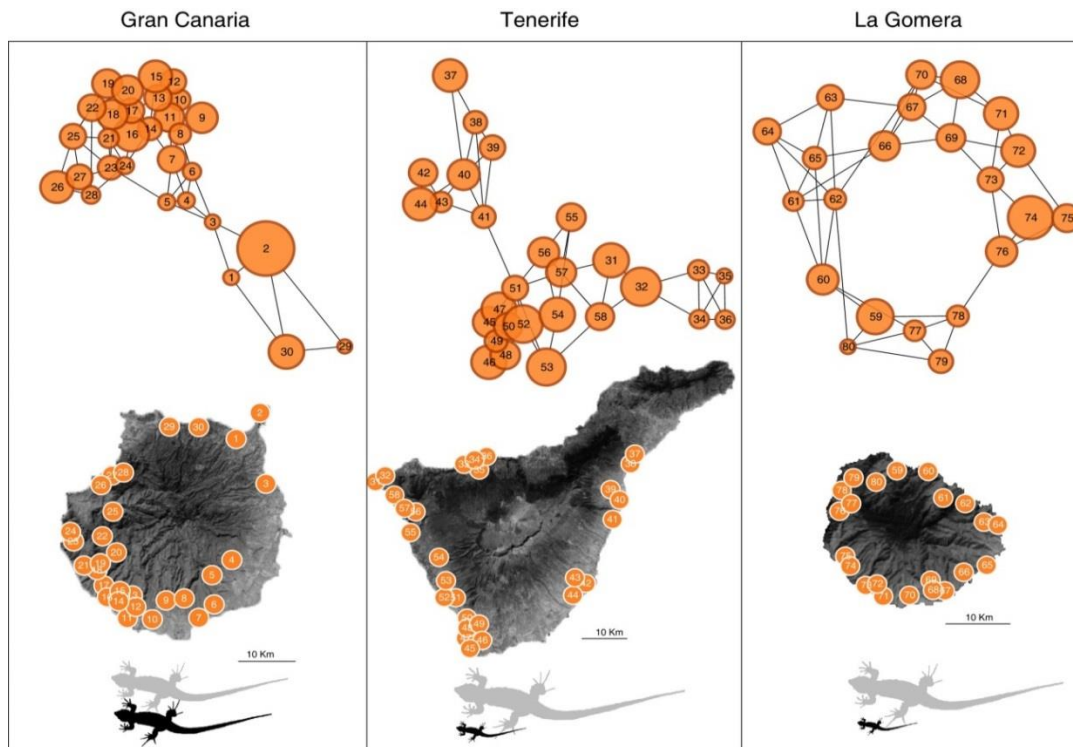


Figure 4. Genetic connectivity of *Neochamaelea pulverulenta* in the three study islands. Each node represents a population and node size is proportional to the genetic diversity in each population. Link length is proportional to conditional genetic distance among populations. Lizards body size reduction is shown at the bottom (grey silhouettes=lizards size in the past; black silhouettes=lizards size nowadays) (Figure from Pérez-Méndez et al., 2018).

The study of pollination networks has also experienced an impressive development. New techniques such as metabarcoding offer the chance to build quantitative pollination networks in a faster and much more detailed way than traditional palynology approaches (e.g. the identification of pollen based on morphological traits). For instance, Richardson et al., (2015) found that metabarcoding was more sensitive than traditional palynology for identifying plant taxa pollinated by honey bees (19 versus eight plant taxa identified, respectively). The metabarcoding of pollen loads of insects has demonstrated to outperform traditional observational visits studies when quantifying plant-pollinator interactions in the field (Pornon et al., 2016). Thereby, the application of these molecular tools in future studies could allow scientists to improve their knowledge about pollination biology at much larger scales, both in space and time (Bell et al., 2016).

Molecular tools also offer the chance to detect impacts of human disturbances on pollinators activity. For example, the impact of managed

pollinators on wild populations has received much attention in recent times (see the review of Mallinger, Gaines-Day, & Gratton, 2017, and references therein). Particularly, the interspecific transmission of pathogens has been highlight as a potentially harmful factor which could entail to severe reductions in pollinators fitness. In that sense, molecular tools enabled to detect the spillover of pathogens in wild bee populations due to the introduction of managed bees in natural ecosystems (e.g. Fürst, McMahon, Osborne, Paxton, & Brown, 2014) thanks to the PCR-amplification of target DNA of pathogens globally dispersed or those considered as emergent (Figure 5). Most of the research effort is currently focused on RNA viruses with 11 out of the 24 known honey bee viruses already detected in wild bees (Fürst et al., 2014; Tehel, Brown, & Paxton, 2016). The application of these approaches is nowadays leading to a significant increase in our knowledge about pathogen-pollinator dynamics in natural ecosystems which facilitates the design of management strategies to mitigate these impacts (Meeus, De Graaf, Jans, & Smagghe, 2010)

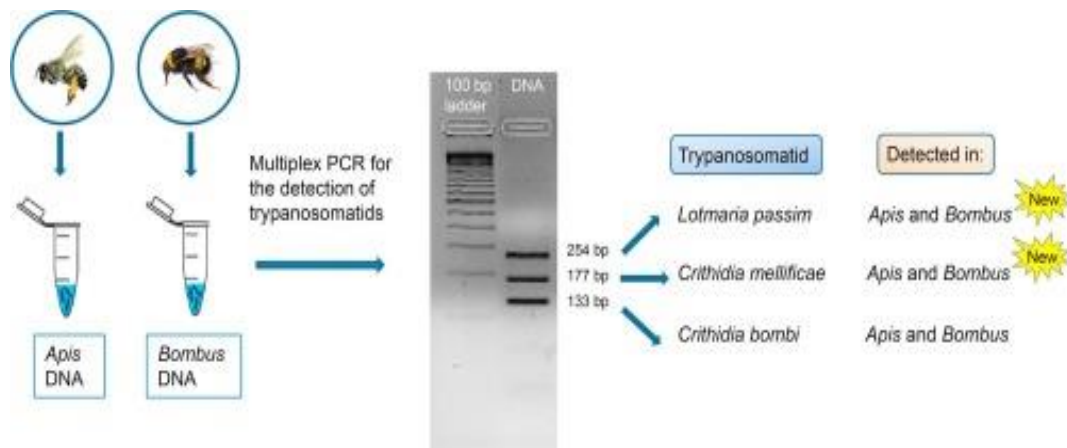


Figure 5. Example of multiplex PCR procedure to detect pathogens in managed and wild bee pollinators (Figure from Bartolomé et al., 2018).

2. Structure of this Thesis

The present thesis is composed of four chapters in which plant-animal mutualisms are studied from different approaches (combining ecological and genetic tools) and at different geographical scales. The chapters themes can be briefly outlined as:

- i) genetic structure patterns of a fleshy-fruited shrub along its entire distribution range spanning the Mediterranean Basin, and the role of long-distance seed dispersal events in those genetics trends;
- ii) effects of extreme fragmentation on the genetic diversity of remnant populations of a bird dispersed fleshy-fruited shrub;
- iii) development of methodological frameworks to assess the contribution of seed dispersers to old fields restoration;
- iv) impact of managed bees (honey bee) on pollination networks under a variable degree of land use and beekeeping intensity.

Following each one of the thesis themes is further explained.

3. Long-distance seed dispersal

Long-distance dispersal has caught the attention of researchers for centuries. Darwin already highlighted the role of long-distance dispersal of organisms as an important process to understand current and past species distribution patterns (Darwin, 1859).

The role of migratory birds as mobile links between continents has been recently reviewed by Viana et al., (2016a). For instance, waterfowls are important long-distance dispersal vectors for many freshwater organisms (e.g. macrophytes and zooplankton) what has important implications for the connectivity among acuatic ecosystems which could present a high level of isolation (Viana, Santamaría, Michot, & Figuerola, 2013; Reynolds & Cumming, 2016). More specifically, there are many examples of birds endozoochorously dispersing organisms at long distances (from hundreds to thousands of kilometres). For instance, bee-eaters have been found as potential dispersers of a honeybee pathogen from the mainland to an island in the southeast of China (Valera et al., 2017), and Van Leeuwen et al., (2012) empirically demonstrated the potential role of birds dispersing aquatic snail species inside their guts.

Long-distance seed dispersal is a key ecological process for plant populations since it shapes their spatial genetic structure and conditions their distribution limits (Nathan, 2006). Thus, under a climate change scenario, it is especially important to understand the functioning of long-distance gene flow processes in long-lived plants, such as shrubs and trees, since they are sessile organisms which can only “move” through pollen and/or seeds (see Kremer et al., 2012 and references therein). For example, seed dispersal, and particularly long-distance seed dispersal mediated by birds, could have played a key role in the survival during glaciations of some of the plant species which evolved in the tertiary (e.g. *Hedera helix* L., see Metcalfe 2005 and references therein). Furthermore, it has been recently demonstrated that animal seed dispersers are helping plants to escape global warming by favouring uphill migrations along mountain slopes (Naoe et al., 2016; González-Varo, López-Bao, & Guitián, 2017a). Many authors have highlighted the potential of frugivorous migratory birds to disperse a big amount of seeds during their displacements (Pulido & Berthold, 2010; Costa et al., 2014). In that sense, Viana et al., (2016b) have recently demonstrated the movement of seeds between Europe and the Canary Islands thanks to migratory birds. However, empirical data are still scarce due to the stochastic nature of these events (Nathan & Muller-Landau, 2000) and the difficulty to track the movement of seeds at such large scales (Viana et al., 2016a). An example of the stochasticity of these processes is shown in Figure 6. According to this model, a successful establishment event at 34 kilometres from the seeds source should occur once every 100 millions of years, but given the stochasticity of long distance dispersal it could happen with a much higher frequency (e.g. once a year).

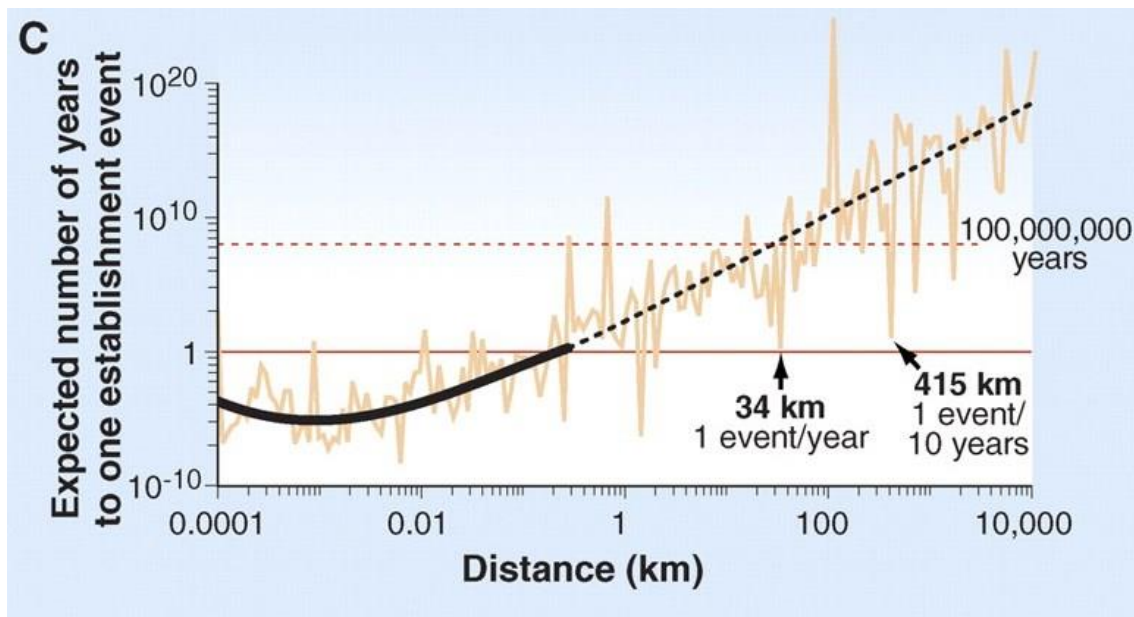


Figure 6. Expected number of years for one plant establishment event in relation to distance. Stochasticity in such events is represented as a light orange line (Figure from Nathan, 2006).

Nathan et al., (2003) proposed a classification of the different methodologies used for assessing long-distance dispersal:

- Biogeographical methods: studies based on the analyses of species distribution to infer its possible drivers, commonly supported by phylogenetic methods based on different genetic markers.
- Movement/redistribution methods: based on estimating rates and ranges of dispersal in plant and animal propagules. However, their application is mostly limited to animals because they usually require the individuals to be marked in order to track their movements.
- Genetic methods: generally used to measure effective dispersal. There are different approaches and applications in this field as for example, patterns of genetic differentiation among populations can be used to infer dispersal parameters (see Nathan et al., 2003 for more details).

For instance, Dyer and Nason (2004) applied genetic methods to study the relationships among cactus populations (*Lophocereus schottii* Engelm.) across the peninsula of Baja California and the Sonoran desert which are separated by Sea of Cortez. Results revealed the presence of two

genetically isolated modules only connected for a single population which, in spite of being in the inland, was more related to the peninsular populations. It was explained by the occurrence of long-distance dispersal events. A similar approach is applied by us in the first chapter of this thesis in order to infer long-distance dispersal processes from patterns of population genetic structure and genetic connectivity of a fleshy-fruited plant in the Mediterranean Basin.

3.1. Chapter I. Intercontinental long-distance seed dispersal explains population genetic structure in a fleshy-fruited shrub spanning the Mediterranean Basin. Goals and hypothesis

In this chapter, we analyse the spatial distribution of genetic variation and the population genetic structure of a widely distributed fleshy-fruited shrub (*Pistacia lentiscus* L.) across its distribution range, which includes the Mediterranean Basin and the Canary Islands. We applied microsatellite-based genetic tools to: i) depict the genetic diversity and structure of 74 populations encompassing the whole distribution range; ii) discern whether the Mediterranean Sea acts as a geographic barrier or, alternatively, if contemporary gene flow attributable to migratory birds connects populations located at both sides of the Mediterranean Basin, as suggested by well-known bird migration routes; and (iii) identify isolation by distance patterns along an east-west axis where migratory routes are less intense, and therefore gene flow would reach shortest distances and/or rely on pollen dispersal. Our hypothesis is that the migration routes of frugivorous birds would promote contemporary gene flow along the North-South axis and result in a significant East-West genetic clustering, and hence, the Mediterranean Sea is not acting as a barrier for gene flow in this species.

4. Genetic consequences of fragmentation in plants in relation to plant-animal mutualisms

Habitat loss and fragmentation caused by human activities threaten biodiversity worldwide (Saunders, Hobbs, & Margules, 1991; Sala et al., 2000; Foley et al., 2005). Those anthropogenic disturbances lead to the isolation of plant populations which could derive in a reduction of genetic diversity and an increase of interpopulation differentiation (Young, Boyle, & Brown, 1996; Lienert, 2004; Willi, Van Buskirk, & Hoffmann, 2006).

However, these effects are not homogeneous among plant species since there are different factors such as evolutionary history and ecological strategies which can lessen or increase the genetic consequences of habitat fragmentation. On the one hand, there are plants which present naturally disjunctive populations and hence, they can successfully buffer the effects of habitat loss (Hopper, Silveira, & Fiedler, 2016). This is in line with the results of the review performed by Aguilar et al., (2008) about the genetic consequences of fragmentation in plant populations. They found that common species having become rare due to fragmentation show higher negative impacts in comparison with naturally rare species facing the same process. On the other hand, different plant life forms also differ in their natural genetic patterns. Long-lived woody species generally maintain higher levels of genetic diversity than other plants (Hamrick, Godt, & Sherman-Broyles, 1992). Specifically, widespread woody plants with outcrossing breeding systems and with seeds dispersed by wind or animals show higher levels of genetic diversity and less differentiation among populations than woody plants with other traits (Hamrick et al., 1992). This is in relation to the generally greater potential of woody species for gene movement that explains their ability to maintain gene flow among populations (Hamrick & Nason, 1996). Plants are sessile organisms, and hence, they rely on external vectors to carry out gene movement via pollen and seed dispersal. Therefore, the way in which plant dispersal agents are affected by fragmentation conditions the consequences of habitat degradation on plant genetic patterns.

Plants mating system might condition the effects of fragmentation on plant reproduction. Some plants are self-compatible (i.e. they can produce viable seeds without exchanging pollen with other conspecifics), and other self-incompatible (i.e. they are obligate outbreeders which need to exchange pollen with another plant individuals in order to obtain viable seeds). Thus, the dependence of the latter on the pollination process is much higher than that of the self-compatible ones. Plants with separate sexes (i.e. dioecious, with female and male plant individuals) are an extreme case of self-incompatible species in which fragmentation can lead to biased sex-ratios (Yu & Lu, 2011) which can negatively impact reproduction and hence, threaten population viability. Indeed, outcrossing plant species are more affected by fragmentation than selfing ones (Aguilar et al., 2008). Self-incompatible plants are more vulnerable to fragmentation due to their

higher dependence from external agents for reproduction (Aguilar, Ashworth, Galetto, & Aizen, 2006). For example, animal pollinated plants can experience pollen limitations due to the fact that small remnants of natural vegetation could sustain less pollinator species than larger ones (Jennersten, 1988; Kearns et al., 1998). In extreme cases fragmentation can lead to the extinction of mutualistic partners (Rathcke & Jules, 1993). However, habitat loss can also affect wind pollinated plants due to the decrease in the density of plants and hence by reducing pollen donors (Knapp, Goedde, & Rice, 2001; Albaladejo, Guzmán, González-Martínez, & Aparicio, 2012).

There is a general assumption that seed dispersal mediated by animals or wind could reduce the impact of fragmentation on plant populations by maintaining gene flow among natural patches of vegetation (e.g. Sork, Nason, Campbell, & Fernandez, 1999). Some authors found that animal dispersed plants were less vulnerable to habitat fragmentation than plants dispersed by other vectors (Hamrick et al., 1992; Montoya, Zavala, Rodríguez, & Purves, 2008). However, there are examples of animal dispersed plants with high dispersal capability that show negative genetic consequences owing to fragmentation (e.g. Dubreuil et al., 2010; Parejo-Farnés et al., 2017). Aguilar et al., (2008) did not detect any significant effect of the seed dispersal vector in relation to the genetic consequences of fragmentation. This is probably due to the fact that the capability of transporting seeds through fragmented landscapes depends on the vector, the plant species and the landscape matrix (Kramer, Ison, Ashley, & Howe, 2008). Indeed, seed dispersal processes are also impacted by landscape, and hence they can be disrupted in highly perturbed matrices (McConkey et al., 2012) which negatively impact on the reproductive cycle of plants (Cordeiro & Howe, 2003; Cancio et al., 2016; de Assis Bomfim, Guimarães, Peres, Carvalho, & Cazetta, 2018; Rey et al., 2018). Conservation measures such as establishing corridors between remnants of natural vegetation can enhance gene flow among plant populations by increasing the seed dispersal activity of frugivorous animals (Tewksbury et al., 2002).

Time since fragmentation is another important factor when assessing genetic diversity erosion. Many plants can live from tens to hundreds of years (especially trees and shrubs) in contrast with the more recent

fragmentation events (20-200 years). Thus, many generations may be needed before detecting any signal of genetic degradation, while ecological or landscape degradation can be rather evident in the short term (Kramer et al., 2008). The loss of ecological interactions should be considered as a wake-up call about the possible future genetic consequences of fragmentation on plant populations. For instance, defaunation processes have been found to correlate with incipient signals of genetic erosion in animal dispersed plants (Carvalho et al., 2016; Pérez-Méndez et al., 2018). These findings lead to a crystal clear conclusion already stated in this general introduction: that ecological interactions should begin to be considered as the basic unit of conservation programmes.

4.1. Chapter II. Ecological and genetic consequences of fragmentation in a semiarid Mediterranean urban forest. Goals and hypothesis

In this study, we combine ecological and genetic approaches to investigate the consequences of fragmentation in the fleshy-fruited shrub *P. lentiscus* in two urban forest fragments located in the suburbs of Murcia (Spain). We assess seed dispersal by frugivorous birds through mist-netting and bird census, and we estimate genetic patterns of wild and planted *P. lentiscus* individuals through microsatellites analysis. Our hypothesis is that fragmentation affects the plant-animal mutualism (i.e. connectivity among patches) and hence the genetic diversity of *P. lentiscus* in patches.

5. The role of seed dispersal mediated by animals in ecosystems restoration

Agroecosystems have demonstrated to support plant and animal biodiversity, and water and soil quality (Antrop, 2005; Blondel, Aronson, Bodiou, & Boeuf, 2010). Indeed, around 50% of all European species depends on agricultural habitats (Kristensen, 2003). Similarly, the provision of ecosystem services to agricultural landscapes is highly dependent on biodiversity (Swift, Izac, & van Noordwijk, 2004; Jackson, Pascual, & Hodgkin, 2007). However, land use changes in the last decades are threatening the functioning of agroecosystems, as well as the services provided by these human-managed landscapes (Salvati & Ferrara, 2014). In the European Union, land abandonment and agricultural intensification stand out among the drivers of such pernicious land use changes (European

Union, 2012; Nainggolan et al., 2012) since both processes lead to habitat homogenization with negative impacts on biodiversity (Robinson & Sutherland, 2002; Klijn, 2004; Rey-Benayas, Martins, Nicolau, & Schulz, 2007; Kati et al., 2010). For instance, many studies have reported negative effects of land abandonment, and the subsequent recovery of natural vegetation, on open habitat bird species (Preiss, Martin, & Debussche, 1997; Vallecillo, Brotons, & Herrando, 2008; Nikolov, 2010; Zakkak et al., 2015). However, these general trends should not be extrapolated to all landscapes since different habitat characteristics and management regimes can affect the results (Nikolov, 2010). In this sense, some studies reported important changes in bird community trends after abandonment due to the lithological substrate, which also is an important factor in plant secondary succession (Robledano-Aymerich et al., 2014, 2016). Those changes in avian and plant communities mirrored in plant-animal interactions such as seed dispersal (Robledano-Aymerich et al., 2014).

Land abandonment has traditionally occurred in marginal or degraded cultivated areas (MacDonald et al., 2000), but a process of fertile land abandonment has been recently experienced owing to socio-economic changes (Hatna & Bakker, 2011). These areas could be recolonized by natural vegetation (Flinn & Vellend, 2005), but secondary succession after abandonment can take from tens of years to centuries to reach the late states of the ecological succession (Lasanta-Martínez, Vicente-Serrano, & Cuadrat-Prats, 2005). Secondary natural succession in old fields is shaped by a wide array of factors such as the availability of seeds in surrounding areas (Pywell et al., 2002; Bonet, 2004), soil characteristics (Bonet, 2004 and references therein), or the type and intensity of disturbance (de Bello, Lepš, & Sebastià, 2007; Ribeiro, Ladero, & Espírito-Santo, 2012). Adverse combinations of those factors can slow down or even stop ecological succession which can entail severe soil erosion processes (Navarro & Pereira, 2015). Thus, crop abandonment itself does not guarantee natural vegetation recovery and active management can be needed to activate secondary ecological succession (Rey-Benayas, 2005; Troani et al., 2016).

The abandonment of large extensions of crops has led to the appearance of the concept of “rewilding”, which is based on an ecological restoration approach whose primary goal is to promote self-sustainability of ecosystems functioning (Svenning et al., 2016). Rewilding involves a wide

variety of measures ranging from no intervention approaches to the reactivation of natural processes areas through the introduction of megafauna, large herbivores and carnivores. However, extreme approaches of this concept such as the introduction of large species are not without controversy owing to the lack of practical knowledge in those novel restoration management measures (e.g. Conti & Fagarazzi, 2005). Thus, the design of new conceptual frameworks to assist practitioners in decision-makings is needed before generalizing those kinds of measures (e.g. “ecological memory concept”, Schweiger, Boulangeat, Conradi, Davis, & Svenning, 2018). In contrast, there is a general consensus about benefits of other views such as passive/spontaneous restoration (Walker, Walker, & Hobbs, 2007; Prach & Hobbs, 2008). Passive restoration of old fields can be considered a special type of rewilding based on “a process in which a formerly cultivated landscape develops without human control” (Höchtl, F., Lehringer, S., & Konold, 2005, p. 86) and “passive management of ecological succession with the goal of restoring natural ecosystem processes and reducing human control of landscapes” (Navarro & Pereira, 2012, p. 904). For instance, passive restoration can rely on natural processes such as seed dispersal for natural vegetation recovery.

Seed dispersal is a key process in plant life cycle (Levine & Murell, 2003) and it is crucial in old fields secondary succession since poor seed supply is often the main limiting factor for their natural regeneration (Holl, Loik, Lin, & Samuels, 2000; Schupp, Jordano, & Gómez, 2010). Particularly, seed dispersal mediated by animals can play a key role in old fields vegetation recovery since they can transport seeds from natural remnants to degraded lands (Cramer, Hobbs, & Standish, 2008). Plant populations are shaped by the behaviour of seed dispersers, as exemplified by frugivores dispersing seeds of fleshy-fruited shrubs. This can be due to the different ability of dispersers to track fruits abundance (García & Pulido, 2004), to their nonrandom use of the space (e.g. the preference for particular microhabitats) (Russo, Portnoy, & Augspurger, 2006), or to the differential ability of frugivores to move seeds through fragmented landscape matrices (Lees, & Peres, 2009; Uriarte et al., 2009; González-Varo, Carvalho, Arroyo, & Jordano, 2017b). Other factors such as body size can be also good predictors of the role of frugivores in seed dispersal networks since larger animals usually disperse more seeds and to longer distances (Wheelwright, 1985; Saavedra et al., 2014). Therefore, traits of frugivores

and habitat characteristics condition the role of seed dispersal in ecosystems restoration, as well as the type of management measures directed to enhance the contribution of animal vectors to old fields secondary succession.

5.1. Chapter III. Barcoding as an ancillary technique to uncover mechanisms of seed dispersal in demanding environments - and to help to restore them. Goals and hypothesis

Here, we apply a methodological framework which combines ecological and genetic tools to measure the species-specific contribution of frugivorous birds to the colonization of fleshy-fruited shrubs in semiarid Mediterranean old fields. We deployed restoration structures (artificial perches and water troughs) to attract birds to abandoned lands, and we also provided microenvironments and prevented herbivory to increase seedling survival opportunities. Specifically, we: i) studied bird community composition in old fields and surrounding natural lands through bird census to identify bird species susceptible of being attracted to the target area; ii) applied indirect detection methods such as camera trapping to determine which species of the local avian community were using our attractors; iii) performed molecular techniques to disentangle the contribution of different bird species to seed dispersal in old fields. We hypothesized that the deployed restoration structures attract avian frugivores as well as enhance plant recruitment to target areas, and our methodological framework identifies the response of different bird species to our restoration structures.

6. Pollination collapse: the role of microsporidia

Pollination is a key ecological process in natural and managed ecosystems since three quarters of all crops depend on pollinators (Aizen et al., 2009). Bees are the main pollination service providers worldwide whose economic value is estimated in 217 billions of dollars owing to food crop production per year (Abrol, 2012). However, functioning of such crucial ecosystem service is currently being threatened by a worldwide pollinators decline, both of wild and managed species (Potts et al., 2010a; Burkle, Marlin, & Knight, 2013), which might put at risk food supply (Kremen & Ricketts, 2000; Steffan-Dewenter, Potts, & Packer, 2005). Different anthropogenic actions such as changes in land use and the use of pesticides have been

identified as causes of this decline (De la Rúa, Jaffé, Dall'Olio, Muñoz, & Serrano, 2009; Vanbergen, & Insect Pollinators Initiative, 2013; Goulson, Nicholls, Botías, Rotheray, 2015; Figure 7). However, the spread of parasites and pathogens worldwide linked to the movement of managed pollinators and the interspecific transmission of infectious agents between managed and wild bees, also stands out among the main drivers of such dramatic situation (Manley, Boots, & Wilfert, 2015; Mallinger et al., 2017).

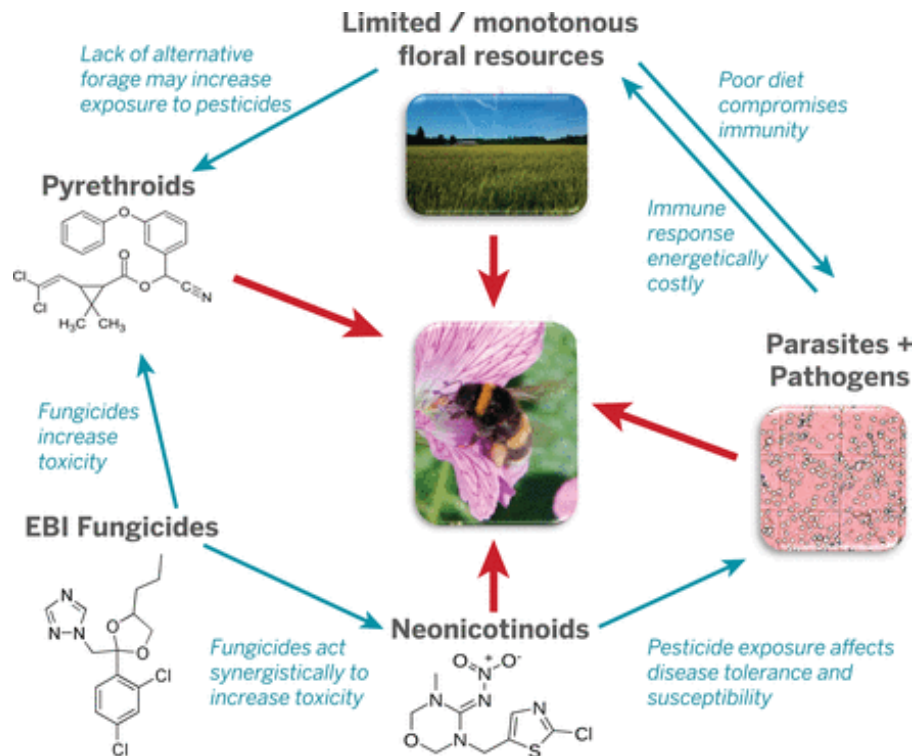


Figure 7. Different drivers of managed and wild pollinators decline (Figure from Goulson et al., 2015).

Crop pollination has traditionally relied on the western honey bee, (*Apis mellifera* L.) (Rucker, Thurman, & Burgett, 2012) due to its adaptability to different environments and its behaviour as a generalist pollinator (Aizen, Morales, & Morales, 2008). Thus, honey bees have been considered able to replace wild pollinators activity (Rucker et al., 2012). However, some studies have documented a complementary role between honey bees and wild bees in crop pollination, in which wild bees can even outperform the pollination service provided by honey bees (Garibaldi et al., 2013). There are at least 17,000 other bee species globally (Michener, 2000), and it would be astonishing to find one single species replacing the ecosystem services supplied by thousands of species. Furthermore, the surface of crops is increasing faster than the production of honey bee colonies needed

to assist their pollination (Aizen & Harder, 2009). Therefore, humanity is concerned about the sustainability of basing all crop pollination services on a single species. Nowadays, around 20 species of social and solitary bees are commercially reared for pollination services (Stout & Morales, 2009). For instance, some species of the genus *Bombus* have been domesticated and they are used in pollination of many crops (Velthuis and Van Doorn, 2006). Among them, *Bombus terrestris* L. is by far the most common and has been exported worldwide leading to its spread in natural ecosystems owing to queens escape (see review of Acosta, Giannini, Imperatriz-Fonseca, & Saraiva, 2016). Indeed the spread of managed bee species worldwide is a common process what alerts about the beneficial outcomes of using local pollinators to provide crops pollination (Stout & Morales, 2009).

Honey bees colonies are declining in large areas of Europe and North America (Ellis, Evans, & Pettis, 2010; Neumann & Carreck, 2010; Potts et al., 2010b). This situation is not new, for instance in Europe between 1970 and 2007 the number of hives decreased from over 21 million to 15.5 million (Aizen & Harder, 2009). However, the situation got much worse between 2005 and 2007, when alarming losses of colonies were registered among large areas of North America and Europe (losses between 30-90% in USA, vanEngelsdorp, Underwood, Caron, & Hayes, 2007). Causes behind such high mortality events were unknown, and hence the syndrome was named Colony Collapse Disorder (CCD). Nowadays, there is a common consensus that the syndrome is triggered by the interaction of different factors such as changes in land use, the massive use of pesticides, the intensification of beekeeping practices, and the increase of the incidence of parasites and pathogens of the honey bee (Ellis et al., 2010 and references therein). However, the role of the latter seems to be somehow determinant (vanEngelsdorp et al., 2009). Among different pathogens, microsporidia play an important role on the disappearance of honey bees, especially in Mediterranean countries (Martín-Hernández et al., 2018).

Microsporidia are a group of fungi which are obligate intracellular parasites of animals, especially insects (Larsson, 1986). Bees get infected when consuming viable spores while feeding (Bailey, 1981), trophalaxis (Webster, 1993), or through water (L'Arrivee, 1965). However, beekeeping tasks and hive management can also influence on the spread of the

pathogen (Fries, 1989). Once the spores get into the bee through the alimentary canal, they travel until the intestine where the infection of the epithelial cells occurs (Figure 8 and Figure 9). In the particular case of honey bees, two microsporidia species from the genus *Nosema* have been identified to affect the viability of honey bee colonies: *Nosema apis*, described by Zander (1909), and *Nosema ceranae*, described by Fries et al., (1996). *N. apis* is a natural pathogen of *A. mellifera* while the Asian bee, *Apis cerana* Fabricius, is the natural host species of *N. ceranae*. Despite the biological similarities of both pathogens, they trigger different pathologies: nosemosis type A produced by *N. apis*, and nosemosis type C produced by *N. ceranae* (COLOSS workshop, 2009), type C being much more aggressive than type A in *A. mellifera* colonies. The nosemosis can affect all individuals in the colony, but it seems to be more common in adult honey bees (Wang & Moeffler, 1970; Fries, 1989). This pathology causes the weakening of the colony through depopulation since heavily infected foraging honey bees do not return to the hive (Higes et al., 2008, 2009). This phenomenon occurs due to *N. ceranae* partial suppression of the humoral and cellular defense mechanisms of the honey bees, a process which does not appear with *N. apis* (Antúnez et al., 2009). Therefore, it is considered that the nosemosis type C has a strong impact on the honey bee colonies that has been associated with the sudden collapse of *A. mellifera* colonies (Higes et al., 2008, 2009; Martín-Hernández et al., 2018 and references therein).

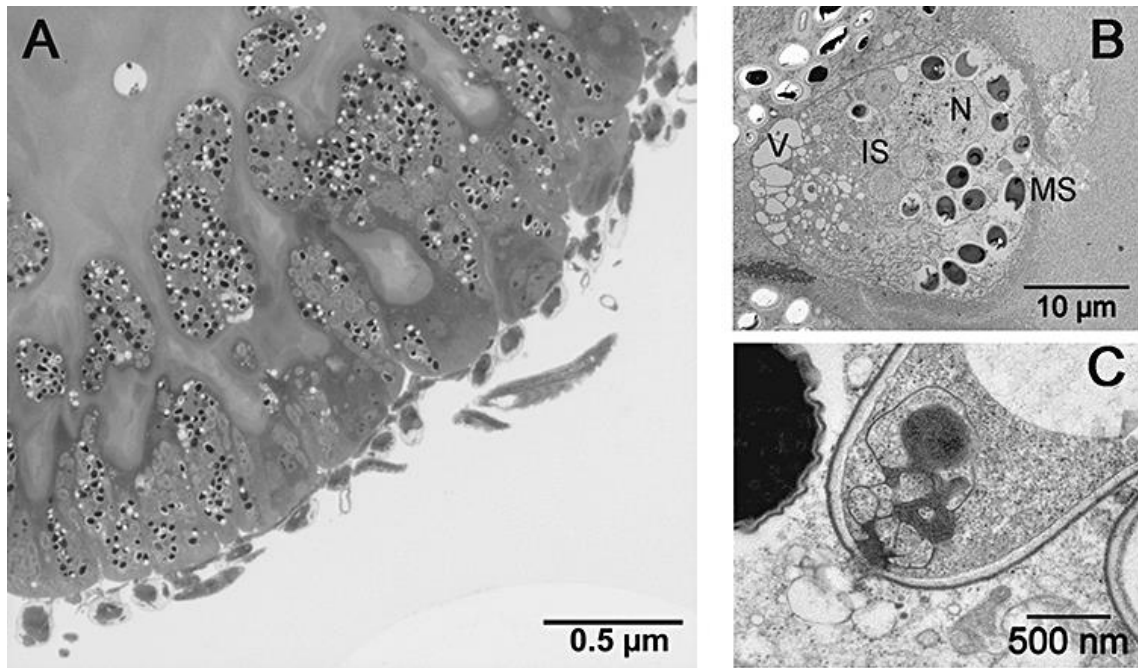


Figure 8. Microscopy images of infected bee ventriculus with *N. ceranae*. A) Epithelial cells infected by *N. ceranae* spores (black dots). B) Infected cell (N=nucleus; IS=immature spores; V= vacuolas; MS= mature spores of the pathogen). C) Spore germinating and infecting a cell (Picture from Higes et al., 2008).

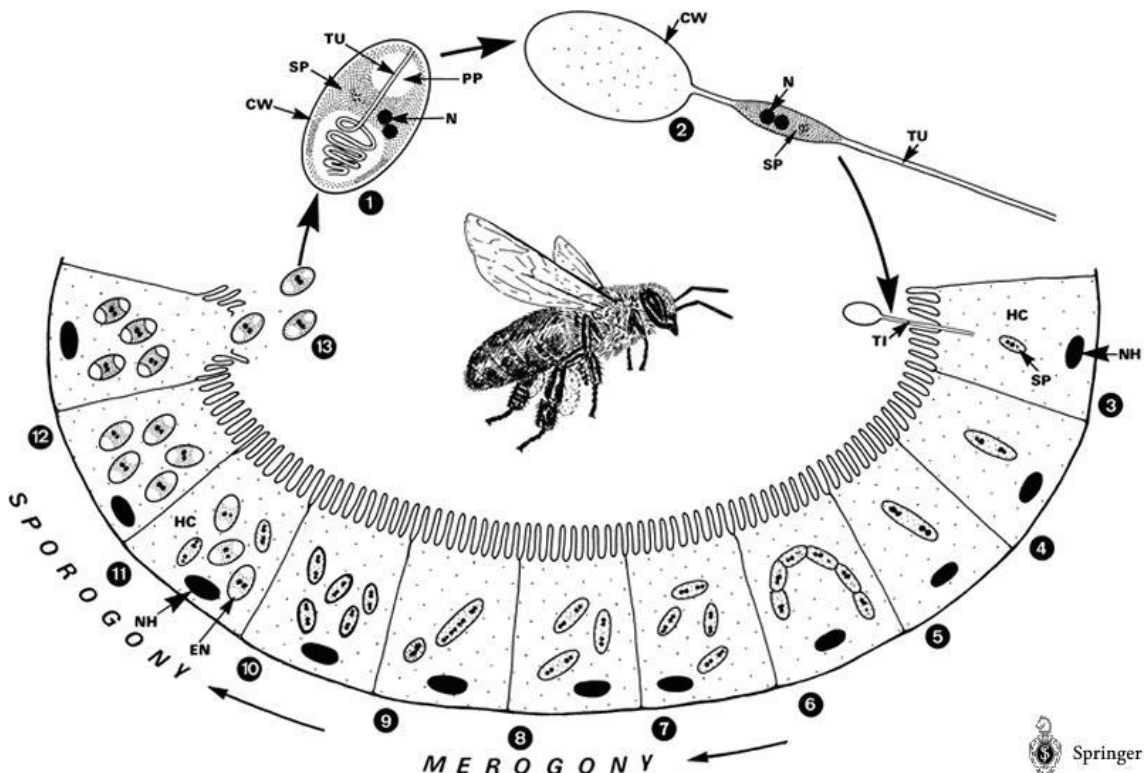


Figure 9. *Nosema* spp. life cycle. Spores of the pathogen arrive at the honey bee (phases 1 and 2), the pathogen infects gut epithelial cells of the insect (phase 3), and it reproduces until new spores are freed into the gut lumen. In addition, the infection can also progress from cell to cell. © Springer Life Sciences Source: <http://scientificbeekeeping.com/images/stories/nosema/nosema1-1.jpg>

Since the ability of *N. ceranae* to infect *A. mellifera* was demonstrated (Fries & Feng, 1995), the pathogen has spread worldwide being detected in honey bees and wild bees (Figure 10). The infestation of the European honey bee by *N. ceranae* (Martín-Hernández et al., 2007) has been related to the CCD in some temperate areas in the world such as Spain (Higes et al., 2008, 2009). Indeed, *N. ceranae* is considered an emerging infectious disease (EID) (Paxton, 2010) whose first record in *A. mellifera* in Europe occurred in Spain and coincided with huge events of colony losses (Higes, Martín-Hernández, & Meana, 2006).

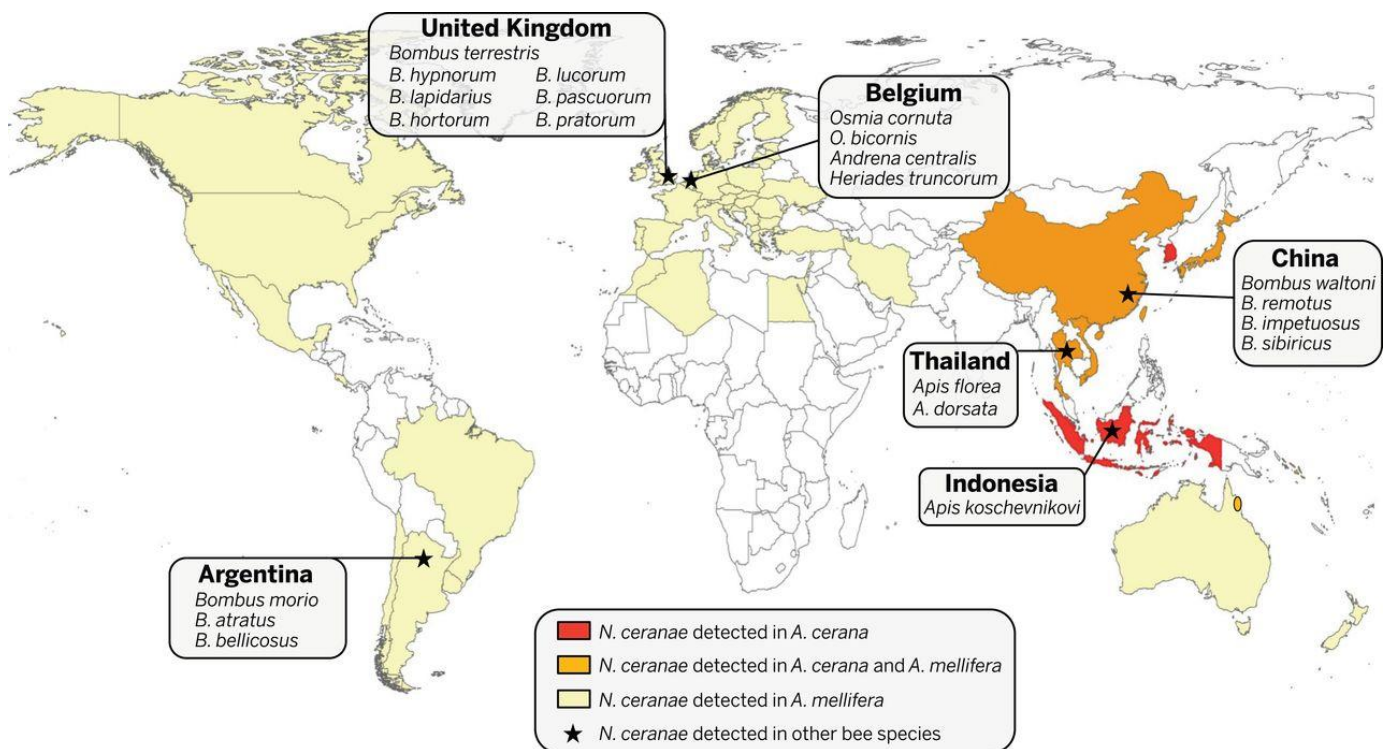


Figure 10. World distribution of *N. ceranae* (Figure from Goulson et al., 2015). After this study, the pathogen has been also detected in stingless bee species from Argentina and Brazil (genera *Melipona*, *Tetragonisca*, and *Scaptotrigona*, Porrini et al., 2017).

Despite the fact that *N. ceranae* has been detected in some wild bees (Martín-Hernández et al., 2018 and references therein), infection has been only demonstrated to occur in *Bombus* spp. with higher virulence than in his native host (Graystock, Yates, Darvill, Goulson, & Hughes, 2013). The family Apidae comprises around 17,000 species (Michener, 2000) and *N.*

ceranae seems not to be host specific unlike *N. apis*, which has only been found in his natural host *A. mellifera*. Therefore, studies assessing the presence of the pathogen at community level are required in order to measure the potential effects of this microsporidium on wild bee assemblages.

6.1. *Chapter IV. Contrasting effects of beekeeping and land use on plant-pollinator networks and pathogen prevalence in a Mediterranean semiarid ecosystem. Goals and hypothesis*

We present a study that investigated the prevalence of microsporidia in plant-pollinator networks of honey bees and wild bees in semiarid Mediterranean areas of the Iberian Southeast under different conditions of land use and beekeeping practices. Climate change is expected to increase aridity in the area, which could aggravate the impacts of *N. ceranae* on honey bees and other potential host species since it seems to prefer hot weather conditions (Paxton, 2010). Thus, this study could shed light on the potential effects of this exotic pathogen at the bee community level, which could help to design strategies to mitigate potential damages. Specifically our goals were: i) to investigate the effect of different factors (land use type and beekeeping), as potential stressor agents, on wild bee communities; ii) to unravel the role of honey bees in the pathogen spillover to the wild bee community under semiarid conditions; iii) to study the structure of bee pollination networks at community level under a variable degree of human disturbance (e.g. intensive agriculture and beekeeping intensity). Our hypotheses are that those areas with higher abundance of honey bees present higher pathogen prevalence, and that plant-pollinator networks are negatively affected by anthropogenic disturbances.

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Chapter I



**Intercontinental long-distance seed dispersal
explains population genetic structure in a
fleshy-fruited shrub spanning the
Mediterranean Basin**

Intercontinental long-distance seed dispersal explains population genetic structure in a fleshy-fruited shrub spanning the Mediterranean Basin

Abstract

Long-distance dispersal (LDD) is a pivotal process for plants that promotes gene flow among distant populations and determines the distribution range of a species. Most fleshy-fruited species rely on frugivorous vertebrates to disperse their seeds across the landscape. LDD events are rare and difficult to record, but a few ecological studies have shown that birds move a sizeable number of ingested seeds across geographic barriers such as sea straits. If this flow of propagules is constant, the spatial distribution of genetic variation and the genetic distances among animal-dispersed plant populations should be better explained by the foraging movement of migrant frugivores than by an isolation by distance (IBD) pattern. Here, we analyse the genetic diversity and structure of *Pistacia lentiscus*, a fleshy-fruited shrub widely distributed in the Mediterranean Basin, to elucidate whether the Mediterranean Sea acts as a geographic barrier or, on the contrary, migratory frugivorous birds promote gene flow among populations located on both sides of the sea. Our results suggest high genetic connectivity among the populations, including the intercontinental populations, and a significant East-West genetic structure. These findings are consistent with the known bird migratory routes that connect the European and African continents from North to South. Our study found that the foraging movements of migrant frugivores are congruent with the spatial genetic structure of animal-dispersed plants. Therefore, anthropic and/or climatic changes that might disrupt the migration routes of frugivorous birds would cascade into genetic consequences for the plant species they feed upon.

Keywords: long-distance seed dispersal, genetic clustering, genetic network, isolation by distance, migratory frugivorous birds, wide distribution range plant species

1. Introduction

Long-distance dispersal (LDD) of seeds is a crucial process that determines spatial genetic structure (SGS) and distribution range in plant species (Nathan, 2006). Frugivorous vertebrates typically provide dispersal services within the range of a few metres up to some kilometres from the seed source (Jordano, García, Godoy, & García-Castaño, 2007; Lavabre, Gilarranz, Fortuna, & Bascompte, 2016; González-Varo, Carvalho, Arroyo, & Jordano, 2017). Nevertheless, empirical LDD data are scarce due to the rarity and stochastic nature of these events (Nathan & Müller-Landau, 2000). However, a few studies have shown that frugivorous birds provide long-distance seed dispersal services across sea barriers (Dyer & Nason 2004) and that they can connect island and continental populations hundreds of kilometres apart (Nogales, Heleno, Traveset, & Vargas, 2012; Viana, Gangoso, Bouten, & Figuerola, 2016). In fact, historical intercontinental LDD events involving the activity of frugivorous birds would explain the extreme bipolar disjunction of some animal-dispersed plant taxa (Popp, Mirré, & Brochmann 2011). In the context of global climate change, bird migration routes might collapse or shift due to warming (WWF Australia, 2006; Pulido & Berthold, 2010) or increased landscape fragmentation (Bairlein, 2016), in turn impacting the genetic connectivity of animal-dispersed plants (Dirzo et al., 2014; Pérez-Méndez, Jordano, García, & Valido, 2016, Martínez-López, De la Rúa, Zapata, & Robledano, 2017; Parejo-Farnés, C., Robledo-Arnuncio, Albaladejo, Rubio-Pérez, & Aparicio, 2017).

Molecular tools and landscape genetics can shed light on the impact of LDD on SGS and gene flow among populations (Ouborg, Piquot, & Van Groenendael, 1999; Cain, Milligan, & Strand, 2000). To date, most of the studies assessing SGS in plants in their entire distribution range have focussed on the genetic diversity and SGS of narrowly distributed plant species, as they typically have high levels of endemism, which make them particularly sensitive to the drivers of global change (e.g., González-Astorga et al., 2004; Medrano & Herrera 2008; Binks et al., 2015). However, these studies are insufficient to fully understand the main drivers that underpin SGS in widely distributed plant species because (i) the populations of widespread species are typically subjected to different environmental conditions (Lesica & Allendorf, 1995; Gapare, Aitken, &

Ritland, 2005), including a variable level of human-driven disturbance, which involves populations adaptation to the local circumstances; (ii) their distribution ranges are shown to correlate with particular demographic and genetic traits (e.g. high levels of genetic diversity and low among population differentiation; Hamrick & Godt, 1989; Hamrick & Godt, 1996; Eckert, Samis, & Loughheed, 2008); and (iii) the impact of LDD events is usually overlooked in studies with narrowly distributed species, but these events are crucial to explain SGS in widely distributed plant species. Therefore, research on genetic variation including the full distribution ranges of widespread plant species is especially needed to understand the impact of LDD underlying the spatial distribution of genetic variation.

The genetic distance between populations typically increases with geographic distance (isolation by distance model, IBD, Wright, 1943), but both geographic barriers and the occurrence of LDD events alter the IBD pattern in a predictable fashion: while barriers hamper the gene flow between populations that show increased levels of genetic structure, LDD events favour connectivity, which increases the genetic similarity between distant populations (Dyer & Nason, 2004; Rozenfeld et al., 2008). Therefore, if the Mediterranean Basin acts as a barrier to gene flow, populations located on both sides of the Mediterranean Basin are expected to be genetically more different than their geographic distances would suggest. In this sense, studies assessing plant taxa migration between Europe and Africa through the Strait of Gibraltar found that it was not a barrier to the dispersal of those species with higher migration capabilities which was conditioned by their traits (Lavergne, Hampe, & Arroyo, 2013; Molina-Venegas, Aparicio, Lavergne, & Arroyo, 2015). LDD in plants can occur by seed or pollen dispersal; however, in our case the later seems unlikely given that distances among populations located on both sides of the Mediterranean Sea are greater than the pollination distances detected in wind-pollinated trees (under ten kilometres, Souza, 2018 and references therein), and a stepping-stone dispersal mode is not possible as in continental and continuous population distributions. However, the bird migratory routes that connect the European and African continents traverse some distant populations of circum-Mediterranean endozoochorous plants (e.g., *Pistacia lentiscus* L., Figure 1). Current bird migratory routes in the Mediterranean Basin have evolved since the last glacial period (10,000-15,000 years) (Bruderer & Liechti, 1999; Newton, 2007). Thus, if

frugivorous birds are carrying seeds across the Mediterranean Sea, the contemporary genetic evidence should show high connectivity, indicating that the Mediterranean Sea does not act as a geographic barrier. The combination of ecological information and genetic tools (i.e., landscape genetics; Manel, Schwartz, Luikart, & Taberlet, 2003) allows testing of this hypothesis by contrasting the observed genetic patterns with those expected under IBD and an isolation by barrier (IBB) scenario. Following this approach, we herein analyse the spatial distribution of genetic variation and the population genetic structure of a widely distributed fleshy-fruited shrub (*P. lentiscus*) across its distribution range, which includes the Mediterranean Basin and the Canary Islands (Figure 1). We applied microsatellites in combination with landscape genetics tools to test whether the Mediterranean Sea acts as a barrier to gene flow among the *P. lentiscus* populations located on opposite shores of the sea or, alternatively, whether contemporary gene flow attributable to migratory birds links the intercontinental populations (Figure 1). To that end, we first described the genetic diversity and structure of 74 populations encompassing the whole distribution range. Then, we tested for IBD patterns across both a North-South axis, where the populations are expected to be connected by migratory frugivorous birds, and an East-West axis, where migratory routes are less intense or absent, and therefore LDD events are less likely to occur. Third, we combined different landscape models (Bayesian clustering, discriminant analysis of principal components (DAPC), and genetic networks) to test whether this directional connectivity resulted in predictable genetic clustering. We expected that the migration routes of frugivorous birds would promote contemporary gene flow along the North-South axis and result in a significant East-West genetic clustering (Figure 1).

2. Materials and Methods

2.1. Study species and sampling design

Pistacia lentiscus is a dioecious and anemophilous evergreen fleshy-fruited shrub widely distributed across the Mediterranean Basin, which also appears in the Canary Islands (Bolós & Vigo, 1990) (Figure 1). The importance of *P. lentiscus* in Mediterranean shrublands has been previously highlighted because it fruits in large amounts for a long period (from August till February), and its lipid-rich fruits are a fundamental component of the diet of migratory birds (Verdú & García-Fayos, 2002). Therefore, *P. lentiscus* acts as a foundation species, sensu Whitham et al., (2006), by structuring the functioning of communities in which it is present (Herrera, 1984).

P. lentiscus fruits are part of the diet of a big variety of bird species (mainly small passerines) (Jordano, 1989; Herrera, 1998). *Sylvia melanocephala* Gmelin, *Sylvia atricapilla* L., *Erithacus rubecula* L. and *Turdus merula* L. have been identified as the main consumers of *P. lentiscus* fruits in Mediterranean environments (Herrera, 1984, 1998; Izhaki & Safriel, 1985; Jordano, 1989). The four species present migrant and resident populations in the European continent; however, *S. melanocephala* and *T. merula* are considered mainly resident in Mediterranean areas (e.g. Herrera, 1984), while *E. rubecula* and *S. atricapilla* show a higher mobility with important contingents of migrant populations which travel southwards from September till November (Leal, Monrós, & Barba, 2004; Arizaga, Alonso, & Barba, 2010) when the ripening peak of *P. lentiscus* fruits occurs (Jordano, 1989). Thus, *E. rubecula* and *S. atricapilla* would be the species with higher potential of contributing to LDD of *P. lentiscus*.

The sampling design encompassed the entire distribution range of the species (Figure 1). A total of 1057 individuals were sampled from 2013 to 2015 (74 populations, Table S1). Leaf tissue was sampled and kept in paper envelopes with silica gel for drying until further work in the lab.

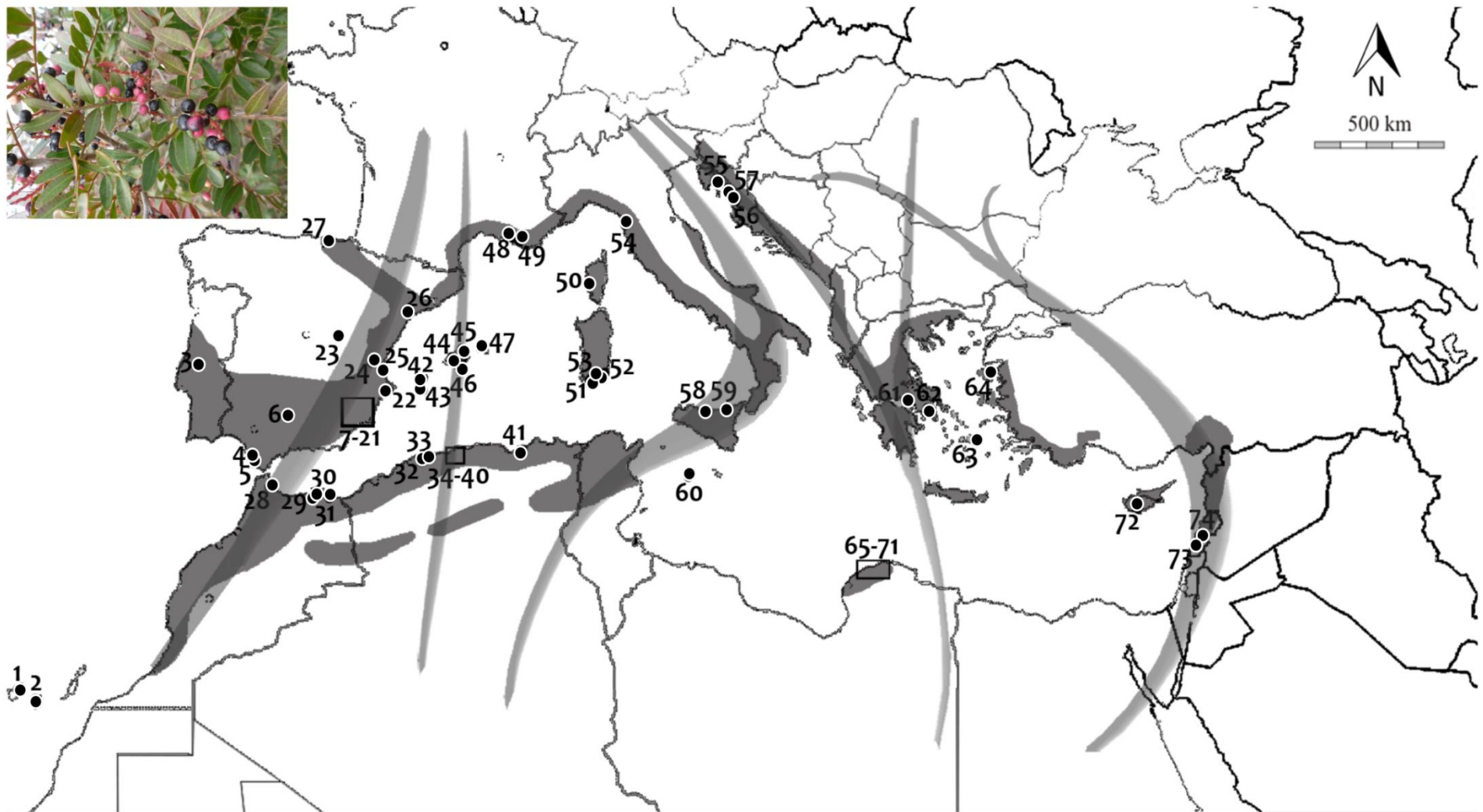


Figure 1. Distribution of *P. lentiscus* (dark grey shadow) (based on Bolós & Vigo 1990) and detail of *P. lentiscus* fruits (upper left photograph). Light grey lines depict the main bird migratory routes from Europe to Africa (based on Bairlein, 2016, Bruderer & Liechti, 1999, and Fiedler & Davidson, 2012). Black dots indicate the locations of the populations sampled for this study, with the ID of each population indicated by a number (see Table S1).

2.2. DNA isolation and microsatellite genotyping

DNA was isolated from dried leaves (0.1 g dry weight) using the NaOH protocol (Werner, Ros, & Guerra, 2002a). We used a set of 15 microsatellites designed for the species (six from Albaladejo et al., 2008, and nine from Motalebipour, Kafkas, Khodaeiaminjan, Çoban, & Gözel, 2016, see Table S2). PCR was performed in total volumes of 10 μ l containing 1 μ l of template DNA; reagent concentrations and PCR profiles are shown in Table S2. PCR products were electrophoretically separated on an ABI 3730 sequencer (Applied Biosystems, Foster City, CA, USA). Allele scoring was carried out manually with GeneMapper v3.7 software (Applied Biosystems). Genotypes were reviewed twice after scoring to reduce genotyping errors. We tested the performance of each marker by estimating the frequency of null alleles as implemented in Micro-Checker software version 2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004) (Table S2, null alleles by marker).

2.3. Data analyses

2.3.1. Population genetic diversity parameters

Data analyses were carried out using R v. 3.2.5 (R Development Core Team 2016). We used the package *diversity* v. 1.9.90 (Keenan, McGinnity, Cross, Crozier, & Prodöhl, 2013) to estimate basic population genetic diversity parameters, including the allelic richness (A_r) and rarefied allelic richness (A_r^*); expected, observed, and unbiased heterozygosity (H_e , H_o , and uH_e , respectively); and inbreeding coefficient (F_{is}). We computed the 95% confidence intervals for A_r and F_{is} with 9999 iterations. We additionally estimated the frequency of private alleles as implemented in GenAlEx 6.5 (Peakall & Smouse, 2012). We tested for Hardy-Weinberg equilibrium (HWE) of populations as implemented in the *diversity* R package (Keenan et al., 2013), and for genotypic linkage disequilibrium with the software Genepop (Raymond & Rousset, 1995).

Bird migration occurs in autumn from northern locations in Europe towards southern wintering places. Thus, seeds are expected to be transported mainly from the North to the South. To test the effect of such process in genetic diversity patterns we have analysed private alleles frequencies

and rarefied allele richness in relation to the latitude and longitude of populations through Pearson correlation.

2.3.2. *Isolation by distance*

We tested for IBD (Wright, 1943) by applying a Mantel test using the pairwise Nei's genetic and geographic distances (Nei, 1972) with the *adeigenet* R package version 2.0.1 (Jombart, 2008). The geographic distance matrix (distances in kilometres) was calculated with the R package *geosphere* version 1.5.5 (Hijmans, Williams, Vennes, & Hijmans, 2016) using the Vincenty ellipsoid method (ellipsoid WGS84) (Vincenty, 1975). We also calculated IBD with the euclidean longitudinal and latitudinal distances to check for different patterns along the East-West and North-South axis. Furthermore, we tested IBD according to the main bird migratory routes which traverse the Mediterranean Sea. Five main migratory routes can be observed in Figure 1; however, we just had three populations in the East extreme one (72-73-74), and hence we analysed together all populations in the East of the Mediterranean Basin. We divided populations in four groups: group one (populations 1-31), group 2 (populations 32-49), group 3 (populations 50-60), and group 4 (populations 61-74). We used 10,000 permutations to test the significance of the IBD test.

2.3.3. *Genetic differentiation across populations*

We first assessed genetic differentiation across populations (F_{st}) with the R package *hierfstat* version 0.04.22 (Goudet, 2005), and confidence intervals and significance of results were calculated with 10,000 bootstrap samples. We have carried out an analysis of molecular variance (AMOVA) based on 999 replicated as implemented in GeneAlex 6.5 (Peakall & Smouse, 2012) to assess how genetic diversity varies within and among populations. Furthermore, we performed a hierarchical F_{st} analysis with the same package to test the impacts of different hierarchical levels of organization on the SGS: i) *continents*; ii) *western-eastern islands/mainland* (populations split into western and eastern portions of the Mediterranean Basin in continents and islands); iii) *geographic accidents* (e.g., peninsulas); and iv) *population* (more information about levels in Table S3). Hierarchical genetic F_{st} analysis was used to test for the effects of different hierarchical grouping factors (ex. continents or populations) in explaining the observed genetic structure. For instance, if we consider the

effect of the level *population* on the level *continents*, we are analysing whether genetic differentiation occurs when populations are grouped by continent (for more details about the technique, see De Meeûs & Goudet, 2007). The significance of these results was tested based on 10,000 repetitions.

2.3.4. Genetic clustering

After testing for a certain degree of hierarchical structure, we evaluated whether this structure gave rise to genetic clusters by applying two types of genetic analysis: (i) a distance-based method called DAPC (Jombart, Devillard, & Balloux, 2010) and (ii) a Bayesian clustering analysis (STRUCTURE, Pritchard, Stephens, & Donnelly, 2000). DAPC is a non-model-based method that builds genetic clusters from genetic data by synthesizing the original variables (alleles), and therefore, it does not assume HWE or linkage disequilibrium (Jombart et al., 2010). The ideal K value (i.e., the number of clusters) is selected by the *Bayesian information criterion* (BIC); the optimal K value is identified through an accompanying decrease in the BIC (Jombart et al., 2010). STRUCTURE is a Bayesian model-based method that presumes HWE and linkage disequilibrium and analyses the individual multilocus genotypes, assigning individuals to clusters according to their probability of assignment. The STRUCTURE analysis was performed with a burn-in period of 50000 iterations followed by 200000 with MCMC, assuming an admixture model of population structure and with K values ranging from 1 to 25 (10 runs per K value). The location of populations was not considered in the analysis. The final value of K was selected according to the Evanno method (Evanno, Regnaut, & Goudet, 2005) as implemented in Structure Harvester (Earl & vonHoldt, 2012). Despite the fact that STRUCTURE generally performs well, its results can be misleading when the presume conditions are not met (i.e. HWE and linkage disequilibrium), or in situations with the presence of isolation by distance (Lombaert, Guillemaud, & Deleury, 2018, and references therein). Thus, we have combined two genetic clustering methods with different methodological approaches to validate and to strengthen our results.

Then, we evaluated the effect of the geographical component on the spatial genetic structure through a spatial principal component analysis (sPCA hereafter) as implemented in the adegenet R package version 2.0.1

(Jombart, 2008). sPCA use Moran's index I (Moran, 1948, 1950) to detect spatial genetic structure comparing allelic frequencies observed at one population to the values observed at surrounding ones. Values of Moran's index are positive when populations at neighbouring places show similar values, and negative when close populations show dissimilar values, which denotes global and/or local genetic structure respectively (Jombart, 2015). Significance of the spatial patterns was tested for 999 permutations with the function `spca_randtest` implemented in the package `adegenet` (Jombart, 2008) and described by Montano and Jombart (2017).

Since recent studies have highlighted the importance of applying different clustering analyses to verify the number of genetic groups (Janes et al., 2017), we performed a third clustering analysis, an unweighted pair group method with arithmetic mean (UPGMA) based on Nei's genetic distance (Sokal & Sneath, 1963) to confirm the overall large-scale genetic structure (i.e., clusters) and to quantify and visualize the pairwise genetic distances among populations, as in the genetic network such relationships are difficult to observe because of the high number of links. This analysis allows us to confirm relationships through a numerical bootstrap values. We used the R package "poppr" (Kamvar, Brooks, & Grünwald, 2015) to obtain bootstrap support with 10,000 replications.

2.3.5. Genetic connectivity

We built a weighted network based on Reynolds genetic distances (Reynolds, Weir, & Cockerham, 1983) to depict the overall distribution of genetic variation (i.e., genetic connectivity) among the populations with the software EDENetwork (Kivelä, Arnaud-Haond, & Saramäki, 2015). Each node of the network represented a single population with a size proportional to its betweenness centrality (i.e., the fraction of shortest paths between pairs of nodes that pass through a given node), while link length was proportional to the genetic distance between populations, and link thickness represented linkage strength. These network metrics provide information about the genetic relationships among populations. We tested for the statistical significance of the results by bootstrapping (i.e., resampling) the network with 85% and 50% of the samples and then by analysing the robustness of the betweenness centrality of the populations (bootstrap with 85% and 50% for 1,000 randomizations). We used Reynolds' distances instead of Nei's distances to statistically test the

network, since the software requires raw genotype data that can be computed with Reynolds' distances but not with Nei's. Nonetheless, a Nei's distances network was also computed with consistent results (Fig. S1a).

We conducted a modularity analysis with the R package *igraph* (Csardi & Nepusz, 2006). Modularity is a measure related to the structure of networks, and it is used to detect substructure inside them (i.e. division inside the network). Such substructures are called modules, and they show more links between nodes inside them than with nodes outside (i.e. in other modules). Modularity was estimated with the Walktrap algorithm (Pons & Latapy, 2005) which has been found to be accurate when detecting modules in small networks (less than 233 nodes) under different networks topologies (Yang, Algesheimer, & Tessone, 2016). Modularity was measured in the global network, and in networks created according to bird migratory routes. Groups in these networks were previously established in the IBD section.

3. Results

The number of alleles per locus varied from 5 to 43 (Table S2), with a total of 260 alleles. The missing data rate was low (average 3.015%, Table S2). The overall average frequency of null alleles per population was 0.104 ± 0.007 (see Table S1, null alleles by population). However, the null allele values were probably overestimated, because population size was limited for most of the locations (72 out of 74 populations contained < 30 individuals, which is the minimum advised number to estimate null alleles, Table S1).

3.1. Population genetic diversity

Across all populations, unbiased expected heterozygosity (uH_e) ranged from 0.42 to 0.71 (average of 0.59 ± 0.06), and rarefied allelic richness (Ar^*) varied from 2.29 to 3.74 (average of 2.81 ± 0.03). The frequency of private alleles ranged from 0 to 0.2 (Table S1). The inbreeding coefficient (F_{is}) was significant for 32 populations, 29 of which had positive values. In total, 45 out of 74 populations showed signals of Hardy-Weinberg disequilibrium (Table S1). Linkage disequilibrium was significant for most pairs of loci (Table S4).

Results showed a negative correlation of private alleles frequencies with the latitude ($\rho=-0.24$, $p\text{-value}<0.05$) and a positive one with the longitude ($\rho=0.25$, $p\text{-value}<0.05$) (private alleles frequency distribution and tendencies according to geographical location are shown in Fig.S2). Similarly, rarefied allelic richness (Ar^*) showed a negative correlation with the latitude ($\rho=-0.23$, $p\text{-value}<0.05$) and a positive one with the longitude ($\rho=0.59$, $p\text{-value}<0.001$) (rarefied alleles richness distribution and tendencies according to geographical location are shown in Fig.S3).

3.2. Genetic differentiation and population structure across the distribution range

The correlation between genetic distance and geographic distance was low but significant ($\rho=0.0001793$, $r^2=0.56$, $p\text{-value}<0.001$) across the entire distribution range (Fig. S4) suggesting the presence of IBD patterns ($p\text{-value}<0.0001$). Similarly, IBD was also detected when latitudinal and longitudinal distances were analysed ($p\text{-value}<0.0001$ in both cases). However, genetic distance was better explained by the distance in longitude (East-West axis, $\rho=0.0001697$, $r^2=0.51$, $p\text{-value}<0.001$) than by the distance in latitude (North-South axis, $\rho=0.0002974$, $r^2=0.20$, $p\text{-value}<0.001$) (Fig. S4). IBD was detected in migratory routes 1 and 4, while not in 2 and 3 (Table 1, Fig. S5).

Table 1. IBD patterns, relationships between genetic and geographic distances, and modularity of networks for all the populations and according to the main bird migratory routes which fly between Europe and Africa (Figure 1) (Significance of results; NS= $p\text{-value}>0.05$; *= $p\text{-value}<0.05$; **= $p\text{-value}<0.01$; ***= $p\text{-value}<0.001$). Populations ID in Table S1.

	Populations	N° of <i>P. lentiscus</i> individuals	IBD	Genetic distance vs geographic distance	Modularity
Migratory route 1	1-31	420	***	$r^2=0.47^{***}$	0.0017
Migratory route 2	32-49	243	NS	$r^2=0.05^{**}$	0.0023
Migratory route 3	50-60	143	NS	$r^2=0.01^{NS}$	0.01
Migratory route 4	61-74	251	**	$r^2=0.25^{***}$	0.11
Global	1-74	1057	***	$r^2=0.56^{***}$	0.048

AMOVA analysis showed that molecular variance was greater inside populations (87%) than among populations (13%). Genetic differentiation (F_{st}) across populations was 0.134 (0.118-0.204, $p\text{-value}<0.001$). Analysis of the hierarchical structure revealed that the highest genetic structure occurred at the level *western-eastern islands/mainland* ($F_{st}=0.073$, $p\text{-value}<0.0001$), followed by *population* ($F_{st}=0.051$, $p\text{-value}<0.0001$) and

geographic accidents ($F_{st}=0.022$, $p\text{-value}<0.0001$) levels. In contrast, the level *continents* did not show significant genetic structure ($p\text{-value}>0.05$) (Table S5).

All clustering analyses (STRUCTURE, DAPC, and UPGMA) concurred showing two main genetic clusters that broadly encompassed the eastern and western populations (Figure 2). The STRUCTURE and DAPC analyses showed the existence of two clusters ($K=2$) (Figure 2a and 2b; DeltaK values of the STRUCTURE analysis in Fig. S6), and the clustering depicted a western group that encompassed the populations from the Canary Islands to the Sicily strait and an eastern group that included the populations from the Cyrenaica Peninsula to Israel, with a mixed assignment to both clusters in populations located in the Balkans (populations 61 and 62) and Aegean Islands (63 and 64). Results of sPCA pointed out the existence of a significant global genetic structure ($p\text{-value}<0.001$) across populations with two main groups that mirror the east-west pattern described above (Figure 2c, Fig. S7). In concordance with all previous results, the UPGMA phylogram split all populations into two main groups that represented the eastern and western populations (Fig. S8).

We also explored the internal structure within each cluster to ensure that we were not underestimating population genetic structure due to the existence of hierarchical genetic structure within each cluster. Genetic differentiation (F_{st}) was 0.084 (0.076-0.095, $p\text{-value}<0.001$) in the western populations and 0.054 (0.041-0.077, $p\text{-value}<0.001$) in the eastern ones. The clustering analyses (STRUCTURE, DAPC, and UPGMA) did not detect any new groups within either the western or the eastern module.

The genetic network based on Reynold's genetic distance revealed two groups (Figure 3). The first included all populations from the western Mediterranean Basin (from the Strait of Gibraltar to the Strait of Sicily) and the Canary Islands, and the second contained eastern Mediterranean locations (Figure 3). Populations from the Balkan Peninsula (Alyhi and Amarousion, 61 and 62 in the network) and Aegean Islands (Amorgos and Lesbos islands, populations 63 and 64 in the network) were those with the highest betweenness centrality in the network, acting as links between the two modules. The Sicilian populations (58 and 59 in the network) and Linosa Island (60 in the network) also play an important role in connecting both groups.

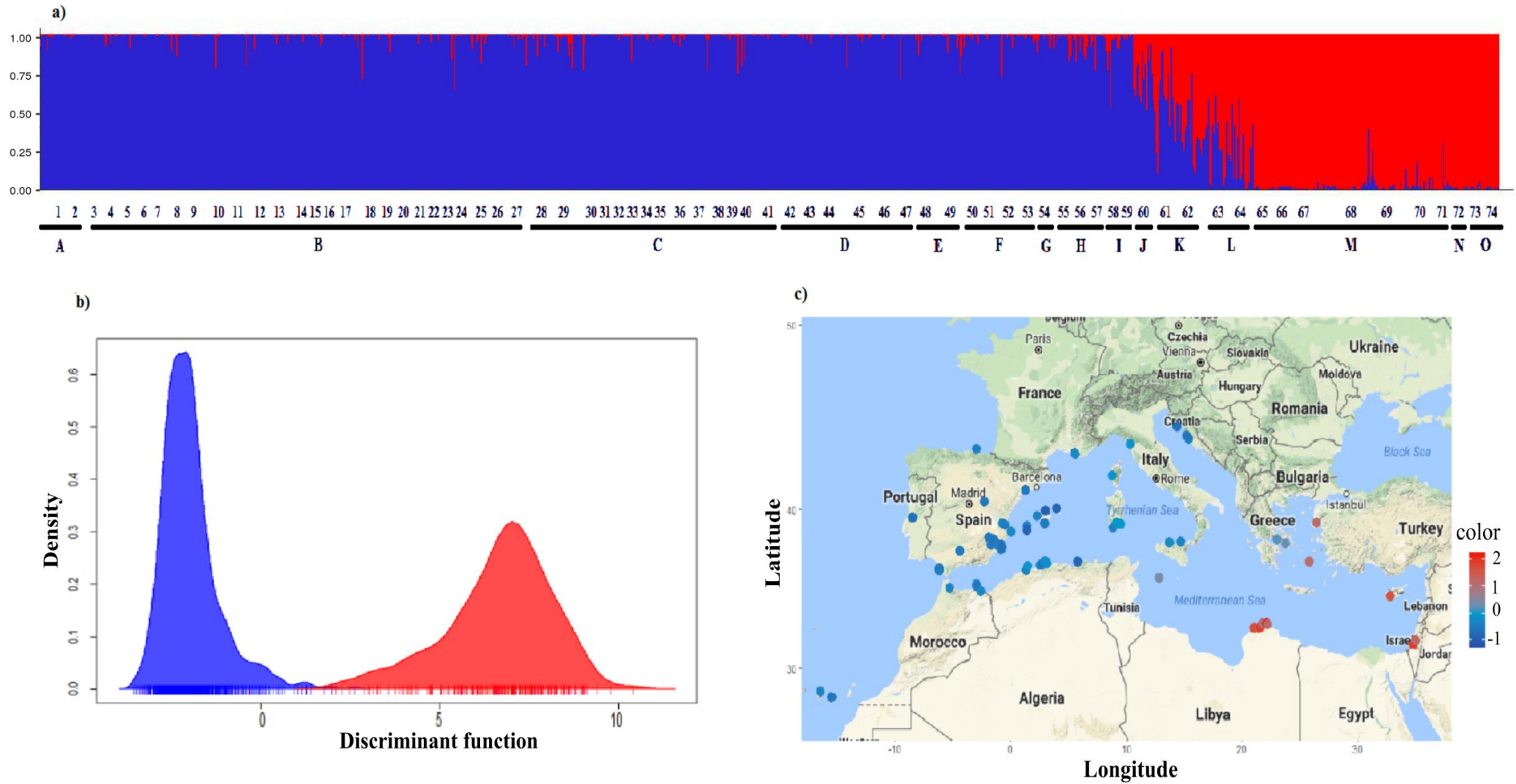


Figure 2. (a) Bar plot showing the STRUCTURE result (blue=group1; red=group2). Numbers refer to population IDs (Table S1). (A=Canary Islands, B=Iberian Peninsula, C=North of Africa, D=Balearic Islands, E=Southeast of France, F=Corsica and Sardinia, G=Italian Peninsula, H=Adriatic Sea, I=Sicily, J=Linosa Island, K=Balkans, L=Aegean Islands, M=Cyrenaican Peninsula, N=Cyprus, O=Middle East); (b) discriminant analysis of principal components (DAPC) for all populations (western populations in blue and eastern ones in red); (c) Spatial principal component analysis (sPCA) displayed according to eigenvalues of the axis 1. Dots depict populations which colours express the spatial distribution of the global genetic structure (Map from Map data © 2018 Google, ORION-ME).

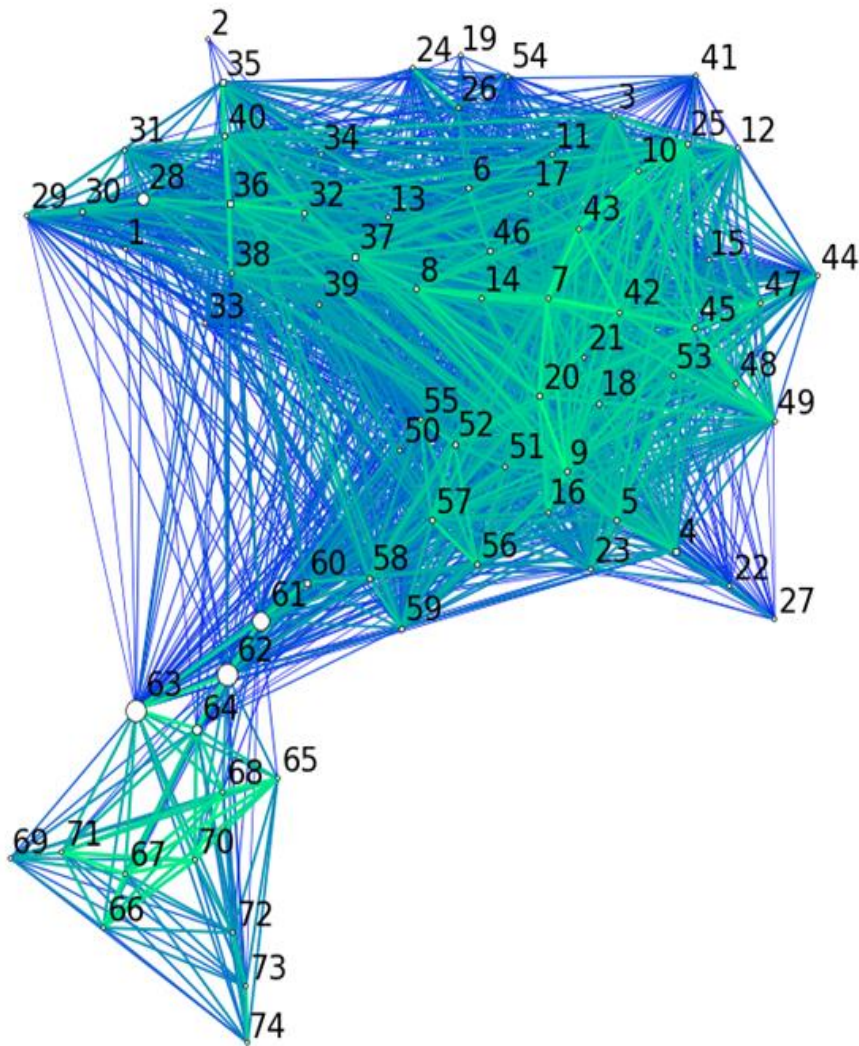


Figure 3. Genetic network obtained based on Reynolds' genetic distances among the sampled populations. The nodes represent the sampled populations, numbered by their IDs (see Table S1). The size of each dot is proportional to the betweenness centrality of the population in the network. The lengths of the links are proportional to the genetic distances between pairwise populations, and their widths represent linkage strength. The visualization of the network is based on the spring-charge-algorithm of Himmeli (see <http://www.finndiane.fi/software/Himmeli> for more details).

These results were confirmed by the betweenness centrality analyses, which demonstrated the importance of the mentioned populations in the topology of the network (populations 61, 63, 45, 60, 58 and 62 appeared most often in the top five nodes of the 50% bootstrap results, Figure 4). The network topology was robust under both 85% and 50% bootstrapping (results of 50% bootstrapping are shown in Fig. S1b). Modularity in the

network was low (0.048) and three modules were detected. The first module included populations from the western Mediterranean Basin (from the Strait of Gibraltar to the Strait of Sicily) and the Canary Islands, and the second one populations from Cyrenaican Peninsula, Cyprus, and Israel. The third module was composed by Aegean population (61-64). With regard to modularity according to bird migratory routes, networks showed a low modularity (Table 1, Fig. S9). Detected groups in routes 1-2-3 were not “real modules” as one of them contained most of the populations, while there was only one population in most of the other groups representing those less connected or with highest betweenness centrality in the networks (Fig. S9, Table S6). However, modularity was higher in route 4 (0.11), and the analysis found two modules. One module included populations from Cyrenaican Peninsula and the Aegean Region (61-71 in the network), and the other populations in Cyprus and Israel (72-74) (Fig. S9d).

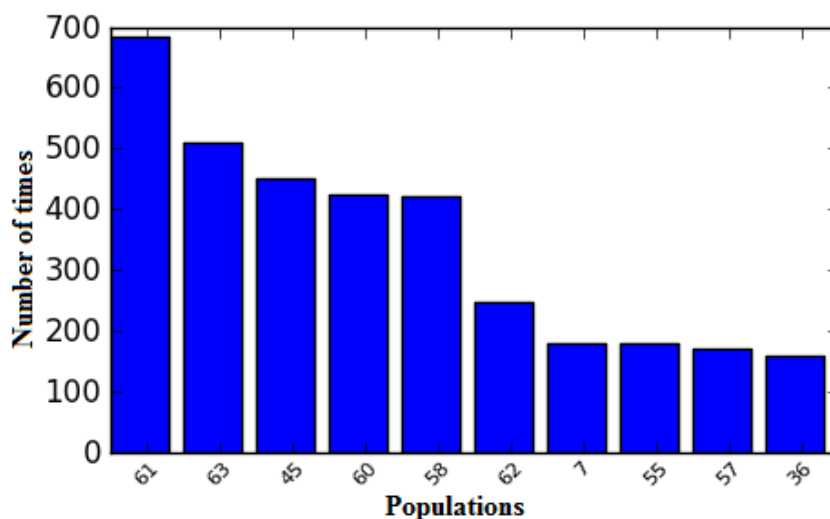


Figure 4. Number of times that a given population showed a high value of betweenness centrality (top 5) in the 50% bootstrap analysis (see population codes in Table S1) (61 and 62=Alyhi and Amarousion, Balkan Peninsula; 63=Amorgos Island, Aegean Islands; 45=Majorca, Balearic Islands; 60=Linosa Island; 58=Palermo, Sicily; 7=Tazona, Iberian Peninsula; 55 and 57=Neresine and Diklo, Adriatic Sea; and 36=Tipaza, North of Africa).

4. Discussion

Overall, our results show that the Mediterranean Sea does not act as a barrier to gene flow among *P. lentiscus* populations, but rather, evidence of connectivity among intercontinental populations was found. Additionally, SGS pattern across an East-West axis was confirmed. These findings support the hypothesis that migrant frugivorous birds mobilize enough seeds to connect distant populations on both sides of the Mediterranean Sea. We discuss next the main lines of evidence that support this general conclusion.

Clustering by several methods (STRUCTURE, DAPC, and UPGMA) concurred in finding two groups. The genetic differentiation among the populations was moderate ($F_{st}=0.134$) and was mainly captured when we grouped the populations according to *western-eastern islands/mainland* locations. Moreover, such molecular variation was much higher inside populations than among populations. These results are consistent with the general patterns observed in woody long-lived plant species, which generally present high levels of variation within populations ($uH_e=0.59\pm 0.06$) but low levels of differentiation among populations (Hamrick et al., 1992; Hamrick & Godt, 1996). Previous studies assessing the genetic diversity distribution of *P. lentiscus* based on random amplification of polymorphic DNA (RAPD) described a high genetic similarity among populations across the Mediterranean Basin (from Spain, Tunisia and Israel) (Barazani, Dudai, & Golan-Goldhirsh, 2003; Nahum, Inbar, Ne'eman, & Ben-Shlomo, 2008). These authors have already suggested that migrant birds could be acting as LDD vectors (Nahum et al., 2008). In contrast, Werner et al., (2002b) described strong differences between populations from the Iberian Peninsula and the North of Africa. Our findings are not consistent with those of any of these previous studies, as we detected strong differences between eastern and western populations in the Mediterranean Basin, and we found genetic similarity in populations from the Iberian Peninsula and the North of Africa. These contrasting results in the study of genetic diversity variation in plant species highlight the importance of including populations from the entire distribution range (Medrano & Herrera, 2008; Hou & Lou, 2011), as otherwise, conclusions can be obscured. As expected, SGS was found to follow a pattern along the East-West axis, because these populations are not connected by migration

routes . IBD was higher in the East-West axis, than in the North-South, although explained a low proportion of the genetic variation (Linear distance: $r^2=0.56$; Longitudinal distance: $r^2=0.51$), partly because other factors, such as contrasting local demographic dynamics, might obscure the effect of the geographic distance. Local conditions can lead to selective pressure in populations. For instance, *P. lentiscus* fruits have been widely used by humans (Rouskas, 1996; Zamora, 1997), and hence, females trees may have been favoured in human-managed landscapes (Verdú & García-Fayos, 1998; Barradas, & Correia, 1999). In addition, longitudinal movements of migratory birds have been detected (Fiedler & Davidson, 2012).

Furthermore, higher connectivity is present in the genetic network among links located within migration routes. The genetic network demonstrated the importance of the Aegean populations as links between both detected modules, and in fact, these are the only populations that directly connect the western and eastern distributions of this species to the main migratory routes (Figure 1). Modularity of the network was low (0.048) and three modules were detected. Such modules coincide with the western and eastern group detected in clustering analysis, and the third group identified the population with higher betweenness centrality in the network (the Aegean populations).

These populations also showed the highest levels of genetic diversity (uH_e), which could be explained by their role as links between western and eastern locations (Figure 3). Aside from this result, we did not observe any spatial patterns in the genetic diversity values among populations, in contrast to the genetic diversity patterns known from other tree and shrub species in the Mediterranean Basin, which tend to increase from West to East (Conord, Gurevitch, & Fady, 2012) and from North to South (Nieto-Feliner, 2014). However, we found that private alleles frequencies and rarefied allelic richness (Ar^*) decreased with the latitude. Such trends support the idea that birds are mobilizing seeds southwards so that these populations have higher allele richness. We also detected an increase in private alleles frequencies and allelic richness according to a longitudinal eastward gradient. These results are congruent with the migratory routes as birds can mobilize seed from populations in the West toward the East but not in the opposite way (Figure 1). Thus, eastern locations present their

local alleles and some from the western ones. With regard to the genetic patterns of each module, the western one showed much higher connectivity (Figure 3). This result is reasonable because the density of migratory routes is higher in the western Mediterranean, and the distribution range of *P. lentiscus* is more extensive (Figure 1). The Balearic Islands (42-47) seem to act as a hub by interconnecting the western populations, which could be explained by their positions with respect to the migratory flows. These observations were confirmed when we analysed modularity according to the main four migratory routes. Modularity was low in the three western routes and the analysis did not detect any module which highlights the high connectivity among populations inside the same route. However, populations in the eastern migratory routes showed higher modularity (between 10 and 100 times more than the western ones), and two modules were detected in the eastern migratory route. Such modules mirror the two main migratory routes existing in the East of the Mediterranean Basin (Figure 1). IBD distance according to migratory routes was found in the West and East extreme of *P. lentiscus* distribution (routes 1 and 4) while not in the other two (routes 2 and 3). The existence of geographical isolation in route 4 can be due to the migratory patterns of *E. rubecula* and *S. atricapilla* (the main consumers of *P. lentiscus* fruits) (Busse, Zaniewicz, & Cofta, 2014 and references therein). On the one hand, most of the birds which migrate in the eastern flyway travel from Asia and the North of Europe, and hence, there is a poor overlap with *P. lentiscus* populations. On the other hand, we have analysed together all populations in the East of the Mediterranean Basin because of the limiting number of populations and there are at least two. Regarding the route 1, it could be attributed to the Canary Islands populations, but we also analysed data omitting these populations and results were consistent (results not shown). Bird migration patterns depend on food supplies, weather conditions and the specific characteristic of every single species (Newton, 2007). Migratory routes that we are considering have been established according to general trends of migration, and hence, species dispersing seeds of *P. lentiscus* could follow specific and particular patterns of migration. For instance, according to data of Migra programme from SEO/Birdlife Spain much more individuals of *E. rubecula* and *S. atricapilla* ringed in Spain have been recapture in Morocco (route 1) than in Algeria (route 2) (58 vs 218) (all available data until 2010) (SEO/Birdlife, 2012). Therefore, studies about species specific

pattern of migration of frugivorous birds are needed in order to fully understand how bird movement can impact genetic diversity of fleshy-fruited plants. Although our results suggest LDD events carried out by frugivores, other factors might impact the observed genetic patterns, such as the following:

- i) Methodological issues. The majority of populations showed Hardy-Weinberg and linkage disequilibria (Tables S1 and S4), which could affect the clustering analysis results. This fact could be related to the small sample size and the existence of rare alleles (i.e., those with low frequency in the populations). Similarly, demographic processes (such as genetic drift and gene flow) could trigger both linkage and Hardy-Weinberg disequilibria. Genetic diversity values might also be biased by the small sample size, so we have used unbiased heterozygosity. Note that our results (uH_e average 0.59 ± 0.06) are high, as expected in woody plant species with outcrossing breeding and medium to large distribution ranges (Hamrick, Godt, & Sherman-Broyles, 1992; Hamrick & Godt, 1996; Nybon, 2004). Previous studies have reported similar levels of genetic diversity for *P. lentiscus* ($H_e=0.66$, Albaladejo, Guzmán, González-Martínez, & Aparicio, 2012; $H_e=0.67-0.73$, Nora, Albaladejo, & Aparicio, 2015; $H_e=0.62-0.63$, Martínez-López et al., 2017) and other fleshy-fruited species, such as *Myrtus communis* (between 0.68 and 0.72, Nora et al., 2015). Both species currently occur in fragmented populations across managed landscapes; they appear to be able to tolerate disturbances and maintain their genetic diversity. Accordingly, previous studies assessing the impact of landscape configuration on the genetic diversity patterns of *P. lentiscus* have concluded that this species is not genetically affected by fragmentation (Aparicio, Hampe, Fernández-Carrillo, & Albaladejo, 2012; Nora et al., 2015).
- ii) Pollen flow. Some studies have suggested that the genetic structure of plants calculated through molecular markers is affected by only the mating system (selfing vs outcrossing), since any seed dispersal signal could be obscured by the movement of pollen (Dumunil et al., 2007). *P. lentiscus* is a wind-pollinated shrub which flowering occurs from March to May (Keynan et al., 1987; Milla, Castro-Díez, & Montserrat-Martí, 2010). Thus, winds direction would condition pollen flow directionality. In the case of the Mediterranean Basin, prevailing winds flow from North-West during flowering months of the species (Soukissian,

Karathanasi, Axaopoulos, Voukouvalas, & Kotroni, 2018). However, angular variance is much greater in the Western Mediterranean than in the Eastern Mediterranean (Soukissian et al., 2018). As winds flow from North to South the influence of pollen in the observed genetic structure patterns of the species can not be discarded. However, some of our results could not be explained as a result of pollen flow. For instance, we have reported some genetic connectivity among Greece and Libyan populations, such connectivity could not occurred by pollen according to the described wind pattern in the area (N-W wind direction and low angular variance). Furthermore, long-distance pollen dispersal for *P. lentiscus* seems unlikely according to the existing information about the species (lack of adaptations to flotation of the grain, Albaladejo, González-Martínez, Heuertz, Vendramin, & Aparicio, 2009; studies to date have found that most pollination events occur under 500 m, Albaladejo et al., 2012), and the pollination distances reported in other wind-pollinated woody plants (under 10 kilometres, Souza, 2018 and references therein). Previous studies of the genetic structure of *P. lentiscus* found that seed dispersal was responsible for local genetic structure, while pollen connected populations at a regional scale (Parejo-Farnés et al., 2017). This pattern has also been found in other wind-pollinated species (Sork, Smouse, Grivet, & Scofield, 2015). Nevertheless, the local population density and mobility of frugivorous birds increase considerably during migration, and thus, so would the capability of dispersing seeds at longer distances. Hence, it is difficult to track such seed dispersal events through local or regional experimental designs. In fact, recent studies have demonstrated that migratory birds can transport seeds for thousands of kilometres (from the European continent to the Canary Islands, Viana et al., 2016). With regard to landscape characteristics, pollen dispersal in *P. lentiscus* is more affected than seed dispersal by fragmentation (Parejo-Farnés et al., 2017). Thus, its contribution to populations genetic connectivity would be expected to be lower than seed dispersal in patchy and discontinuous species distributions as the case of *P. lentiscus* in the eastern of the Mediterranean Basin. This difference could be caused by the tolerance of Mediterranean bird communities (the main dispersers of *P. lentiscus*) to fragmentation (González-Varo, 2010). Therefore, seed dispersal could have had greater importance in the dispersal of the species across the Mediterranean Basin, which has been perturbed by humans for thousands of years (Blondel & Aronson, 1999). Our

hypothesis of long-distance seed dispersal of *P. lentiscus* through migratory birds is supported by both previous information and the results obtained in this study; notwithstanding, future studies should include chloroplast markers (i.e. maternally inherited) to undoubtedly discern among seed and pollen dispersal contribution to gene flow.

- iii) Historical migration routes (biogeography). *P. lentiscus* appeared in the Tertiary, when the current Mediterranean Basin had a tropical climate, and is assumed to have evolved very little (Verdú & García-Fayos, 2002 and references therein). The East-West split that we detected has been commonly found in studies assessing the phylogeographic patterns of plants in the Mediterranean Basin (Nieto-Feliner, 2014). For example, clustering analysis and genetic networks concurred in finding Aegean population as pivotal among the eastern and western groups. However, these results could be caused by different processes as genetic connectivity inferred from genetic networks is different from the genetic connectivity detected through clustering analysis. Furthermore, private alleles frequencies and allelic richness distributions could be also mirroring *P. lentiscus* recolonization movements northwards after glaciations. Consequently, we cannot disregard the influence of past events on our results, and future phylogeographic studies should address these issues to fully understand the present *P. lentiscus* genetic structure distribution.
- iv) Frequent transportation across the Mediterranean Sea by ancestral cultures. This species has been widely used by many civilizations across the Mediterranean Basin for millennia (Piluzza, Viridis, Serralutzu, & Bullitta, 2015 and references therein; Alonso, Jordà, Rovira, & Reyes, 2016 and references therein), so humans may also have affected the current genetic diversity patterns of the species.

Our results highlight the role of migratory birds acting as vectors between distant fleshy-fruited shrub populations. As a consequence, every driver affecting bird migratory patterns would potentially impact the demographic and genetic patterns of plant species dispersed by these frugivores. Recent studies (Bairlein, 2016) have shown that climate change, fragmentation and poaching present threats to European migratory birds. On the one hand, a change in the fruiting phenology of *P. lentiscus* or a temporal change in bird migration behaviour (e.g., starting migration sooner or later) because

of climate change may cause a decoupling of this bird-shrub interaction (Zalakevicius, Bartkeviciene, Raudonikis, & Janulaitis, 2006; Thackeray et al., 2010). On the other hand, if the climate becomes warmer, birds could stop needing to migrate in winter, or they could travel shorter distances, which has, in fact, already been reported (WWF Australia, 2006; Pulido & Berthold, 2010). For instance, recent studies have described a reduction in migrant populations of *E. rubecula* in some areas of the Iberian Peninsula (one of the main seed dispersers of *P. lentiscus*) which was attributed to changes in the migratory behaviour due to global warming (Tellería, 2014). These changes may cause a disruption in the observed genetic connectivity. Therefore, plant conservation strategies ought to consider both the species itself and its dispersers.

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Supporting information

Fig. S1 a) Network based on Nei's genetic distances. b) Network based on Reynolds genetic distances with a bootstrap of 50%.

Fig. S2 Private alleles frequency distribution in *P. lentiscus* populations along the Mediterranean Basin

Fig. S3 Rarefied allelic richness (Ar^*) distribution in *P. lentiscus* populations along the Mediterranean Basin

Fig. S4 Isolation by distance graph.

Fig. S5 Isolation by distance graphs according to bird migratory routes.

Fig. S6 DeltaK values for the STRUCTURE analysis.

Fig. S7 sPCA eigenvalues.

Fig. S8 UPGMA phylogram.

Fig. S9 Networks based on Reynolds genetic distance according to bird migratory routes.

Table S1 Populations information and genetic parameters.

Table S2 Information about microsatellites

Table S3 Levels used to the population hierarchical structure analyses.

Table S4 Linkage disequilibrium for each pair of locus across all populations.

Table S5 Results of the hierarchical F_{st} analysis.

Table S6 Modules detected by the modularity analysis.

Table S7 Plant collectors.

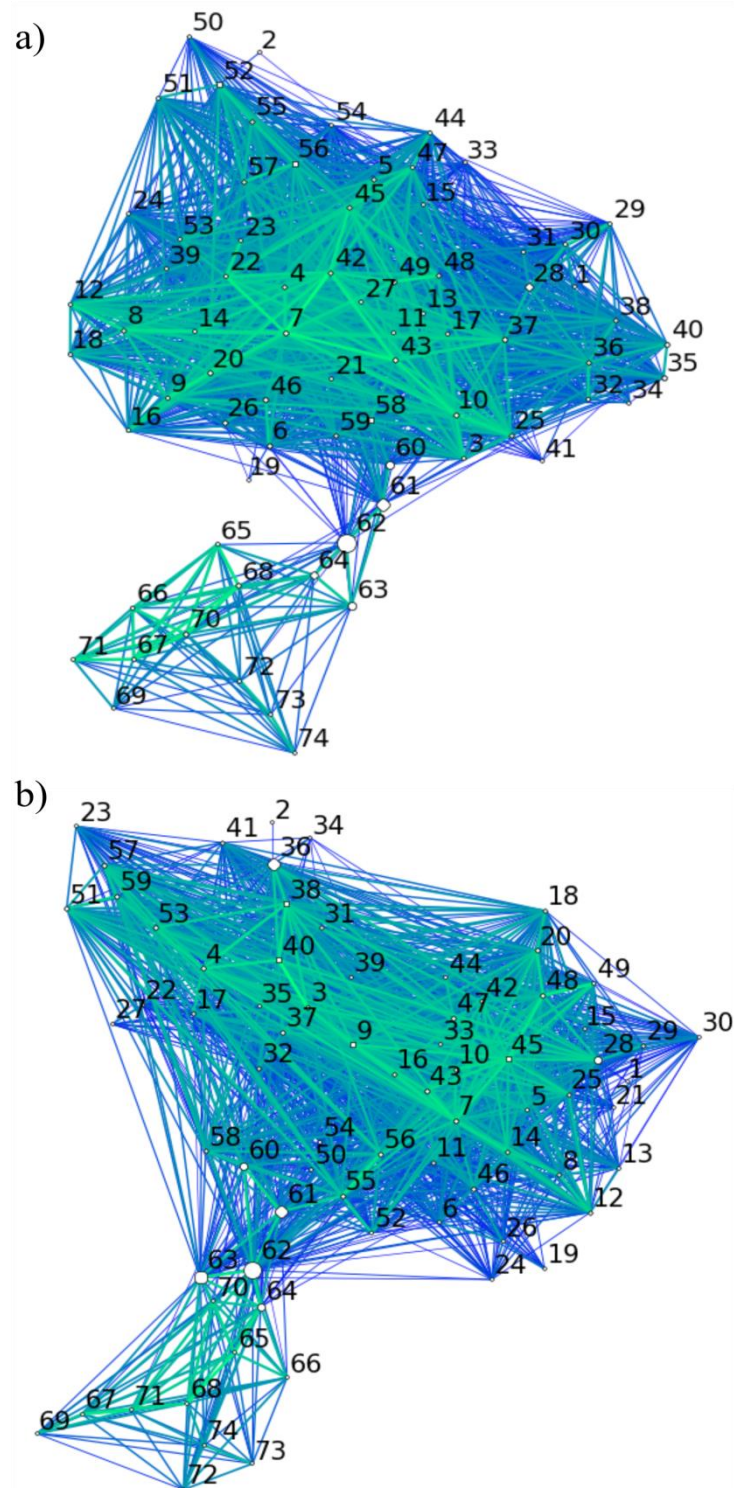


Fig. S1. a) Network based on Nei's genetic distance. b) Network based on Reynolds genetic distance with a bootstrap of 50%. Nodes represent sampled populations numbered by their ID. The size of the dot is proportional to the betweenness centrality of the population in the network. The length of the links is proportional to the genetic distance among pairwise populations and their width represent linkage strength. The visualization of the networks is based on the spring-charge-algorithm of Himmeli (see <http://www.finndiane.fi/software/Himmeli> for more details).

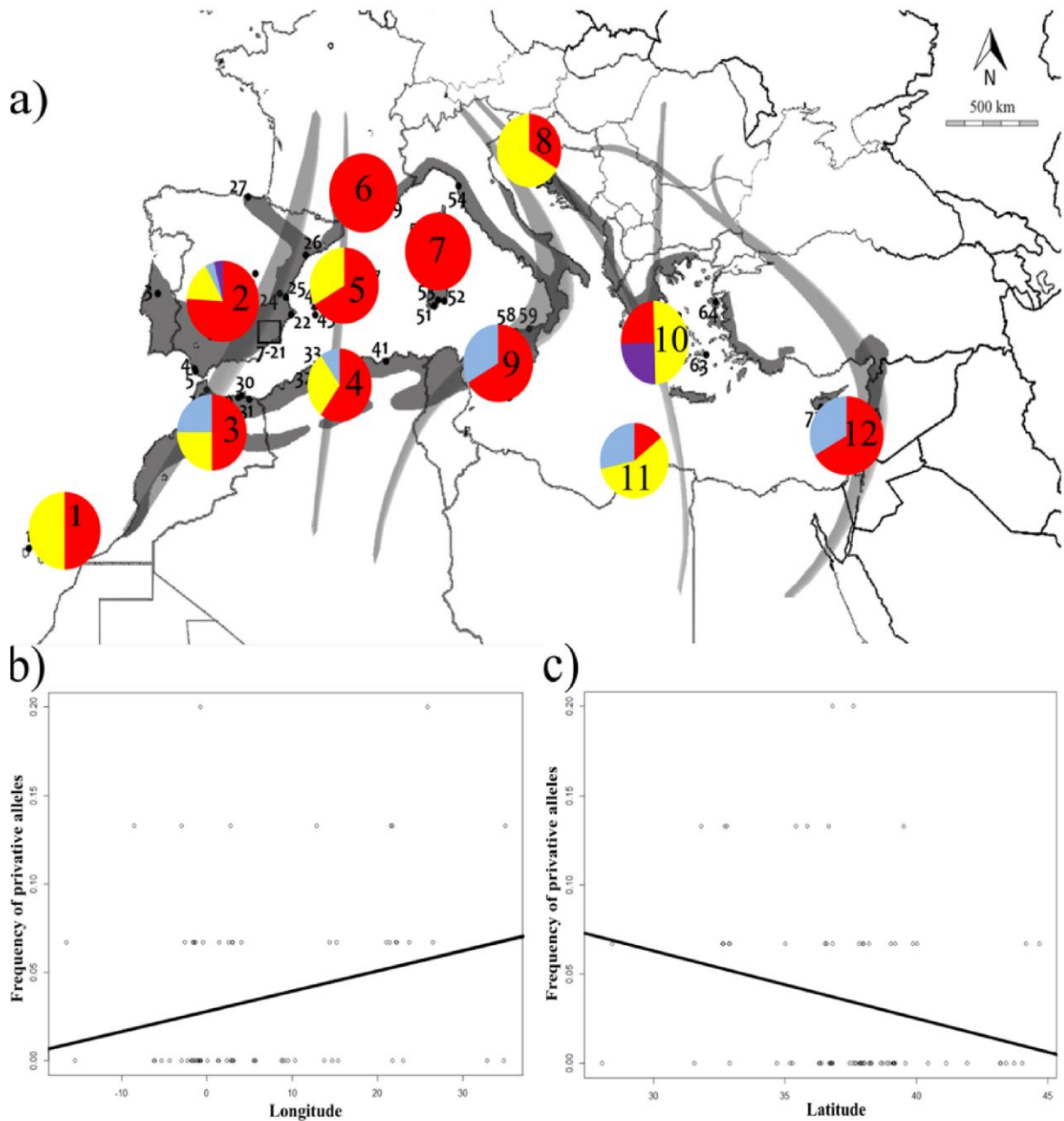


Fig. S2. Privative alleles frequency distribution in *P. lentiscus* populations along the Mediterranean Basin. (a) Circle graphs represent the percentage of populations in the area in each one of the categories considered according to privative alleles frequency in each population (Table S1) (red=0; yellow=0.067; blue=0.133; purple=0.2). Numbers inside circle graphs refer to the groups in which populations has been represented in the map (1=1-2; 2=3-27; 3=28-31; 4=32-41; 5=42-47; 6=48-49; 7=50-54; 8=55-57; 9=58-60; 10=61-64; 11=65-71; 12=72-74; population IDs in Table S1). (b) Relationship between the longitude of populations and their frequency of privative alleles. (c) Relationship between the latitude of populations and their frequency of privative alleles.

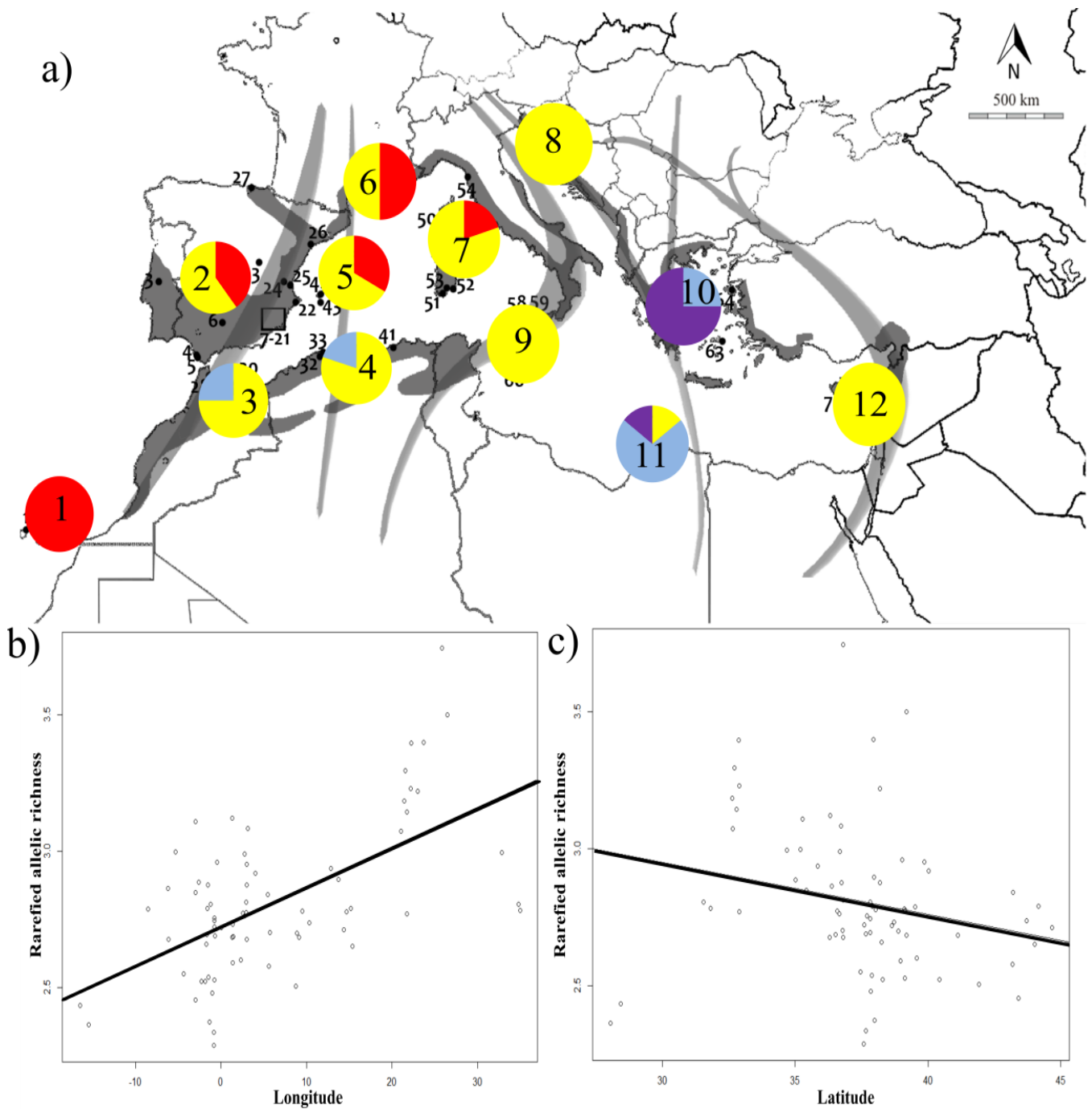


Fig. S3. Rarefied allelic richness (Ar^*) distribution in *P. lentiscus* populations along the Mediterranean Basin. (a) Circle graphs represent the percentage of populations in the area in each one of the categories considered according to the rarefied allelic richness in each population (Table S1) (red=2.287-2.651; yellow=2.652-3.016; blue=3.017-3.380; purple=3.381-3.745). Numbers inside circle graphs refer to the groups in which populations has been represented in the map (1=1-2; 2=3-27; 3=28-31; 4=32-41; 5=42-47; 6=48-49; 7=50-54; 8=55-57; 9=58-60; 10=61-64; 11=65-71; 12=72-74; population IDs in Table S1). (b) Relationship between the longitude of populations and their rarefied allelic richness. (c) Relationship between the latitude of populations and their rarefied allelic richness.

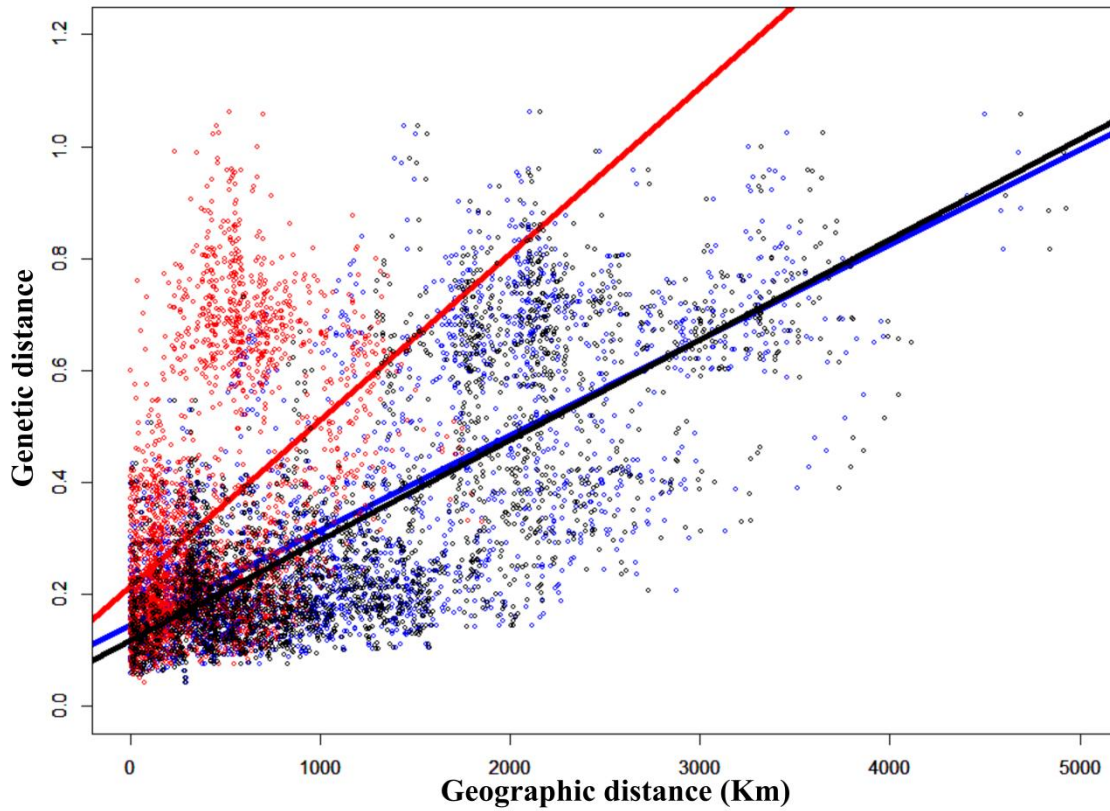


Fig. S4. Isolation by distance patterns in *P. lentiscus* populations (Nei's genetic distance vs geographic distance in km). Black dots and black regression line=Linear geographic distance-Genetic distance ($y=0.1174+0.0001793x$, $r^2=0.56$, $p\text{-value}<0.001$). Red dots and red regression line=Latitudinal geographic distance-Genetic distance ($y=0.2144+0.0002974x$, $r^2=0.20$, $p\text{-value}<0.001$). Blue dots and blue regression line=Longitudinal geographic distance-Genetic distance ($y=0.1453+0.0001697x$, $r^2=0.51$, $p\text{-value}<0.001$).

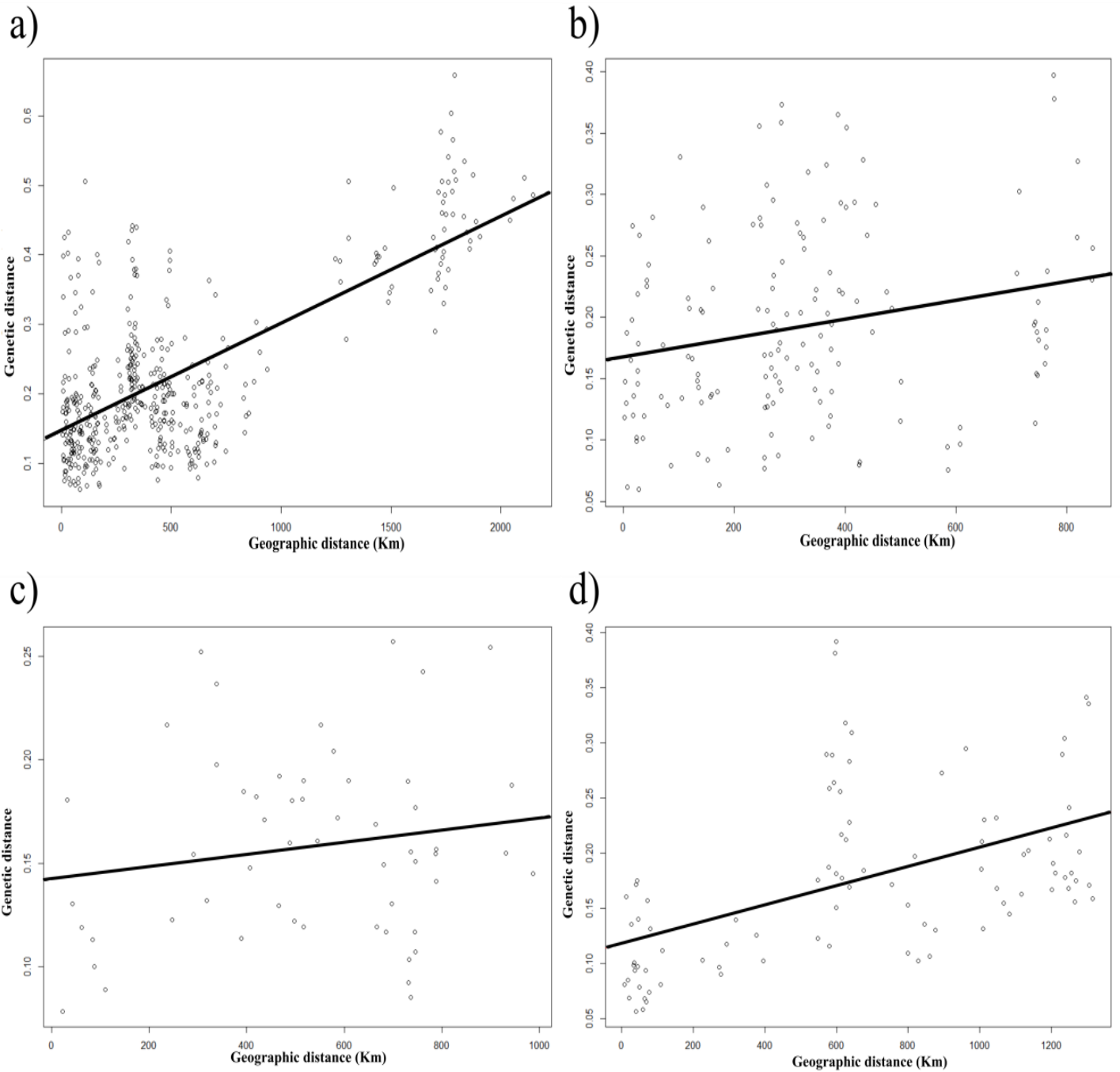


Fig. S5. Isolation by distance patterns in *P. lentiscus* populations according to bird migratory routes (Nei's genetic distance vs geographic distance in km); (a) Route 1 (populations 1-31) ($y=0.1450+0.000155x$, $r^2=0.47$, $p\text{-value}<0.001$); (b) Route 2 (populations 32-49) ($y=0.1678+0.0000760x$, $r^2=0.05$, $p\text{-value}<0.01$); (c) Route 3 (populations 50-60) ($y=0.1426+0.00002923x$, $r^2=0.01$, $p\text{-value}>0.05$); (d) Route 4 (populations 61-74) ($y=0.1178+0.0000872x$, $r^2=0.25$, $p\text{-value}<0.001$).

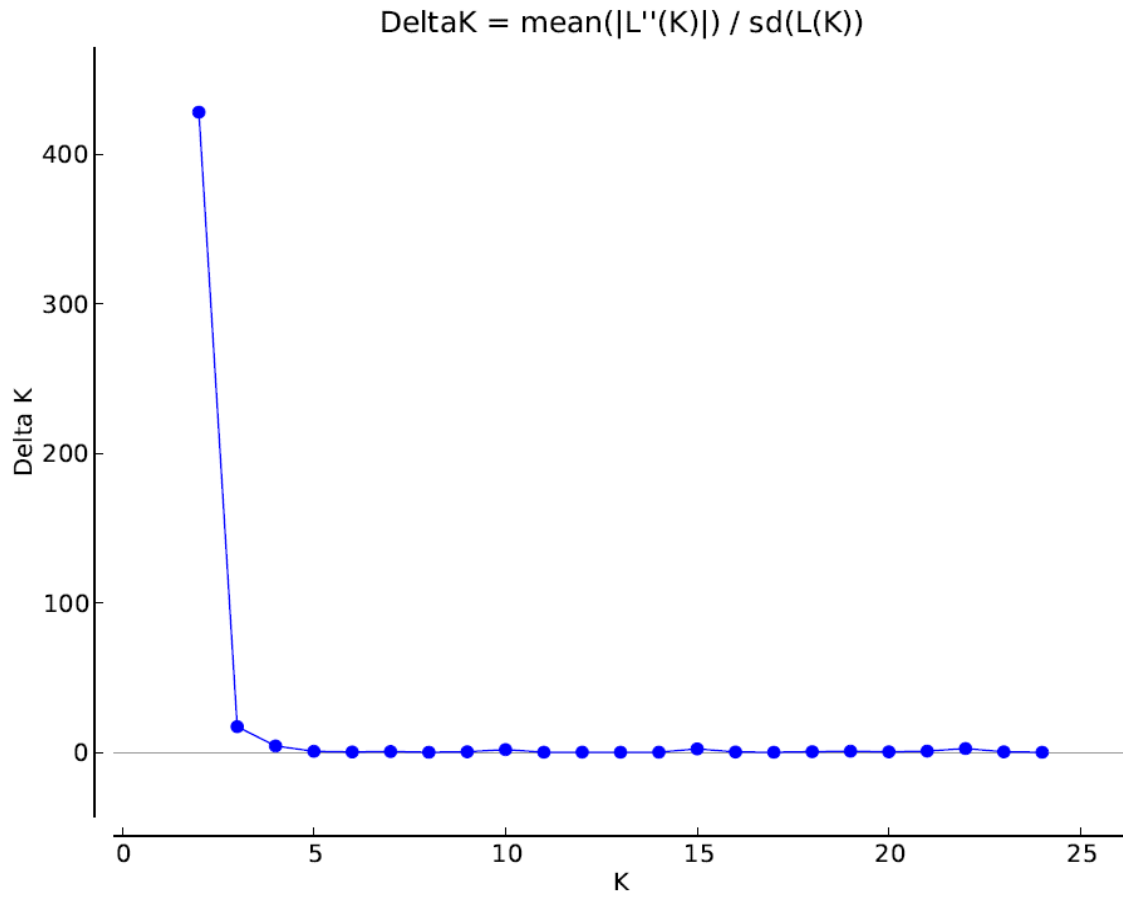


Fig. S6. DeltaK values for the STRUCTURE analysis. This value is used to select the optimal number of groups in the STRUCTURE analysis (i.e. K). The best K value is the one with higher Delta K, in our case the best value is two.

Eigenvalues of sPCA

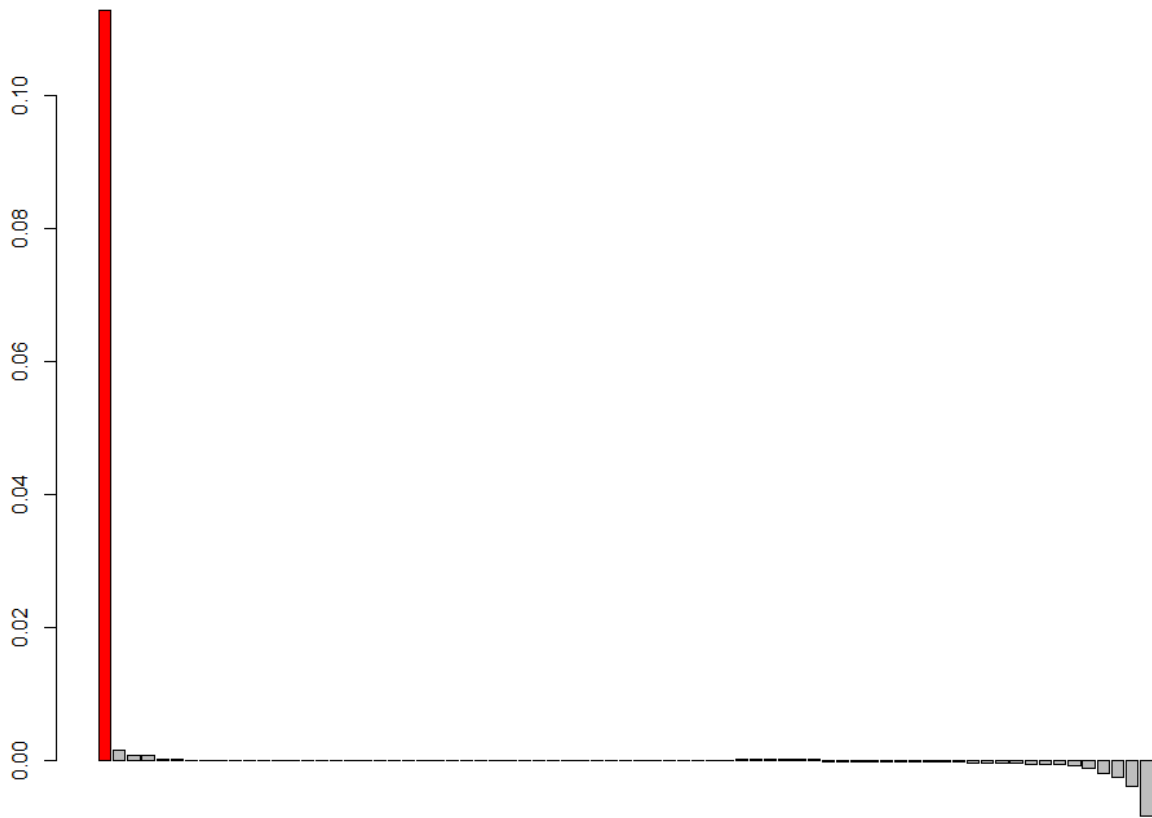


Fig. S7. sPCA eigenvalues obtained from the alleles frequencies. Positive eigenvalues (on the left) indicate global structures, while negative ones (on the right) represent the local structures. In our case, there is only one single global structure for all populations. Red colour points out the retained axis.

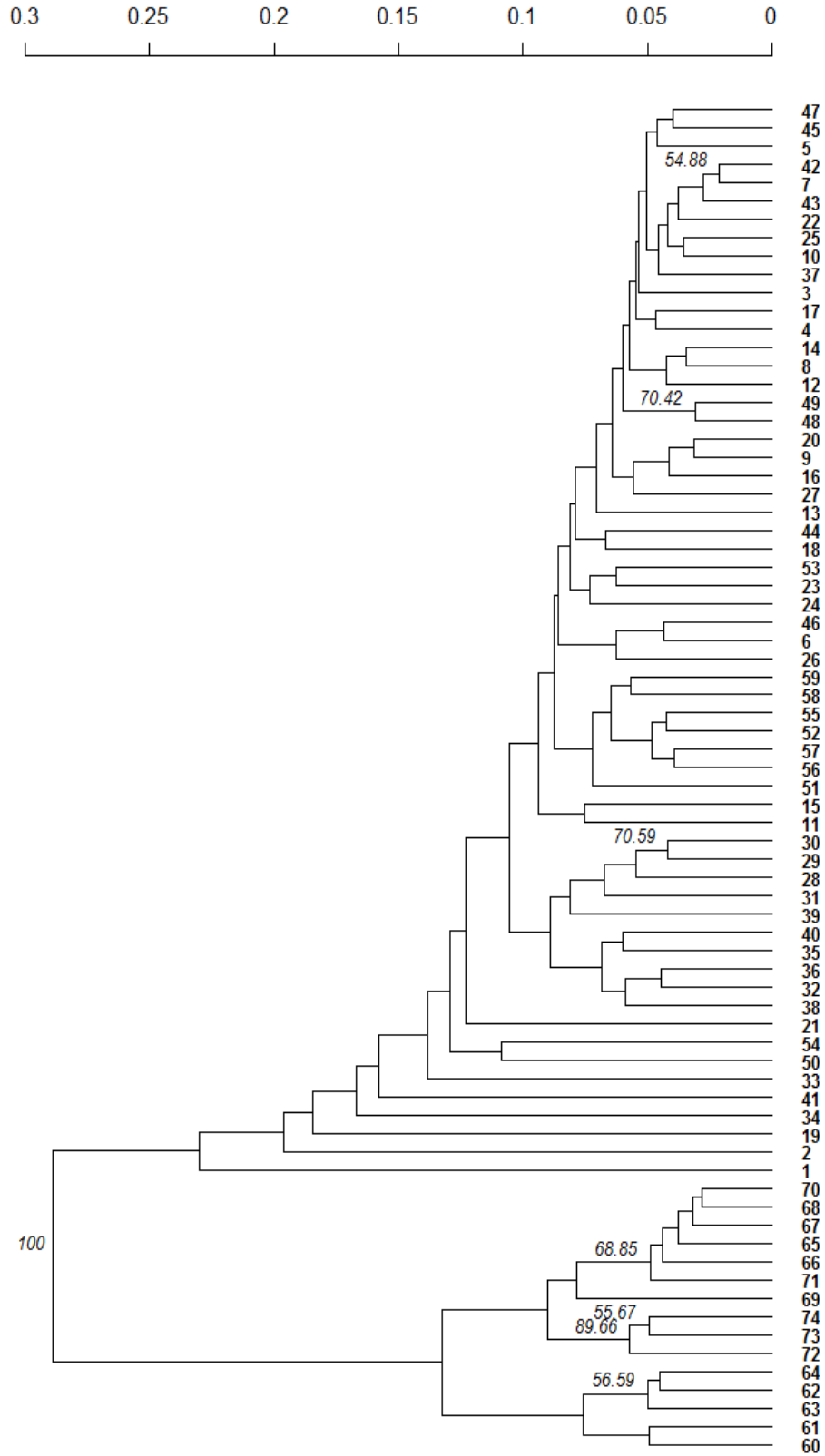


Fig. S8. UPGMA phylogram for population separation according to Nei genetic distance. Only bootstrap over 50% are shown. Populations are represented with the Pop ID (Table S1).

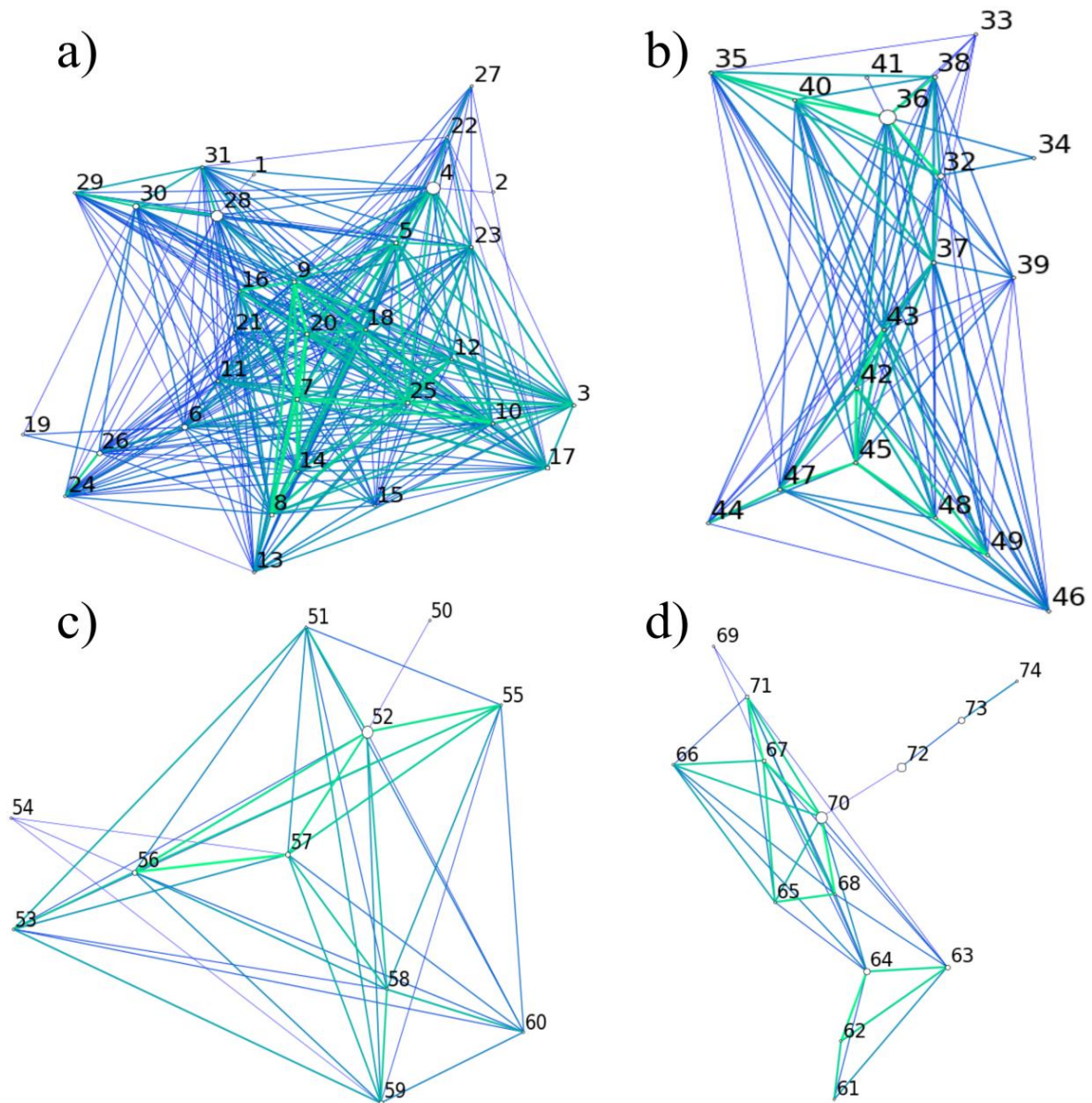


Fig. S9. Networks based on Reynolds genetic distance according to bird migratory routes. The visualization of the networks is based on the spring-force algorithm of Himmeli (see <http://www.finndiane.fi/software/Himmeli> for more details). Populations IDs in Table S1; (a) Results of route one (populations 1-31); (b) Results of route two (populations 32-49); (c) Results of route three (populations 50-60); (d) Results of route four (populations 61-74).

Table S1. Population data and population genetic parameters for each sample site. Populations are listed geographically sorted from West to East. Coordinates are provided in WGS84 system. (N=number of samples; Ho=observed heterozygosity; He=expected heterozygosity; uHe=unbiased heterozygosity; Ar=allelic richness; Ar*=rarefied allelic richness; Fis=inbreeding coefficient (significance with confidence interval of 95% *); HW=Hardy-Weinberg equilibrium test (^{NS}=p-value>0.05; *=p-value<0.05; **=p-value<0.01;***=p-value<0.001); Standard errors (SD) are shown for all parameters.

Pop ID	Pop location	Pop name	N	Ho (±SD)	He (±SD)	Uhe (±SD)	Ar* (rarefied)	Ar	Fis	HW	Average Null alleles(±SD)		Privative alleles (±SD)	
1	Tenerife (Canary Islands)	Canary Islands1	19	0.543 0.070	0.520 0.058	0.534 0.060	2.436	2.674	-0.05	0***	0.040	0.012	0.067	0.067
2	Gran Canaria (Canary Islands)	Canary Islands2	6	0.27 0.071	0.459 0.054	0.523 0.063	2.363	2.106	0.382	0.028*	NA	NA	0.000	0.000
3	Torres Novas (Portugal)	Iberian Peninsula1	13	0.426 0.060	0.570 0.054	0.595 0.057	2.788	3.042	0.272*	0.001***	0.161	0.053	0.133	0.091
4	Chiclana (Cádiz,Andalusia, Spain)	Iberian Peninsula2	13	0.489 0.056	0.583 0.055	0.609 0.058	2.864	3.206	0.171*	0.172 ^{NS}	0.178	0.038	0.000	0.000
5	Conil (Cádiz,Andalusia, Spain)	Iberian Peninsula3	13	0.464 0.056	0.590 0.047	0.616 0.049	2.676	3.042	0.235*	0.065 ^{NS}	0.189	0.046	0.000	0.000
6	Córdoba (Andalusia, Spain)	Iberian Peninsula4	11	0.429 0.058	0.502 0.071	0.529 0.076	2.550	2.750	0.098	0.894 ^{NS}	0.212	0.041	0.000	0.000
7	Tazona (Region of Murcia, Spain)	Iberian Peninsula5	15	0.441 0.068	0.528 0.069	0.547 0.072	2.523	2.937	0.222	0.284 ^{NS}	0.139	0.027	0.000	0.000
8	Viveros (Region of Murcia, Spain)	Iberian Peninsula6	14	0.429 0.055	0.548 0.062	0.569 0.065	2.660	3.013	0.194*	0.024*	0.103	0.020	0.000	0.000
9	Cieza (Region of Murcia, Spain)	Iberian Peninsula7	14	0.423 0.055	0.588 0.060	0.61 0.063	2.876	3.161	0.289*	0***	0.140	0.022	0.067	0.067

Pop ID	Pop location	Pop name	N	Ho (\pm SD)	He (\pm SD)	Uhe (\pm SD)	Ar* (rarefied)	Ar	Fis	HW	Average Null alleles(\pm SD)	Privative alleles (\pm SD)
10	Lorca(Region of Murcia, Spain)	Iberian Peninsula8	14	0.407 0.054	0.550 0.063	0.57 0.065	2.695	3.027	0.289*	0.028*	0.098 0.016	0.067 0.067
11	El Berro (Region of Murcia, Spain)	Iberian Peninsula9	14	0.499 0.071	0.527 0.061	0.547 0.064	2.538	2.855	0.1	0.497 ^{NS}	0.141 0.024	0.000 0.000
12	Casas Nuevas (Region of Murcia, Spain)	Iberian Peninsula10	16	0.415 0.054	0.538 0.060	0.557 0.062	2.791	2.989	0.238*	0.016*	0.201 0.042	0.000 0.000
13	Los Guillemos (Region of Murcia, Spain)	Iberian Peninsula11	16	0.406 0.056	0.463 0.060	0.479 0.062	2.373	2.606	0.102	0.347 ^{NS}	0.192 0.035	0.067 0.067
14	La Murta (Region of Murcia, Spain)	Iberian Peninsula12	13	0.415 0.056	0.554 0.057	0.576 0.059	2.807	3.168	0.25*	0***	0.086 0.017	0.000 0.000
15	La Tercia (Region of Murcia, Spain)	Iberian Peninsula13	13	0.422 0.051	0.485 0.059	0.505 0.062	2.480	2.694	0.097	0.243 ^{NS}	0.087 0.029	0.000 0.000
16	San Pedro (Region of Murcia, Spain)	Iberian Peninsula14	12	0.483 0.065	0.521 0.052	0.545 0.054	2.746	2.906	0.092	0.097 ^{NS}	0.172 0.032	0.000 0.000
17	Lo Poyo (Region of Murcia, Spain)	Iberian Peninsula15	12	0.439 0.064	0.467 0.067	0.489 0.070	2.337	2.636	0.047	0.566 ^{NS}	0.145 0.028	0.000 0.000
18	Calblanque (Region of Murcia, Spain)	Iberian Peninsula16	15	0.421 0.035	0.576 0.038	0.596 0.040	2.722	3.015	0.264*	0.001***	0.174 0.020	0.200 0.107
19	Portman (Region of Murcia, Spain)	Iberian Peninsula17	5	0.407 0.075	0.377 0.057	0.422 0.063	2.287	2.136	- 0.061*	1 ^{NS}	NA NA	0.000 0.000
20	Isla del Barón (Region of Murcia, Spain)	Iberian Peninsula18 (island)	15	0.443 0.063	0.568 0.056	0.589 0.058	2.755	3.152	0.232*	0***	0.170 0.031	0.000 0.000
21	Cala del Pino (Region of Murcia, Spain)	Iberian Peninsula19	15	0.422 0.056	0.513 0.056	0.531 0.058	2.690	2.946	0.197*	0***	0.061 0.013	0.000 0.000
22	Calpe (Alicante, Spain)	Iberian Peninsula20	9	0.551 0.082	0.564 0.068	0.532 0.075	2.721	2.747	- 0.059*	0.905 ^{NS}	NA NA	0.000 0.000

Pop ID	Pop location	Pop name	N	Ho (\pm SD)	He (\pm SD)	Uhe (\pm SD)	Ar* (rarefied)	Ar	Fis	HW	Average Null alleles (\pm SD)		Privative alleles (\pm SD)	
23	(Cuenca, Castilla la Mancha, Spain)	Iberian Peninsula21	11	0.412 0.056	0.551 0.050	0.579 0.052	2.523	2.812	0.332*	0.001***	0.212	0.041	0.000	0.000
24	Quesa (Valencia, Valencian Community, Spain)	Iberian Peninsula22	7	0.316 0.063	0.484 0.062	0.53 0.067	2.528	2.537	0.342	0.294 ^{NS}	NA	NA	0.000	0.000
25	Xàtiva (Valencia, Valencian Community, Spain)	Iberian Peninsula23	15	0.54 0.055	0.610 0.041	0.632 0.042	2.959	3.339	0.13	0.002**	0.151	0.026	0.067	0.067
26	Tarragona (Catalonia, Spain)	Iberian Peninsula24	13	0.491 0.058	0.532 0.043	0.586 0.053	2.685	2.704	0.081	0.79 ^{NS}	0.126	0.027	0.000	0.000
27	Plencia (Vizcaya, Basque Country, Spain)	Iberian Peninsula25	15	0.444 0.080	0.492 0.061	0.517 0.064	2.456	2.553	0.156	0.014*	0.227	0.072	0.000	0.000
28	El Aaiún (Morocco)	North of Africa1	15	0.551 0.055	0.612 0.050	0.633 0.052	2.999	3.417	0.091	0.344 ^{NS}	0.048	0.013	0.000	0.000
29	Cape Three Forks (Morocco)	North of Africa2	15	0.498 0.063	0.569 0.064	0.588 0.066	2.850	3.259	0.125	0***	0.065	0.017	0.133	0.133
30	Melilla (Spain)	North of Africa3	29	0.504 0.049	0.620 0.055	0.632 0.056	3.108	3.374	0.178*	0***	0.175	0.040	0.000	0.000
31	Kebdana (Morocco)	North of Africa4	13	0.479 0.060	0.596 0.057	0.62 0.059	2.888	3.210	0.205*	0***	0.083	0.023	0.067	0.067
32	Ténès (Chlef , Algeria)	North of Africa5	10	0.476 0.058	0.611 0.053	0.646 0.056	3.123	3.223	0.225	0***	0.178	0.034	0.000	0.000
33	Boucheghal (Chlef , Algeria)	North of Africa6	6	0.356 0.063	0.525 0.061	0.575 0.067	2.688	2.748	0.29	0.014*	NA	NA	0.067	0.067
34	Berar (Tipaza, Algeria)	North of Africa7	6	0.5 0.075	0.531 0.070	0.585 0.078	2.772	2.904	0.004	0.083 ^{NS}	NA	NA	0.067	0.067
35	Fouka (Tipaza, Algeria)	North of Africa8	7	0.432 0.066	0.579 0.051	0.626 0.055	2.764	2.888	0.218*	0.002**	NA	NA	0.133	0.091

Pop ID	Pop location	Pop name	N	Ho (\pm SD)	He (\pm SD)	Uhe (\pm SD)	Ar* (rarefied)	Ar	Fis	HW	Average Null alleles(\pm SD)		Privative alleles (\pm SD)	
36	Litoral (Tipaza, Algeria)	North of Africa9	17	0.499 0.056	0.621 0.057	0.64 0.059	2.990	3.459	0.181*	0.006**	0.194	0.033	0.000	0.000
37	Bainem (Argel, Algeria)	North of Africa10	14	0.497 0.056	0.580 0.057	0.604 0.059	2.813	3.100	0.124	0***	0.160	0.041	0.067	0.067
38	Benaknoun (Argel, Algeria)	North of Africa11	11	0.493 0.056	0.593 0.054	0.622 0.057	2.876	3.248	0.165	0***	0.111	0.022	0.000	0.000
39	Rais-Hamidou (Argel, Algeria)	North of Africa12	13	0.502 0.068	0.578 0.051	0.603 0.054	2.677	3.054	0.133	0***	0.193	0.033	0.000	0.000
40	El-Harrach (Argel, Algeria)	North of Africa13	9	0.506 0.050	0.628 0.045	0.666 0.047	3.085	3.383	0.205*	0.422 ^{NS}	0.105	0.022	0.000	0.000
41	Jijel (Algeria)	North of Africa14	24	0.597 0.054	0.605 0.030	0.618 0.030	2.702	3.049	0.025	0***	0.090	0.025	0.000	0.000
42	Ibiza (Balearic Islands, Spain)	Balearic Islands1	12	0.417 0.064	0.504 0.070	0.526 0.073	2.592	2.940	0.168	0.829 ^{NS}	0.060	0.014	0.000	0.000
43	Formentera (Balearic Islands, Spain)	Balearic Islands2	14	0.424 0.066	0.549 0.064	0.569 0.066	2.734	3.060	0.254*	0.005**	0.057	0.014	0.000	0.000
44	Dragonera (Balearic Islands, Spain)	Balearic Islands3	12	0.501 0.057	0.540 0.048	0.564 0.050	2.601	2.923	0.052	0.093 ^{NS}	0.090	0.025	0.000	0.000
45	Mallorca (Balearic Islands, Spain)	Balearic Islands4	30	0.529 0.055	0.622 0.051	0.633 0.051	2.953	3.532	0.184*	0***	0.066	0.014	0.067	0.067
46	Cabrera (Balearic Islands, Spain)	Balearic Islands5	14	0.494 0.052	0.579 0.052	0.601 0.054	2.776	3.177	0.181*	0.477 ^{NS}	0.071	0.016	0.000	0.000
47	Menorca (Balearic Islands, Spain)	Balearic Islands6	14	0.519 0.067	0.578 0.059	0.599 0.061	2.920	3.315	0.105	0.024*	0.054	0.017	0.067	0.067
48	Cassis (France)	Southeast of France1	14	0.495 0.060	0.576 0.055	0.597 0.057	2.842	3.260	0.133	0.035*	0.059	0.015	0.000	0.000
49	Route des Crêtes (France)	Southeast of France2	16	0.492 0.070	0.558 0.055	0.576 0.057	2.578	3.086	0.177	0.004**	0.064	0.014	0.000	0.000

Pop ID	Pop location	Pop name	N	Ho (\pm SD)	He (\pm SD)	Uhe (\pm SD)	Ar* (rarefied)	Ar	Fis	HW	Average Null alleles(\pm SD)		Private alleles (\pm SD)	
50	Campo dell'oro (Corsica, France)	Corsica island	13	0.385 0.054	0.507 0.057	0.542 0.062	2.506	2.611	0.255*	0.067 ^{NS}	0.183	0.052	0.000	0.000
51	Chia (Sardinia, Italy)	Sardinia island1	11	0.49 0.052	0.571 0.058	0.599 0.061	2.700	3.086	0.117	0.883 ^{NS}	0.113	0.028	0.000	0.000
52	Villasimius (Sardinia, Italy)	Sardinia island2	10	0.522 0.065	0.562 0.047	0.592 0.050	2.782	3.019	0.12	0.058 ^{NS}	0.113	0.028	0.000	0.000
53	Cagliari (Sardinia, Italy)	Sardinia island3	8	0.492 0.065	0.554 0.055	0.591 0.059	2.685	3.068	0.133	0.02*	NA	NA	0.000	0.000
54	San Rossone Massaciucoli (Italy)	Italian Peninsula	14	0.425 0.043	0.590 0.032	0.62 0.035	2.738	2.791	0.263*	0***	0.109	0.037	0.000	0.000
55	Neresine (Lussino Island, Croatia)	Adriatic Sea1 (island)	14	0.419 0.049	0.537 0.058	0.557 0.060	2.712	2.968	0.18*	0***	0.077	0.016	0.067	0.067
56	Donje Rastane (Croatia)	Adriatic Sea2	16	0.492 0.061	0.556 0.056	0.574 0.057	2.652	3.057	0.107	0.054 ^{NS}	0.081	0.019	0.000	0.000
57	Diklo (Croatia)	Adriatic Sea3	15	0.538 0.042	0.617 0.038	0.639 0.039	2.792	3.298	0.131	0.385 ^{NS}	0.109	0.024	0.067	0.067
58	Palermo (Italy)	Sicily island1	13	0.514 0.061	0.583 0.059	0.607 0.061	2.898	3.263	0.115	0.202 ^{NS}	0.106	0.032	0.000	0.000
59	San Marco D'Alunzio (Sicily, Italy)	Sicily island2	15	0.5 0.065	0.580 0.067	0.6 0.069	2.779	3.270	0.126	0.005**	0.177	0.047	0.000	0.000
60	Linosa Island (Italy)	Linosa island	14	0.504 0.071	0.581 0.068	0.603 0.071	2.937	3.450	0.163*	0.002**	0.120	0.024	0.133	0.091
61	Alyhi (Voiotia, Greece)	Balkans Peninsula1	14	0.497 0.071	0.601 0.068	0.623 0.070	3.220	3.538	0.172*	0***	0.098	0.026	0.000	0.000
62	Amarousion (Attiki, Greece)	Balkans Peninsula2	15	0.536 0.065	0.655 0.063	0.678 0.065	3.399	3.931	0.181*	0***	0.082	0.021	0.067	0.067
63	Amorgos Island (Greece)	Aegean Sea islands1	28	0.563 0.053	0.692 0.064	0.705 0.066	3.745	4.307	0.174*	0***	0.103	0.016	0.200	0.107
64	Lesvos Island (Greece)	Aegean Sea islands2	15	0.529 0.061	0.645 0.067	0.667 0.069	3.502	3.981	0.175*	0.004**	0.070	0.014	0.067	0.067

Pop ID	Pop location	Pop name	N	Ho (\pm SD)	He (\pm SD)	Uhe (\pm SD)	Ar* (rarefied)	Ar	Fis	HW	Average Null alleles(\pm SD)	Privative alleles (\pm SD)
65	Talmaithah (Al-Marj, Libya)	Cyrenaican1	12	0.515 0.071	0.568 0.068	0.593 0.071	3.074	3.493	0.098	0.218 ^{NS}	0.113 0.030	0.067 0.067
66	Qasir (Al-Jabal Al-Akhdar, Libya)	Cyrenaican2	14	0.567 0.077	0.581 0.073	0.603 0.076	3.184	3.685	0.01	0.001 ^{***}	0.122 0.032	0.067 0.067
67	Wadi Al-Kouf (Al-Jabal Al-Akhdar, Libya)	Cyrenaican3	25	0.573 0.070	0.619 0.074	0.632 0.076	3.296	3.933	0.059	0 ^{***}	0.124 0.028	0.133 0.091
68	Wardamah (Al-Jabal Al-Akhdar, Libya)	Cyrenaican4	41	0.538 0.077	0.603 0.079	0.611 0.080	3.144	3.973	0.113*	0 ^{***}	0.048 0.012	0.133 0.091
69	Wadi Al-Mahboul (Susah, Libya)	Cyrenaican5	13	0.615 0.066	0.572 0.056	0.594 0.059	2.770	3.284	- 0.095*	0 ^{***}	0.029 0.012	0.000 0.000
70	Ras Al-Hilal (Dernah, Libya)	Cyrenaican6	25	0.554 0.069	0.595 0.072	0.607 0.074	3.230	3.859	0.073	0 ^{***}	0.051 0.013	0.067 0.067
71	Wadi Al-Mahboul (Al-Jabal Al-Akhdar, Libya)	Cyrenaican7	15	0.576 0.065	0.617 0.066	0.638 0.068	3.396	3.854	0.071	0 ^{***}	0.051 0.015	0.067 0.067
72	Sotira (Limassol, Cyprus)	Cyprus island	13	0.509 0.080	0.559 0.078	0.582 0.081	2.995	3.331	0.095	0.193 ^{NS}	0.065 0.021	0.000 0.000
73	Lachisch (Israel)	Middle East1	10	0.466 0.065	0.537 0.068	0.568 0.072	2.807	3.011	0.12	0.251 ^{NS}	0.155 0.038	0.000 0.000
74	Neve Shalom (Israel)	Middle East2	11	0.509 0.069	0.533 0.071	0.560 0.075	2.784	3.059	0.018	0.928 ^{NS}	0.090 0.038	0.133 0.091

Table S2. Information about microsatellite loci analysed in this study. PCR reagents concentration was 10X PCR buffer, 0.2 mM dNTPs, 1 U Taq polymerase (KAPATaq DNA Polymerase, Kapa Biosystems), 0.6 mg/mL of BSA (bovine serum albumin) and 0.2 μ M of each primer. In the case of multiplex 3 and 4 we doubled the concentration of reverse primers (up to 0.4 μ M) to increase PCR yield. PCR profiles: profile A with 94 °C for 4 min, 10 touchdown cycles of 94 °C for 30 s, 60 °C for 30 s (–1 °C/cycle), 72 °C for 1 min, 30 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min and a final step of 72 °C for 7 min; profile B with 94 °C for 4 min, 10 touchdown cycles of 94 °C for 30 s, 60 °C for 30 s (–0.5 °C/ cycle), 72 °C for 1 min, 30 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min and a final step of 72 °C for 7 min.

Locus name	GenBank accession number	Author	Dye (forward primer)	Type of amplification	PCR profile	Alleles ranges	Total number of alleles	Missing data (%)	Average null alleles (with standard error)
Pislen 21	EU263300	Albaladejo et al., (2008)	FAM	Individually	B	219-311	43	5.393	0.099 (\pm 0.014)
Pislen 114	EU263301	Albaladejo et al., (2008)	HEX	Individually	A	148-193	13	3.406	0.114 (\pm 0.015)
Pislen 501	EU263303	Albaladejo et al., (2008)	FAM	Individually	A	155-199	23	6.339	0.122 (\pm 0.018)
Pislen 510	EU263304	Albaladejo et al., (2008)	HEX	Multiplex1	A	196-246	26	1.041	0.051 (\pm 0.009)
Pislen 526	EU263305	Albaladejo et al., (2008)	HEX	Multiplex1	A	115-130	5	0.378	0.045 (\pm 0.008)
Pislen R05	EU263306	Albaladejo et al., (2008)	FAM	Multiplex1	A	167-197	11	1.608	0.109 (\pm 0.013)
CUPVSiirt660	KX223468	Motalebipour et al., (2016)	FAM	Multiplex2	B	102-130	9	1.892	0.132 (\pm 0.011)
CUPVSiirt129	KX223413	Motalebipour et al., (2016)	HEX	Multiplex2	B	112-154	11	1.987	0.086 (\pm 0.013)
CUPVSiirt1120	KX223526	Motalebipour et al., (2016)	HEX	Multiplex2	B	173-205	6	3.500	0.141 (\pm 0.018)
CUPVSiirt1278	KX223549	Motalebipour et al., (2016)	PET	Multiplex3	A	160-211	15	4.825	0.132 (\pm 0.017)
CUPVSiirt1400	KX223562	Motalebipour et al., (2016)	NED	Multiplex4	B	123-185	27	2.554	0.151 (\pm 0.014)
CUPVSiirt1402	KX223563	Motalebipour et al., (2016)	NED	Multiplex3	A	157-202	11	3.690	0.156 (\pm 0.016)
CUPVSiirt1405	KX223564	Motalebipour et al., (2016)	FAM	Multiplex4	B	156-226	35	2.460	0.131 (\pm 0.015)
CUPVSiirt1734	KX223592	Motalebipour et al., (2016)	VIC	Multiplex3	A	149-179	8	2.649	0.134 (\pm 0.014)
CUPVSiirt1764	KX223597	Motalebipour et al., (2016)	FAM	Multiplex3	A	101-176	17	7.379	0.158 (\pm 0.003)

Table S3. Levels used in the population hierarchical structure analyses. In level 1 (*continents*) populations are organised by continents (islands are included in the nearest continent). In level 2 (*western-eastern islands/mainland*) populations are splitted out in west and east of the Mediterranean Basin in continents and islands (the two populations in the Macaronesian islands, and the three in the Adriatic Sea have been included in their own level because of its locations which make difficult to include them in the other levels). In level 3 (*geographic accidents*) locations are grouped according to geographic accidents (i.e. peninsulas, archipelago, isolated islands, etc), while level 4 (*population*) refers to populations name which are described in Table S1.

Level1 (continents)	Level2 (western-eastern islands/mainland)	Level3 (geographic accidents)	Level4 (population)
Africa (1)	Macaronesian islands (1)	Macaronesian islands (1)	1
Africa (1)	Macaronesian islands (1)	Macaronesian islands (1)	2
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	3
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	4
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	5
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	6
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	7
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	8
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	9
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	10
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	11
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	12
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	13
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	14
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	15
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	16
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	17
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	18
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	19
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	20
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	21
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	22
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	23
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	24
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	25
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	26
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	27
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	28
Europe (2)	Western Mediterranean mainland (2)	Iberian Peninsula (2)	29
Africa (1)	Western Mediterranean mainland (2)	North of Africa (3)	30
Africa (1)	Western Mediterranean mainland (2)	North of Africa (3)	31
Africa (1)	Western Mediterranean mainland (2)	North of Africa (3)	32
Africa (1)	Western Mediterranean mainland (2)	North of Africa (3)	33
Africa (1)	Western Mediterranean mainland (2)	North of Africa (3)	34
Africa (1)	Western Mediterranean mainland (2)	North of Africa (3)	35

Level1 (continent)	Level2 (western-eastern islands/mainland)	Level3 (geographic accidents)	Level4 (population)
Africa (1)	Western Mediterranean mainland (2)	North of Africa (3)	36
Africa (1)	Western Mediterranean mainland (2)	North of Africa (3)	37
Africa (1)	Western Mediterranean mainland (2)	North of Africa (3)	38
Africa (1)	Western Mediterranean mainland (2)	North of Africa (3)	39
Africa (1)	Western Mediterranean mainland (2)	North of Africa (3)	40
Africa (1)	Western Mediterranean mainland (2)	North of Africa (3)	41
Europe (2)	Western Mediterranean islands (3)	Balearic Islands (4)	42
Europe (2)	Western Mediterranean islands (3)	Balearic Islands (4)	43
Europe (2)	Western Mediterranean islands (3)	Balearic Islands (4)	44
Europe (2)	Western Mediterranean islands (3)	Balearic Islands (4)	45
Europe (2)	Western Mediterranean islands (3)	Balearic Islands (4)	46
Europe (2)	Western Mediterranean islands (3)	Balearic Islands (4)	47
Europe (2)	Western Mediterranean mainland (2)	Southeast of France (5)	48
Europe (2)	Western Mediterranean mainland (2)	Southeast of France (5)	49
Europe (2)	Western Mediterranean islands (3)	Corsica and Sardinia (6)	50
Europe (2)	Western Mediterranean islands (3)	Corsica and Sardinia (6)	51
Europe (2)	Western Mediterranean islands (3)	Corsica and Sardinia (6)	52
Europe (2)	Western Mediterranean islands (3)	Corsica and Sardinia (6)	53
Europe (2)	Western Mediterranean mainland (2)	Italian Peninsula (7)	54
Europe (2)	Adriatic Sea (4)	Adriatic Sea (8)	55
Europe (2)	Adriatic Sea (4)	Adriatic Sea (8)	56
Europe (2)	Adriatic Sea (4)	Adriatic Sea (8)	57
Europe (2)	Western Mediterranean islands (3)	Sicily (9)	58
Europe (2)	Western Mediterranean islands (3)	Sicily (9)	59
Africa (1)	Western Mediterranean islands (3)	Linosa Island (10)	60
Europe (2)	Eastern Mediterranean mainland (6)	Balkan Peninsula (11)	61
Europe (2)	Eastern Mediterranean mainland (6)	Balkan Peninsula (11)	62
Europe (2)	Eastern Mediterranean islands (5)	Aegean Islands (12)	63
Europe (2)	Eastern Mediterranean islands (5)	Aegean Islands (12)	64
Africa (1)	Eastern Mediterranean mainland (6)	Cyrenaican Peninsula (13)	65
Africa (1)	Eastern Mediterranean mainland (6)	Cyrenaican Peninsula (13)	66
Africa (1)	Eastern Mediterranean mainland (6)	Cyrenaican Peninsula (13)	67
Africa (1)	Eastern Mediterranean mainland (6)	Cyrenaican Peninsula (13)	68
Africa (1)	Eastern Mediterranean mainland (6)	Cyrenaican Peninsula (13)	69
Africa (1)	Eastern Mediterranean mainland (6)	Cyrenaican Peninsula (13)	70
Africa (1)	Eastern Mediterranean mainland (6)	Cyrenaican Peninsula (13)	71
Middle East (3)	Eastern Mediterranean islands (5)	Cyprus (14)	72
Middle East (3)	Eastern Mediterranean mainland (6)	Middle East (15)	73
Middle East (3)	Eastern Mediterranean mainland (6)	Middle East (15)	74

Table S4. Linkage disequilibrium for each pair of loci across all populations.

Locus		P-value
21	510	Highly significant
21	526	0.148677
510	526	Highly significant
21	R05	Highly significant
510	R05	Highly significant
526	R05	Highly significant
21	114	Highly significant
510	114	Highly significant
526	114	0.148757
R05	114	Highly significant
21	501	Highly significant
510	501	Highly significant
526	501	0.293466
R05	501	Highly significant
114	501	Highly significant
21	SSR1	Highly significant
510	SSR1	Highly significant
526	SSR1	0.029336
R05	SSR1	Highly significant
114	SSR1	Highly significant
501	SSR1	Highly significant
21	SSR2	0.686513
510	SSR2	0.901329
526	SSR2	0.414318
R05	SSR2	0.476466
114	SSR2	0.947339
501	SSR2	0.999450
SSR1	SSR2	Highly significant
21	SSR3	Highly significant
510	SSR3	0.984295
526	SSR3	0.999970
R05	SSR3	0.455500
114	SSR3	0.676785
501	SSR3	0.999487
SSR1	SSR3	0.297052
SSR2	SSR3	0.700876
21	SSR5	Highly significant
510	SSR5	Highly significant
526	SSR5	0.850771
R05	SSR5	0.944322
114	SSR5	Highly significant
501	SSR5	Highly significant
SSR1	SSR5	Highly significant
SSR2	SSR5	Highly significant
SSR3	SSR5	0.924490
21	SSR6	Highly significant
510	SSR6	Highly significant
526	SSR6	Highly significant
R05	SSR6	Highly significant
114	SSR6	Highly significant
501	SSR6	Highly significant
SSR1	SSR6	Highly significant
SSR2	SSR6	Highly significant
SSR3	SSR6	Highly significant
SSR5	SSR6	Highly significant
21	SSR7	Highly significant
510	SSR7	0.000065

Locus		P-value
526	SSR7	0.424286
R05	SSR7	0.574435
114	SSR7	Highly significant
501	SSR7	Highly significant
SSR1	SSR7	Highly significant
SSR2	SSR7	0.365069
SSR3	SSR7	0.839797
SSR5	SSR7	Highly significant
SSR6	SSR7	Highly significant
21	SSR8	Highly significant
510	SSR8	Highly significant
526	SSR8	0.394880
R05	SSR8	Highly significant
114	SSR8	Highly significant
501	SSR8	Highly significant
SSR1	SSR8	Highly significant
SSR2	SSR8	0.946389
SSR3	SSR8	0.999983
SSR5	SSR8	Highly significant
SSR6	SSR8	Highly significant
SSR7	SSR8	Highly significant
21	SSR9	0.173448
510	SSR9	0.001945
526	SSR9	0.128575
R05	SSR9	0.158529
114	SSR9	0.000109
501	SSR9	0.509457
SSR1	SSR9	0.000559
SSR2	SSR9	0.709772
SSR3	SSR9	0.996962
SSR5	SSR9	Highly significant
SSR6	SSR9	0.494189
SSR7	SSR9	Highly significant
SSR8	SSR9	0.720426
21	SSR10	0.204692
510	SSR10	Highly significant
526	SSR10	0.814181
R05	SSR10	0.333511
114	SSR10	Highly significant
501	SSR10	Highly significant
SSR1	SSR10	Highly significant
SSR2	SSR10	0.927000
SSR3	SSR10	0.572903
SSR5	SSR10	Highly significant
SSR6	SSR10	Highly significant
SSR7	SSR10	Highly significant
SSR8	SSR10	Highly significant
SSR9	SSR10	0.574361

Table S5. Results of the hierarchical F_{st} analysis. Details about levels in Table S3. Bold values are the ones referenced in the manuscript which significance has been tested (NS=non significant; *=p-value<0.001).

	Level1 <i>(continents)</i>	Level2 <i>(western-eastern islands/mainland)</i>	Level3 <i>(geographic accidents)</i>	Level4 <i>(population)</i>	Individuals
Total	0.019^{NS}	0.091	0.111	0.156	0.307
Level1 <i>(continents)</i>	0.000	0.073*	0.093	0.139	0.293
Level2 <i>(western-eastern islands/mainland)</i>	0.000	0.000	0.022*	0.071	0.237
Level3 <i>(geographic accidents)</i>	0.000	0.000	0.000	0.051*	0.220
Level4 <i>(population)</i>	0.000	0.000	0.000	0.000	0.179

Table S6. Modules detected by the modularity analysis in network with populations grouped according to bird migratory routes. Populations ID in Table S1.

	Populations ID	N° of <i>P. lentiscus</i> individuals	Number of groups	Group 1	Group 2	Group 3	Group 4	Group 5
Migratory route 1	1-31	420	5	3 5-27 29-31	1	2	4	28
Migratory route 2	32-49	243	5	35 37-40 42-49	32-33	34	36	41
Migratory route 3	50-60	143	3	51 53-60	50	52	-	-
Migratory route 4	61-74	251	2	61-71	72-74	-	-	-

Table S7. Plant collectors. Populations not included in the table were collected by the authors.

Pop ID	Pop location	Name
1	Tenerife (Canary Islands)	María Llaría
2	Gran Canaria (Canary Islands)	Ruth Jaén
3	Torres Novas (Portugal)	María Villa
4	Chiclana (Cádiz, Andalusia, Spain)	Francisco Hortas
5	Conil (Cádiz, Andalusia, Spain)	Francisco Hortas
6	Córdoba (Andalusia, Spain)	María Ángeles Pérez and José Manuel Zamora
23	La Hoz de Priego (Cuenca, Castilla la Mancha, Spain)	Pedro Abellán
25	Xátiva (Valencia, Valencian Community, Spain)	Paqui Carreño
27	Plencia (Vizcaya, Basque Country, Spain)	Eduardo Berriatua
28	El Aaiún (Morocco)	Fran García Castellanos
29	Cape Three Forks (Morocco)	Adrián Ruíz Rocamora
30	Melilla (Spain)	Manuel Tapia
31	Kebdana (Morocco)	Adrián Ruíz Rocamora
32	Ténès (Chlef, Algeria)	Nora Chahbar
33	Boucheghal (Chlef, Algeria)	Nora Chahbar
34	Berar (Tipaza, Algeria)	Nora Chahbar
35	Fouka (Tipaza, Algeria)	Nora Chahbar
36	Litoral (Tipaza, Algeria)	Nora Chahbar
37	Bainem (Argel, Algeria)	Nora Chahbar
38	Benaknoun (Argel, Algeria)	Nora Chahbar
39	Rais-Hamidou (Argel, Algeria)	Nora Chahbar
40	El-Harrach (Argel, Algeria)	Nora Chahbar
41	Jijel (Algeria)	Nora Chahbar
42	Ibiza (Balearic Islands, Spain)	Joan Mayol
43	Formentera (Balearic Islands, Spain)	Joan Mayol
44	Dragonera (Balearic Islands, Spain)	Joan Mayol
45	Mallorca (Balearic Islands, Spain)	Joan Mayol and Pere Tomas
46	Cabrera (Balearic Islands, Spain)	Joan Mayol
47	Menorca (Balearic Islands, Spain)	Joan Mayol
48	Cassis (France)	Óscar Belmar
49	Route des Crêtes (France)	Óscar Belmar
50	Campo dell'oro (Corsica, France)	Jennifer Mejean
51	Chia (Sardinia, Italy)	Carmen Balibrea Escobar
52	Villasimius (Sardinia, Italy)	Carmen Balibrea Escobar
53	Cagliari (Sardinia, Italy)	Carmen Balibrea Escobar
54	San Rossone Massaciucoli (Italy)	Raffaella Dall'Olio
55	Neresine (Lussino Island, Croatia)	Janja Filipi
56	Donje Rastane (Croatia)	Janja Filipi
57	Diklo (Croatia)	Janja Filipi
58	Palermo (Sicily, Italy)	Eugenia Oliveri
59	San Marco D'Alunzio (Sicily, Italy)	Alessandro Tanania
60	Linosa Island (Italy)	Raffaella Dall'Olio

Pop ID	Pop location	Name
61	Alyhi (Voiotia, Greece)	Christos Barboutis
62	Amarousion (Attiki, Greece)	Maria Bouga
63	Amorgos Island (Greece)	Maria Bouga
64	Lesvos Island (Greece)	Christos Barboutis
65	Talmaithah (Al-Marj, Libya)	Taher Shaibi
66	Qasir (Al-Jabal Al-Akhdar, Libya)	Taher Shaibi
67	Wadi Al-Kouf (Al-Jabal Al-Akhdar, Libya)	Taher Shaibi
68	Wardamah (Al-Jabal Al-Akhdar, Libya)	Taher Shaibi
69	Wadi Al-Mahboul (Susah, Libya)	Taher Shaibi
70	Ras Al-Hilal (Dernah, Libya)	Taher Shaibi
71	Wadi Al-Mahboul (Al-Jabal Al-Akhdar, Libya)	Taher Shaibi
72	Sotira (Limassol, Cyprus)	Paraskevi C. Manolaki
73	Lachisch (Israel)	Victoria Soroker
74	Neve Shalom (Israel)	Victoria Soroker

Chapter II



Ecological and genetic consequences of fragmentation in a semiarid Mediterranean urban forest

Ecological and genetic consequences of fragmentation in a semiarid Mediterranean urban forest

Abstract

Urbanization threatens biodiversity worldwide and is expected to increase in the future. This involves intensification of fragmentation and increase of urban forest remnants. Thus, it is important to understand the functioning of these patches for the preservation of local biodiversity. Mutualistic interactions such as seed dispersal have a key role in the dynamics of plant populations and could help to mitigate the negative effects of fragmentation on wild plant populations. In this context, we have used the mastic *Pistacia lentiscus*, a common Mediterranean shrub, as a model to study the interaction between seed dispersal by birds and the local genetic diversity of individuals in a highly fragmented semiarid forestry area of the Iberian Southeast (Murcia, Spain). Results reveal a high genetic diversity and a lack of differentiation between shrub patches of *P. lentiscus* in the area, probably as a result of a strong, but rather recent fragmentation, and an apparently poor contribution of birds to the dispersal of *Pistacia* seeds. However, the existence of latent impacts cannot be discarded, since at least one of the patches shows some signs of inbreeding.

Keywords: plant-animal mutualisms, fragmentation, birds, urban areas, genetic diversity, mist-netting

1. Introduction

Urbanization is growing worldwide due to the increase of human population, which involves a negative impact on biological diversity (McDonald, Kareiva, & Forman 2008) since it causes biotic homogenization (McKinney, 2006; Olden & Rooney, 2006). This term was defined by McKinney and Lockwood (1999) as “the replacement of local biotas with non-indigenous species” which involves the loss of endemic species. Thus, it is important to promote the conservation of native species inside urban areas in order to preserve local biodiversity (Goddard, Dougill, & Benton 2010; Kowarik, 2011).

In the Mediterranean Basin, human activities, such as agriculture and urbanization, have caused fragmentation of habitats (Grove & Rackham, 2001) potentially leading to a loss of genetic diversity and to increased genetic drift and inbreeding (Young, Boyle, & Brown, 1996). Plant-animal mutualisms such as seed dispersal promote gene flow across and among plant populations (Jordano, 2010; Nora, Albaladejo, González-Martínez, Robledo-Arnuncio, & Aparicio, 2011) which might mitigate the negative effects of habitat fragmentation. Hence, it is necessary to increase the knowledge of plant-animal interactions to keep these processes running and to improve their application in the management and restoration of fragmented habitats (Briggs, 2009; Manel & Holderegger, 2013).

Frugivorous vertebrates make a key contribution to seed dispersal, plant regeneration and community patterns (Cousens, Dytham, & Law, 2008). For instance, in tropical and subtropical forests more than 90% of woody species rely on vertebrates for the dispersal of their seeds, while in Mediterranean scrublands this value ranges from 50 to 70% (Jordano, 2000). Mutualistic interactions such as seed dispersal have received much attention in the last decade due to its role for biodiversity conservation and ecosystem functioning (Bascompte & Jordano 2007; Schleuning, Fründ, & García, 2014; Valiente-Banuet et al., 2014).

In temperate habitats, studies about the effects of fragmentation on seed dispersal revealed its negative effect on seed-dispersers diversity and in the number of interactions (Fontúrbel et al., 2015), that may result in a limited dispersal of plant species relying on animal vectors. It is assumed that in plant-animal mutualisms, plants can be less vulnerable to fragmentation

than animals since they are only affected by changes in their immediate surroundings (Corlett & Turner, 1997; Memmot, Waser, & Price, 2004), which seems to be true in mutualistic systems composed by long-lived plant taxa and short-lived animal dispersers (e.g. shrubs and birds). However, research carried out in disturbed landscapes of the Iberian Peninsula obtained contrasting results: some studies found a reduction in the diversity of birds and fruit mobility (Rey & Alcántara, 2014), whereas others concluded that birds in warm Mediterranean areas are generally tolerant to fragmentation (González-Varo, 2010). This probably reflects other factors also affecting the community of dispersers, such as landscape structure and/or fruit abundance (Tellería, Ramírez, & Pérez-Tris, 2005). With regard to the genetic consequences of habitat degradation for plant populations, Aparicio et al., (2012) studied how four plant species (*Cistus salviifolius*, *Myrtus communis*, *Pistacia lentiscus* and *Quercus coccifera*) were affected by fragmentation in the South of the Iberian Peninsula. They found that genetic diversity was higher in long-lived species (*Quercus*>*Pistacia*>*Myrtus*>*Cistus*) and that *Myrtus*, *Cistus* and *Quercus* seemed to be affected by fragmentation in some way. These authors concluded that the same fragmentation process could affect the genetic structure of plant populations in different ways, since it depends on the particular traits of each species. The work of Nora et al., (2015) about the genetic diversity of *P. lentiscus* and *M. communis* in the Southwest of the Iberian Peninsula revealed that landscape configuration does not determine genetic patterns of the study species.

Previous studies carried out in southern Spain (Zapata, Robledano, Ramos, & Martínez-López, 2014) revealed a fragmented distribution and low regeneration of *P. lentiscus* in forest patches close to urban areas, probably related to factors limiting dispersal, among them dioecy (that may lead to an inadequate proportion of sexes). In turn, this may reduce pollination success and thus, limit seed production and dispersal. Studies about pollination of the species in fragmented populations suggest that related genotypes can be generated at a local scale due to the low number of effective pollen donors and scarce seed dispersal (Albaladejo, Guzmán, González-Martínez, & Vendramin, 2012). Moreover, low genetic diversity of populations might decrease the reproductive success (e.g. less viable seeds). Empirical data show that the number of effective parentals is lower in smaller and fragmented populations (Nora et al., 2011).

In this study, we combine ecological and genetic approaches to investigate the consequences of fragmentation in the fleshy-fruited shrub *P. lentiscus* in two urban forest fragments located in the suburbs of Murcia (Spain). To this aim, we (1) estimate the genetic diversity and quantify the degree of differentiation of *P. lentiscus* individuals from patches (wild and planted), and (2) evaluate the relative contribution of frugivorous birds to the seed dispersal. Our hypothesis is that fragmentation affects the plant-animal mutualism (i.e. connectivity among patches) and hence the genetic diversity of *P. lentiscus* patches.

2. Materials and Methods

2.1. Study area

The research was carried out in two close forest fragments remnants (1.5 km of distance) of a more continuous past distribution, Montepinar Municipality Forest Park (38° 2' 11'' N, 1° 5' 40'' W), and Cantalares (38° 2' 49'' N, 1° 5' 54'' W), both located in the Municipality of Murcia, Spain (Figure 1). The two sites represent isolated forest patches within the intensively developed lowlands of the municipality, surrounded by crops and urban areas. Climatic conditions are representative of the semiarid Mediterranean climate. Mean annual rainfall is 290 mm, distributed mainly in spring and autumn, and mean annual temperature is 18 °C.

The tree layer in the forest patches is dominated by dense reforestation with Aleppo Pine (*Pinus halepensis*) (Zapata et al., 2014). The understory is composed of typical Mediterranean scrubland species such as *Asparagus albus*, *Chamaerops humilis*, *Lavandula multifida*, *Rhamnus lycioides*, *Withania frutescens* and *Thymelaea hirsuta*.

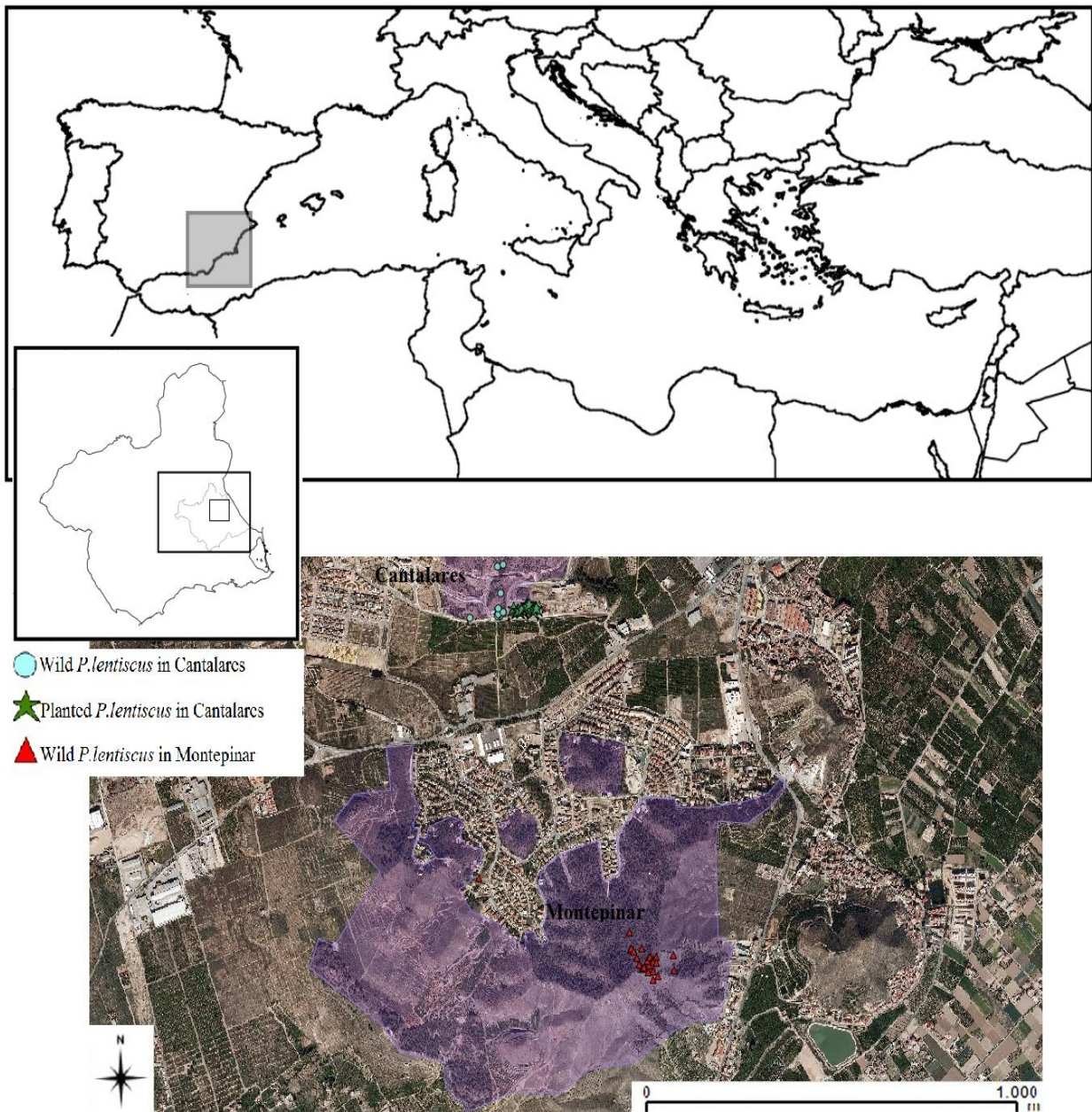


Figure 1. Map of the area and patches studied. Shaded polygons represent the area surveyed for *P. lentiscus*; circles, stars and triangles represent *P. lentiscus* individuals sampled.

2.2. Study species

Pistacia lentiscus (Anacardiaceae) is a typical Mediterranean shrub with a key role in Mediterranean landscapes for several phenological reasons. First, the ripening of fruits covers autumn and winter (Herrera, 1984), which makes them an important resource for the community of wintering birds. Secondly, *P. lentiscus* is an evergreen shrub that protects the soil from erosion throughout the year and favors decomposition of organic

matter and humus formation (Naveh & Lieberman, 1993). Further, it is a dioecious and anemophilous species, hence its pollination depends on the intensity and direction of the wind, and on the density of pollen in the vicinity of female plants (Jordano, 1988).

2.3. *Sampling and characterization of P. lentiscus*

After surveying about 140 ha (Figure 1) we found 12 native individuals in a natural patch and around 60 planted individuals in a public garden in Cantalares, and 24 native individuals in Montepinar (distribution shown in Figure 1).

Individuals in the garden were planted ca. 10 years ago, as seedlings grown from seeds collected in a wild population located 80 km away from the study area. Shrub patches were considered isolated since previous bibliography cited pollination distances for *P. lentiscus* up to 500 m in most cases (Albaladejo et al., 2012) and seed dispersal carried out by small birds, as in our study area, occurring mostly in the first 100 m from the source (Jordano, García, Godoy, & García-Castaño, 2007).

For each plant individual, we measured height and perimeter to calculate the biovolume in order to classify individuals as adults or juveniles. We used the formula of Blanco and Navarro (2003) to estimate the aerial biomass of shrubs. We considered the cylindrical morphotype:

$$V = \pi * \left[\frac{D}{2}\right]^2 * h$$

“D” = width of the plant and “h” = height

We considered as juvenile individuals those with a biovolume equal or less than 0.1 m³. Individuals with higher values were considered adults. We based this approach on previous observations after analyzing data from different Mediterranean areas of Southeast Iberia (authors' unpublished data). Planted shrubs from Cantalares were excluded for this analysis because of non-natural growth due to irrigation.

For wild individuals, we also determined their sex in order to calculate the effective population size (Ne) with the formula of Fontdevila and Moya (2000):

$$N_e = \frac{4N_{em} * N_{eb}}{N_{em} + N_{eb}}$$

“Nem” = number of male individuals and “Neb” = number of female individuals

Plant individuals with fruits were considered as female, and those with pollen as male. Undetermined sex was assigned to juveniles.

2.4. Genetic analyses

In the analyses, samples were distributed in three groups: Montepinar, wild Cantalares and planted Cantalares (we randomly sampled 12 of the latter for the genetic analyses). DNA was extracted from leaves using the NaOH method (Werner, Ros, & Guerra, 2002). Seven microsatellite markers (Pislen 20B, Pislen 21, Pislen 114, Pislen 501, Pislen 510, Pislen 526 and Pislen R05) designed for the species (Albaladejo et al., 2008) were amplified. Pislen 20B was removed due to failed amplification. PCR reactions were performed in 10 µl total volume containing 1µl of template DNA, 10X PCR buffer, 0.2 mM dNTPs, 1 U Taq polymerase (KAPATaq DNA Polymerase, Kapa Biosystems), 1.5 mM of MgCl₂, 0.15% of milk powder (as a substitute of bovine serum albumin, BSA) and 0.2 µM of each primer. Two PCR profiles were used: profile A with 94 °C for 4 min, 10 touchdown cycles of 94 °C for 30 s, 60 °C for 30 s (-1 °C/cycle), 72 °C for 1 min, 30 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min and a final step of 72 °C for 7 min; profile B with 94 °C for 4 min, 10 touchdown cycles of 94 °C for 30 s, 60 °C for 30 s (-0.5 °C/cycle), 72 °C for 1 min, 30 cycles of 94 °C for 30 s, 50 °C for 30 s and 72 °C for 1 min and a final step of 72 °C for 7 min. PCR products were electrophoretically separated on an ABI-3730 sequencer. Alleles were subsequently scored using GeneMapper v3.7 software.

Population-genetics parameters (heterozygosity, allelic diversity, Hardy-Weinberg test, genetic and genotypic structure) were obtained with GenAlEx version 6.1 (Peakall & Smouse, 2006) and Genepop (Raymond and Rousset, 1995). Population differentiation was evaluated with Arlequin (Excoffier, Laval, & Schneider, 2005).

2.5. *Bird species richness and abundance*

From November 2012 to January 2013, we monitored the community of birds through point counts (Blondel et al., 1981; Coreau & Martin, 2007; Rost, Clavero, Bas, & Pons, 2010) in the surroundings of the studied shrubs within a radius of 50 m. We recorded all visual and auditive contacts with birds, avoiding double counting of the same individual through on the spot judgment of the positions of sighted and singing individuals (two observers did the point counts at each study site). We carried out three morning surveys (10 min each) on sunny days in each place during December of 2012 and January of 2013. Based on the point counts we calculated the bird species richness and the abundance (individuals/ha) per species in the community. We focused on legitimate frugivorous birds that eat fruits but do not digest the seed thus contributing to the dispersal flow (Herrera 2004). In this analysis, we considered two groups (Montepinar and Cantalares) due to the close proximity of wild and planted individuals in Cantalares (Figure 1), which made difficult to discriminate their respective bird assemblages.

Data on bird activity were obtained by mist-netting from November 2012 to January 2013 in six different days (three days per site and 16 h each, 32 h in total). Our objective was to collect bird faeces in order to confirm the consumption of fleshy fruits to evaluate the seed dispersal flow. We used eight nets (111 m) in Cantalares (planted garden and wild patches) and only seven (87 m) in Montepinar due to the higher density of Aleppo pines in the last area. For each faecal sample collected from a bird caught in the nets, we recorded the species of disperser, the number of seeds carried and the species of plants to which they belonged. The content of faeces was examined with a binocular microscope.

2.6. *Statistical analyses*

Due to the different number of plant individuals per plant group (native and planted individuals from Cantalares and native individuals from Montepinar), we performed a rarefaction analysis (Hurlbert, 1971; Smith & Grassle, 1977; Leberg, 2002) using the software HP-RARE 1.0 (Kalinowski, 2005). We used t-Student tests to compare the values of

genetic diversity (estimated through the values of expected heterozygosity, H_e) between the three plant groups studied.

To compare richness and abundance of birds between the two areas studied (Montepinar and Cantalares) we used Kruskal-Wallis test. All statistical tests were performed with the R statistical package (R Development Core Team, 2012).

3. Results

3.1. Physiognomic characterization of *P. lentiscus*

The sex ratio (male: female) in the wild population of Montepinar (24 individuals) and Cantalares (12 individuals) was 0.5 (8: 15) and 1.4 (7: 5), respectively (Table S1). The effective population size was 20.9 and 11.9 for Montepinar and Cantalares, respectively (excluding juvenile individual with undetermined sex) (Figure 2).

3.2. Genetic characterization

We analyzed 48 individuals, but four (three from Montepinar and one from Cantalares) were discarded due to lack of amplification. Genetic diversity values (estimated from H_e) were 0.621 ± 0.043 in Montepinar, 0.634 ± 0.049 in the wild population and 0.616 ± 0.045 in the planted population both in Cantalares (Figure 2). These values were not significantly different between the three studied sites (Cantalares-Montepinar, $t = 0.22$, $p = 0.423$; Cantalares-planted, $t = 0.27$, $p = 0.397$; Montepinar-planted, $t = 0.47$, $p = 0.470$).

Allelic richness was 5.00 in Montepinar, 4.83 in wild Cantalares and 4.67 in planted Cantalares. Private allele numbers were 0.510 ± 0.166 in Montepinar, 0.590 ± 0.190 in wild Cantalares, and 0.460 ± 0.099 in the planted Cantalares.

Hardy-Weinberg equilibrium was observed in both wild and planted groups from Cantalares, but no in the patch from Montepinar ($p = 0.047$). Linkage disequilibrium was significant for between loci Pisen 501 and Pisen R05 in the population of Montepinar ($p = 0.004$). Inbreeding coefficient (F_{is}) was in general positive (Table S4). No signs of population differentiation were observed (wild Cantalares-planted, $F_{st} = -0.035$, $p = 0.991$; wild

Cantalaes-Montepinar, $F_{st} = -0.018$, $p = 0.883$; planted Cantalaes-Montepinar, $F_{st} = -0.012$, $p = 0.863$).

3.3. Bird community

Point counts recorded eight and 17 bird species in Montepinar and Cantalaes, respectively. In Montepinar three bird species were legitimate frugivores (*Erithacus rubecula*, *Sylvia melanocephala* and *Sylvia undata*) (Table S2), while in Cantalaes we recorded seven species of this type (*E. rubecula*, *Phoenicurus ochruros*, *Sturnus unicolor*, *Sylvia atricapilla*, *S. melanocephala*, *S. undata* and *Turdus merula*) (Table S2). The Kruskal-Wallis test did not show a significant difference in bird species richness between sites. The abundance of legitimate frugivores was the same in both areas (Table 1, Figure 2).

Table 1. Total richness (number of species) and abundance (individuals/ha) of the birds community in Montepinar and Cantalaes from point count data

	Montepinar		Cantalaes	
	Community	Legitimate frugivores	Community	Legitimate frugivores
Total richness	8	3	17	7
%		37.50		41.18
Total abundance	15.92±3.55	6.79±1.66	25.25±5.65	6.79±2.26
%		42.65		26.89

3.4. Bird faeces

We obtained 21 faeces from mist-netted legitimate frugivorous birds (14 in Cantalaes and 7 in Montepinar). We only found six seeds in the four faeces collected from these birds, four of them were of *P. lentiscus* (in three faeces) (Table S3).

4. Discussion

Contrary to expectations, mastic patches showed in this study high genetic diversity values and a lack of differentiation between them, most probably due to the established contribution of birds to seed dispersal in these patches as seeds of *P. lentiscus* were found in faeces. The design of the study and the interpretation of its results are, however, conditioned by the

limited availability of representative sites of fragmented populations in the municipality of Murcia. This is a common feature of small-scale studies of highly fragmented plant populations, although such research can serve as a basis for more general interpretations (Aguilar et al., 2008). On the other hand, by using a double framework to evaluate the issue (including genetic structure and the seed dispersal analyses), we have been able to give more support to our inferences about the biotic and anthropogenic processes explaining current genetic patterns.

4.1. Sex ratio and genetic diversity of *P. lentiscus* populations

Deviation of the sex ratio from 1:1 brings about a decrease in the effective population size (Fontdevila & Moya, 2000). However, the sex ratio did not significantly affect the effective population size in our study populations. Hence, this is not a limiting factor for the dispersion of the species.

The patches of *P. lentiscus* studied showed a high genetic diversity, typical of woody plants with anemophilous pollination (Nybom, 2004). Thus, genetic diversity should not be a limiting factor for the dispersion of this plant species in the study area. Furthermore, there was not population differentiation between patches. Individuals from Montepinar were not in Hardy-Weinberg equilibrium, which could indicate the effect of the genetic drift. This hypothesis is reinforced by the significant linkage disequilibrium values between loci Pislén R05 and Pislén 501 in Montepinar as well as by the generally positive values of the hybridization coefficient, which suggest the existence of inbreeding (Beebee & Rowe, 2004). Our findings support the results of similar studies on genetic effects of fragmentation on *P. lentiscus* populations in the south of the Iberian Peninsula, which found high values of genetic diversity for the species and no relationship between habitat fragmentation and genetic patterns (Aparicio et al., 2012; Nora et al., 2015). They also found signs of some degree of inbreeding and no population differentiation (Aparicio et al., 2012).

Both study sites, Montepinar and Cantalares, have suffered fragmentation processes for hundreds of years, initially due to agriculture, and at present due to housing and infrastructure development (highways and roads). Even so, our results did not show population differentiation or genetic diversity

reduction. This could indicate the existence of genetic flow between sites owing to pollen or seed dispersal. Animals such as birds (Herrera, 1984, 1998, 2004; Cuadrado, 1988; Verdú & García-Fayos, 2002) and mammals (Rosalino & Santos-Reis, 2009; Suárez-Esteban, Delibes, & Fedriani, 2013) are considered important consumers of fruits of *P. lentiscus*. In our case, the high density of barriers (roads, fences, etc.) may difficult the contribution of the latter. Another hypothesis is that fragmentation is too recent to affect these populations (at least at a genetic level) since *P. lentiscus* can live more than 200 years (Aparicio et al., 2012). Therefore, the observed genetic patterns can be the result of a past scenario under different conditions of fragmentation. The current situation could nevertheless hide a latent “extinction debt” (Tilman, May, Lehman, & Nowak, 1994) whose consequences would appear in these isolated populations in the mid- to long-term.

The planted individuals from Cantalares (originated 80 km far from the study area) were also genetically homogeneous with regard to wild individuals of the same site, in congruence to the high genetic similarity of *P. lentiscus* observed in the Mediterranean Basin (Barazani, Dudai, & Golan-Goldhirsh, 2003; Nahum, Inbar, Ne’eman, & Ben-Sholomo, 2008). These authors attributed the phenomenon to the role of migrant birds that act as long-distance dispersive vectors. Our results, despite the difference of scale, could be explained by a similar mechanism of interpatch dispersal.

4.2. *Disperser role of birds in the area*

With regard to the composition of the bird community, the values of winter abundance of legitimate frugivores recorded during the study period (6.79 individuals/ha in both Montepinar and Cantalares) were lower than the results obtained in a Mediterranean sclerophyllous scrubland in southwestern Spain by Jordano (1985) with ca. 10 individuals/ha but a great interannual variability (e.g. between 8.5 and 15 individuals/ha in consecutive years). These strong fluctuations have been also detected in Montepinar in previous studies that recorded densities of 10.9 individuals/ha (Zapata et al., in preparation), comparable to the values of the study of Jordano. The reason for interannual fluctuations is unclear. They could be related to variations in the availability and composition of fruits (Jordano 1985; Rodríguez de los Santos et al., 1986; Cuadrado et al.,

1995), although in other studies the relationship is not direct, which could indicate different responses of birds depending on the type of habitat (Herrera 2004). In any case, the abundance of frugivorous birds seems to be a no limiting factor *a priori* for the dispersive flow.

The faeces collected from the mist-netted birds contained seeds, albeit not in large quantity (four out of the six seeds were from *P. lentiscus* in 21 faecal samples) (Table S3). Furthermore, samples collected in a parallel study under perches in Cantalares had a higher content of seeds (52 seeds, all of them from *P. lentiscus*), most of them in the close vicinity of *P. lentiscus* shrubs. These results match our records in Montepinar in the previous year, when we found only 10 seeds from fleshy-fruited shrubs in the 38 faecal samples collected by mist-netting, while 100 seeds were found under perches (Zapata, Martínez-López, Ramos, & Robledano, 2017). Previous studies showed that birds tend to remain in shrubs where they forage for long periods of time, favouring local deposition (Jordano & Godoy, 2002 and references therein). Thus, it seems that birds are favouring seed dispersal near the maternal shrubs, where the recruitment is less feasible, instead of colonizing new environments. On the other hand, the role of gardens in the functioning of seed dispersal in these urban forests needs to be studied in the future, since there are frequent movements of birds between forest patches and gardens (author's personal observations). Although it has been generally assumed that planting native species in urban green areas helps to conserve biodiversity (Alvey, 2006), these urban gardens might attract birds, and hence yield seed dispersal, reducing the amount of seeds directed to other areas susceptible of regeneration.

5. Conclusions

For *P. lentiscus*, the fragmentation experienced in the study area is not noticeable at genetic level despite some signs of inbreeding in one of the patches of shrubs (Montepinar). There is no genetic differentiation between the three groups (planted and wild Cantalares and Montepinar), probably due to genetic flow (by pollination or seed dispersal) or to a recent fragmentation of the wild populations. Seed dispersal has been detected (seeds of the species were found in faeces from mist-netted birds), and both areas seem to have a similar dispersal potential (same frugivore

abundance). These results encourage undertaken more studies on the interaction between plants and dispersers in natural remnants and green spaces, to define more precisely the capacity of birds to link patches through seed dispersal (Gasperin & Pizo, 2009) as a tool for the preservation or restoration of urban forest fragments.

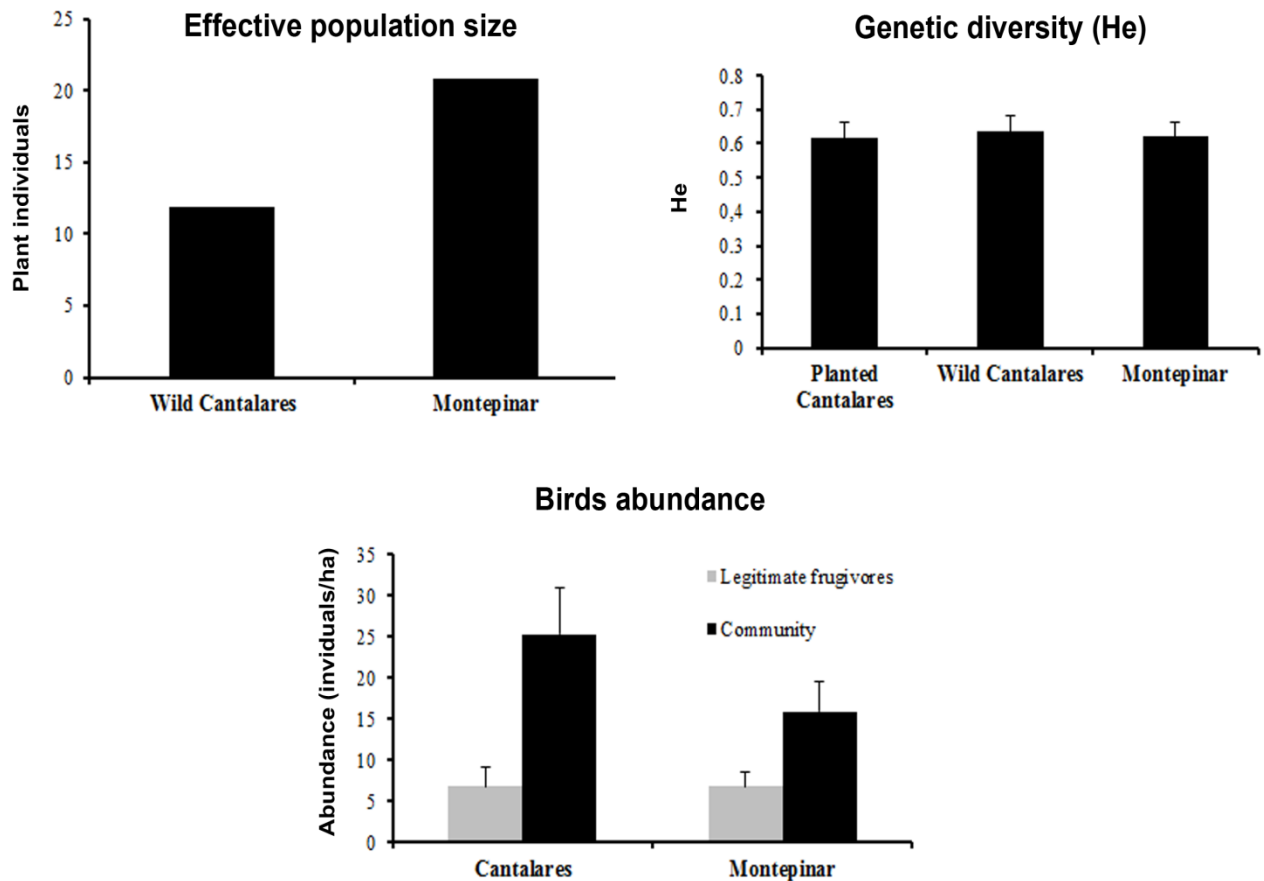


Figure 2. Summary of comparisons between the two areas studied. “Effective population size” was calculated only for wild patches. In “Birds abundance” wild and planted patches of Cantalares are analysed together. Only in the case of “Genetic diversity” we consider three groups (Cantalareas planted, Cantalares wild and Montepinar).

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Supporting information

Table S1 Biovolume and sex of sampled *P. lentiscus* individuals.

Table S2 Bird species detected in Montepinar and Cantalares and their respective densities.

Table S3 Data from the analysis of droppings collected from mist-netted birds.

Table S4 Data of genetic diversity per locus.

Table S1. Biovolume and sex of sampled *P. lentiscus* individuals.

ID	Population	Biovolume (m³)	Age types	Sex
1	Cantatares natural	1.44	Adult	Male
2	Cantatares natural	2.19	Adult	Female
3	Cantatares natural	2.36	Adult	Male
4	Cantatares natural	4.33	Adult	Male
5	Cantatares natural	13.41	Adult	Female
6	Cantatares natural	1.85	Adult	Male
7	Cantatares natural	4.29	Adult	Female
8	Cantatares natural	1.24	Adult	Female
9	Cantatares natural	3.55	Adult	Male
10	Cantatares natural	0.65	Adult	Male
11	Cantatares natural	5.51	Adult	Female
12	Cantatares natural	5.04	Adult	Male
1	Montepinar	20.96	Adult	Male
2	Montepinar	11.99	Adult	Female
3	Montepinar	18.78	Adult	Female
4	Montepinar	3.31	Adult	Female
5	Montepinar	0.64	Adult	Male
6	Montepinar	5.67	Adult	Female
7	Montepinar	0.20	Adult	Female
8	Montepinar	2.31	Adult	Female
9	Montepinar	8.45	Adult	Female
10	Montepinar	3.52	Adult	Female
11	Montepinar	0.50	Adult	Female
12	Montepinar	7.29	Adult	Female
13	Montepinar	7.26	Adult	Female
14	Montepinar	1.22	Adult	Female
15	Montepinar	0.27	Adult	Male
16	Montepinar	0.48	Adult	Male
17	Montepinar	5.83	Adult	Male
18	Montepinar	272.63	Adult	Female
19	Montepinar	109.51	Adult	Male
20	Montepinar	0.04	Juvenile	Unknown
21	Montepinar	1.44	Adult	Male
22	Montepinar	1.05	Adult	Female
23	Montepinar	1.36	Adult	Female
24	Montepinar	0.31	Adult	Male

Table S2. Bird species detected in Montepinar and Cantalares and their respective densities (Hirundinidae and other flight-feeding species excluded). ⁽¹⁾ Species of passerines (order Passeriformes). Within these, legitimate (*) and illegitimate frugivores (***) are differentiated from non-frugivores, according to Herrera (2004).

Species	Montepinar			Cantalares		
	Presence (P)	Abundance (per ha)	±SE	Presence (P)	Abundance (per ha)	±SE
<i>Accipiter nisus</i>	-	-	-	P	0.21	0.17
<i>Aegithalus caudatus</i> ⁽¹⁾	P	1.49	1.21	-	-	-
<i>Anthus pratensis</i> ⁽¹⁾	P	0.21	0.17	P	0.21	0.17
<i>Columba palumbus</i>	-	-	-	P	0.64	0.52
<i>Erithacus rubecula</i> ^{(1)*}	P	0.64	0.30	P	0.21	0.17
<i>Falco tinnunculus</i>	-	-	-	P	0.21	0.17
<i>Fringilla coelebs</i> ^{(1)**}	-	-	-	P	1.06	0.62
<i>Galerida sp.</i> ⁽¹⁾	-	-	-	P	0.42	0.35
<i>Motacilla alba</i> ⁽¹⁾	-	-	-	P	0.21	0.17
<i>Parus major</i> ^{(1)**}	-	-	-	P	0.42	0.17
<i>Phoenicurus ochruros</i> ^{(1)*}	-	-	-	P	1.49	0.46
<i>Phylloscopus collybita</i> ⁽¹⁾	P	2.97	0.17	P	2.33	1.05
<i>Regulus ignicapillus</i> ⁽¹⁾	P	0.21	0.17	-	-	-
<i>Serinus serinus</i> ^{(1)**}	P	4.24	0.17	P	12.73	0.00
<i>Sturnus unicolor</i> ^{(1)*}	-	-	-	P	0.64	0.30
<i>Sylvia atricapilla</i> ^{(1)*}	-	-	-	P	0.21	0.17
<i>Sylvia melanocephala</i> ^{(1)*}	P	4.88	0.76	P	3.18	0.30
<i>Sylvia undata</i> ^{(1)*}	P	1.27	0.60	P	0.85	0.69
<i>Turdus merula</i> ^{(1)*}	-	-	-	P	0.21	0.17
Total abundance		15.92	3.55		25.25	5.65

Table S3. Legitimate frugivorous birds (Herrera, 2004) captured by mist-nettings. Data from the analysis of droppings collected from mist-netted birds. (PIS= *Pistacia lentiscus*; ASP= *Asparagus albus*).

Species	Date	Period	Location	Feaces samples	Number of seeds	Plant species
<i>Erithacus rubecula</i>	11-15-12	Morning	Cantatares	Yes	0	-
<i>Sylvia melanocephala</i>	11-15-12	Morning	Cantatares	No	-	-
<i>Erithacus rubecula</i>	11-15-12	Morning	Cantatares	Yes	0	-
<i>Erithacus rubecula</i>	11-15-12	Morning	Cantatares	Yes	0	-
<i>Phoenicurus ochruros</i>	11-15-12	Morning	Cantatares	Yes	0	-
<i>Phoenicurus ochruros</i>	11-15-12	Morning	Cantatares	Yes	0	-
<i>Turdus merula</i>	11-15-12	Morning	Cantatares	No	-	-
<i>Erithacus rubecula</i>	12-13-12	Morning	Montepinar	No	-	-
<i>Sylvia melanocephala</i>	12-13-12	Morning	Montepinar	No	-	-
<i>Erithacus rubecula</i>	12-13-12	Morning	Montepinar	No	-	-
<i>Erithacus rubecula</i>	12-13-12	Morning	Montepinar	Yes	0	-
<i>Sylvia melanocephala</i>	12-13-12	Morning	Montepinar	No	-	-
<i>Phoenicurus ochruros</i>	12-13-12	Morning	Montepinar	Yes	0	-
<i>Erithacus rubecula</i>	12-13-12	Morning	Montepinar	No	-	-
<i>Sylvia melanocephala</i>	12-13-12	Morning	Montepinar	Yes	2	PIS
<i>Sylvia melanocephala</i>	12-13-12	Morning	Montepinar	No	-	-
<i>Erithacus rubecula</i>	12-15-12	Afternoon	Cantatares	No	-	-
<i>Sylvia atricapilla</i>	12-15-12	Afternoon	Cantatares	Yes	0	-
<i>Sylvia melanocephala</i>	12-15-12	Afternoon	Cantatares	No	-	-
<i>Erithacus rubecula</i>	12-15-12	Afternoon	Cantatares	Yes	0	-
<i>Sylvia atricapilla</i>	12-15-12	Afternoon	Cantatares	Yes	1	PIS
<i>Erithacus rubecula</i>	12-15-12	Afternoon	Cantatares	Yes	0	-
<i>Sylvia melanocephala</i>	12-15-12	Afternoon	Cantatares	No	-	-
<i>Sylvia melanocephala</i>	12-15-12	Afternoon	Cantatares	Yes	1	PIS
<i>Erithacus rubecula</i>	12-20-12	Morning	Cantatares	Yes	0	-
<i>Sylvia atricapilla</i>	12-20-12	Morning	Cantatares	No	-	-
<i>Erithacus rubecula</i>	12-20-12	Morning	Cantatares	Yes	0	-
<i>Phoenicurus ochruros</i>	12-20-12	Morning	Cantatares	Yes	0	-
<i>Sylvia atricapilla</i>	12-20-12	Morning	Cantatares	No	-	-
<i>Sylvia melanocephala</i>	12-20-12	Morning	Cantatares	Yes	0	-
<i>Sylvia melanocephala</i>	12-26-12	Afternoon	Montepinar	No	-	-
<i>Erithacus rubecula</i>	12-26-12	Afternoon	Montepinar	Yes	0	-
<i>Erithacus rubecula</i>	12-26-12	Afternoon	Montepinar	Yes	0	-
<i>Sylvia melanocephala</i>	01-15-13	Morning	Montepinar	No	-	-
<i>Sylvia atricapilla</i>	01-15-13	Morning	Montepinar	No	-	-
<i>Sylvia atricapilla</i>	01-15-13	Morning	Montepinar	Yes	2	ASP
<i>Erithacus rubecula</i>	01-15-13	Morning	Montepinar	Yes	0	-
<i>Erithacus rubecula</i>	01-15-13	Morning	Montepinar	No	-	-

Table S4. Data of genetic diversity per locus. Number of alleles; H_o , observed heterozygosity; H_E , expected heterozygosity; F_{is} , hibrydization coefficient by locus; Hardy-Weinberg equilibrium test by locus (NS=p-value>0.05; *=p-value<0.05; **=p-value<0.01;***=p-value<0.001); and privative alleles.

	N of alleles	H_o	H_E	F_{is}^{HW}	Privative alleles
Montepinar					
Pislen 21	7	0.667	0.654	0.009 ^{NS}	3
Pislen 114	4	0.714	0.609	-0.149 ^{NS}	0
Pislen 501	8	0.714	0.783	0.112 ^{NS}	0
Pislen 510	6	0.467	0.649	0.312 ^{NS}	0
Pislen 526	2	0.421	0.465	0.122 ^{NS}	0
Pislen R05	4	0.381	0.562	0.344 ^{**}	1
Average	5.00	-	-	-	0.510
Cantalaes					
Pislen 21	6	0.500	0.720	0.352 ^{NS}	2
Pislen 114	4	0.727	0.607	-0.151 ^{NS}	0
Pislen 501	8	0.900	0.805	-0.658 ^{NS}	2
Pislen 510	6	0.700	0.640	-0.041 ^{NS}	1
Pislen 526	2	0.364	0.463	0.258 ^{NS}	0
Pislen R05	3	0.636	0.566	-0.077 ^{NS}	0
Average	4.83	-	-	-	0.190
Planted					
Pislen 21	5	0.667	0.660	0.033 ^{NS}	1
Pislen 114	4	0.714	0.541	0.040 ^{NS}	1
Pislen 501	7	0.714	0.792	0.200 ^{NS}	0
Pislen 510	5	0.467	0.667	0.043 ^{NS}	1
Pislen 526	3	0.421	0.497	0.203 ^{NS}	1
Pislen R05	3	0.381	0.538	0.416 ^{NS}	0
Average	4.67	-	-	-	0.460

Chapter III



Barcoding as an ancillary technique to uncover mechanisms of seed dispersal in demanding environments - and to help to restore them

Barcoding as an ancillary technique to uncover mechanisms of seed dispersal in demanding environments - and to help to restore them

Abstract

Mediterranean semiarid areas are suffering important changes in land use. In particular, large extensions of agricultural fields are experiencing a growing process of abandonment that represents a challenge for restoration. Drought and the scarcity of suitable microhabitats make it difficult for plants to colonize these environments. Increased seed dispersal through animal vectors could enhance ecological succession, but precise knowledge of the agents involved is fundamental to take advantage of such interactions for restoration. Otherwise, efforts to foster the processes of interest may be misdirected.

We propose and test a methodological framework based on the combination of ecological (bird censuses and camera trapping) and genetic tools (DNA barcoding), to assess seed dispersal of fleshy-fruited shrub species by birds towards old fields in one of the driest areas of South-eastern Spain (yearly rainfall < 250 mm). We assisted natural restoration with artificial bird attractors (perches and water troughs) in two abandoned areas with different age. Furthermore, we provided microenvironments (rocks) and prevented herbivory (by fencing) to enhance plant recruitment. Results revealed strong differences among assessment techniques in the characterization of dispersal interactions. While bird censuses only allowed for the detection of frugivore occurrence, camera trapping and barcoding enabled the identification of species responses to attractors. The restoration structures deployed proved to be effective for increasing seed arrival to the study areas. However, so far, a subsequent plant establishment has not been observed.

Our study points out the importance of implementing different techniques, including molecular studies, to characterize and assess avian seed disperser performance. Water was undoubtedly an effective attractor which increased seed arrival to study plots. However, our restoration design failed to enhance plant recruitment in the old fields. Thus, the next logical step towards restoration of abandoned Mediterranean fields will be the

optimization of conditions in restored fields for seed germination and successful plant establishment.

Keywords: camera trapping, DNA barcoding, frugivorous birds, old field restoration, seed dispersal networks

1. Introduction

Land abandonment is increasing worldwide (Cramer & Hobbs, 2007), especially in temperate areas such as the Mediterranean Basin (Rey-Benayas, Martins, Nicolau, & Schulz, 2007). This trend particularly affects developed countries (Mottet, Ladet, C  que, & Gibon, 2006), where models predict an increase in such process in coming decades (Rounsevell et al., 2006). Old fields represent an opportunity for the recovery of natural vegetation (Schr  ter et al., 2005) and hence, these environments are considered target areas for restoration policies (Rey-Benayas & Bullock, 2012; Navarro & Pereira, 2015; Queiroz, Beilin, Folke, & Lindborg, 2014). Old fields secondary succession in Mediterranean areas has been widely studied under mesic conditions (Pausas, Bonet, Maestre, & Climent, 2006 and references therein), but there is a lack of studies focusing on this process under semiarid conditions (but see Pausas et al., 2006; Robledano-Aymerich et al., 2014). Old fields in Mediterranean demanding environments (i.e. long drought period and extreme rainfall events) show slow succession and recovery of native plant communities (Midoko-Iponga, Krug, & Milton, 2005; Pugnaire, Luque, Armas, & Guti  rrez, 2006; Ca  nadas et al., 2015; Rey-Benayas, Mart  nez-Baroja, P  rez-Camacho, Villar-Salvador, & Holl, 2015) which can entail severe soil erosion (Romero-D  az, Ruiz-Sinoga, Robledano-Aymerich, Brevik, & Cerd  , 2017). Therefore, the design of practical restoration strategies for activating and accelerating successional processes in such environments is urgently needed (Mendez, Garc  a, Maestre, & Escudero, 2008).

Frugivorous animals can play a key role in the restoration of abandoned patches as they can disperse seeds of plants from natural remnants (Cramer, Hobbs, & Standish, 2008). Frugivorous birds have been widely studied as drivers of restoration in degraded lands. Studies carried out in Mediterranean environments found that birds tend to deposit seeds beneath the isolated remnant trees in old fields (Debussche, Escarr  , & Lepart, 1982; Debussche & Isenmann, 1994) as these places are used for perching

by birds. Furthermore, they provide better conditions for the establishment of seedlings due to higher humidity and less insolation (i.e. facilitation effect, Verdú & García-Fayos, 1998). Hence, birds produce a nucleation effect concentrating seeds in suitable microenvironments (Verdú & García-Fayos, 1996; Pausas et al., 2006). Such effect has been observed in old fields of Mediterranean areas as well as in those located in other biomes (e.g. Willson & Crome, 1989; McClanahan & Wolfe, 1993). Pausas et al., (2006) recommended taking advantage of such effect in management policies of degraded landscapes, enhancing vegetation recovery in old fields by providing artificial perches. These practical experiences were recently reviewed by Guidetti et al., (2016) who found that artificial perches increase seed arrival to target restoration habitats, and hence they are considered one of the cheapest and more effective nucleation techniques. However, the authors pointed out some research gaps as the fact that few studies have evaluated recruitment of plants, and particularly, the lack of practical studies in temperate areas such as the Mediterranean biome (e.g. Heelemann, Krug, Esler, Reisch, & Poschlod, 2012).

The role of frugivorous birds in restoration of old fields can be limited in absence of the above mentioned perch effect, which implicates a poor contribution of birds to seed dispersal in open areas with low vegetation cover (as many semiarid Mediterranean degraded habitats) (Debussche et al., 1982) where other animal vectors can be more effective (e.g. carnivorous mammals, Escribano-Ávila et al., 2014; Escribano-Ávila, Pías, Escudero, & Virgós, 2015; González-Varo, Fedriani, Guitián, López-Bao, & Suárez-Esteban, 2015). Furthermore, old fields are unattractive environments to frugivorous birds since the low vegetation coverage involves high predation risk and low supply of trophic resources (i.e. few fruits) (Santos, Tellería, & Virgós, 1999; García, Zamora, & Amico, 2011). However, most of the fleshy-fruited plants species in Mediterranean habitats are only bird-dispersed (Herrera, 1989 and references therein). Contribution of birds to habitat restoration, as well as their response to restoration initiatives, has been investigated mostly at community level because of the difficulty of establishing species-specific interactions (bird-plant interactions) through classical methods like bird censuses or observational studies (e.g. Lavabre, Gilarranz, Fortuna, & Bascompte, 2016). The study of such species-specific relationships, however, is crucial as different bird species provide complementary seed dispersal services

(García & Martínez, 2012; García et al., 2013; Lavabre et al., 2016; Bai, Li, Li, & Lu, 2017; González-Varo, Carvalho, Arroyo, & Jordano, 2017). On one hand, non-intrusive tracking methods such as camera trapping can be used to conduct research on animal community composition (Kays et al., 2011; Burton et al., 2015), its associated processes (e.g. seed removal, Mokotjomela, Musil, & Esler, 2016), and its response to restoration policies (e.g. Boone IV, McCleery, & Reichert, 2017). On the other hand, molecular techniques can help to shed light on seed dispersal processes (e.g. seed dispersal distances and spatial patterns of seed rain, Godoy & Jordano, 2001; Jordano, García, Godoy, & García-Castaño, 2007; Lavabre et al., 2016; effects of fragmentation, Martínez-López, De la Rúa, Zapata, & Robledano, 2017; Parejo-Farnés, Robledo-Arnuncio, Albaladejo, Rubio-Pérez, & Aparicio, 2017) and to build species-specific seed dispersal networks of bird communities (González-Varo, Arroyo, & Jordano, 2014; González-Varo et al., 2017). However, these genetic tools have been rarely applied to the study of restoration processes (Galimberti et al., 2016).

Here, we develop and test a methodological framework to measure frugivorous bird contribution to the colonization of fleshy-fruited shrubs in semiarid Mediterranean old fields, as well as to study species-specific relationships (bird-plant interactions) in relation to restoration activities. We installed artificial perches and water troughs (a limiting resource in arid conditions; Degen, Pinshow, & Alkon, 1983) to attract birds to abandoned lands and we created microenvironments and prevented herbivory to increase seedling survival opportunities. Since not all species are expected to respond in the same way to our attractors, we combined ecological and genetic tools to get a comprehensive picture of the bird-seed dispersal pattern and its relevance for restoration. First, we studied bird community composition in a ‘natural restoration unit’ involving sink and source areas (i.e. old fields and surrounding natural land) through classical methods (bird census) to know which species could be potentially attracted to the target area. Then, we used non-intrusive detection methods (camera trapping) in old field patches to determine which species of the local bird community were actually using our attractors. Finally, we used molecular techniques (DNA barcoding) to identify which species were dispersing seeds into old fields and to build species-specific bird-seed dispersal networks. Our hypotheses are, first, that this methodological framework detects differential species-specific responses of the frugivores in the bird

community of areas subjected to restoration activities; second, that the restoration framework deployed by us attracts frugivorous birds to old fields thus providing a seed flow directed to water troughs; and third, the microenvironments provided, as well as herbivory exclusion, promote seedling and subsequent plant establishment.

2. Materials and methods

2.1. Methodological framework

We first describe the proposed methodological framework to assess the effect of restoration activities mediated by birds. It combines three different tools (ranging from classical census methods to molecular techniques) to answer the key questions that arise when restoration relying on frugivorous birds is going to be implemented:

- a) *Which avian seed dispersers are there in the area?* Disperser abundance data are hardly ever included in seed dispersal networks (Pigot et al., 2016). However, this is the first question that has to be addressed when assessing the potential role of seed dispersers to assist degraded land restoration. Previous research highlighted the importance of bird abundance predicting seed dispersal in temperate ecosystems (García, Zamora, & Amico, 2010). Classical census methods such as point counts have been widely used to monitor bird communities (e.g. Blondel, Ferry, & Frochot, 1981; Rost, Clavero, Bas, & Pons, 2010; Martínez-López et al., 2017). This method is based on points from which all visual and auditive contacts with birds are recorded considering a fixed radius and/or a delimited period of sampling. Data obtained through this method allow researchers to estimate bird densities, frequencies of occurrence and/or number of individuals per unit of time. However, we are not simply interested in the identity and/or relative abundance of the birds using the target areas, but in the seed dispersal service they can provide to assist their restoration. To this aim, count data can be combined into a dispersal index. This is an unbounded comparative index used to simplify data and make easier to research on differences between study units. Such indexes have been previously used to measure the potential seed dispersal function of birds in Mediterranean semiarid areas, in order to assess the consequences of

land abandonment (Robledano-Aymerich et al., 2014, 2016), and to investigate the effect of fragmentation (Zapata & Robledano, 2014). The index applied in these studies, adapted from Pons et al., (2003), takes into account the total abundance of birds and their role as seed dispersers by classifying them in three categories: legitimate (those that consume fruits but do not damage the seeds which are deposited in faeces or regurgitations; Herrera, 1984), illegitimate (also consuming fruits but destroying the seeds; Herrera, 1984), and facultative (those that can only occasionally consume and disperse fruits, e.g. *Phylloscopus collybita* Vieillot; Cramp, 1998; and those acting as legitimate seed dispersers only in their interaction with some plant species, e.g. *Columba palumbus* L. which is generally considered an illegitimate frugivore but can disperse fruits of *Olea europaea* L. since seeds are hard enough to resist gut passage; Perea & Gutiérrez-Galán, 2016). Nevertheless, the approach followed in such studies was too simplistic since the ability of birds to disperse seeds is also conditioned by other factors such as body mass or dietary preferences (i.e. how important are fruits in the diet of a species). Indeed, recent studies assessing the contribution of frugivorous bird species in seed dispersal networks found that these factors were the best predictors of their importance regarding the interaction strength between birds and plants (Pigot et al., 2016). Therefore, we propose a new index (I_{DISP}), based on that used in the previous studies (details about the index parameters in Table S1a), but including additional features shaping the dispersal potential of species:

$$I_{DISP} = \sum_{i=1}^k [\log(A_i + 1) \times DISP \times B_m \times F]$$

“ k ” is the species richness in a study area “ i ”

A_i = relative abundance of each bird species (e.g. individuals counted per unit of time).

$DISP$ = a coefficient reflecting the role of each species as seed disperser (legitimate = 1, illegitimate = -1, and facultative = 0.5).

B_m = a coefficient that modulates the dispersal ability according to body size (i.e. an standardization of the body mass of each species with regard to the lighter species of the community) (data of body mass from Dunning, 2008).

F = a coefficient that reflects the importance of fruits in each species' diet (i.e. percentage of frugivory) (data taken from the database of Willman et al., 2014).

b) *Which seed dispersers are being affected by our restoration design?*

It is necessary to understand how each species from the disperser assemblage reacts to 'restoration facilities' (e.g. water, perches...). Among all detection systems, camera trapping is increasingly being used as a non-intrusive method which allows researchers to study animal communities without affecting their natural behaviour (Burton et al., 2015). Therefore, we decided to install cameras with automatic detection in the study sites which make possible to obtain data of species occurrence in the target area. Such occurrence data (number of birds of each species recorded per unit of time) can be used to calculate a dispersal index which can be compared with that obtained from point counts. However, it is important to consider the relative abundance of species in the area when their occurrence is approached through camera trapping (see Burton et al., 2015). Thus, we included such factor in the seed dispersal index for camera trapping (I_{DISPc}) data (details about the index parameters in Table S1a-b):

$$I_{DISPc} = \sum_{i=1}^k [\log ((A_i/d) + 1)) \times DISP \times B_m \times F]$$

D = a factor of abundance obtained from point counts (i.e. a standardization of each species abundance with regard to the most abundant one). The remaining factors are the same than in normal I_{DISP} . For non-detected species this factor was considered 1 (i.e. species which did not appear in bird census in some of the sampling dates).

c) *Which seed dispersers are effectively contributing to habitat restoration?* Uncovering the species preferences in relation to

attractors and other components of the restoration design, does not imply knowing which ones are actually carrying seeds to the target area. In fact, recent studies highlight the existence of uneven roles among avian frugivores in seed dispersal across natural and fragmented landscapes (González-Varo et al., 2017; Lavabre et al., 2016). Therefore, genetic tools as DNA barcoding are adequate to determine which birds dispersed the seeds found in the study areas (González-Varo et al., 2014). This technique is based on the amplification of a specific region of the mitochondrial DNA (*cox1*: cytochrome c oxidase subunit I) (Hebert, Ratnasingham, & de Waard, 2003) which show high specificity among animal species, and hence can be used as “barcode” to identify them. Thus, the method can be used to amplify remaining avian DNA in seed surfaces after defecation or regurgitation to identify bird species (González-Varo et al., 2014).

2.2. A case study

2.2.1. Study area

The study was conducted in “Castillo de Chuecos”, a rural estate located in a coastal semiarid Mediterranean mountain area of the Southeast of the Iberian Peninsula (Region of Murcia, Spain) (37° 31'57.25"N, 1° 36'2.89"O; 550-600 m a.s.l.) (Figure 1a). We studied seed dispersal in the area from mid-November to late-January in 2016-2017 (16.2 ± 2.9 days between sampling dates) and 2017-2018 (14.0 ± 0.4 days between sampling dates). The sampling period was selected according to the peak of fructification for most of the Mediterranean fleshy-fruited shrub species (Jordano, 1988). Furthermore, abiotic conditions in such period are better for plant recruitment as temperatures are cooler than in summer and precipitation is more likely to occur (rainfall in Mediterranean areas is concentrated in autumn and spring). The study area is characterized by a matrix of rain-fed crops with almond and olive trees, and natural remnants of Mediterranean forest and shrublands. The area represents an ecotone between the more humid and somewhat colder mesomediterranean layer and the warmer and drier termomediterranean layer (biolimatic classification; Rivas-Martínez, 1983). It is characterized by scarce rainfall (yearly average 218 mm) and high temperatures (average 17.8 °C) (climatic data from L051 station, Tébar, Águilas (Murcia), SIAM 1980-2015).

Vegetation consists of a Mediterranean semiarid shrubland with a sparse tree layer dominated by *Pinus halepensis* L. which only forms a forest canopy in troughs and shady hillsides, and a shrub layer dominated by species such as *Anthyllis cytisoides* L., *Asparagus albus* L., *Asparagus horridus* L., *Cistus clusii* Dunal, *Juniperus oxycedrus* L., *Rhamnus lycioides* L., *Rhamnus alaternus* L., *Rosmarisnus officinalis* L., *Osyris lanceolata* Hochst & Steud.

The area suffered the previously described land abandonment process, which in the Iberian Peninsula reached its maximum intensity in the last decades of the XXth century (García-Ruiz & Lana-Renault, 2011), resulting in many old fields whose age since abandonment ranges from 30 to less than one year. In most cases, these areas have turned into shrublands dominated by *A. cytisoides* which is known to have inhibitory and competitor effects towards other potential plant colonizers (Robledano-Aymerich et al., 2014 and references therein). Therefore, specific restoration strategies are needed if the colonization of these areas by other plant species wants to be enhanced. For instance, promoting the colonization of fleshy-fruited shrubs would increase the presence of frugivores in old fields, which in the long-run could favour plant diversification through internal dispersal.

2.2.2. Restoration design

We applied a bird-based restoration scheme in two abandoned almond fields, in which fleshy-fruited shrubs were lacking, from September 2015 till January 2018. One of the fields remained abandoned for more than 20 years (old abandonment, OA, hereafter) and presented a shrubland cover over 90%, mostly of *A. cytisoides*. The other field was abandoned just before starting the experiment (recent abandonment, RA, hereafter) with less than 10% of vegetation cover (mainly annual species and chamaephytes) (Fig. S1). We established 16 square plots of 64 m² (eight in each abandonment type), half of them fenced for preventing from the effect of herbivory on plant recruitment. However, only 15 plots were considered by the end of the study since water troughs of one of them were stolen in 2016 (unfenced plot 3 in RA, Figure 1b). Two plots (one fenced and one unfenced) were kept as a control in each study area (OA and RA) (Figure 1b). In each of the remaining plots we established water troughs (three replicates per plot) (a drum connected to a cement container) which were

surrounded by three artificial crossbars perches (90 cm high with a 20 cm long crossbar) (Fig. S1c) to increase bird use of the plots (Figure 1c). A plastic tray (44 cm x 28 cm, 7 cm height) was located under one of this artificial perches in each replicate to collect bird-dispersed seeds. Trays were covered with wire mesh (1 cm light) to prevent post-dispersal seed predation and small holes were made on the bottom (1 mm diameter) to allow water drainage. Some rocks (around 10) were placed under one of the perches to provide better microenvironments (i.e. shade and wind protection) for fleshy-fruited plant establishment, whereas the other was kept in bare soil. Rocks have been found to act as effective facilitator element increasing plant recruitment (Peters, Martorell, Ezcurra, 2008; Fujita & Mizuno, 2015). In every plot we also had a control (i.e. only with the three perches but without water trough) to assess the attractant effect of water and its consequences in seed deposition patterns mediated by birds (Figure 1c). Three plastic trays were located in each control plot to assess seed arrival in absence on perches and water troughs.

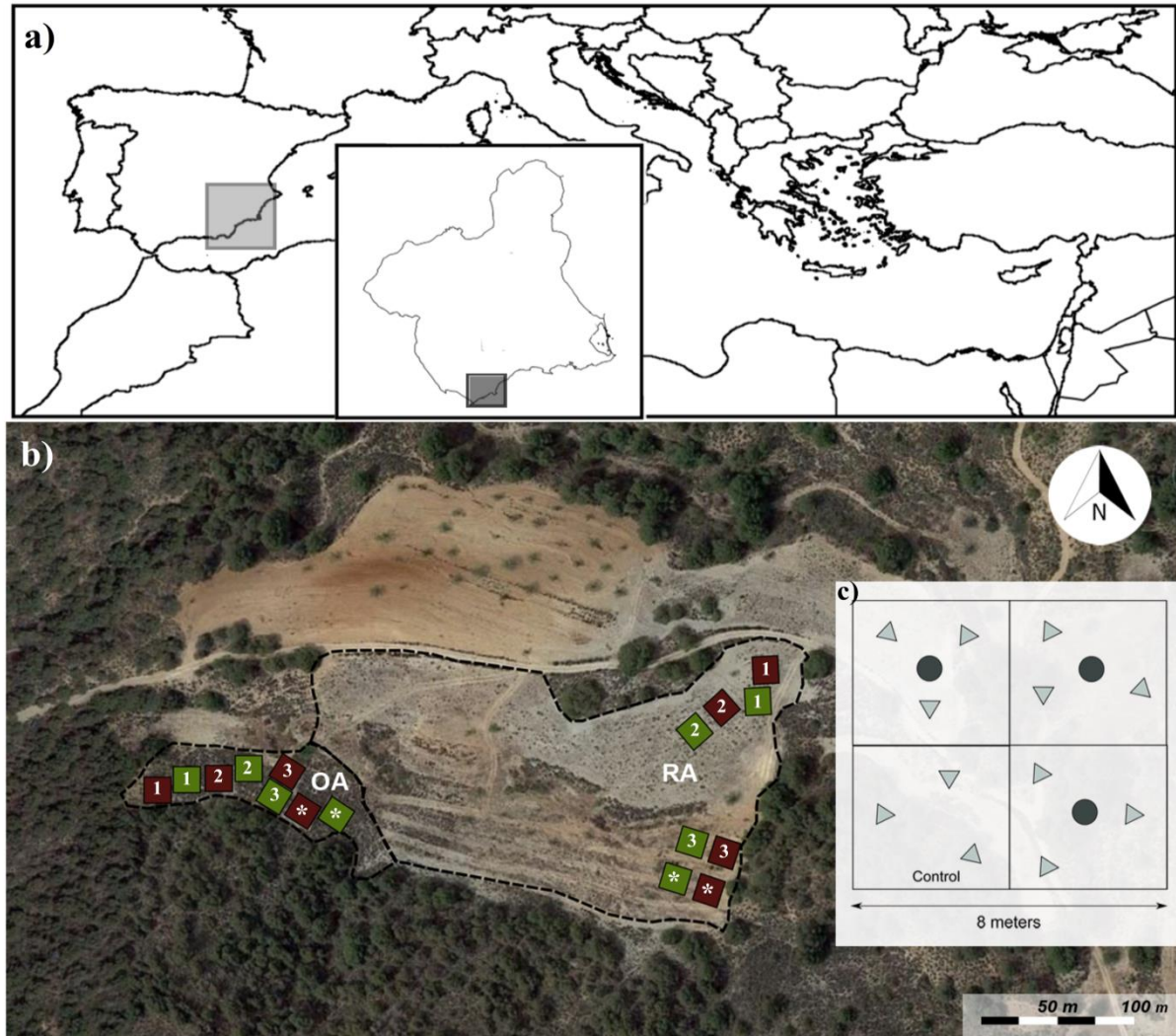


Figure 1. (a) Study area. (b) Restoration design (OA = old abandonment; RA = recent abandonment). Plots of study are represented with squares (Green squares = unfenced; Brown squares = fenced; * = control plots). (c) Experiment design inside each plot (Circles = water trough; triangles = artificial perches).

2.2.3. Plant sampling

We recorded data on shrubs both as seed sources and as colonizers (individuals established from seeds germinating in the plots) (Table S2). In 2016 and 2017, we recorded natural vegetation in the surroundings of the study fields to assess the density of fleshy-fruited species and fruit abundance in the area. Such information is crucial since we did not supply additional propagules (e.g. through seeding or planting), thus plant colonization depended exclusively on these wild vegetation remnants. We recorded the density of fleshy fruited shrub species (individuals/surface unit) in 16 circular units of 100 m² and the abundance of fruits they contained (total number of fruits per individual visually counted). These

circular units were located in the first 100 m from our studied old fields since most of the seed dispersal events mediated by birds has reported to occur in such distances (Jordano et al., 2007). To assess seed arrival effect on vegetation recovery, we fortnightly checked fleshy-fruited plant establishment in eight perches per plot (two perches per water trough (those without tray) and two in the control point) during the three years of study (2015 onwards).

2.2.4. Identification of the seed dispersing birds via censuses and camera trapping

First, we recorded the presence of birds in the area through nine point counts of ten minutes each considering a radius of 50 m (as described above; Blondel et al., 1981): three in the old fields and six in the surrounding remnants of natural vegetation. Data collected (individuals of each species per unit of time, birds/hour) were used to calculate seed dispersal indexes. The seed dispersal index for each period was calculated from the average bird abundance of the nine point counts. Then, we identified bird species using water troughs by camera trapping. We set six cameras (three in RA and three in OA) which were randomly rotated among plots of each abandoned category (we only used data from five cameras for sampling periods 1 and 5 (2016-2017) and sampling periods 2 and 3 (2017-2018) because one of the cameras failed in each of them, see Table S3). Cameras were focused on the water trough and programmed to record 10 sec. video when activated by movement. We also calculated seed dispersal indexes based on birds using water troughs (in this case number of birds of each species per unit of time, birds/hour). Bird abundance was calculated by dividing the number of individuals detected for each species between the number of hours that each camera was functioning (see details in Table S3). The seed dispersal index of each period was calculated as an average of the seed dispersal indexes of cameras in each category (RA and OA). Finally, we checked for seed arrival to the study plots by sampling plastic trays under perches and water troughs (from mid-November to late-January 2015-2016, 2016-2017, and 2017-2018).

2.2.5. Identification of seed dispersing birds via DNA barcoding

Seeds were directly taken with Eppendorf plastic tubes in the deposition places avoiding any contact that could contaminate samples before molecular analysis. Once in the lab, samples were kept frozen (-20 °C) until DNA extraction. Bird dispersers were identified through DNA barcoding

following González-Varo et al., (2014). DNA extraction from seeds was performed with the Kit NucleoSpin Tissue for the isolation of genomic DNA from tissue of MACHEREY-NAGEL (Düren, Germany). After DNA extraction, the dispersed plants were identified through the seed's external morphology. PCR reactions were carried out in a volume of 12.5 µl: 2.5 µl of 5X PCR buffer, 0.5 µl of each 0.04 µM primer (COI-fsdF and COI-fsdR; González-Varo et al., 2014), 0.312 µl of 0.5 mg/ml BSA (bovine serum albumin, Roche Diagnostics, Barcelona, Spain), 0.2 µl of Taq polymerase (Bioline, London, UK) (1 U/Tube), and 6.48 µl of distilled water (ThermoFisher Scientific, Waltham, Massachusetts, USA). PCR conditions followed the description of González-Varo et al., (2014). For those samples that did not successfully amplify due to DNA degradation we used primers COI-fsd-degF and COI-fsd-degR (González-Varo et al., 2017). Such primers perform better when DNA is not well preserved (generally because of high humidity in the sampling area, for example after rainfall events) since they amplify shorter regions. Most of the samples (93.74%) were one strand sequenced with COI-fsdF. Obtained sequences were long enough for species identification (percentage of identification over 99.57%, details about strand amplified and sequence length in Data accessibility). Sequences were edited with Mega7 (Kumar, Stecher, & Tamura, 2016) and identification was done through "BOLD: The Barcode Life of Data System" (Ratnasingham & Hebert, 2007). This platform allows researchers to identify species by uploading sequences and comparing them with those stored in the system.

2.2.6. Data analysis

Data analyses were performed using R v. 3.2.5 (R Development Core Team 2016). The presence of statistically significant differences among dispersal indexes in the area (i.e. data of point counts) and dispersal indexes in the old fields with water troughs (i.e. camera trapping) was checked using Mann–Whitney U tests. For these analysis we pooled data of 2016-2017 and 2017-2018. Data from different years were considered independent as we assumed that bird behaviour is affected by spatio-temporal variation of resources abundance (similar approach as in Tellería, Ramirez, & Pérez-Tris, 2008). We also tested for statistically significant differences between years.

Water through attractant effect to birds would be conditioned by water availability in the area of study. Thus, we also checked for the effect of precipitation on seed dispersal indexes of camera trapping by linear regression. Weighted seed dispersal networks between birds and dispersed seeds were plotted using *bipartite* R package (Dormann, Fründ, Blüthgen, & Gruber, 2009).

3. Results

3.1. *Fleshy-fruited shrubs and fruits in the area*

Five fleshy-fruited species were detected in the area (no. of individuals per ha \pm standard error): *A. horridus* (18.75 ± 6.25), *Dhapne gnidium* L. (3.12 ± 3.12), *J. oxycedrus* (100.00 ± 12.50), *R. alaternus* (6.25 ± 0), and *R. lycioides* (21.87 ± 3.12) (Table S4). *Juniperus oxycedrus* was by far the most abundant species. All observed fruits were from *J. oxycedrus*. Their abundance was higher in 2016-2017 than in 2017-2018 (3593.50 fruits/ha and 1131.20 fruits/ha, respectively; Table S4)

3.2. *Seed dispersers bird community*

We detected 24 frugivorous bird species through point counts. Seven of these species were legitimate seed dispersers (*Erithacus rubecula* L., *Phoenicurus ochruros* S. G. Gmelin, *Sylvia melanocephala* Gmelin, *Sylvia undata* Boddaert, *Turdus merula* L., *Turdus philomelos* Brehm, and *Turdus viscivorus* L.) and five were facultative (*Aegithalos caudatus* L., *Alectoris rufa* L., *C. palumbus*, *P. collybita*, and *Pica pica* L.). Seed dispersal indexes ranged from 0.005 to 0.017 in 2016-2017, and from 0.005 to 0.034 in 2017-2018. There were not significant differences between both years (Census_{16-17 vs 17-18} p-value = 0.8413).

Camera trapping detected 16 bird species using the water troughs (2303 individuals, Table S3); among them only six species were legitimate seed dispersers (*E. rubecula*, *P. ochruros*, *S. melanocephala*, *S. undata*, *T. merula* and *T. viscivorus*) and two facultative (*A. caudatus* and *P. pica*). Seed dispersal indexes ranked from 0.151 ± 0.095 to 2.202 ± 0.947 in 2016-2017, and from 0.148 ± 0.078 to 3.830 ± 1.575 in 2017-2018. There were not significant differences neither between years (OA_{16-17 vs 17-18} p-value = 0.095, n=10; RA_{16-17 vs 17-18} p-value = 0.841, n=10) nor between abandonment categories (i.e. when data from different years were pooled,

OA-RA p-value = 0.421, n=20, Figure 2b). Nevertheless, seed dispersal indexes of bird censuses were significantly different from seed dispersal indexes of camera trapping (p-value<0.001, n=30, Figure 2a). Seed dispersal indexes were not significantly related to precipitation (p-value = 0.499, n=10). However, we explored associations between the two factors by graphically representing seed dispersal indexes evolution versus rainfall in studied periods (Fig. S2). These graphs show that the seed dispersal index decreases according to the increment of precipitation in 2016-2017, whereas the index increases along the study period in 2017-2018 when precipitation was scarce.

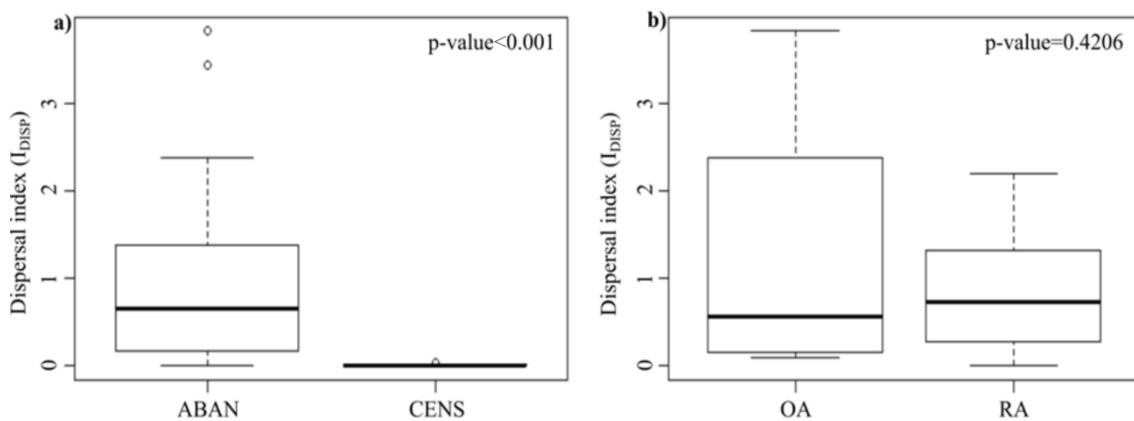


Figure 2. Seed dispersal indexes in the study area and in the plots where restoration activities have been applied with pooled data for the ten sampling periods (five in 2016-2017 and five in 2017-2018). Boxplots show the median, the quartiles and the outliers (white dots). Significance of Mann-Whitney U tests are shown upper right. (a) Comparison between seed dispersal indexes of camera trapping (ABAN; n=20) and point counts (CENS, n=10). (b) Comparison between seed dispersal indexes of camera trapping in old abandonment (OA, n=10) and recent abandonment (RA, n=10).

3.3. Frugivorous bird seed dispersal to old fields

We collected 256 seeds during the three sampling campaigns (2015-2016, 2016-2017, and 2017-2018). Seed arrival to study plots was quite heterogeneous among years. No seed was detected in 2015-2016, whereas most (255) were found in 2016-2017, and only one in 2017-2018. No seed was detected in the control plots along the study period.

DNA barcoding allowed us to identify the dispersers of 248 seeds (96.87% of those collected): *E. rubecula* (1.21% of dispersed seeds), *P. ochruros* (6.45% of dispersed seeds), and *T. viscivorus* (92.34% of dispersed seeds).

Four plant species were dispersed into old fields: *A. albus*, *A. horridus*, *J. oxycedrus*, and *O. lanceolata* (Figure 3). When we looked at the seed rain pattern in old fields in 2016-2017, we observed that most seeds were deposited in fenced RA plots (Figure 4). Furthermore, seed arrival to controls inside study plots (i.e. perches without water trough, Figure 1c), expressed as the number of seeds found in each sampling campaign averaged for the three study plots in fenced RA and two study plots in unfenced RA, was negligible ($RA_{\text{control}} \text{ fenced} = 0.33 \pm 0.33$; $RA_{\text{control}} \text{ unfenced} = 0$) when compared with seeds found close to the water troughs ($RA \text{ fenced} = 26.00 \pm 10.45$; $RA \text{ unfenced} = 1.67 \pm 0.67$). Regarding places where seeds arrived, most of them were deposited in the water trough (95.92%) mainly by *T. viscivorus* (Fig. S3).

With respect to plant establishment in the study plots, we did not detect any recruitment (no seedling emerged from the seeds deposited) throughout the sampling period. However, we can confirm the usage of perches by birds as we detected faeces on the rocks placed beneath them (Fig. S4).

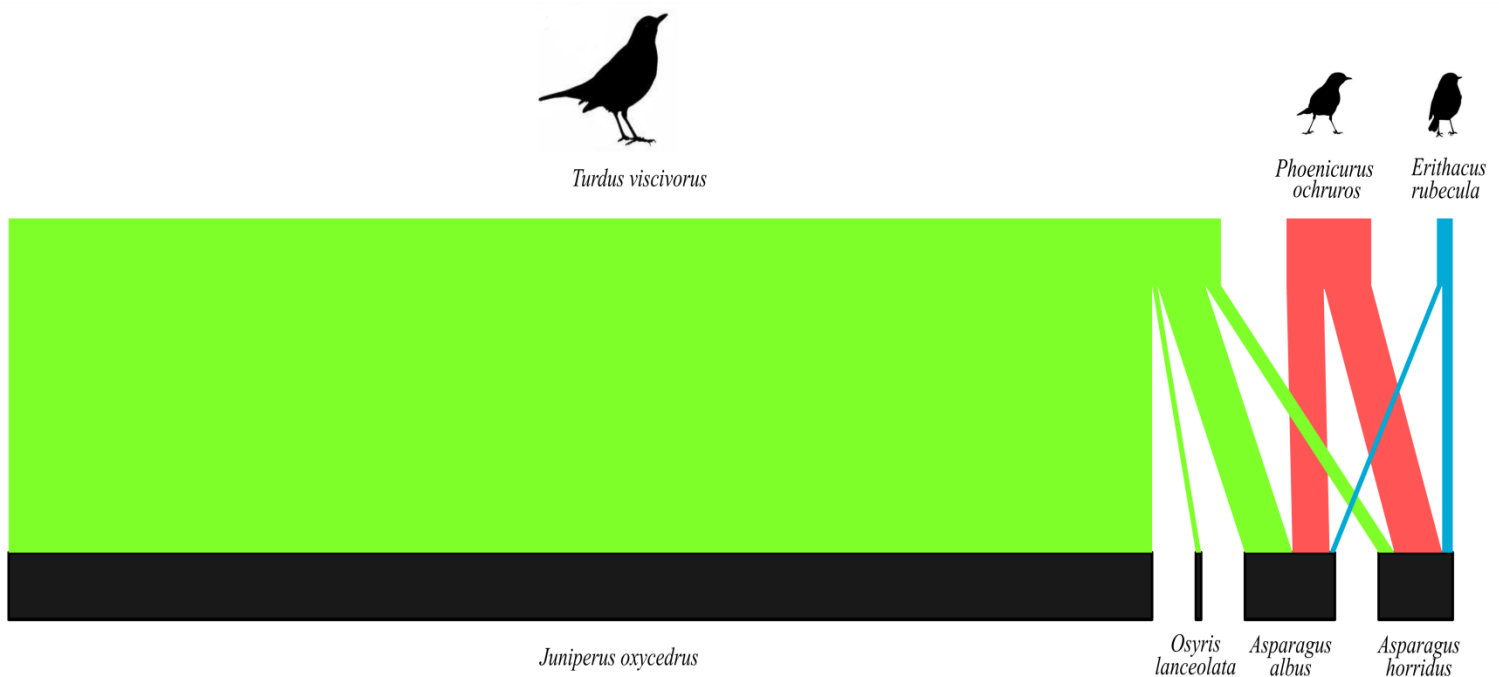


Figure 3. Seed dispersal network among frugivorous birds and fleshy-fruited dispersed plants in the target area after applying our restoration design. Bird-plant interactions have been established through DNA barcoding (network based on 248 seeds).

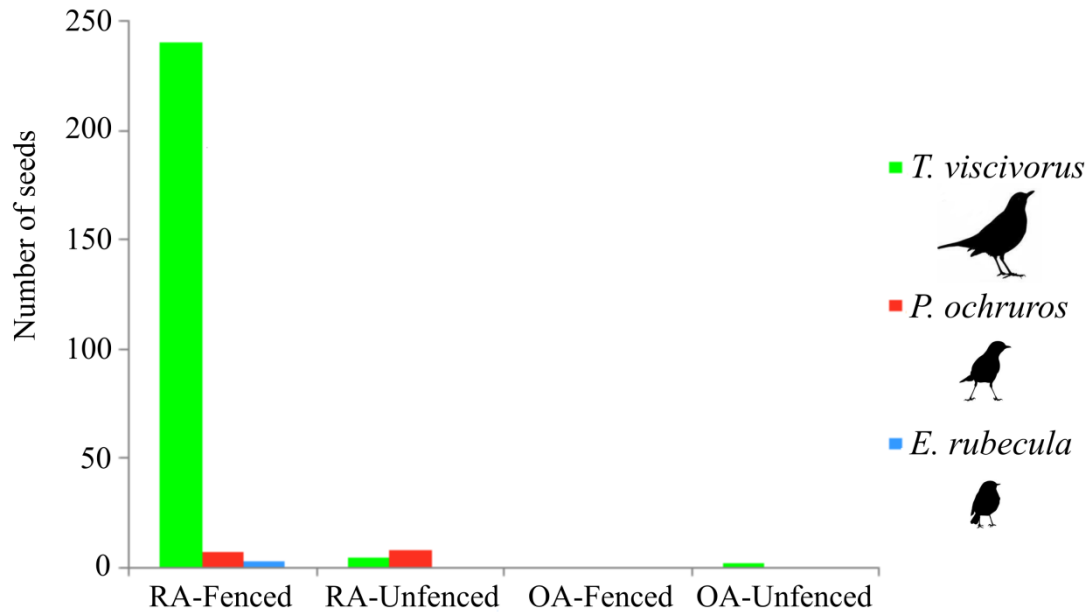


Figure 4. Seed rain mediated by each identified disperser inside the different sampled plots in old fields during 2016-2017 campaign (RA = Recent abandonment; OA = Old abandonment).

4. Discussion

The proposed methodological framework based on the combination of ecological and genetic tools succeeded in identifying bird species-specific responses to habitat restoration activities. The combination of perches and water troughs proved effective in attracting frugivores to the target areas, which resulted in an increase of seed arrival in these areas. However, no positive impact on colonization of old fields was detected, since seedling establishment did not occur. In addition, we identified which bird species of the community were effectively contributing to seed dispersal towards the study area.

García et al., (2010) studied which factors better predicted seed dispersal in different temperate ecosystems. They found that fleshy-fruit abundance, vegetation cover and frugivore abundance were significant related to seed dispersal in the study areas. Among all factors, these authors suggested to use fruits availability as a signal of the seed dispersal in a study area, since frugivores show a high capability of tracking such trophic resources in Mediterranean ecosystems, even foraging in patches with poor vegetation cover (García & Pulido, 2004; Tellería, Ramírez, & Pérez-Tris, 2005). In

the present study, fruit availability was low and highly variable between years (3,593.5 fruits/ha in 2016-2017 and 1,131.2 fruits/ha in 2017-2018), given that a single shrub of *J. oxycedrus* or *R. lycioides* could carry from hundreds to thousands of fruits (authors personal observation). Furthermore, all fruits detected belonged to a single species (*J. oxycedrus*, whose fruit diameter in the study area is around 10 mm, authors personal observation). Fruits that birds can consume are restricted to those they can swallow. In other words it depends on the gape width of each species (Wheelwright, 1985). In our system, the gape width of the legitimate seed disperser species ranges from 6.48 mm (*S. undata*) to 13.52 mm (*T. merula*) (data from Pigot et al., 2016), with only three species (*Turdus* spp) exceeding the fruit diameter of *J. oxycedrus*. Thus, only a small fraction of the frugivorous community can use these resources (but see González-Varo & Traveset, 2016). Indeed, our results mirror such limitation since only *T. viscivorus* dispersed seeds of *J. oxycedrus* into the old field. However, other plant species were also dispersed into the habitat under restoration (*A. albus*, *A. horridus*, and *O. lanceolata*), which highlights the known ability of birds to disperse seeds from more distant source populations than those sampled by us (>100 m, Jordano et al., 2007; González-Varo et al., 2017).

Seed rain mediated by frugivores conditions the chance for fleshy-fruited plant recovery in abandoned lands. Thus, it represents a challenge for colonization by bird-dispersed shrubs since such open areas are generally avoided by the frugivorous bird community due to scarce trophic resources and low vegetation cover (Santos et al., 1999; García et al., 2011). In our case study, most of the legitimate seed dispersers in the landscape also occurred in old fields (six out of seven, with the only exception of *T. philomelos*). Seed dispersal service, measured through seed dispersal indexes, was significantly higher in the study plots (data from camera trapping) than in the surrounding landscape (data from point counts). This fact indicates the effectiveness of our structures (perches and water troughs) directing birds toward old fields. Therefore, our restoration framework managed to overcome the barrier that open areas constitute for many birds increasing seed dispersal services to target areas. Seed dispersal indexes did not significantly change between study years (neither in the landscape nor in the old field) in spite of fruit availability variation. Frugivores can track changes in fruit abundance within heterogeneous landscapes (García & Pulido, 2004; Tellería et al., 2005). However, such

capability is not homogeneous for all avian seed dispersers. Some species, such as *Sylvia atricapilla* L. and *T. philomelos*, have shown to perform better when tracking fruit availability across heterogeneous Mediterranean landscapes (Rey, 1995; Tellería et al., 2008; González-Varo, 2010). In fact, *T. philomelos* occurrence was really low during the study period and it was the only species that did not use the water troughs, which could be possibly explained by the low abundance of fruits in the area. The attractant role of water troughs was not significantly affected by rainfall; however, we observed that seed dispersal indexes from camera trapping showed a decreasing trend in the year with higher precipitations (2016-2017). Other studies did not find a relationship between precipitation and water consumption by wild animals (Gaudioso et al., 2009). Previous studies assessing the role of cattle water troughs in the Iberian Southeast found a negative correlation between precipitation and bird attendance to water troughs (García-Castellanos, Robledano-Aymerich, Zapata-Pérez, Martínez-López, & González-Barberá, 2016). However, such correlation was not found when they analyzed only data from the frugivorous bird community. In arid ecosystems, diet seems to determine water requirements of birds to a greater degree than climatic conditions (Smyth & Coulombe, 1971). The diet of frugivorous birds shows a high content of water (over 80%) what probably lessens their water requirements and hence dependency on artificial water supply (Denslow, Levey, Moermond, & Wentworth, 1986).

The above described seed dispersal service was mirrored in the arrival of propagules to the study plots. Seed rain in the old field was highly heterogeneous between study years (2015-2016, 2016-2017 and 2017-2018) with one of the sampling campaigns accounting for almost all seeds (255 out of 256 in 2016-2017). This could be ascribed to the observed fluctuation in fruits abundance between years since this is the key factor when predicting seed dispersal in temperate ecosystems (García et al., 2010; González-Varo, 2010). Interannual changes in fruit availability are common in ecosystems even within the same landscape (Herrera, 1998; García, Martínez, Herrera, & Morales, 2013). Such temporal dynamics may trigger the increase of seed dispersal into degraded areas (Martínez & García, 2015). The fact that no seed arrived at the control plots is an evidence of the effectiveness of our structures (perches and water troughs) enhancing seed deposition in open areas. Our results are in line with other

studies revealing that artificial perches and water troughs enhance seed dispersal (for perches, see the review by Guidetti et al., 2016 and references therein; for water troughs, García-Castellanos et al., 2016). Seed arrival was much greater in perches close to water troughs than in controls only with perches inside each plot, which shows that the attraction of water outperforms the effect of artificial perches used alone. Most seeds were deposited in RA-fenced plots what contrasts with the absence of differences between abandonment categories in terms of seed dispersal service. Such differences may occur due to habitat preferences of different bird species (forest species vs open habitat species, González-Varo et al., 2017) as OA presents a much higher vegetation cover than RA. *T. viscivorus* is by far the most important disperser in our system and it has been described to use open areas more than other *Turdus* species (e.g. *T. merula*) (García et al., 2013). *Phoenicurus ochruros*, the second species with higher contribution to seed rain, is also a common disperser in open habitats (González-Varo et al., 2017). Indeed, open habitat species abundance was markedly greater in RA than in OA in 2016-2017 (Fig. S5). Seed deposition was higher in fenced plots due to *T. viscivorus* disperser activity. This phenomenon could be related to the use of the fence as intermediate perch when birds fly toward water troughs since we observed them using these structures. Here, we identified dissimilar seed rain in the two monitored restoration structures (i.e. water troughs, 236 seeds, and perches, 12 seeds). These results point out the importance of identifying species-specific interactions with attractors, in order to adapt the restoration design to bird preferences as seed fate can influence the chance of seedling survival (Rey et al., 2004). Our perches were smaller than those used in previous studies (e.g. 6 m high, Holl, 1998; 8 m high, Graham & Page, 2012; 1.8 m high; de Almeida, Marques, de Fátima Ceccon-Valente, Vicente-Silva, & Mikich, 2016). The idea behind this design was to make their usage more practical by future practitioners as we considered that the size was enough to be used by birds (vegetation cover in RA was low and the height of vegetation in OA was less than the height of the perch). Furthermore, we combined them with another attractor (water) unlike previous studies which only relied on the perches. In fact, perches were used by birds so that the design seems to be suitable. Morphology can predict the role of frugivores in seed dispersal networks (Dehling, Jordano, Schaefer, Böhning-Gaese, & Schleuning, 2016). For instance, species with

greater body mass and wider gapes are more important in seed dispersal networks in terms of interaction strength (i.e. the number of plant species they can disperse) (Saavedra et al., 2014; Pigot et al., 2016). Results of our seed dispersal network are consistent with such trends since most of the seeds were dispersed by *T. viscivorus* whose body mass and gape width are one of the greatest in our study system (Table S1 for body mass and database form Pigot et al., 2016 for gape width). Other studies highlighted the role of this thrush in seed deposition in degraded landscapes (García et al., 2013).

For activating old fields secondary succession, seed arrival needs to be translated into plant recruitment. Our restoration measures improved seed arrival to the target area, but plant recruitment can not be granted by this fact (Reid & Holl, 2013). Indeed, we did not detect any seedling in our plots in spite of seed arrival. Different biotic and abiotic factors can limit plant recruitment after seed dispersal. With regard to the biotic factors, post-dispersal seed predation by small mammals and herbivory can act as a barrier for successful vegetation recovery in fragmented landscapes (Santos & Tellería, 1994; MacDougall & Wilson, 2007). However, we did not observe any signal of seed predation in our sampling (e.g. seed coats) and herbivory was prevented by fencing half of the plots. Therefore, the seedlings seem to be limited by abiotic factors (for example, light, nutrients, water, etc.) that are conditioned in turn by the microhabitat where the seeds arrive (Gómez-Aparicio, 2008). Our results suggest that the microenvironment provided in our system (rocks) was not fully effective in spite of its demonstrated facilitator effect in other studies (Peters et al., 2008; Fujita & Mizuno, 2015). Although these microhabitats could represent safe sites (e.g. against seed predation), microclimate amelioration was probably insufficient to allow germination and seedling survival, at least during the years of the study.

5. Concluding remarks and future research lines

Many studies have highlighted the importance of taking advantage of frugivorous animals to restore degraded lands (Pausas et al., 2006; Mendez et al., 2008). However, there is a gap of knowledge regarding practical experiences in Mediterranean environments (but see Rey-Benayas et al., 2015; Fedriani et al., 2017; Castillo-Escrivà, López-Iborra, Cortina, & Tormo, 2018). This information is crucial to develop management policies

that promote vegetation recovery of degraded lands in Mediterranean semiarid areas threatened by desertification processes such as the Southeast of the Iberian Peninsula (López-Bermúdez & Albaladejo, 1990; Romero-Díaz, 2016).

We have monitored the three critical stages through which old field vegetation colonization has to be assessed. However, our measures were mainly directed at increasing frugivore occurrence and seed arrival to the area under restoration, and we supplied only one type of microhabitat (rocks) for enhancing seedling establishment, which proved to be insufficient (Reid & Holl, 2013). In the future, they could be oriented to the implementation of facilitating elements for the restoration. For example, many studies report an enhancement of recruitment when shrub species are located beneath perching sites as seeds arrive to suitable environments with proper conditions for plant establishment (Gómez-Aparicio, Gómez, Zamora, & Boettinger, 2005; Smit, Den Ouden, & Müller-Schärer, 2006; Martínez & García, 2017; Fujita & Yamashina, 2018). Hence, active planting of some vegetation under artificial perches could help old field plant colonization. On the other hand, landscape configuration (e.g. fruit abundance and vegetation cover) can condition the success of restoration. Restoration ecologists and practitioners should monitor such variables as the amount and variety of fruits, and vegetation cover when determining the role of different frugivorous species as restoration agents in the system. Restoration frameworks can also artificially increase propagule abundance in the area by supplying fruits which could be a solution in those cases of extreme landscape degradation with absence of seed sources (i.e. when the shrub layer is absent from surrounding areas). Indeed, other studies documented that an increase in resource availability could prevent the loss of mutualistic interactions in fragmented landscapes (Fontúrbel, Salazar, & Medel, 2017).

Our methodological framework proved to be effective in assessing species attraction and usage of restoration structures (perches and water troughs in our case), as well as seed dispersal provided by different frugivores. The identification of species-specific relationships between plant and seed dispersers through the recent development of molecular techniques (González-Varo et al., 2014) has allowed researchers to unravel the ecological role of species in seed dispersal across fragmented landscapes

(González-Varo et al., 2014, 2017). The application of such approach in restoration practices makes it possible to adapt them to the target frugivores according to particular conditions (e.g. different landscape characteristics such as fruit abundance and distribution, or vegetation cover). With regard to our study case, to our knowledge it represents the first example of applied research on the use of perches and water troughs for enhancing frugivore-mediated old field restoration in the Mediterranean Basin. Thereby, our results provide restoration ecologists and practitioners with practical knowledge that can be used as a tool in the management of abandoned lands in demanding environments where habitat restoration remains as a challenge.

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Supporting information

Fig. S1 Study areas and experimental design.

Fig. S2 Temporal evolution in seed dispersal service measured through camera trapping.

Fig. S3 Seed dispersal networks according to the number of seeds that arrived at each structure.

Fig. S4 Proof of the use of perches by birds.

Fig. S5 Main seed dispersers abundances in 2016-2017 sampling campaign.

Table S1 Parameters for the calculation of seed dispersal indexes.

Table S2 Information about sampling variables in the study area.

Table S3 Information about birds per hour detected in camera trapping.

Table S4 Fleshy-fruited shrubs and fruits in the area.

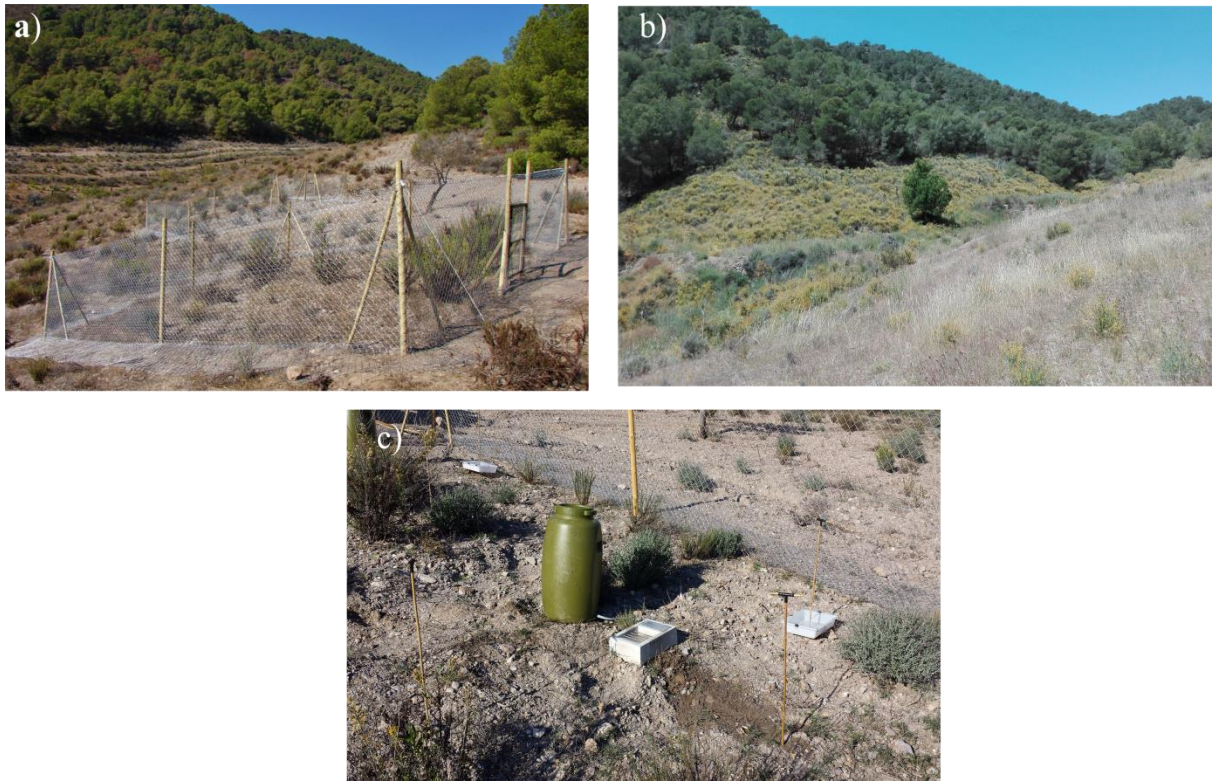


Fig. S1. Study areas and experimental design. (a) Recent abandonment area just after fencing; (b) View of the old abandonment area after the experiment was deployed; (c) Water troughs and artificial perches.

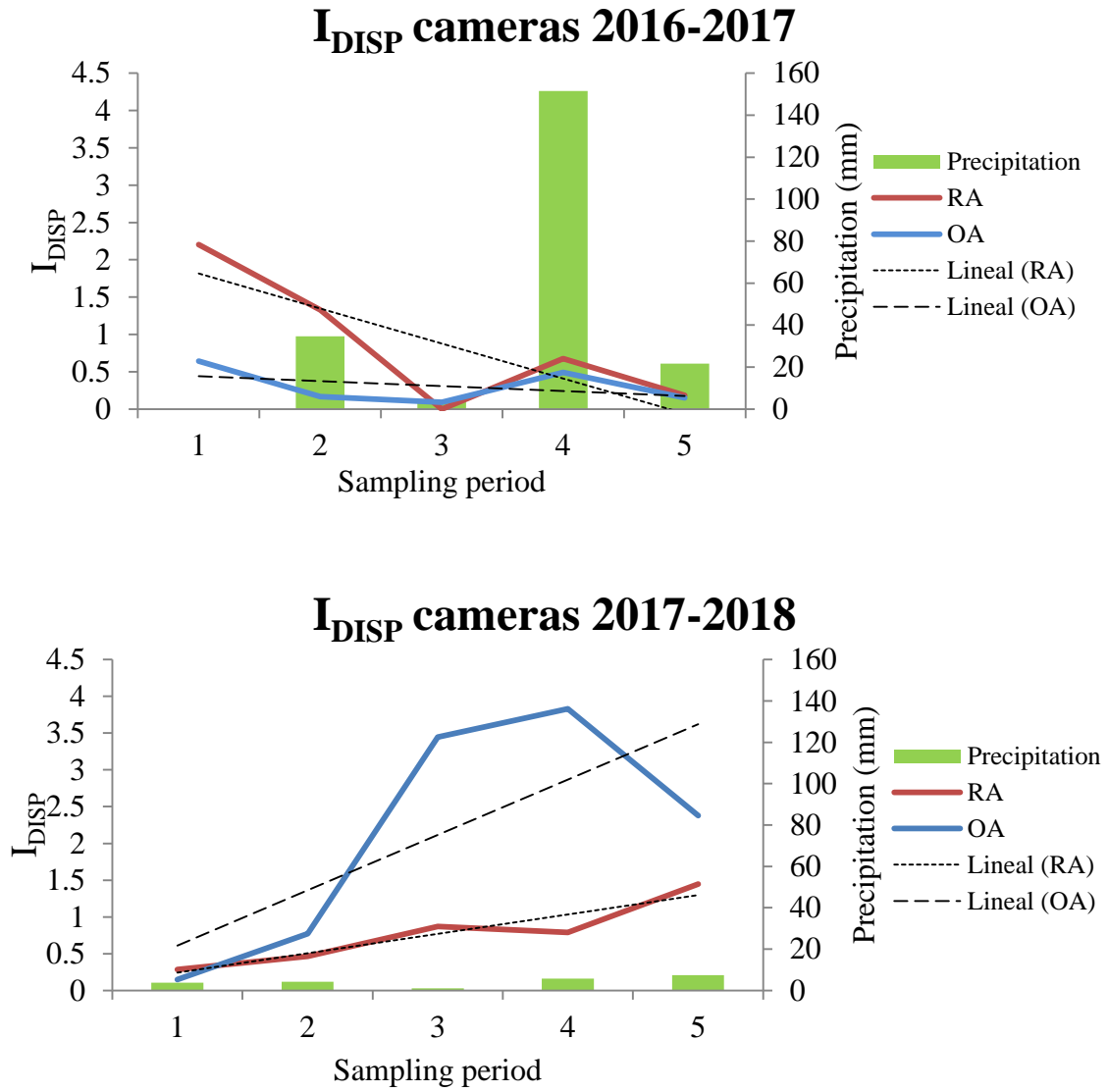


Fig. S2. Seed dispersal service in study plots from camera trapping data (I_{DISPc}) in relation to precipitation in the two study periods (OA=Old abandonment; RA=Recent abandonment).

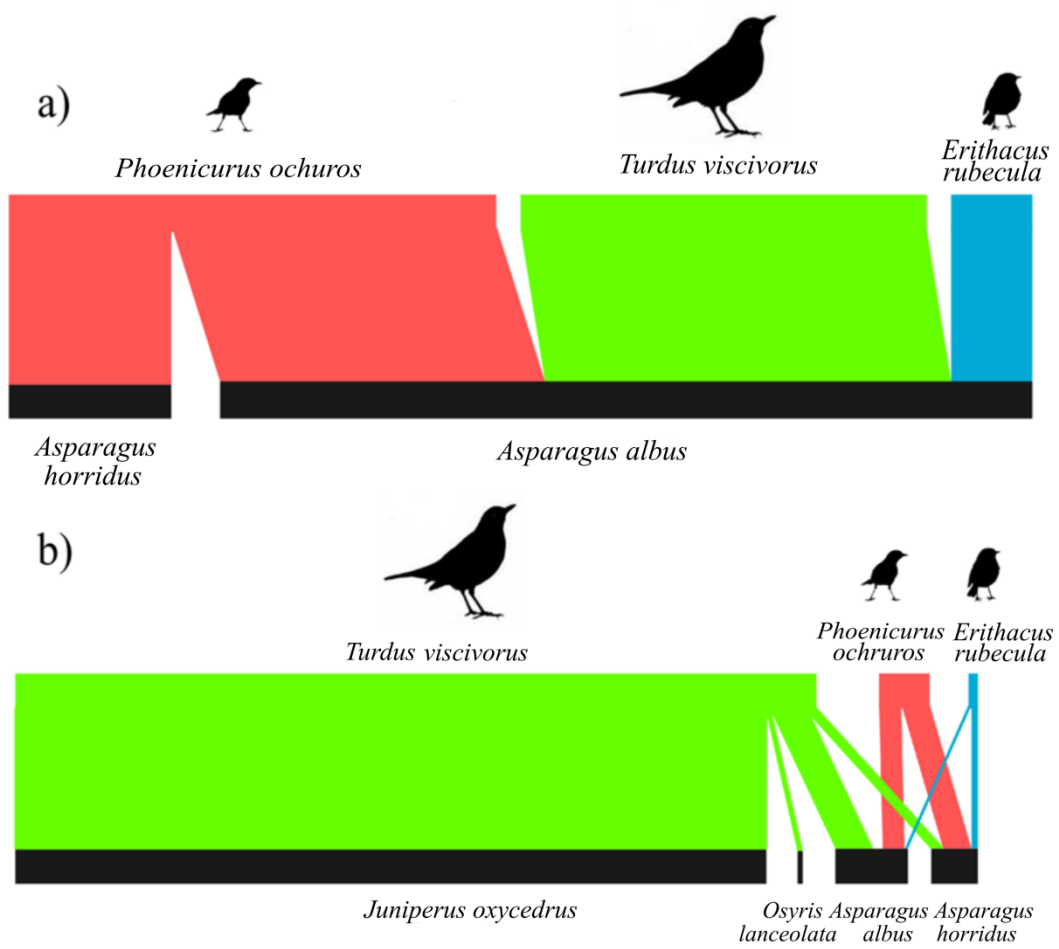


Fig. S3. Seed dispersal networks according to the number of seeds that arrived at each structure. (a) Seed dispersal network of seeds collected in plastic trays under perches (based on 12 seeds); (b) Seed dispersal network of seeds deposited in the water trough (based on 236 seeds).



Fig. S4. Proof of the use of perches by birds. Examples of faeces deposited beneath different perches.

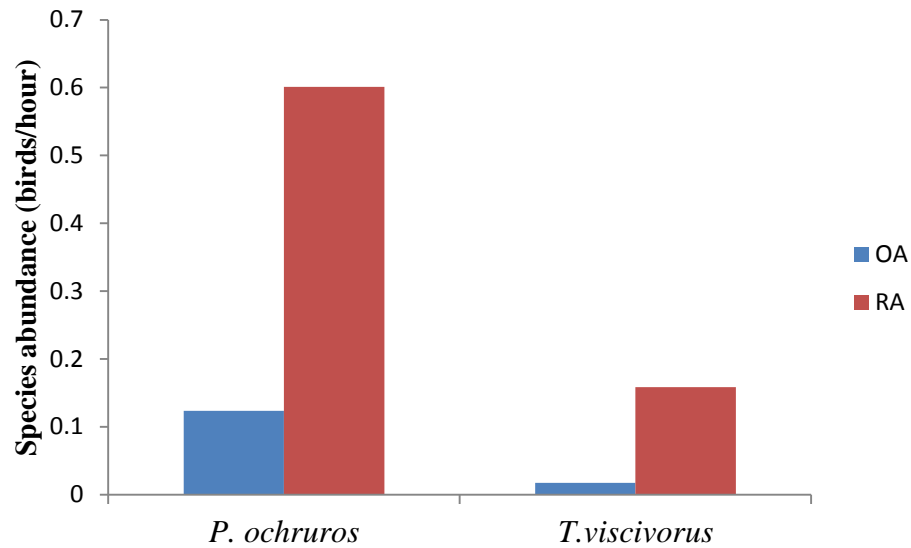


Fig.S5. Abundance of the main seed dispersers in our system (expressed as the sum of abundances of the five periods) according to camera trapping (*PHO OCH*=*Phoenicurus ochruros*; *TUR VIS*=*Turdus viscivorus*) in 2016-2017 sampling campaign (OA=Old abandonment; RA=Recent abandonment).

Table S1. Parameters for the calculation of seed dispersal indexes. Frugivory (i.e. importance of fruit in the diet) in species detected in our study system (data from Wilman et al., 2014). Body mass and body mass standardization with regard to the lower value (i.e. species body mass divided by the lighter species) (Data from Dunning, 2008). (AEG CAU=*Aegithalos caudatus*; ALE RUF=*Alectoris rufa*; CAR CAN=*Carduelis cannabina*; CAR CAR=*Carduelis carduelis*; CAR CHL=*Carduelis chloris*; COL PAL=*Columbus palumbus*; CYA CAE=*Cyanistes caeruleus*; EMB CIA=*Emberiza cia*; EMB CIR=*Emberiza cirulus*; ERI RUB=*Erithacus rubecula*; FRI COE=*Fringilla coelebs*; LOP CRI=*Lophophanes cristatus*; LOX CUR=*Loxia curvirostra*; PAR MAJ=*Parus major*; PER ATE=*Periparus ater*; PHO OCH=*Phoenicurus ochruros*; PHY COL=*Phylloscopus collybita*; PIC PIC=*Pica pica*; SER SER=*Serinus serinus*; SYL MEL=*Sylvia melanocephala*; SYL UND=*Sylvia undata*; TUR MER=*Turdus merula*; TUR PHI=*Turdus philomelos*; TUR VIS=*Turdus viscivorus*). Facultative (*), Legitimate (**), and illegitimate frugivores (***) are differentiated according to Herrera (1984).

Abbreviation	Frugivory (<i>F</i>)	Body mass	Seed disperser (<i>DISP</i>)	Body mass standardization (Lower limit) (<i>Bm</i>)
AEG CAU*	1	8.60	0.5	1.04
ALE RUF*	1	527.86	0.5	63.60
CAR CAN***	2	19.53	-1	2.35
CAR CAR***	2	16.00	-1	1.93
CAR CHL***	0	26.00	-1	3.13
COL PAL*	3	490.00	0.5	59.04
CYA CAE***	2	13.30	-1	1.60
EMB CIA***	0	23.43	-1	2.82
EMB CIR***	0	25.60	-1	3.08
ERI RUB**	2	17.70	1	2.13
FRI COE***	0	23.81	-1	2.87
LOP CRI***	2	11.04	-1	1.33
LOX CUR***	0	38.29	-1	4.61
PAR MAJ***	2	16.25	-1	1.96
PER ATE***	0	9.20	-1	1.11
PHO OCH**	3	16.50	1	1.99
PHY COL*	1	8.30	0.5	1.00
PIC PIC*	1	217.48	0.5	26.20
SER SER***	0	11.20	-1	1.35
SYL MEL**	4	11.70	1	1.41
SYL UND**	3	10.80	1	1.30
TUR MER**	2	102.73	1	12.38
TUR PHI**	3	67.74	1	8.16
TUR VIC**	2	117.37	1	14.14

Table S2. Information about sampling variables in the study area.

	Plants	Birds	Seeds
Surrounding area	Density of fleshy-fruited plants Fruit abundance	Point counts	-
OA and RA*	-	Point counts	-
Study plots	Seedling establishment**	Camera trapping DNA barcoding	Seed arrival to perches***and water troughs

*The full area (Figure 1).

**Under perches without trays (with and without microenvironments).

***Those with tray

Table S3. Birds per hour detected with camera trapping in the different plots and study periods (Data about Plot ID in Figure 2) (AEG CAU=*Aegithalos caudatus*; CAR CHL=*Carduelis chloris*; EMB CIA=*Emberiza cia*; EMB CIR=*Emberiza cirulus*; ERI RUB=*Erithacus rubecula*; FRI COE=*Fringilla coelebs*; LOP CRI=*Lophophanes cristatus*; LOX CUR=*Loxia curvirostra*; PAR MAJ=*Parus major*; PHO OCH=*Phoenicurus ochruros*; PIC PIC=*Pica pica*; SER SER=*Serinus serinus*; SYL MEL=*Sylvia melanocephala*; SYL UND=*Sylvia undata*; TUR MER=*Turdus merula*; TUR VIS=*Turdus viscivorus*). Facultative (*), Legitimate (**), and illegitimate frugivores (***) are differentiated according to Herrera (1984).

2016-2017				N° of visits recorded for each species																	N° detected birds	N° of species
Sampling	Plot	Installation date	Recording hours	AEG CAU *	CAR CHL ***	EMB CIA ***	EMB CIR **	ERI RUB **	FRI COE ***	LOP CRI ***	LOX CUR ***	PAR MAJ ***	PHO OCH **	PIC PIC *	SER SER ***	SYL MEL **	SYL UND **	TUR MER **	TUR VIS **			
1	2-OA-fenced	11/10/16	116.98	0	0	1	0	14	0	0	0	0	2	0	3	0	0	0	0	20	4	
	2-RA-fenced	11/10/16	116.00	0	0	1	2	3	0	1	0	0	19	0	19	0	0	0	13	58	7	
	1-RA-unfenced	11/10/16	115.52	0	0	0	0	0	0	0	0	0	2	0	8	0	1	0	0	11	3	
	1-RA-fenced	11/10/16	112.28	0	0	2	0	1	0	1	2	0	30	0	55	0	0	0	3	94	7	
	3-OA-unfenced	11/10/16	116.35	0	0	6	0	4	0	0	2	0	0	0	16	0	0	0	2	30	5	
2	1-OA-unfenced	11/21/16	130.47	0	0	1	0	5	0	0	5	0	0	0	1	0	0	0	0	12	4	
	2-OA-fenced	11/21/16	130.27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	3-OA-fenced	11/21/16	130.67	0	0	2	0	0	0	0	0	0	4	0	5	0	0	0	0	11	3	
	2-RA-unfenced	11/21/16	130.67	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	2-RA-fenced	11/21/16	131.10	0	0	0	0	0	0	0	0	0	7	0	0	0	0	0	0	7	1	
	3-RA-fenced	11/21/16	130.58	0	0	0	1	0	0	0	0	0	3	0	6	0	0	0	2	12	4	

Sampling	Plot	Installation date	Recording hours	N° of visits recorded for each species																	N° detected birds	N° of species
				AEG CAU *	CAR CHL ***	EMB CIA ***	EMB CIR ***	ERI RUB **	FRI COE ***	LOP CRI ***	LOX CUR ***	PAR MAJ ***	PHO OCH **	PIC PIC *	SER SER ***	SYL MEL **	SYL UND **	TUR MER **	TUR VIS **			
3	2-OA-fenced	12/05/16	93.00	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	3-OA-fenced	12/05/16	93.00	0	0	1	0	0	0	0	0	0	2	0	2	0	0	0	0	5	3	
	2-RA-unfenced	12/05/16	93.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	2-RA-fenced	12/05/16	93.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	3-RA-fenced	12/05/16	93.25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	1-OA-unfenced	12/15/16	236.35	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	2	2	
4	1-RA-unfenced	12/15/16	235.47	0	0	0	0	1	0	0	0	0	3	0	2	0	0	0	6	3		
	1-RA-fenced	12/15/16	235.57	0	0	1	0	0	0	0	0	2	0	9	0	0	0	12	3			
	2-OA-fenced	12/15/16	235.28	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1			
	3-OA-fenced	12/15/16	235.50	0	0	4	0	1	0	0	0	3	10	0	29	0	1	1	49	7		
	3-RA-fenced	12/15/16	235.43	0	0	0	0	2	0	1	2	0	2	0	5	0	0	1	13	6		
	5	1-OA-fenced	01/09/17	226.65	0	0	0	0	5	0	4	57	0	0	0	0	0	0	66	3		
1-RA-unfenced		01/09/17	226.03	0	0	1	1	1	0	0	0	0	0	0	0	0	0	3	3			
2-RA-unfenced		01/09/17	228.07	0	0	1	0	0	0	0	1	0	1	0	5	0	0	8	4			
3-OA-unfenced		01/09/17	227.70	0	0	1	0	1	0	0	1	0	0	0	3	0	0	6	4			
3-RA-fenced		01/09/17	227.97	0	0	10	0	1	0	3	3	0	2	0	0	0	0	19	5			

2017-2018

Sampling	Plot	Installation date	Recording hours	N° of visits recorded for each species																N° detected birds	N° of species
				AEG CAU *	CAR CHL ***	EMB CIA ***	EMB CIR* **	ERI RUB **	FRI COE ***	LOP CRI ***	LOX CUR ***	PAR MAJ ***	PHO OCH **	PIC PIC *	SER SER ***	SYL MEL **	SYL UND **	TUR MER **	TUR VIS **		
1	1-OA-fenced	11/15/17	144.25	0	0	0	0	0	0	0	0	0	0	0	2	0	1	1	0	4	3
	1-RA-fenced	11/15/17	145.03	0	0	3	0	2	0	0	0	0	3	0	5	0	0	0	0	13	4
	2-OA-fenced	11/15/17	144.53	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2-RA-fenced	11/15/17	145.37	0	0	0	0	1	0	0	0	0	0	0	6	0	0	0	0	7	2
	3-OA-fenced	11/15/17	144.92	0	0	0	0	4	0	0	0	0	1	0	4	0	3	0	0	12	4
	3-RA-fenced	11/15/17	143.23	0	0	4	0	6	0	1	0	4	6	0	2	0	2	0	0	25	7
2	1-RA-unfenced	11/30/17	139.53	0	0	4	0	2	0	0	0	0	0	6	0	2	0	0	14	4	
	2-OA-unfenced	11/30/17	138.75	0	0	4	0	0	0	2	0	2	0	82	0	1	1	0	92	6	
	2-RA-unfenced	11/30/17	141.93	0	0	8	0	4	0	0	0	0	0	59	0	1	0	0	72	4	
	3-OA-unfenced	11/30/17	138.62	0	0	0	0	15	0	0	0	5	0	22	0	0	0	0	42	3	
	3-RA-unfenced	11/30/17	139.45	0	0	1	0	5	2	0	0	2	5	1	0	3	0	0	19	7	
3	1-RA-unfenced	12/13/17	141.87	0	0	1	0	3	0	0	0	0	0	66	0	0	0	0	70	3	
	2-OA-unfenced	12/13/17	140.95	0	0	3	0	0	0	0	1	0	0	114	0	0	7	0	125	4	
	2-RA-unfenced	12/13/17	141.83	0	0	2	0	1	0	0	0	4	0	358	0	4	0	0	369	5	
	3-OA-unfenced	12/13/17	143.53	0	0	6	0	8	0	0	0	3	0	86	0	1	2	0	106	6	
	3-RA-unfenced	12/13/17	142.03	0	0	3	0	0	6	1	0	0	1	16	0	8	0	0	35	6	

Sampling	Plot	Installation date	Recording hours	N° of visits recorded for each species																	N° detected birds	N° of species
				AEG CAU *	CAR CHL ***	EMB CIA ***	EMB CIR* **	ERI RUB **	FRI COE ***	LOP CRI ***	LOX CUR ***	PAR MAJ ***	PHO OCH **	PIC PIC *	SER SER ***	SYL MEL **	SYL UND **	TUR MER **	TUR VIS **			
4	1-OA- unfenced	12/27/17	161.23	0	0	1	0	2	0	1	0	3	0	0	10	0	0	1	0	18	6	
	1-RA- fenced	12/27/17	160.95	0	0	1	0	0	3	0	0	0	3	0	200	0	0	0	0	207	4	
	2-OA- fenced	12/27/17	161.02	0	0	1	0	0	0	0	0	0	1	0	13	0	0	8	0	23	4	
	2-RA- unfenced	12/27/17	160.83	0	0	1	0	0	0	0	0	0	1	0	2	0	1	0	0	5	4	
	3-OA- fenced	12/27/17	160.15	0	8	27	2	5	2	0	0	4	17	0	183	3	7	0	0	258	10	
	3-RA- fenced	12/27/17	160.77	0	1	15	0	14	2	6	0	2	0	0	43	0	5	0	0	88	8	
	1-OA- unfenced	01/11/18	131.22	0	0	2	0	2	0	0	0	2	4	0	1	0	0	1	0	12	6	
5	1-RA- unfenced	01/11/18	131.17	0	1	2	0	1	1	0	0	0	6	1	98	0	0	0	0	110	7	
	2-OA- unfenced	01/11/18	131.22	2	0	0	0	0	0	0	0	3	0	0	13	0	0	2	0	20	4	
	2-RA- unfenced	01/11/18	131.35	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	2	1	
	3-OA- unfenced	01/11/18	131.05	0	0	1	0	7	0	0	0	0	0	0	8	0	6	0	0	22	4	
	3-RA- fenced	01/11/18	131.47	0	0	9	0	4	1	4	0	3	3	0	61	0	2	0	0	87	8	

Table S4. Fleshy-fruited shrub density and fruit abundance in the natural vegetation remnants surrounding the studied old fields.

2016						
Species	N°in div	Density (ind/m ²)	Density (ind/ha)	Total amount of fruits	Fruits density (fruit/m ²)	Fruit density (fruit/ha)
<i>Asparagus horridus</i>	4	0.0025	25	0	0	0
<i>Dhapne gnidium</i>	0	0.0025	0	0	0	0
<i>Juniperus oxycedrus</i>	14	0.00875	87.5	575	0.359375	3593.75
<i>Rhamnus alaternus</i>	1	0.000625	6.25	0	0	0
<i>Rhamnus lycioides</i>	3	0.001875	18.75	0	0	0
2017						
Species	N°in div	Density (ind/m ²)	Density (ind/ha)	Total amount of fruits	Fruits density (fruit/m ²)	Fruit density (fruit/ha)
<i>Asparagus horridus</i>	2	0.00125	12.5	0	0	0
<i>Dhapne gnidium</i>	1	0.000625	6.25	0	0	0
<i>Juniperus oxycedrus</i>	18	0.01125	112.5	181	0.113125	1131.25
<i>Rhamnus alaternus</i>	1	0.000625	6.25	0	0	0
<i>Rhamnus lycioides</i>	4	0.0025	25	0	0	0

Chapter IV



**Contrasting effects of beekeeping and land use on
plant-pollinator networks and pathogen
prevalence in a Mediterranean semiarid
ecosystem**

Contrasting effects of beekeeping and land use on plant-pollinator networks and pathogen prevalence in a Mediterranean semiarid ecosystem

Abstract

The knowledge about mutualistic interaction networks between pollinators and plants is crucial to establish protection measures given the current decline of populations of insect pollinators. Land use changes and interspecific transmission of pathogens stand among the different stressors related to such decrease. However, most of the research effort has been focused on specific taxa and rarely at a community level, particularly in the less-studied semiarid ecosystems.

Here, we investigate plant-pollinator (Apoidea) and pathogen-pollinator networks in the Iberian Southeast during late winter and spring of 2017. We selected nine areas under a variable degree of land use and beekeeping intensity. We studied the effect of these two variables on the structure of pollination networks as well as the role of honey bees in the spillover of pathogens (*Nosema apis* and *Nosema ceranae*) to the wild bee community.

Interaction diversity in plant-pollinator networks was greater at the end of the spring while weighted connectance slightly decreased. Land use had a weak impact on plant-pollinator networks; however, those impacts were generally positive in the case of agriculture, whereas mainly negative in the case of shrublands. Density of honey bees decreased along the study period and so the prevalence of *N. ceranae*. Contrastingly, density of wild bee communities did not vary in time, but the prevalence of pathogens increased in spring by the end of the study.

Results corroborate the impact of honey bees on wild bees also in semiarid ecosystems by promoting the spread of pathogens in the landscape even after honey bee density decreases.

Keywords: wild bees, honey bees, beekeeping, land use, pathogens interspecific transmission

1. Introduction

Pollination mediated by animal vectors is a crucial ecosystem service for plants since around the 88% of the 352,000 species of angiosperm plants are pollinated by animal guilds (Ollerton, Winfree, & Tarrant, 2011). Such mutualistic interaction is also crucial for humans as many crops depend on animal pollination (Klein et al., 2007). Insects, and especially bees, stand out among animals as plant pollinators. Thus, there is a global concern about the worldwide decline of pollinators (Potts et al., 2010; Goulson, Nicholls, Botías, & Rotheray, 2015) which could threaten ecosystems functioning by affecting plant reproductive success (Biesmeijer et al., 2006). Different stressing factors have been identified as drivers of such decline, for example natural ecosystems surface reduction due to changes of land use, and the interspecific transmission of pathogens (Goulson et al., 2015). However, it is not well known how those factors interact among them (Potts et al., 2006; Ratnieks & Carreck, 2010), and hence research assessing the effect of different stressors on pollinators is needed (Vanbergen & Insect Pollinators Initiative, 2013).

The honey bee (*Apis mellifera* L.) has been traditionally considered the main supplier of pollination services in crop production (Morse, 1991; Rucker, Thurman, & Burgett, 2012). However, recent studies described a complementary and independent role of honey bees and wild bees in crop pollination with some wild bees being twice as effective as honey bees increasing fruit sets (Garibaldi et al., 2013). Nowadays, there is an open debate in science about the effect of honey bees on wild pollinators (Geslin et al., 2017; Geldmann & González-Varo, 2018). In that sense, recent reviews about the competition between honey bees and wild bees for floral resources have reported controversial results (Mallinger, Gaines-Day, & Gratton, 2017; Wojcik, Morandin, Davies Adams, & Rourke, 2018). Nevertheless, their authors pointed to a lack of research on this topic, and therefore encouraged conservative approaches when establishing bee hives in natural areas (Wojcik et al., 2018). Similarly, contrasting results have been described in relation to possible changes in the composition of the vegetation community induced by the honey bee (Mallinger, Gaines-Day, & Gratton, 2017). However, there is more evidence about the role of honey bees on the spillover of pathogens to wild bee pollinators communities as around 70% of the studies reported the presence of honey bee pathogens in

wild bees (Mallinger et al., 2017). Therefore, both, the settlement of honey bee hives in protected areas to obtain honey, as well as the movement of honey bees from agricultural lands to surrounding natural areas (González-Varo & Vilá, 2017), expose wild bees to the contact with honey bees and their associated pathogens (Graystock, Blane, McFrederick, Goulson, & Hughes, 2016). Those pathogens have been related to Emerging Infectious Diseases (EID, Manley, Boots, & Wilfert, 2015), among them, microsporidia play an important role on the disappearance of honey bees, especially in Mediterranean countries (Martín-Hernández et al., 2017). The effects on wild bees of the microsporidia from the genus *Nosema* have been studied in wild bees in Central Europe (Fürst, McMahon, Osborne, Paxton, & Brown, 2014; Ravoet et al., 2014) and South America (Plischuk et al., 2009; Porrini et al., 2017). For instance, it is known that *N. bombi* reduce bumblebee colonies and reproductive success (Otti & Schmid-Hempel, 2008), and its spread has been related to the decline of *Bombus* spp in North America (Cameron et al., 2011). However, most of the research effort has been conducted in a specific genus (*Bombus*) or species and studies involving entire wild bee communities are lacking, especially in semi-natural arid areas.

Semi-natural areas are appropriate for wild bee populations because of the food and nesting resources they provide (Goulson, Lye and Darvill, 2008; Winfree, Aguilar, Vázquez, LeBuhn and Aizen, 2009), but under arid climatic conditions floral resources become scarce which could lead to an increase of the interaction between managed and wild bees and hence, to pathogen transmission since the flowers have been identified as a contact point for pathogen exchange (Graystock, Goulson, & Hughes, 2015; Tehel, Brown, & Paxton, 2016). However, these areas are disappearing because of changes in land use and agricultural intensification (Robinson & Sutherland, 2002). Such intensification has affected Mediterranean areas mainly in those places suitable for irrigation, thereby threatening the functioning of Mediterranean ecosystems (Aronson et al., 1998). This kind of intensive farming usually involves an intense use of pesticides potentially affecting pollinators. It has been proved that some insecticides can cause mortality of honey bees (Henry et al., 2012) and also of wild bees (Whitehorn, O'connor, Wackers, & Goulson, 2012).

Studies of pollination networks are an important tool to explore the relationships between the structure and functioning of communities (Heleno et al., 2014). This approach allows scientists to estimate the resilience of pollinator communities to anthropogenic disturbances or climate change (Burkle, Marlin, & Knight, 2013). In that sense, previous studies about plant-pollinator networks have described a high resilience to human disturbances due to generalist and well connected species as well as to the redundancy of species and behavioral plasticity (Vanbergen, 2014 and references therein). Networks specialization would involve higher vulnerability (Aizen, Sabatino, & Tylianakis, 2012); however, Mediterranean pollinator communities show low specialization levels (Bosch, Retana, & Cerda, 1997; Bosch, Martín González, Rodrigo, & Navarro, 2009). Notwithstanding such robustness, many studies have reported that there is a critical point of disturbance in which pollinator communities suddenly collapse (Kaiser-Bunbury, Muff, Memmott, Müller, & Caflisch, 2010; Lever, van Nes, Scheffer, & Bascompte, 2014). Therefore, studies about plant-pollinator networks across different landscapes and biomes are needed in order to unravel those critical points.

Herein, we present a study in which the pollination networks of honey bees and wild bees were investigated in Mediterranean semiarid areas of the Iberian Southeast under different conditions of land use and beekeeping practices. This area is a distribution limit for many species wherein an increase in aridity conditions is expected to occur due to current climate change. Thus, our study can provide crucial information for future management policies in less studied semiarid landscapes based on plant-pollinator interactions. Specifically our goals were: i) to investigate the effect of different stressors (land use and beekeeping) on wild bee communities; ii) to disentangle the role of honey bees in the pathogen spillover to the wild bee community under semiarid conditions; iii) to study bee pollination networks structure at community level under a variable degree of human disturbance (e.g. intensive agriculture and beekeeping intensity). Our hypotheses are that those areas with higher abundance of honey bees present higher pathogen prevalence, and that plant-pollinator networks are affected by anthropogenic disturbances.

2. Materials and Methods

2.1. Study site

Four samplings were conducted from February to May 2017 in Murcia Region in the Southeast of the Iberian Peninsula ($37^{\circ} 49' 57.55''$ N, $1^{\circ} 28' 35.24''$ W). We selected nine plots of 100x100 m distributed along a transect of ca. 20 km (municipalities of Totana, Alhama de Murcia, and Mula) (Figure 1). The plots are in the limits and surrounding areas of a protected area (Regional Park) which is also Site of Community Importance (SCI of Sierra Espuña, ES0000173).

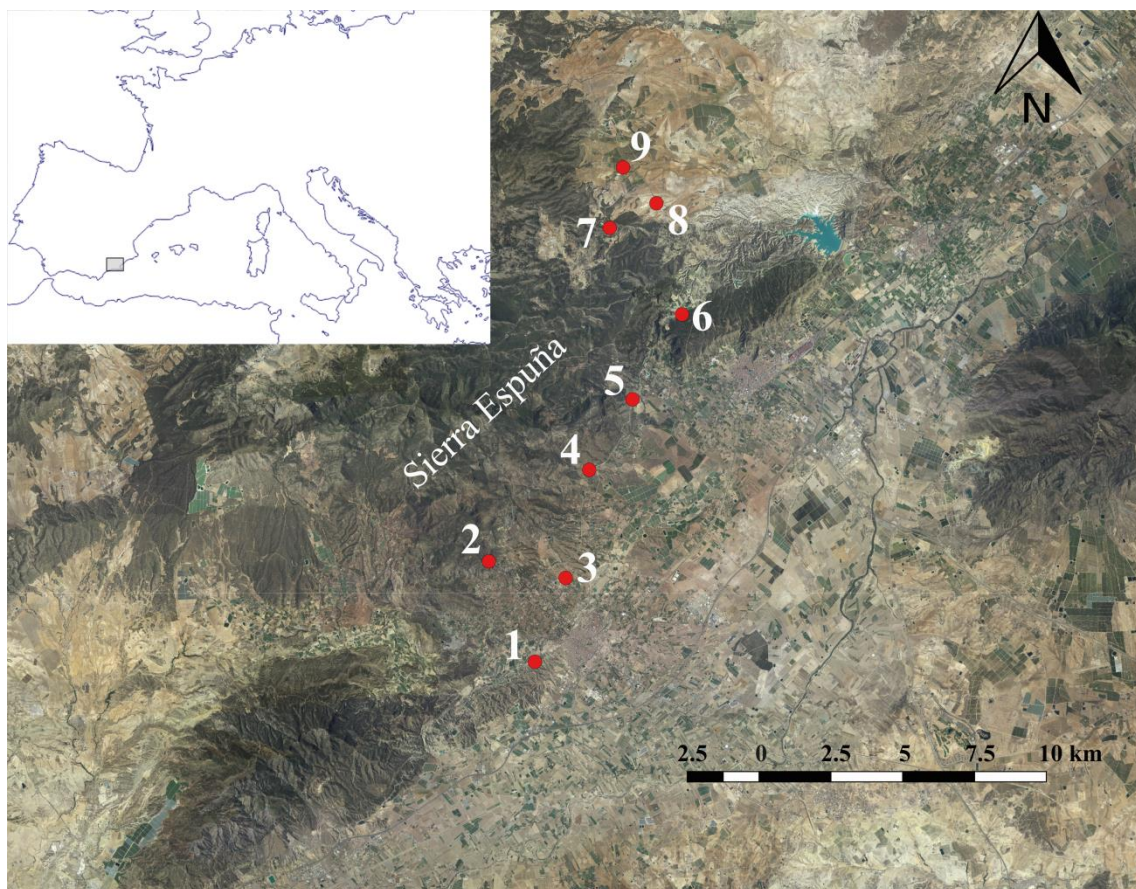


Figure 1. Study site. Red dots represent the surveyed plots.

The area is characterized by a Mediterranean semiarid climate with scarce precipitations and warm temperatures (243.51 ± 16.27 mm and 17.22 ± 0.12 °C, climatic data from AL41 station, La Calavera, Alhama de Murcia (Murcia), SIAM 1994-2018). Landscape consists of a matrix of cultivated lands (irrigated and rainfed crops), patches with natural vegetation (pinewoods and shrublands), and anthropogenic structures (roads,

urbanizations, etc.). The most common irrigated crops in the area are citrus trees, table grapes and vegetables, whereas non-irrigated are mainly almond trees, olive trees and cereals. With regard to the natural vegetation layer, we can find reforested pinewoods of *Pinus halepensis* Mill. dating from the last decades of the XIX century, and shrublands of Mediterranean species such as *Anthyllis cytisoides* L., *Helianthemum syriacum* (Jacq.) Dum. Cours., *Helianthemum violaceum* (Cav.) Pau, *Rhamnus lycioides* L., *Rosmarinus officinalis* L., *Sideritis murgetana* Obón & Rivera, *Stipa tenacissima* L., and *Thymus hyemalis* Lange.

2.2. Interaction sampling

The abundance and species richness of flowers is positively related to the abundance and species richness of bees (Bosch et al., 1997; Potts, Vulliamy, Dafni, Ne'eman, & Willmer, 2003; Steffan-Dewenter & Tschardt, 2001), since higher flower richness means a greater variety of foraging niches for different functional groups of bees (Fenster, Armbruster, Wilson, Dudash, & Thomson, 2004). Thus, we monitored the number of flowers and species in the study plots in the four samplings. Those parameters were estimated by counting flowers in ten square plots (1x1 m) randomly distributed in the study plots. Flowers of the family Asteraceae were considered as one floral unit. Plants were identified at species level when possible. Only in those cases in which species determination was uncertain we used the genus level.

Managed and wild bee pollinators were collected in four samplings from February to May (late winter, early spring, mid spring, and late spring). Field work was conducted in sunny windless days from 10:00 to 18:00 h. The sampling effort was uniform for each study plot (1 hour/sampling). Samplings consisted on random transects inside the 100 x 100 m plots where bees were collected with hand nets and the plant species in which bees were foraging recorded to construct the plant-pollinator networks. When honey bees were too abundant we collected only one in ten, but their total number was recorded for future density calculations (i.e. number of honey bees per square metre). Densities of wild bees were estimated at community level (i.e. pooling all species in a single group). Some bees were collected outside the sampling scheme but within plots in order to obtain more data on pathogens prevalence and plant-pollinator networks, but they were not included in density estimations.

2.3. *Pollinators identification*

Species identification was carried out under an integrative approach combining both classical taxonomic and molecular tools. Bees collected were identified in the field in order to discern between wild bees and honey bees. Once in the laboratory samples were individually photographed with a camera integrated in a binocular stereomicroscope Stemi 2000-C (ZEISS®). Pictures were sent to an expert taxonomist for their identification. Additionally, we also identified wild bee species through DNA barcoding. This technique consists on amplifying a specific region of the mitochondrial DNA (*cox1*: cytochrome c oxidase subunit I) (Hebert, Ratnasingham, & de Waard, 2003) which makes possible to identify species given its high specificity among animal taxa.

DNA extraction was performed individually for each sampled bee using the abdomen and following the protocol of Ivanova et al., (2006). We used the primers designed by Françoso & Arias (2013) for bee identification. PCR was performed in a volumen of 12.5 µl: 1 X buffer (Bioline, London, UK), 0.1 µM of forward primer (Barbee-F, Françoso & Arias, 2013), 0.1 µM of reverse primer (MtD9, Simon et al., 1994), 0.3 U of Taq polymerase (Bioline, London, UK), 2 µl of template DNA and distilled water (ThermoFisher Scientific, Waltham, Massachusetts, USA). PCR profile was: 94 °C for 5 min, 35 cycles of 94 °C for 1 min, 46 °C for 1 min 20 s, and 64 °C for 2 min, and a final extension step of 10 min at 64 °C. Amplicons of *cox1* were Sanger sequenced (Secugen SL, Madrid, Spain). Obtained sequences were edited with Mega7 (Kumar, Stecher, & Tamura, 2016) and identified in “BOLD: The Barcode Life of Data System” (Ratnasingham & Hebert, 2007). This platform allows researchers to compare their sequences with all those stored in the system, and hence to identify species as long as their sequences have been previously uploaded to the database.

2.4. *Bipartite network analysis*

We used the R package bipartite (Dormann, Gruber, & Fründ, 2008) to build quantitative plant-pollinator networks with bees collected while foraging in plants during the four samplings. We calculated structural parameters of networks in order to obtain information of the functioning of plant-pollinator interactions in the study plots. Network parameters are

briefly described below (further descriptions of can be found in Bascompte & Jordano (2013) and references therein):

- Number of animal species (A). Number of bee pollinators collected when foraging.
- Number of plant species (P). Number of plant species visited by the collected bees.
- Linkage density. Average number of interactions per species.
- Interaction Shannon diversity. Shannon diversity of interactions.
- Interaction evenness (Es). Shannon evenness of interactions. It is a measure about how similar are interactions in the network among different species.
- Web asymmetry (W). Balance between richness of animals (A) and plants (P) ($W = (P-A)/(A+P)$). It ranges from -1 to 1, negative values point out that there are more animals and positive values indicate that there are more plants.
- Weighted connectance (LDq). It is a measure of the complexity of the network. Connectance is the number of interactions divided by the possible potential interactions. However, weighted connectance includes the “weights” of the interactions (i.e. the intensity of the interaction in quantitative terms). A high value of connectance implies greater robustness to the loss of species in the network due to disturbances, or in other words, a greater stability of networks.
- Nestedness (NODF). It is a measure of the level of interaction among generalist species, which usually are core in networks interacting with them, and specialist species which interact with some generalist species but not with other specialists (Bascompte, Jordano, Melián, & Olesen, 2003). We use the index of Almeida & Ulrich (2011) which has been described to be more suitable for quantitative pollination networks (Santamaría et al., 2018). The index ranges from 0 (non-nested networks) to 100 (fully nested networks)
- Specialization (H_2'). It is a measure of niche complementarity among species (Blüthgen, Menzel, & Blüthgen, 2006). It ranges from 0 to 1 (poor to high specialization). High values of this index indicate that species in the network are highly selective, in our case that pollinators tend to interact only with some species but not with the

other. Thus, it can be considered a measure of selectiveness in the network.

2.5. *Pathogen prevalence detection*

Prevalence of microsporidia *N. ceranae* and *N. apis* in the collected samples was assessed through a multiplex PCR (Martín-Hernández et al., 2007). DNA was extracted individually from the abdomen of collected bees (DNA extraction as described above). The reliability of the analysis was determined using an internal PCR control (Botías et al., 2012). Negative controls were included in all PCR reactions and DNA extraction. PCR reactions were carried out in a total volume of 25 µl: 12.5 µl of Fast Start PCR Master (No. 04710452001 Roche Diagnostic), 0.4 µM of each primer (218 CER-F/218 CER-R) and 0.03 µM of primers COI-F/R (Martín-Hernández et al., 2007), 0.2 mg/ml of BSA, 0.1% Tritón X-100 and 2.5 µl of DNA template. PCR profile was: 95 °C for 10 min, 35 cycles of 95 °C for 30 s, 61.8 °C for 30 s, and 72 °C for 45 s, and a final extension step at 72 °C for 7 min. PCR products were analyzed in a QIAxcel System (Qiagen) using a QIAxcel DNA High Resolution Kit (Qiagen, No. 929002).

2.6. *Land use analysis*

Land use was assessed in the nine study plots. For that we used the information included in the Information System of Land Occupancy in Spain (Sistema de Información de Ocupación del Suelo en España, SIOSE) (Instituto Geográfico Nacional, 2015). In each plot we established four buffers at different distances from the centre of the plot: 250 m - 500m - 750m - 1000m (Figure 2). Buffers and surface calculation of polygons inside them were done with QGIS version 2.14.3 (QGIS Development Team, 2016). For our study we have considered five categories:

- Irrigated crops: watered cultures.
- Rainfed crops: not watered cultures. We also considered in this category those abandoned irrigated crops which had still not turned into a shrubland.
- Shrubland: remnants of natural vegetation with shrubby-physiognomy.

- Pinewood: remnants of natural vegetation in which the tree layer is the dominant cover.
- Anthropogenic structures: structures built by humans such as roads, houses, etc.

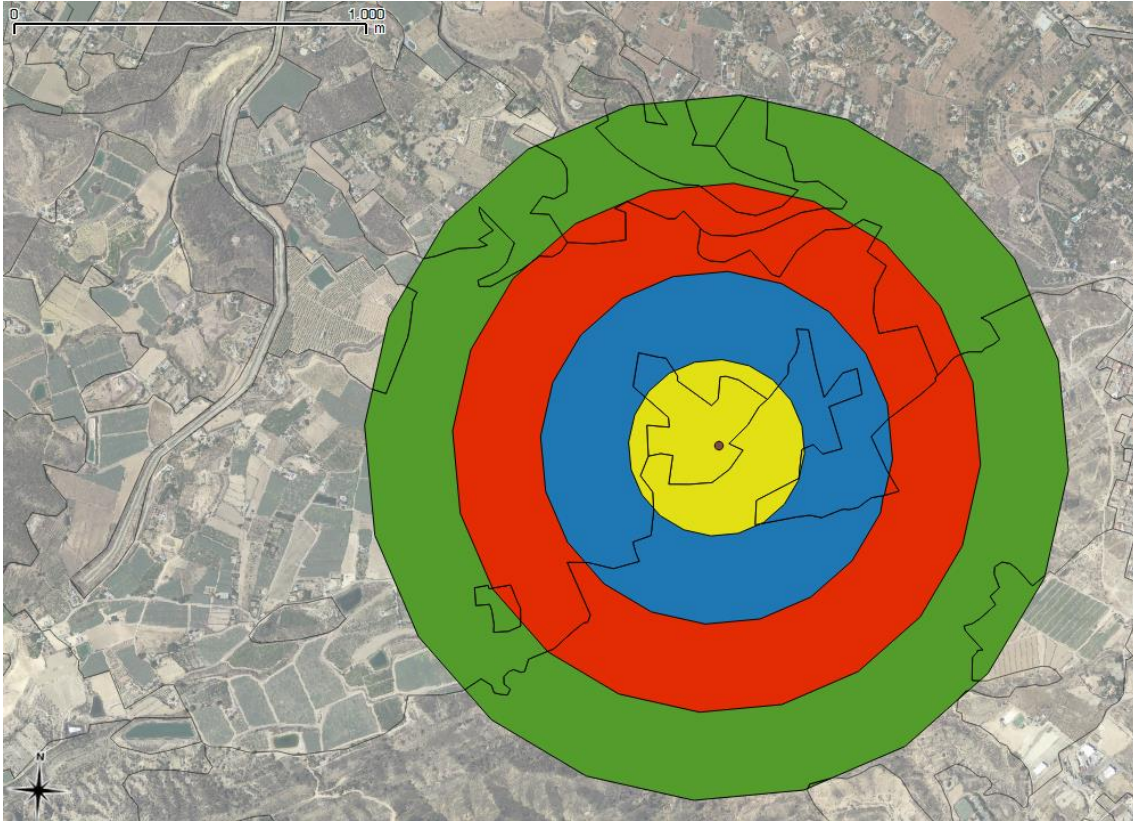


Figure 2. Example of the four buffers established in the study plot 1 (green circle=buffer 1000 m; red circle=buffer 750 m; blue circle=500 m; yellow circle=250 m).

With this information we calculated the percentage of each land use considered into each of the four buffers in the nine study plots. We also calculated the land use percentages inside the study plot in order to take into account its effects on the wild bee community.

2.7. *Data analysis*

All statistical analyses were performed in R version 3.4.4 (R Development Core Team 2018). We performed a Bray-Curtis dissimilarity analysis to check for differences in plant and bees communities composition between sampling dates and study plots (Bray & Curtis, 1957). Significance of Bray-Curtis dissimilarity analyses was tested through a Permutational Multivariate Analysis of Variance (PERMANOVA) with 999 permutations. We also performed a repeated measures ANOVA with the R

package ez version 4.4-0 (Lawrence, 2016) to test for statistical differences in the number of flowers among samplings and study plots. We log-transformed data of number of flowers to get a normal distribution. We tested the effect of sampling and study plots on honey bee and wild bee density through one way ANOVA analyses. Then, we performed generalized linear model (GLMs) with Gaussian distribution and identity link function to explore the relationship between bee and flower density. We considered bee density values in each study plot and sampling period as independent data because pollinators are able to track resources in terms of quality (kind of resource) and quantity (number of flowers) (Fowler, Rotheray, & Goulson, 2016). Thus, bee density in each period and location would vary in relation to resource availability.

We assessed the effect of the sampling period and study plot on the pathogens prevalence through repeated measures ANOVA's with the R package ez version 4.4-0 (Lawrence, 2016). We used GLMs to: i) explore the relationship of honey bee density on wild bee density; ii) test the impact of honey bee density in network structure parameters; iii) checked for relationships in the prevalence of pathogens in honey bees (the host species) and in wild bees. Flowers have been identified as the contact point between wild bees and honey bees for the transmission of pathogens (Graystock et al., 2015; Tehel et al., 2016). Thus, we performed a Principal Component Analysis (PCA) with the software PAST v.3.1.4 (Hammer, Harper, & Ryan, 2001) to assess the effect of floral resources on pathogen transmission by comparing plants visited by honey bees (healthy and with the pathogen) and wild bees (healthy and with the pathogen). Then, we assessed the effect of land use on wild bee density and the obtained pollination networks through GLMs. We did not include honey bee density as they are located by humans in those landscapes with higher flower richness, and hence it would not be related to land uses. For those analyses we used data of nine plant-pollinator networks (data of the four sampling periods for each study plot).

We used Gaussian distribution and identity link function for all GLMs as after testing different link functions it was the one with better residuals distribution. GLMs and one way Anovas were performed with the R package vegan (Oksanen et al., 2013).

3. Results

3.1. *Plant-pollinator interactions*

We collected 815 bee individuals (505 wild bees and 310 honey bees). Only 20 individuals of wild bees could not be identified at genus or species level, and they were excluded for the plant-pollinator network analysis. We finally registered 519 plant-pollinator interactions. We found significant differences in community composition of plants in relation to sampling dates ($F= 3.287$, $R^2= 0.236$, $p\text{-value}=0.001$), but there was only a marginal relationship with the study plot ($F=1.176$, $R^2=0.258$, $p\text{-value}=0.1$). The communities of pollinators significantly varied among samplings ($F= 1.752$, $R^2= 0.141$, $p\text{-value}= 0.011$) and among study plots ($F= 1.6642$, $R^2= 0.330$, $p\text{-value}=0.003$). The density of honey bees decreased with time (Figure 3a), only with a marginal statistical significance ($F= 2.570$, $p\text{-value}= 0.071$, $n= 36$), but not according to the study plot ($F= 1.259$, $p\text{-value}= 0.305$, $n=36$). Contrastingly, the density of wild bees did not show temporal variation ($F= 0.289$, $p\text{-value}= 0.833$, $n= 36$) (Figure 3b), but spatial differences ($F= 4.123$, $p\text{-value}= 0.002$, $n= 36$). Post-hoc Tukey tests showed that those spatial differences were due to the interaction between units 5-1, 9-4, and 9-5 ($p\text{-value}<0.05$). The number of flowers decreased significantly along the study period (Figure 3c) ($F=3.718$, $p\text{-value}=0.025$, $n= 360$). The number of flowers also varied among study units ($F=2.432$, $p\text{-value}=0.044$, $n=360$). Density of honey bees was significantly related to the density of flowers in the study plots ($p\text{-value}= 0.024$, $n=36$), whereas density of wild bees was not ($p\text{-value}= 0.340$, $n=36$).

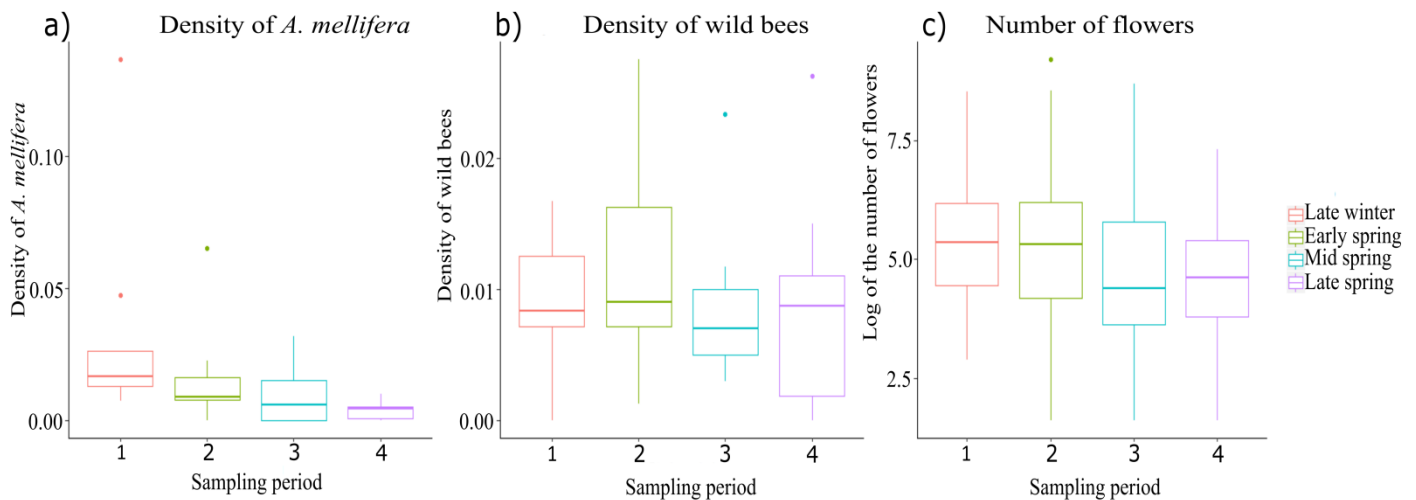


Figure 3. (a) Variation in the density of *A. mellifera*; (b) Variation in the density of wild bees; (c) Variation in the log-transformed number of flowers. Boxplots show the median, the quartiles (horizontal lines in the box), minimum and maximum (vertical lines) and the outliers (dots outside boxes).

We built nine pollination networks (one per study plot) with the plant-pollinator interactions recorded during the four samplings (Table S1). The number of bee species in networks was higher in early spring, whereas the maximum richness of plant species was observed in mid spring (Figure 4). Regarding to specialization, it increased along the study period while weighed connectance slightly decreased (Figure 4).

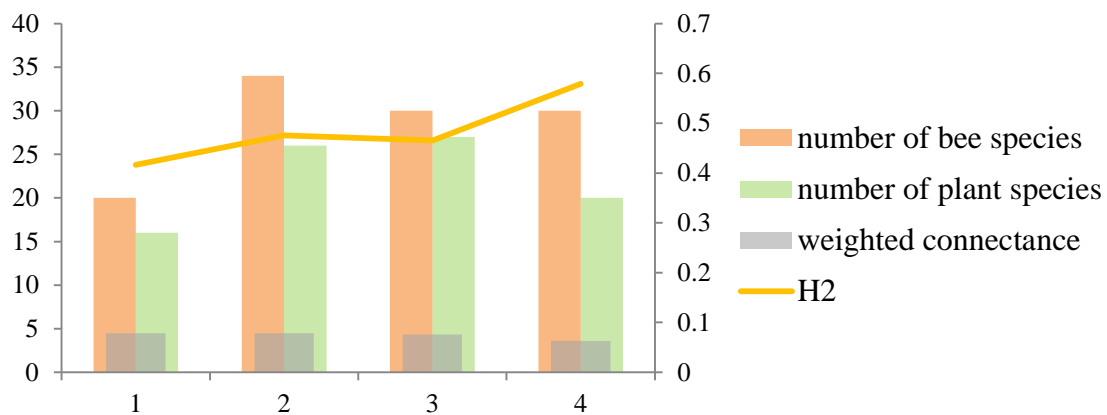


Figure 4. Variation in network structure parameters according to the sampling period (1= late winter; 2= early spring; 3= mid spring; 4= late spring). Left axis points the number of bee and plant species and right axis the weighted connectance and H_2 .

Network structure patterns were heterogeneous among study plots (Figure 5). For instance, bee pollinators richness was lower in study plots 3, 4, 5 and 7. Thus, some of these areas (3, 4 and 5) also present the higher values

of weighed connectance as there are less possible links (i.e. less species involves less possible interactions). Network specialization ranged from 0.403 in study plot 6 to 0.711 in plot 2.

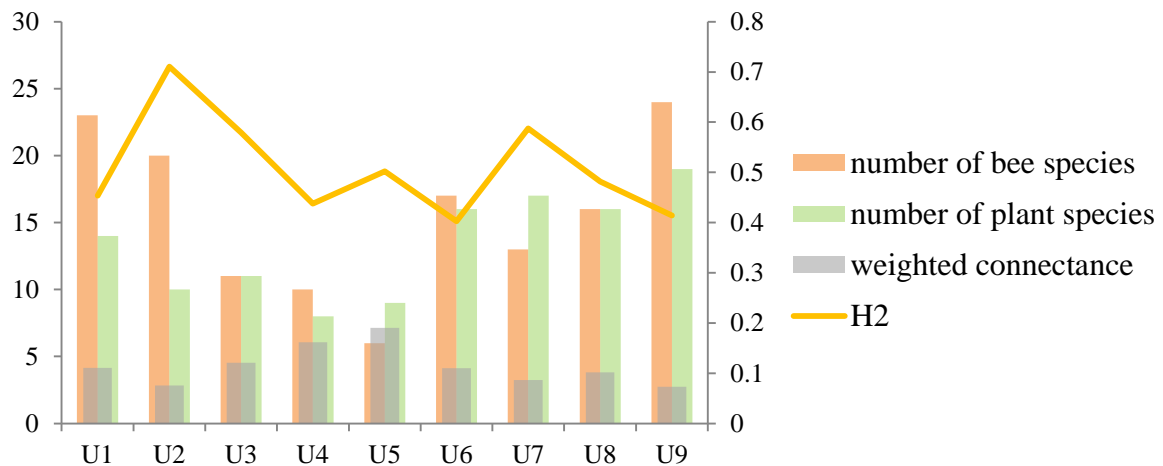


Figure 5. Variation in network structure parameters according to the study plot (numbers shown in Figure 1). Left axis points the number of bee and plant species and right axis the weighted connectance and H₂.

3.2. Anthropogenic impacts on plant-pollinator interactions: beekeeping

Density of honey bees did not affect wild bee densities ($t = 0.659$, $p\text{-value} = 0.514$). Regarding to network structure parameters, the density of honey bees affected specialization negatively, but increased the number of bee species in the network, although significance in both cases was marginal (Table S2). The prevalence of *N. ceranae* and *N. apis* was assessed for 815 bee individuals. We obtained two positive samples for *N. apis* in late winter sampling (a honey bee and a wild bee) and 238 samples positive for *N. ceranae* presence (142 and 96 individuals of honey bees and wild bees, respectively). The 96 wild bee samples with presence of *N. ceranae* involved 36 species (Fig. S1) of 20 genus (Fig. S2). *N. apis* was only detected in a *Eucera* species. All statistical analyses onwards refer to *N. ceranae* data as the prevalence of *N. apis* was low. Prevalence of *N. ceranae* decreased in honey bees along the study period, while it increased in wild ones (Figure 6). However, such tendencies were only significant for the prevalence of the pathogen in relation to the sampling period in honey

bees ($F= 16.150$, $p\text{-value}<0.001$), but not in wild bees ($F= 0.829$, $p\text{-value}= 0.586$). Study plots did not have a significant effect either for prevalence in honey bees ($F= 0.593$, $p\text{-value}=0.774$), or in wild bees ($F= 1.885$, $p\text{-value}=0.159$).

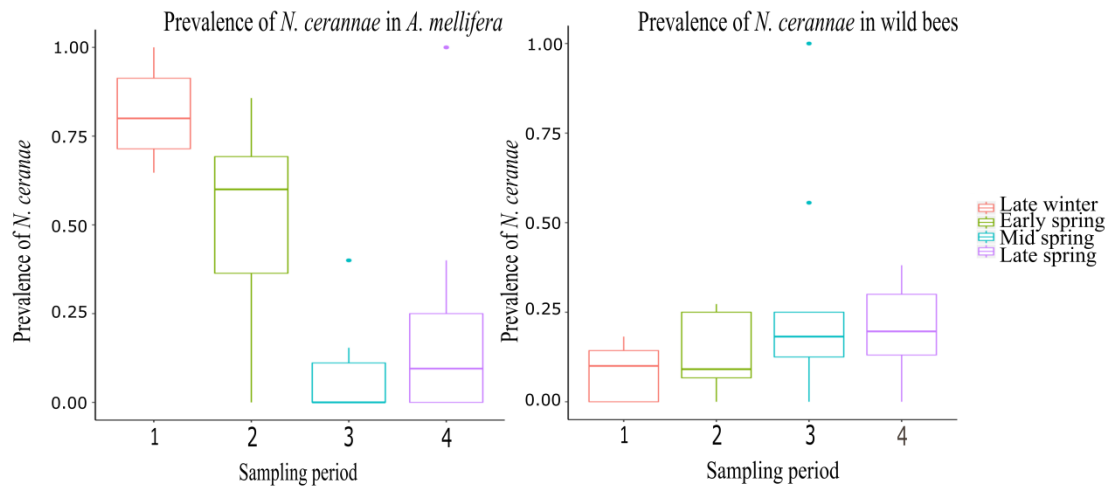


Figure 6. Prevalence of *N. ceranae* in *A. mellifera* and wild bees according to the sampling period. Boxplots show the median, the quartiles (horizontal lines in the box), minimum and maximum (vertical lines) and the outliers (dots outside boxes).

The prevalence of *N. ceranae* in *A. mellifera* was negatively and significantly related to the prevalence of the pathogen in wild bees ($t= -3.193$, $p\text{-value}= 0.003$, $D^2= 23.607\%$, $AIC= -50.844$, $n= 35$; Figure 7a). Contrastingly, the tendency was significant and positive when we analysed the accumulative effect of the pathogen (i.e. prevalence of the pathogen in each study plot as the sum of the prevalence in the sampling plus the prevalence in previous samplings) ($t= 2.882$, $p\text{-value}= 0.007$, $D^2= 20.114\%$, $AIC= -49.278$, $n=35$); Figure 7b).

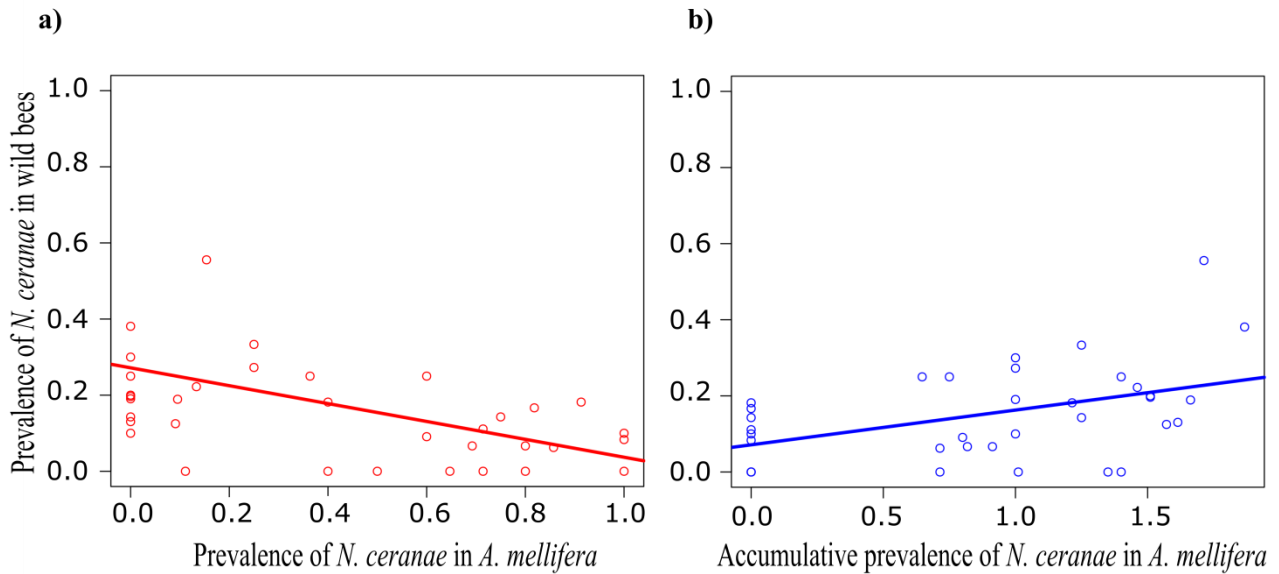


Figure 7. GLMs for the prevalence of *N. ceranae* in wild bees related to the prevalence in *A. mellifera*. Data of prevalence in study plot 5 for the third sampling was eliminated from the analyses because it was identified as an outlier. However, results were significant with and without the outlier (a) GLM of the prevalence of the pathogen in wild bees in relation to the prevalence in *A. mellifera* ($t = -3.193$, $p\text{-value} = 0.003$, $D^2 = 23.607\%$, $AIC = -50.844$, $n = 35$); (b) GLM of the prevalence of the pathogen in wild bees in relation to the accumulative prevalence in *A. mellifera* ($t = 2.882$, $p\text{-value} = 0.007$, $D^2 = 20.114\%$, $AIC = -49.278$, $n = 35$).

PCA analysis of the number of visits of healthy bees and bees with the pathogen explained 88.64% of the overall variability (Figure 8). PC1 accounted for the 50.24% of the variability, and it showed plants that healthy honey bees visited in the negative part, whereas those more visited by healthy wild bees were in the positive part of the axis. PC2 captured 38.40% of the variability. Positive values of the axis were related to plants visited by honey bees with the pathogen, while the negative part refers to plants visited by wild bees with the pathogen. Results showed a poor overlap of plants visited by honey bees and wild bees with the pathogen. *Rosmarinus officinalis* and *Diploaxis erucoides* were the two plant species more related to the prevalence of the pathogen in honey bees, but no plant seems to be especially implicated in the prevalence of the pathogen in wild bees (Figure 8).

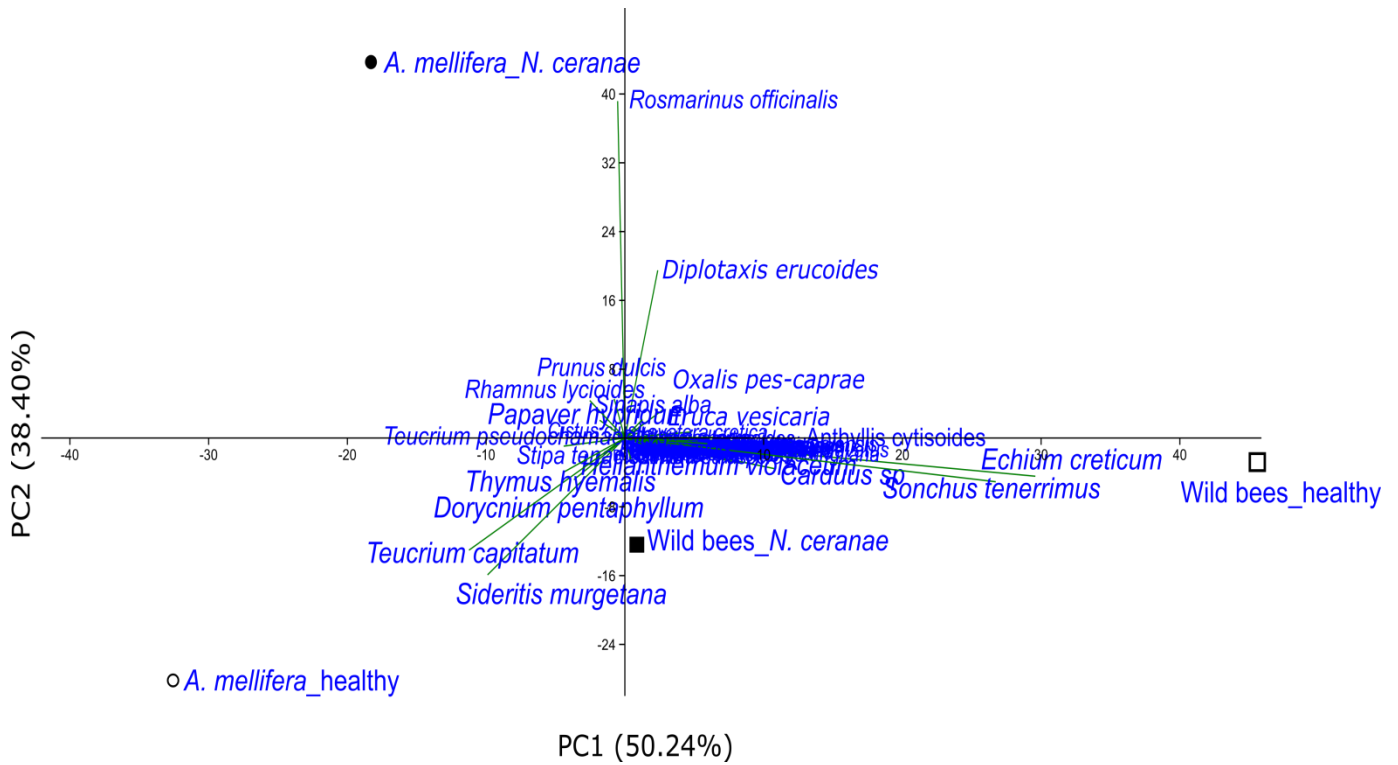


Figure 8. Principal Component Analysis (PCA) showing those plants visited by healthy honey bees and wild bees, and by honey bees and wild bees with the pathogen. Green lines express the influence of each particular plant species. PC1 and PC2 show the variability captured by each axis (while circle= Healthy *A. mellifera*; black circle= *A. mellifera* with *N. ceranae*; white square= healthy wild bees; black square= wild bees with *N. cerane*).

3.3. Anthropogenic impacts on plant-pollinator interactions: land use

We obtained land use percentages for the nine study plots and the four buffers analysed (Table S3). Significance values of the GLMs analyses are shown in Table S2. Rainfed crop surface had a significant positive impact on wild bee densities and several network structure parameters (the number of interactions, web asymmetry, interaction Shannon diversity, interaction evenness and the number of bee species) at plot level, but negative in the weighted connectance. However, such effects disappeared at larger scales. Regarding irrigated crop land use, it positively affected wild bee densities at all scales (from 250 till 1000 m). Likewise, this land use significantly increased the number of interactions and the number of bee species at all studied scales. Additionally, irrigated crops had a positive although

marginal influence in the linkage density and interaction Shannon diversity. We did not find any negative impact of such land use on network structure parameters. Shrubland cover had a negative impact on the density of wild bees and the number of plant species from the plot level to the 500 m buffer. Furthermore, it reduced the number of interactions, interaction Shannon diversity, interaction evenness and the number of bee species at plot level. Contrastingly, shrubland surface benefitted the weighted connectance at all scales and the weighted nestedness from 250 m onwards. No effects of anthropogenic structures or pinewoods were found.

4. Discussion

Overall, our results showed that beekeeping impact wild pollinator communities by leading to spill over of pathogens in the landscape even after honey bees density and pathogen load decrease. However, honey bee density did not significantly affect network structure parameters. On the contrary, some human land uses (rainfed and irrigated crops) seem to benefit wild bee densities as well as the number of bee species in pollination networks at different scales.

Plant-pollinator networks show seasonality patterns in Mediterranean environments. For instance, Bosch et al., (1997) found that honey bees were the main group in early spring, but not in other periods when other taxa were more abundant. The contribution of other Hymenoptera steadily remains for longer periods. We have also observed this pattern in our study as density of honey bees was higher in late winter and early spring but not in mid and late spring. This phenomenon is probably related to the movement of hives to track flower resources. The period in which honey bees are abundant in our area coincides with flowering of almond trees and other fruit trees, and so hives are masively concentrated in the area in order to take advantage of these resources. This could also explain why density of honey bees was significantly related to flower density in our study. However, densities of wild bees were more or less constant during the four samplings and they were not affected by flower density which denotes that other factors such as landscape configuration affect their abundance. In that sense, we found that significant differences in wild bee densities among study plots were mainly due to changes in land use composition between study plots: i.e. those with high shrubland surface (plots 4-5) versus those with high agricultural surface (plot 1-9). Network structure parameters also

mirror differences among study plots with more patches of natural vegetation (study plots 3-4-5 with higher weighted connectance and higher NOFD nestedness, but less species and links) and those with higher diversity of land uses (remaining study plots with more species in the network and more number and diversity of links). Specialization was low as expected in Mediterranean communities (Bosch et al., 2009) which makes plant-pollinator networks more robust to disturbances as pollinators do not depend on specific floral resources.

The interaction among different stressors has been identified as the driver of the current decline in wild bee pollinators (Goulson et al., 2015). Many studies assessed the role of managed bees in this multifactorial scheme, for example, due to competition for resources. Results in that sense have demonstrated to be controversial with studies reporting neutral, negative, or mixed effects (Mallinger et al., 2017; Magrach, González-Varo, Boiffier, Vila, & Bartomeus, 2017). However, the impact of managed bees, specially the honey bee, on wild bee pollinators through the interespecific transmission of pathogens seems to be generally accepted with most of the research reporting negative effects (Mallinger et al., 2017). Most of those investigations were performed in northern latitudes such as North America and the Central Europe (e.g. Fürst et al., 2014). Thus, there is a lack of studies assessing this effect under semiarid conditions. It is important to take into account that the interaction among different factors can lead to different host-pathogen dynamics (Meeus, Pisman, Smagghe, & Piot, 2018). Hence, conclusions obtained under particular climatic conditions can not be extrapolated to others as different weather conditions may lead to distinct transmission and infection rates of pathogens (Neidel, Steyer, Schafellner, & Hoch, 2017). For instance, the microsporidia of *N. ceranae* prefers warm temperatures whereas *N. apis* seems to prefer colder weather (Gisder et al., 2010). This is probably the reason why *N. apis* prevalence was so low in our study with only two positive samples detected in the cooler months of sampling (late-winter). However, it is remarkable the fact that the pathogen was detected in a wild bee species (*Eucera* sp3) since interespecific transmission of this pathogen has been rarely described. *N. ceranae* had been previously detected in wild bee species of genera as *Bombus*, *Osmia*, *Heriades*, *Andrena*, *Melipona*, *Tetragonisca*, and *Scaptotrigona* (Martín-Hernández et al., 2017, and references therein). Herein, we identified the pathogen in 20 genera of bee species (17 of them

for the first time) (Fig. S1 and Fig. S2). *N. ceranae* prevalence in honey bees decreased along the samplings. Seasonal patterns in the prevalence of *N. ceranae* was also found in previous studies about the pathogen in Spain, with peaks of infection in winter (Higes et al., 2008) which is congruent with the pattern that we found. On the contrary, wild bees showed a tendency to prevalence increase of the pathogen along the sampling period (although it was no statistically significant). GLMs showed a significant but negative relationship between the prevalence of the pathogen in honey bees and wild bees ($t = -3.193$, $p\text{-value} = 0.003$); however, such relationship was positive and significant when we used accumulated prevalence instead of prevalence in each sampling ($t = 2.882$, $p\text{-value} = 0.007$). These results suggest that the pathogen is able to remain in the landscape even after the host species (honey bee) presence decreases (in density and pathogen prevalence). The deviance (D^2) (i.e. how data fit to the model) of both GLMs was low (under 24% in both cases) what might be due to the differential sanitary state of honey bee hives in the area. For instance, poor management of hives can derive in a high pathogen prevalence (Martín-Hernández et al., 2017) and hence, a few poorly managed honey bee colonies could outperform the contribution of larger number of hives in the pathogen spill over. The mechanisms by which the pathogen could keep spreading might be related with its capability of remaining in flowers and/or the role of secondary vectors (e.g. other pollinators) disseminating the spores of the microsporidian through the landscape. Flowers have been identified as a vector in the pathogen transmission among pollinators (Graystock et al., 2015; Tehel et al., 2016). However, we found a poor overlap between flowers visited by honey bees and wild bees with the pathogen. Plant species flowering at the end of the winter and early spring (*R. officinalis* and *D. erucoides*) were related to honey bees with the pathogen, what is not surprising as this is the period with higher prevalence of *N. ceranae* in honey bees. There was not any plant particularly related to wild bees with the pathogen. The complementarity among pollinated plant species between honey bees and wild bee has been previously reported (Garibaldi et al., 2013; Rollin et al., 2013). Thus, it is not surprising that we did not detect strong significative effects of honey bees in pollination networks as the resources they use are different from those used by wild bees. However, negative impacts could have not been detected because honey bees were present in all the study plots (although in different

densities), so we did not have control plots without honey bees to study network structure parameters in absence of the managed pollinator. In fact, we found a negative (although marginally significant) impact of honey bees on specialization in networks which makes sense given that the honey bee is considered a supergeneralist pollinator. This behaviour could have negative consequences for the reproduction of plants, as it has been proved that pollinator effectiveness of specialists can be higher than generalist ones. Our results point the capability of pathogen transmission even under low resource overlap scenarios. Transmission routes of the pathogen are unpredictable once introduced in the landscape, as other insect pollinators can act as secondary vectors in the pathogen spillover. For example, recent studies reported the presence of bee viruses in hoverflies (Bailes et al., 2018) which reinforced the capability of wild pollinators (not only bee species) of acting as host and/or secondary vectors. In fact, we have also detected *N. ceranae* in a non-Apoidea Hymenoptera species (*Athalia ancilla*, unpublished results). Therefore, it would be necessary to monitor the entire insect-pollinator community in order to detect the overlap in plants visited by different pollinators.

Intensive land use practices can derive in wild bee populations decline and the local extinction of more vulnerable species (Vanbergen & Insect Pollinators Initiative, 2013). Nevertheless, the maintenance of matrices of diverse land uses including patches of natural vegetation and local management practices could offset the effect of intensive land uses such as urbanization and intensive agriculture (e.g. monoculture agriculture) (Kennedy et al., 2013; Tscharntke, Klein, Kruess, Steffan-Dewenter, & Thies, 2005). These trends could be related to the fact that different sort of habitats would provide a wider variety of resources to bees (Kennedy et al., 2013; Westrich, 1996). The metaanalysis performed by Winfree et al., (2009) about the effects of human disturbances on wild bees revealed that only the loss of habitat has a significant negative impact on wild bee abundance and species richness. Furthermore, such adverse effects were only detected in cases of extreme fragmentation. Our results are in line with previous research since we did not detect any remarkable negative impact of anthropogenic land uses on wild bee densities and plant-pollinator networks. On the contrary, our results revealed positive impacts of the rainfed crops at plot level (higher wild bee densities, more bee species in networks, and more abundant and diverse interactions), and of irrigated

crop land use at all scales (higher wild bee densities, and more bee species and interactions in networks). The positive effects of mixed crops including low-intensity irrigated cultures have been demonstrated in animals ranging from wild bees (Baños-Picón, Torres, Tormos, Gayubo, & Asís, 2013) to medium-sized carnivores (Lara-Romero et al., 2012). Natural shrubland land use had a negative effect on wild bee densities, the number of species in plant-pollinator networks as well as in the number and diversity of interactions in networks. Thus, shrublands improved weighted connectance and weighted nestedness since less diversity of species in network benefits those network structure parameters. Other studies also found higher abundance and species richness of wild bees in agricultural than in natural areas (Westphal, Steffan-Dewenter, & Tschamntke, 2003; Winfree, Griswold, & Kremen, 2007). This could be due to the higher offer of floral resources in agricultural patches. This trend can be especially important in semiarid landscapes, as our study system, since drought reduces floral resources for bees (Phillips et al., 2018). Therefore, irrigated crops would provide more feeding resources which turn into an increase in bee densities and species richness. However, the attractant effect of irrigated crops can be a double edged sword since these crops are usually related to the use of high amounts of pesticides which could lead to negative consequences in wild bee populations because of higher exposures to those chemical agents.

In conclusion, our results suggest to be cautious when introducing honey bees in protected areas as they can promote the spillover of microsporidian pathogens to the wild bee community. Furthermore, the current climate change scenario with a generalized increase in temperatures could aggravate the situation as warmer temperatures benefit *N. ceranae* (Gisder et al., 2010). We have only detected the presence of the pathogen in these wild bee species; however, upcoming studies should analyse if the pathogen is actually capable of infecting these species, or if they are only acting as vectors. The land use matrix in our study system seems to be suitable for pollinators with enough percentage of different patches to support wild bee communities. Thus, land use change drivers leading to landscape structure simplification might negatively impact on wild bees. For instance, land abandonment of agricultural patches and the subsequent colonization by shrubland vegetation in absence of proper management could reduce bee species richness and density, as it occurs with other wildlife (Russo, 2007; Plieninger, Hui, Gaertner, & Huntsinger, 2014;

Queiroz, Beilin, Folke, & Lindborg, 2014; Robledano et al., 2014; Uchida & Ushimaru, 2014). These processes of agricultural land use losses are increasing worldwide (Cramer & Hobbs, 2007), with special impact on temperate areas such as the Mediterranean. Therefore, management policies should address these trends and display adequate measures in order to enhance wild bee communities.

5. Acknowledgements

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Supporting information

Fig. S1 Bipartite network of pathogens and bee species.

Fig. S2 Prevalence of *N. ceranae* wild bees.

Table S1 Network structure parameters for the nine obtained pollination networks.

Table S2 Results of GLMs among anthropogenic disturbances and plant-pollinator interactions.

Table S3 Percentage of land use for the study plots.

Fig S1. Bipartite network of pathogens (two species, right column) and bee species (36 species and four genera in which species could not be confirmed, left column) in our study. Network based on 236 interactions. Notice that there were 238 interactions but two wild bee individuals could not be identified, and hence they were excluded from the network.

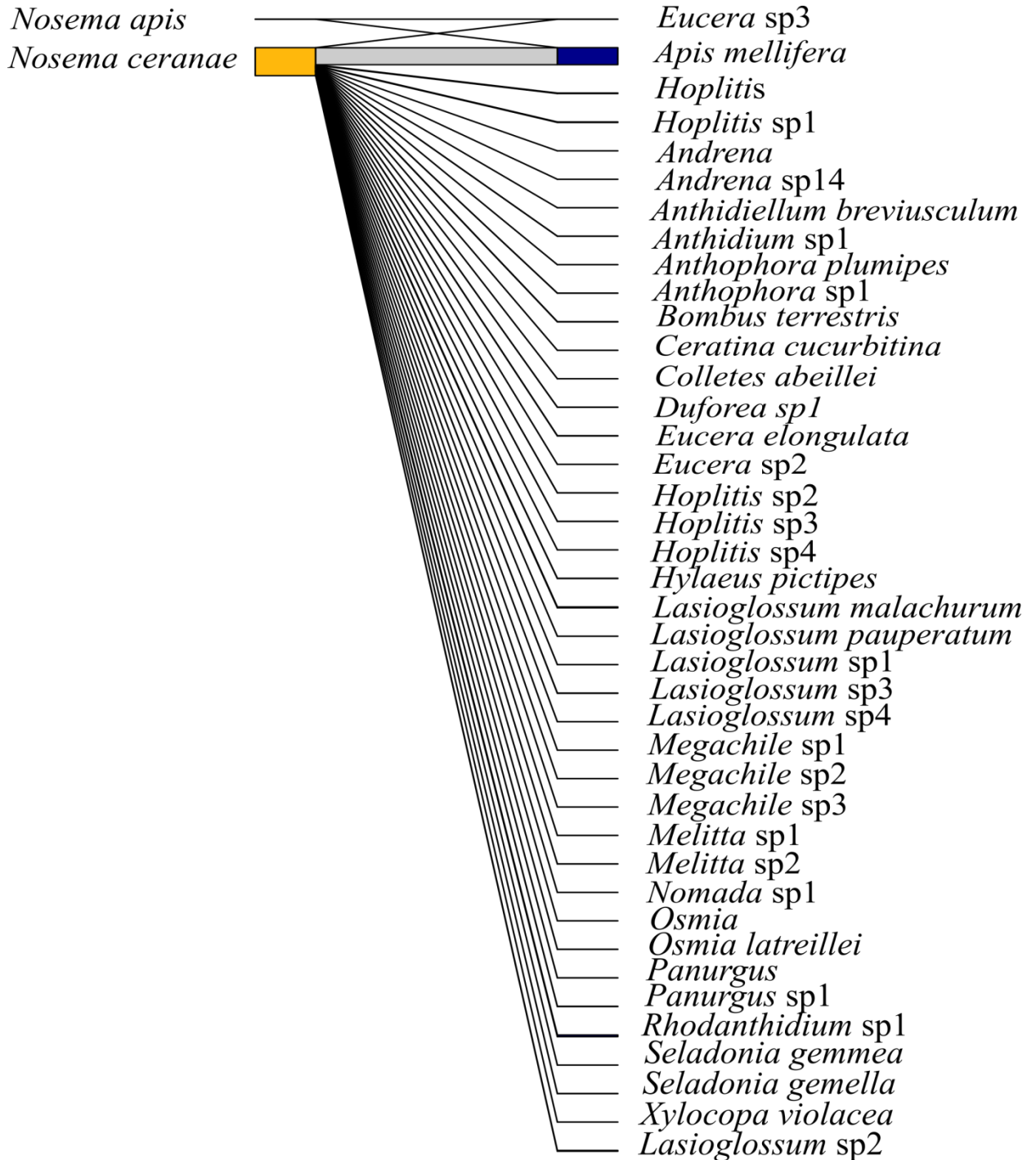


Fig S2. Prevalence of *N. ceranae* wild bees. Taxonomic identification done at the genus level.

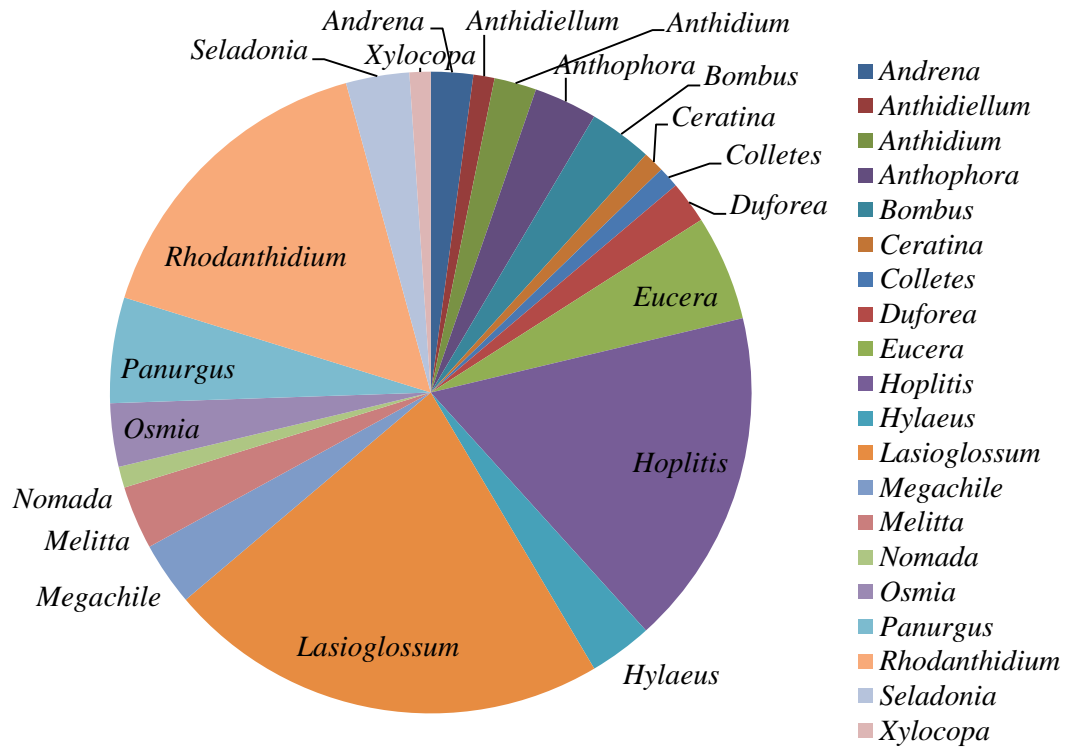


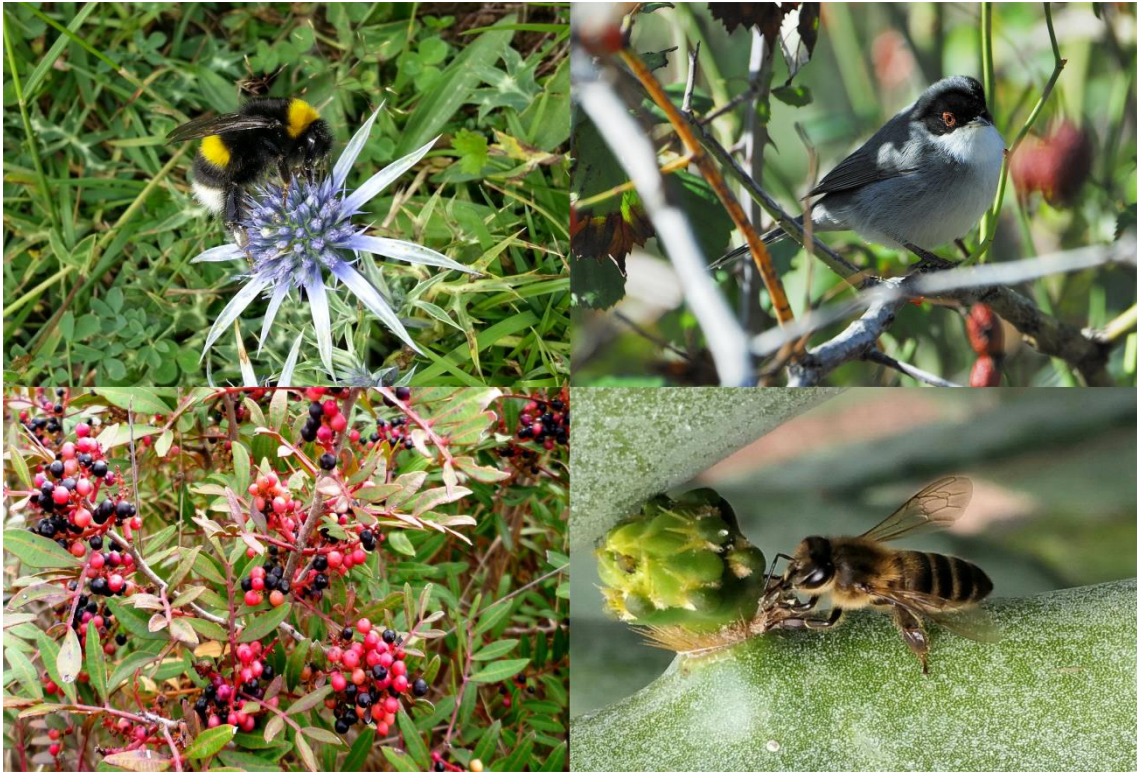
Table S1. Network structure parameters for the nine obtained pollination networks. (U1= study plot 1; U2= study plot 2; U3= study plot 3; U4= study plot 4; U5= study plot 5; U6= study plot 6; U7= study plot 7; U8= study plot 8; U9= study plot 9).

	U1	U2	U3	U4	U5	U6	U7	U8	U9
n° of interactions	97	40	39	43	41	77	42	56	83
web asymmetry	0.243	0.333	0.000	0.111	-0.200	0.030	-0.133	0.000	0.116
weighted NODF	13.421	1.277	7.121	15.525	13.725	9.082	6.269	6.736	7.532
linkage density	4.083	2.266	2.655	2.914	2.860	3.640	2.590	3.254	3.152
weighted connectance	0.110	0.076	0.121	0.162	0.191	0.110	0.086	0.102	0.073
Shannon diversity	3.541	3.033	2.423	2.342	2.038	3.257	2.939	2.977	3.193
interaction evenness	0.613	0.572	0.505	0.534	0.511	0.581	0.544	0.537	0.522
specialization	0.454	0.711	0.579	0.438	0.502	0.403	0.587	0.482	0.414
number of plant species	14	10	11	8	9	16	17	16	19
number of bee species	23	20	11	10	6	17	13	16	24

Table S3. Percentage of land use for the study plots and for each one of the buffers considered (NA=pinewood; NM=shrubland; CS=fedrain crop; CR=irrigated crop; NA=anthropogenic structures)

	Land use in plots					Buffer 250m					Buffer 500m					Buffer 750m					Buffer 1000m				
	NA	NM	CS	CR	AN	NA	NM	CS	CR	AN	NA	NM	CS	CR	AN	NA	NM	CS	CR	AN	NA	NM	CS	CR	AN
Plot 1	0	10	90	0	0	2.26	40.44	3.28	46.61	7.40	1.88	37.16	4.52	50.77	5.68	1.97	38.42	9.14	44.56	5.91	2.01	38.19	14.68	38.82	6.31
Plot 2	0	30	70	0	0	40.91	25.09	14.74	14.68	4.59	35.10	24.76	14.77	20.64	4.72	28.21	32.57	12.54	19.20	7.48	27.87	38.49	10.73	15.91	6.99
Plot 3	0	100	0	0	0	1.97	19.67	21.78	12.51	44.07	2.03	18.87	26.05	19.77	33.29	3.80	15.54	28.61	25.46	26.59	3.65	17.69	30.24	27.00	21.42
Plot 4	0	80	20	0	0	12.84	72.87	0.00	6.65	7.64	22.59	62.81	0.07	6.91	7.62	25.03	52.82	4.96	10.45	6.74	23.69	51.22	6.29	12.92	5.88
Plot 5	0	100	0	0	0	7.23	68.88	0.00	16.89	7.00	15.91	60.89	0.87	17.54	4.79	19.31	64.10	1.27	11.35	3.96	19.48	65.41	2.15	8.86	4.11
Plot 6	0	30	70	0	0	34.62	19.93	3.48	37.63	4.34	38.60	17.04	4.34	36.19	3.83	45.58	22.64	5.30	23.88	2.60	51.72	23.01	5.57	17.70	2.00
Plot 7	10	50	40	0	0	23.02	40.91	31.98	4.09	0.00	26.70	39.80	30.91	2.56	0.02	30.72	37.62	29.81	1.77	0.08	31.84	37.29	29.49	1.24	0.14
Plot 8	5	65	30	0	0	0.00	2.13	97.87	0.00	0.00	0.00	9.65	90.35	0.00	0.00	0.80	15.01	84.06	0.10	0.03	3.93	18.10	76.36	1.09	0.52
Plot 9	0	20	80	0	0	0.19	20.02	28.05	51.75	0.00	1.82	23.68	23.44	49.05	2.01	2.43	26.54	24.22	43.75	3.06	2.43	26.93	29.28	38.22	3.13

GENERAL CONCLUSIONS



General conclusions

Chapter I. Intercontinental long-distance seed dispersal explains population genetic structure in a fleshy-fruited shrub spanning the Mediterranean Basin

1. Spatial genetic structure patterns in *Pistacia lentiscus* showed a high genetic connectivity among populations at both shores of the Mediterranean Sea, including mainland and island populations. However, a significant East-West genetic structure was found.
2. These findings are consistent with the known bird migratory routes that connect the European and African continents from North to South.
3. Anthropogenic and/or climatic changes that might disrupt the migration routes of frugivorous birds could cascade into genetic consequences for the plant species they feed upon.

Chapter II. Ecological and genetic consequences of fragmentation in a semiarid Mediterranean urban forest

1. We observed a high genetic diversity and a lack of differentiation between shrub patches of *P. lentiscus* in the study area, probably as a result of a strong but rather recent fragmentation.
2. Frugivorous birds seemed to contribute poorly to seed dispersal among *P. lentiscus* patches in the area, as inferred from the low number of seeds found in faeces collected by mist-netting in comparison with the high number of seeds deposited near maternal shrubs.
3. Population genetics parameters in Montepinar suggest the existence of latent impacts which can negatively affect the viability of this population in the long term.

Chapter III. Barcoding as an ancillary technique to uncover mechanisms of seed dispersal in demanding environments - and to help to restore them

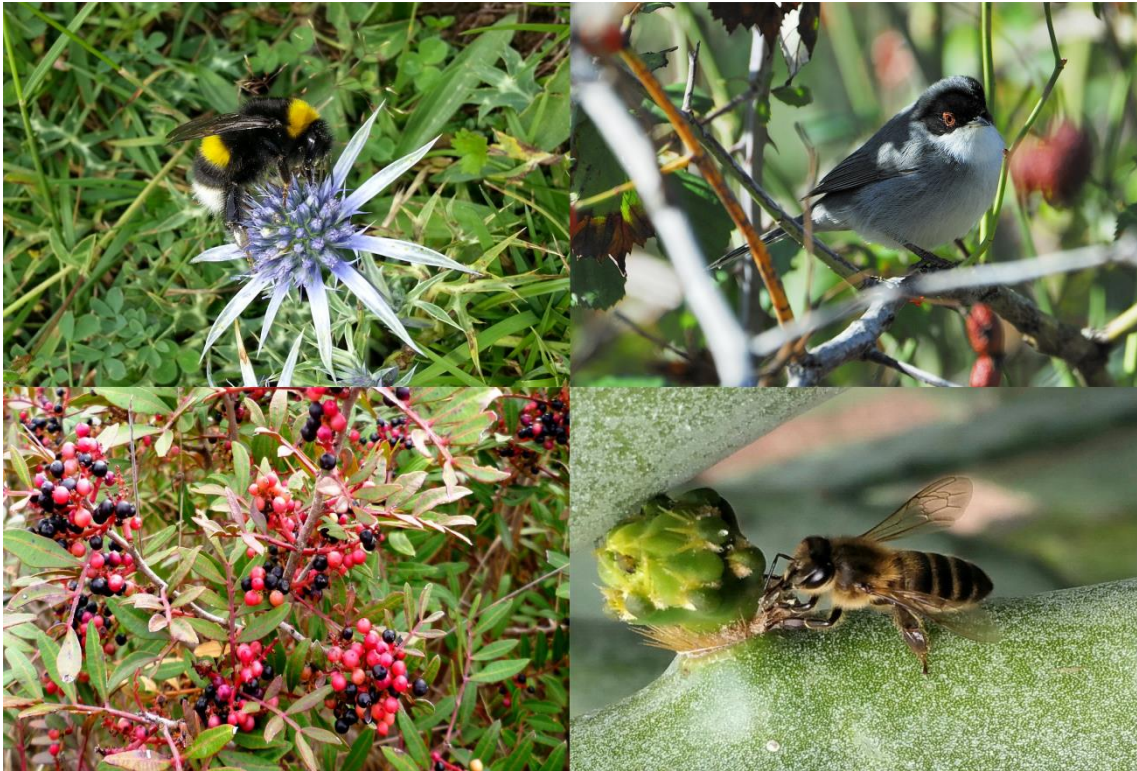
1. The proposed methodological framework, based on the combination of ecological (bird census and camera trapping) and genetic (DNA barcode) tools, has proven to be effective in identifying which species are attracted and what their role is in habitat restoration.
2. The deployed bird attractors (water troughs and perches) succeeded in increasing seed rain towards old fields. However, our design failed in enhancing plant recruitment since no seedlings were found during the study.
3. Thus, the next logical step towards restoration of abandoned Mediterranean fields will be the optimization of conditions in restored fields for seed germination and successful plant establishment.

Chapter IV. Contrasting effects of beekeeping and land use on plant-pollinator networks and pathogen prevalence in a Mediterranean semiarid ecosystem

1. Anthropogenic land uses such as irrigated crops had a generalized positive impact on plant-pollinator networks structural parameters. Honey bee density did not condition properties of plant-pollinator networks.
2. *Nosema ceranae* was the most common pathogen and its prevalence in honey bees decreased over the study period as did the density of honey bees; however, its prevalence increased in wild bees, whose density remained constant during sampling. Flowers seem not to be the contact point for the transmission of pathogens between honey bees and wild bees, as the plants visited by honey bees and wild bees in which *N. ceranae* were detected did not overlap.
3. *Nosema apis* has been detected for the first time in a wild pollinator (*Eucera* sp). *N. ceranae* was detected in 20 genera of bee species (17 of them for the first time).

4. Our findings corroborate the impact of honey bees on wild bees also in semiarid ecosystems, by promoting the spread of pathogens in the landscape even after honey bee density decreases.

Acknowledgements (Agradecimientos en español)



Agradecimientos

Bueno por fin hemos llegado a la que sin ninguna duda es la sección más leída de cualquier tesis que se precie. Llegados a este punto, se me hace muy difícil plasmar en unas pocas líneas todas las sensaciones que me produce pensar en estos cinco años, pero como no podría ser de otra manera, empezaré por el principio.

Desde siempre me había gustado la biología, y en particular la ecología. Esa fue la razón principal que me llevó a iniciar mis estudios en Ciencias Ambientales allá por 2007. Mi interés por la ciencia no tardó en manifestarse, lo que me llevó a implicarme en tareas de investigación como alumno interno ya en segundo de carrera. Empecé por la geología, para más tarde probar con la microbiología y finalmente llegar a la ecología. Durante la carrera pude cursar una asignatura que me marcaría mucho más de lo que yo pensaba, Técnicas de Ecología Molecular. De mano de los profesores Pepe Galián y Pilar De la Rúa conocí el potencial de las técnicas moleculares en los estudios ecológicos. Sin embargo, no me veía en ese campo, me decía a mí mismo “esto es trabajo demasiado fino para mí”, “a mí me va el campo”, “esto del ADN no es lo mío, a mí me gustan las cosas que se ven”. Quién me iba a decir entonces los millares de PCRs que iba a hacer. Más tarde, hacia el final de la carrera, decidí que la ecología era lo mío, y en particular me atraían las aves. Viendo las líneas de investigación del departamento me llamaron la atención las de Paco Robledano, me había dado clase, pero no sabía nada de sus investigaciones. Después de un tiempo colaborando, llegó el momento de hacer mi proyecto fin de carrera, y Paco me ofreció hacerlo sobre dispersión de semillas mediada por aves. Una vez más, quién me iba a decir que esto sería el primer paso de un largo viaje.

Poco a poco me fui introduciendo en la temática y empezaron a surgir preguntas, hipótesis, teorías, etc. Necesitaba saber más, quería buscar la respuesta a mis preguntas, pero en muchos casos las técnicas ecológicas clásicas se me quedaban cortas. Tenía que buscar algo más, herramientas que me ayudasen a testar eficientemente mis hipótesis. Ahí fue cuando empecé a recordar lo que había aprendido en Técnicas de Ecología Molecular, así que pese a mis reticencias con el ADN, me fui al Área de Biología Animal para hablar con los profesores que me la habían impartido. Empecé a hablar con Pilar sobre los microsatélites, era un

campo nuevo, pero las piezas empezaban a encajar, esto era lo que necesitaba. Por tanto, decidí aplicar este enfoque de ecología molecular en mi trabajo fin de máster. Sin embargo, mis preguntas no desaparecieron, sino que aparecieron más. Mi mente ya no se conformaba con intentar comprender lo que pasaba en un parche forestal ¿qué sucedería a escalas más grandes? ¿en otros ambientes? ¿con otros dispersores? ¿y si aplicamos otra técnica? De este modo, empezó a gestarse lo que acabaría convirtiéndose en la Tesis que aquí presento.

Estos años han supuesto muchísimo trabajo en los que he tenido al peor jefe que se puede tener “yo”. La curiosidad por saber más y por resolver las preguntas que se me ocurrían me han llevado a involucrarme en temáticas muy diversas, a olvidar durante la mayor parte del tiempo que lo que hacía era un trabajo, y por tanto, a desdibujar los límites entre la vida personal y laboral. He tenido la enorme suerte de tener a dos directores que me han dejado desarrollar mis propias líneas, mis propias ideas, por lo que en muchos sentidos mis investigaciones eran mucho más que un trabajo o una tesis, “necesitaba saber más”. Agradezco enormemente a Pilar y Paco por haber fomentado mi independencia siempre, por supuesto con su consejo y apoyo, pero dejándome siempre llevar a mí las riendas de mis investigaciones. Después de todos estos años en el mundo científico, soy consciente de la enorme confianza que depositaron en mí cuando me respaldaron para realizar una tesis doctoral que constituía, en gran medida, una línea nueva para ambos, y además sin un proyecto específico para financiarla.

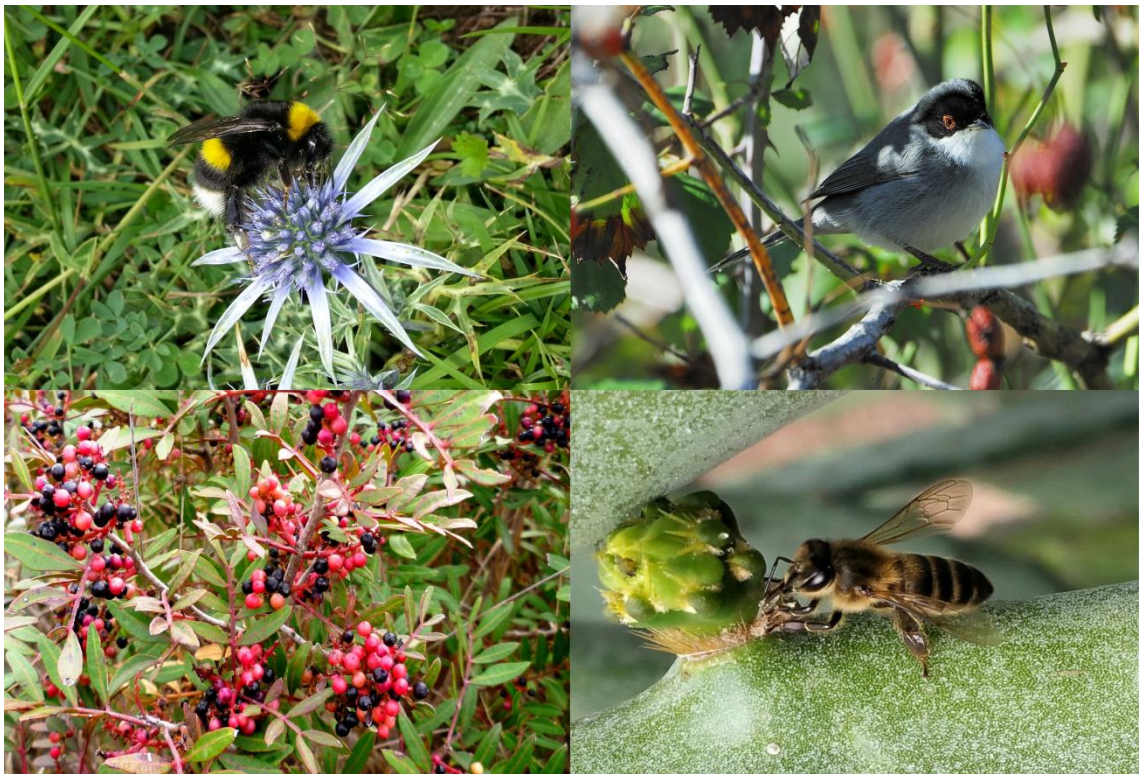
Doy las gracias a mis padres y a mi hermana por el apoyo recibido durante estos años de tanto trabajo y estrés. Hubiera sido imposible llevar a cabo este Tesis sin la estabilidad que proporciona la familia. Muchas gracias a Tatiana por su comprensión, y por echarme una mano en más de una ocasión en las interminables jornadas de trabajo. No podía olvidar mencionar a Pepe Galián y Pepe Serrano, que con su experiencia y saber hacer han sido de gran ayuda en muchas ocasiones. También quiero agradecer a Ana y a Obdulia por estar siempre dispuestas a echar una mano. ¡Ay Obdulia! Quién me iba a decir cuando te conocí que aquella señora que se me antojaba un poco desequilibrada (y no me equivocaba...) acabaría siendo tan buena amiga mía.

No podría olvidarme de dar las gracias a las Dras. Cristina García y Nina Farwig por acogerme en las estancias que realicé en Portugal y Alemania. Estos periodos supusieron un gran avance en mi formación científica tanto por las técnicas adquiridas, como por el desarrollo de otro tipo de aptitudes igual de importantes en la labor de un investigador, como son el pensamiento crítico y la capacidad de adaptación a diferentes grupos y dinámicas de trabajo.

Realizar la Tesis doctoral en dos departamentos me ha dado la oportunidad de tener contacto con muchas personas que sin lugar a dudas han hecho este camino mucho más llevadero. En primer lugar quiero dar las gracias a Carlos, Irene, Paqui y Viki por estar siempre dispuestos a echarme una mano con su experiencia. Saber que podía contar con vuestro consejo ha sido un alivio en más de una ocasión. Estos cinco años no hubieran sido lo mismo sin todos los compañeros/as a los que he tenido el placer de conocer, muchas gracias por el apoyo recibido y por compartir conmigo tantos momentos de altibajos: Andrés, Claudia, Maridol, Marta, Alberto, Laura, Juli, Alejandro, Lury, Diego, Javi, Toni, Nuria, Mica, Txuby, Pablo Farinos, Mario, Isa, Raquel, Nora, Martín, Víctor, María Alcaraz, Etoe, Rubén, María Sánchez, Marisa, Pereñíguez, Irene, María Botella, Susana, Tano, Dani, Miguel, Carbo, Félix, Simone, Nuria García, Pili, Miriam, Antonio Zamora, Jose Manuel Zamora, Fátima y Ana.

ANNEX I.

Curriculum Vitae of the author (Spanish)





MINISTERIO
DE EDUCACIÓN,

SECRETARÍA GENERAL DE

DIRECCIÓN GENERAL

DE POLÍTICA UNIVERSITARIA

Currículum vitae Impreso normalizado

Número de hojas que contiene: 20

Nombre: Vicente Martínez López

Fecha: 06-09-2018

Firma: Vicente Martínez López



El arriba firmante declara que son ciertos los datos que figuran en este currículum, asumiendo en caso contrario las responsabilidades que pudieran derivarse de las inexactitudes que consten en el mismo.

Resumen curriculum

- Publicaciones científicas: 5 (4 en revistas indexadas en el JCR).
- Comunicaciones a congresos: 28 (13 internacionales).
- Capítulos de libro: 6
- Participación en proyectos de investigación: 5 (2 cómo colaborador, 3 cómo miembro del equipo y 1 como IP).
- Trabajos Fin de Grado dirigidos: 2
- Docencia: 12 créditos ECTS en la Universidad de Murcia (Grado Ciencias Ambientales, Grado en Biología, Grado en Veterinaria, y Grado en Ciencia y Tecnología de los alimentos).
- Otros méritos:
 - Revisor en revistas científicas (American Journal of Botany, Molecular Ecology, Ecosistemas)
 - Organización de eventos científicos (congresos internacionales: 1; congresos nacionales: 2; otros: 5)

DATOS PERSONALES

APELLIDOS: Martínez López

NOMBRE: Vicente

DNI: 48549512P

FECHA DE NACIMIENTO: 04-11-1989

SEXO: Varón

DIRECCIÓN PARTICULAR: Calle Compositor Albéniz, nº3, 1ª

CIUDAD: Alcantarilla

CÓDIGO POSTAL: 30820

TELÉFONO MÓVIL 628489189

CORREO ELECTRÓNICO: vicente.martinez2@um.es

DATOS DEL CENTRO DE ADSCRIPCIÓN

ORGANISMO: Universidad de Murcia

FACULTAD, ESCUELA o INSTITUTO DEL C.S.I.C.: Facultad de Veterinaria

DEPT./SECC./ UNIDAD ESTR.: Zoología y Antropología Física. Área de Biología Animal

DIRECCIÓN POSTAL: Campus de Espinardo, Murcia, 30100

TELÉFONO (indicar prefijo, número y extensión): 868 88 80 31

FORMACIÓN ACADÉMICA REGLADA

LICENCIATURA/INGENIERIA: Ciencias Ambientales

CENTRO: Facultad de Biología. Universidad de Murcia

FECHA: Julio 2012

MASTER: Máster en Gestión de la Biodiversidad en Ambientes Mediterráneos

CENTRO: Facultad de Biología. Universidad de Murcia

FECHA: Julio 2013

DOCTORADO: Doctorado en el programa de Biodiversidad y Gestión Ambiental

CENTRO: Facultad de Veterinaria. Universidad de Murcia

FECHA: 12 de Septiembre de 2014 hasta la actualidad

FORMACIÓN ACADÉMICA NO REGLADA

CURSO: Pollen and Plant Metabarcoding Workshop 2016

CENTRO: Universidad de Würzburg, Alemania

DURACIÓN: 25 horas (2,5 créditos ECTS)

CERTIFICADO/DIPLOMA/TÍTULO: CERTIFICADO

FECHA: Mayo 2016

CURSO: Diseño de Experimentos y Fundamentos de Análisis de datos (IV. Métodos de Análisis de Datos Multivariantes)
CENTRO: Facultad de Biología. Universidad de Murcia. España
DURACIÓN: 25 horas
CERTIFICADO/DIPLOMA/TÍTULO: CERTIFICADO
FECHA: Mayo 2016

CURSO: Diseño de Experimentos y Fundamentos de Análisis de Datos (V. Creación, manejo y explotación de datos espaciales)
CENTRO: Facultad de Biología. Universidad de Murcia. España
DURACIÓN: 25 horas
CERTIFICADO/DIPLOMA/TÍTULO: CERTIFICADO
FECHA: Junio 2016

CURSO: Diseño de Experimentos y Fundamentos de Análisis de Datos (III. Métodos de contrastes de hipótesis y diseño de experimentos)
CENTRO: Facultad de Biología. Universidad de Murcia. España
DURACIÓN: 20 horas
CERTIFICADO/DIPLOMA/TÍTULO: CERTIFICADO
FECHA: Mayo 2018

OTRAS BECAS/AYUDAS DISFRUTADAS

INSTITUCIÓN: Beca de Colaboración. Departamento de Ecología e Hidrología. Universidad de Murcia
FECHA: Curso académico 2011-2012

INSTITUCIÓN: Becario de Inicio a la investigación. Departamento de Ecología e Hidrología. Universidad de Murcia
FECHA: 1-09-2012 a 31-12-2012

INSTITUCIÓN: Becario de Inicio a la investigación modalidad B. Departamento de Zoología y Antropología Física. Universidad de Murcia
FECHA: 1-10-2013 a 31-12-2013

INSTITUCIÓN: Investigador Contratado. Departamento de Ecología e Hidrología. Universidad de Murcia
FECHA: 19-06-2014 a 18-08-2014

INSTITUCIÓN: Becario predoctoral FPU MECD. Departamento de Zoología y Antropología Física. Universidad de Murcia
FECHA: 12-09-2014 hasta la actualidad

IDIOMAS (Indicar el nivel. Excelente, Bueno, Básico)

IDIOMA: Inglés
LECTURA: Excelente
ESCRITURA: Bueno
EXPRESIÓN ORAL: Bueno
Certificados que lo acreditan: Certificado B2 Escuela Oficial de Idiomas de Murcia

Incluidas en el JCR

1. AUTORES (p.o. de firma): Zapata V., Robledano F., Ramos V., **Martínez-López V**
TÍTULO: *Bird-mediated seed dispersal of fleshy fruits of mediterranean shrubs in semiarid forest patches: the role of Pinus halepensis Miller trees as seed receptors*

NOMBRE DE LA REVISTA: *Plant Ecology*

Volumen: 215

Páginas (inicial y final): 1337-1350

Editorial: Springer

País de publicación: Países Bajos

Año de publicación: 2014

ISSN: 1573-5052

2. AUTORES (p.o. de firma): Robledano F., Romero A., Belmonte F., Zapata V., Martínez-Hernández C., **Martínez-López V**
TÍTULO: *Ecogeomorphological consequences of land abandonment in semiarid Mediterranean areas: Integrated assessment of physical evolution and biodiversity*

NOMBRE DE LA REVISTA: *Agriculture, Ecosystems and Environment*

Volumen: 197

Páginas (inicial y final): 222-242

Editorial: Elsevier

País de publicación: Países Bajos

Año de publicación: 2014

ISSN: 0167-8809

3. AUTORES (p.o. de firma): Farinós, P., Zapata, V., **Martínez-López, V.** & Robledano, F.
TÍTULO: *Consumption of honey bees by Merops apiaster Linnaeus, 1758 (Aves: Meropidae) in Mediterranean semiarid landscapes: a threat to beekeeping?*

NOMBRE DE LA REVISTA: *Journal of Apicultural Research*

Volumen: 55

Páginas (inicial y final): 193-201

Editorial: Taylor & Francis

País de publicación: Reino unido

Año de publicación: 2016

ISSN: 2078-6913

4. AUTORES (p.o. de firma): **Martínez-López, V.**, De la Rúa, P., Zapata, V. & Robledano, F.
TÍTULO: *Ecological and genetic consequences of fragmentation in a semiarid Mediterranean urban forest*

NOMBRE DE LA REVISTA: *Urban Ecosystems*

Volumen: 20

Páginas (inicial y final): 1161-1168

Editorial: Springer US
País de publicación: USA
Año de publicación: 2017
ISSN: 1573-1642

No incluidas en el JCR

1. AUTORES (p.o. de firma): *Farinós, P., Zapata, V., **Martínez-López, V.**, Jiménez-Franco, MV. & Robledano, F*
TÍTULO: *El abejaruco (Merops apiaster L., AVES: MEROPIDAE) y el sector apícola de la Región de Murcia (SE España): percepción de su impacto y modelo ecogeográfico de distribución*

NOMBRE DE LA REVISTA: Papeles de Geografía

Volumen: 59-60
Páginas (inicial y final): 83-89
Editorial:
País de publicación: Murcia
Año de publicación: 2014
ISSN: 0213-1781

LIBROS/CAPÍTULOS DE LIBROS

1. AUTORES (p.o. de firma): *Robledano-Aymerich, F., Zapata-Pérez, V.M., **Martínez-López, V.**, García-Meseguer, A.J., García-Castellanos, F.A., Zamora-López, A. & Pérez-Navarro, M.A*
TÍTULO DEL LIBRO: *Abandono de cultivos en la Región de Murcia. Consecuencias ecogeomorfológicas (Ed.: A. Romero-Díaz)*
TÍTULO CAPÍTULO: *Indicadores de biodiversidad en campos abandonados: flora y fauna*
Volumen: *I*
Editorial: *Servicio de Publicaciones de la Universidad de Murcia (Editum)*
Páginas (inicial y final): *111-138*
País de Publicación: *España*
Año de publicación: *2016*
ISBN (si lo tiene)/Depósito Legal: *978-84-16551-37-8*
2. AUTORES (p.o. de firma): *Zapata-Pérez, V., Robledano-Aymerich, F. & **Martínez-López, V.***
TÍTULO DEL LIBRO: *Abandono de cultivos en la Región de Murcia. Consecuencias ecogeomorfológicas (Ed.: A. Romero-Díaz)*
TÍTULO CAPÍTULO: *Dinámica recolonizadora de la vegetación*
Volumen: *I*
Editorial: *Servicio de Publicaciones de la Universidad de Murcia (Editum)*
Páginas (inicial y final): *139-160*
País de Publicación: *España*
Año de publicación: *2016*
ISBN (si lo tiene)/Depósito Legal: *978-84-16551-37-8*

3. AUTORES (p.o. de firma): Zapata, V., **Martínez-López, V.**, Ramos, V. & Robledano, F.
TÍTULO DEL LIBRO: *Advances in Environmentl Research*
TÍTULO CAPÍTULO: *Seed Dispersal Systems in the Regeneration of Semiarid Mediterranean Forests*
Volumen: 54
Editorial: *Nova Science Publisher*
Páginas (inicial y final): 59-100
País de Publicación: USA
Año de publicación:2017
ISBN (si lo tiene)/Depósito Legal: 978-1-53610-681-7

4. AUTORES (p.o. de firma): García Castellanos, F.A., Robledano Aymerich, F., Zapata-Pérez, V., **Martínez-López, V.** & González-Barberá, G.
TÍTULO DEL LIBRO: *Biodiversidad y Procesos Ecológicos en el Sureste Ibérico*
TÍTULO CAPÍTULO: *Bebederos de aves: dinámica de uso y función en la dispersión ornitócora*
Volumen:
Editorial: *Servicio de Publicaciones de la Universidad de Murcia (Editum)*
Páginas (inicial y final): 118-126
País de Publicación: España
Año de publicación:2017
ISBN (si lo tiene)/Depósito Legal: 978-84-617-7235-3

5. AUTORES (p.o. de firma): Jiménez-Franco, M.V., Robledano-Aymerich, F., Hernández, I., Zapata-Pérez, V.M. & **Martínez-López, V.**
TÍTULO DEL LIBRO: *Cuestiones sobre paisaje, patrimonio natural y medio ambiente en el sureste ibérico*
TÍTULO CAPÍTULO: *Propuestas para la gestión de fincas agroforestales ubicadas en espacios Natura 2000: el Convenio de colaboración Universidad de Murcia - Castillo de Chuecos*
Volumen:
Editorial: *Servicio de Publicaciones de la Universidad de Murcia (Editum)*
Páginas (inicial y final): 218-225
País de Publicación: España
Año de publicación:2017
ISBN (si lo tiene)/Depósito Legal: 978-84-617-5939-2

6. AUTORES (p.o. de firma): Robledano-Aymerich, F., Romero-Díaz, A., Belmonte-Serrato, F., Zapata-Pérez, V.M., Martínez-Hernández, C. & **Martínez-López, V.**
TÍTULO DEL LIBRO: *Cuestiones sobre paisaje, patrimonio natural y medio ambiente en el sureste ibérico*
TÍTULO CAPÍTULO: *La renaturalización de cultivos abandonados como contribución a la infraestructura verde del sureste ibérico*
Volumen:
Editorial: *Servicio de Publicaciones de la Universidad de Murcia (Editum)*
Páginas (inicial y final): 255-262
País de Publicación: España
Año de publicación:2017
ISBN (si lo tiene)/Depósito Legal: 978-84-617-5939-2

PARTICIPACIÓN EN PROYECTOS DE INVESTIGACIÓN FINANCIADOS

1. *TÍTULO DEL PROYECTO: EVALUACION Y EFECTOS DEL ABANDONO DE CULTIVOS EN LA DEGRADACION O RECUPERACION DE SUELOS EN LA REGION DE MURCIA (CGL 2010-21425-C02-02)*

ENTIDAD FINANCIADORA: TRAGSA-Confederación Hidrográfica del Segura
TIPO DE CONVOCATORIA (Nacional o Internacional): Nacional
ENTIDADES PARTICIPANTES: Universidad de Murcia
DURACIÓN: Desde 01/01/2011 Hasta 31/12/2011
INVESTIGADOR PRINCIPAL: Asunción Romero Díaz
GRADO DE RESPONSABILIDAD/PARTICIPACIÓN: Colaborador

2. *TÍTULO DEL PROYECTO: CONSECUENCIAS ECOGEOFORMOLÓGICAS DEL ABANDONO DE CAMPOS DE CULTIVO EN LA REGIÓN DE MURCIA (15233/PI/10)*

ENTIDAD FINANCIADORA: Fundación Séneca
TIPO DE CONVOCATORIA (Nacional o Internacional): Nacional
ENTIDADES PARTICIPANTES: Universidad de Murcia
DURACIÓN: Desde 01/01/2012 Hasta 31/12/2015
INVESTIGADOR PRINCIPAL: Asunción Romero Díaz
GRADO DE RESPONSABILIDAD/PARTICIPACIÓN: Colaborador

3. *TÍTULO DEL PROYECTO: EVALUACIÓN DE LAS REDES DE POLINIZACIÓN Y DISPERSIÓN DE SEMILLAS COMO FACTORES CLAVE PARA LA CONSERVACIÓN DEL HÁBITAT 5220* DE LA COSTA DE CARTAGENA*

ENTIDAD FINANCIADORA: Autoridad Portuaria de Cartagena
TIPO DE CONVOCATORIA (Nacional o Internacional): Regional
ENTIDADES PARTICIPANTES: Universidad de Murcia
DURACIÓN: Desde 21/09/2016 Hasta 21/09/2017
INVESTIGADOR PRINCIPAL: Pilar de la Rúa Tarín
NÚMERO DE INVESTIGADORES: 5
IMPORTE TOTAL DEL PROYECTO: 19.849,52€
GRADO DE RESPONSABILIDAD/PARTICIPACIÓN: Miembro del equipo investigador

4. *TÍTULO DEL PROYECTO: POLINIZADORES EN RIESGO: ANÁLISIS DE REDES DE POLINIZACIÓN Y DISPERSIÓN DE PATÓGENOS EN ZONAS SEMIÁRDAS*

ENTIDAD FINANCIADORA: Asociación Española de Ecología Terrestre (AEET)
TIPO DE CONVOCATORIA (Nacional o Internacional): Nacional
ENTIDADES PARTICIPANTES: Universidad de Murcia
DURACIÓN: Desde 01-01-2017 Hasta 31-12-2017
INVESTIGADOR PRINCIPAL: Vicente Martínez López
NÚMERO DE INVESTIGADORES: 1
IMPORTE TOTAL DEL PROYECTO: 2000€
GRADO DE RESPONSABILIDAD/PARTICIPACIÓN: Investigador principal

5. **TÍTULO DEL PROYECTO:** *APLICACIÓN DE NUEVAS HERRAMIENTAS GENÓMICAS AL ESTUDIO DE LA RESISTENCIA A NOSEMA y ESTRÉS TÉRMICO EN LA ABEJA IBÉRICA RTA2015-00013-C03-00*

ENTIDAD FINANCIADORA: INIA/ Ministerio de Economía y Competitividad.

TIPO DE CONVOCATORIA (Nacional o Internacional): Nacional

ENTIDADES PARTICIPANTES: Centro Apícola Regional de Marchamalo, UMU, Universidad de Valladolid.

DURACIÓN: 2017-2020

INVESTIGADOR PRINCIPAL: Raquel Hernández Martín (del proyecto), Pilar de la Rúa Tarín (del subproyecto)

NÚMERO DE INVESTIGADORES: 4

IMPORTE TOTAL DEL PROYECTO: 90.000 €

GRADO DE RESPONSABILIDAD/PARTICIPACIÓN: Miembro del equipo investigador

ASISTENCIAS/CONTRIBUCIONES A CONGRESOS (Comunicaciones, Actas, Publicaciones...)

CONTRIBUCIONES A CONGRESOS

1. *AUTOR/ES:* **Martínez-López V.**, Zapata V., Robledano F.

TÍTULO: *Papel de las interacciones plantas-frugívoros en la regeneración de especies forestales en el sureste de España*

ENTIDAD ORGANIZADORA: SEO/BirdLife

TIPO DE PARTICIPACIÓN: Comunicación oral

TÍTULO DEL CONGRESO: XXI Congreso español y V Ibérico de ornitología

ÁMBITO (Nacional/internacional): Internacional

LUGAR DE CELEBRACIÓN: Vitoria-Gasteiz, País Vasco (España)

FECHA: 06-12-2012 al 09-12-2012

2. *AUTOR/ES:* Zapata V., Robledano F., **Martínez-López V.**, García-Castellanos F., Martínez-Hernández C.

TÍTULO: *Papel de las interacciones plantas-frugívoros en la regeneración de especies forestales en el sureste de España*

ENTIDAD ORGANIZADORA: SEO/BirdLife

TIPO DE PARTICIPACIÓN: Comunicación oral

TÍTULO DEL CONGRESO: XXI Congreso español y V Ibérico de ornitología

ÁMBITO (Nacional/internacional): Internacional

LUGAR DE CELEBRACIÓN: Vitoria-Gasteiz, País Vasco (España)

FECHA: 06-12-2012 al 09-12-2012

3. *AUTOR/ES:* **Martínez-López V.**, Zapata V., Robledano F.

TÍTULO: *Papel de las interacciones plantas-frugívoros en la regeneración de especies forestales en el sureste de España*

ENTIDAD ORGANIZADORA: CONAMA 2012 – Congreso Nacional del Medio Ambiente

TIPO DE PARTICIPACIÓN: Póster

TÍTULO DEL CONGRESO: 11º Congreso Nacional del Medio Ambiente, CONAMA 2012
ÁMBITO (Nacional/internacional): Nacional

Volumen: 1

Editorial:

Páginas (inicial y final):

País de Publicación: España

Año de publicación: 2012

ISSN/ISBN (si lo tiene)/Depósito Legal: 978-84-695-6377-9

LUGAR DE CELEBRACIÓN: Madrid

FECHA: 26-11-2012 al 30-11-2012

4. AUTOR/ES: Martínez-López V., Robledano-Aymerich F., Zapata-Pérez V, García-Castellanos F.

TÍTULO: Aves frugívoras y su papel en la regeneración de arbustos mediterráneos productores de frutos carnosos

ENTIDAD ORGANIZADORA: Asociación de Jóvenes Investigadores de la Universidad de Murcia (AJIUM) y Facultad de Biología de la Universidad de Murcia

TIPO DE PARTICIPACIÓN: Comunicación oral

TÍTULO DEL CONGRESO: II Jornadas de Inicio a la Investigación de Estudiantes de la Facultad de Biología

ÁMBITO (Nacional/internacional): Nacional

DATOS DE LA PUBLICACIÓN:

Volumen: 1

Editorial: Servicio de publicaciones de la Universidad de Murcia

Páginas (inicial y final): 15-25

País de Publicación: España

Año de publicación: 2013

ISSN/ISBN (si lo tiene)/Depósito Legal: 978-84-695-9296-0

LUGAR DE CELEBRACIÓN: Murcia

FECHA: 11-12 Abril 2013

5. AUTOR/ES: Martínez-López V., Robledano F., Zapata V.

TÍTULO: Papel de las interacciones plantas-frugívoros en la regeneración de arbustos mediterráneos en el sureste español

ENTIDAD ORGANIZADORA: Sociedad Española de Ciencias Forestales

TIPO DE PARTICIPACIÓN: Comunicación oral

TÍTULO DEL CONGRESO: VI Congreso Forestal Español

ÁMBITO (Nacional/internacional): Nacional

Volumen: 6CFE01-014

Editorial: DIALNET

Páginas (inicial y final): 1-13

País de Publicación: España

Año de publicación: 2013

ISSN/ISBN (si lo tiene)/Depósito Legal: 978-84-937964-9-5

LUGAR DE CELEBRACIÓN: Vitoria-Gasteiz, País Vasco (España)

FECHA: 10-06-2013 a 14-06-2013

6. AUTOR/ES: Zapata V., Robledano F., Romero A., Belmonte F., **Martínez-López V.**, Martínez-Hernández C.

TÍTULO: *Condicionantes ambientales para la reforestación natural de cultivos de secano abandonados en el sureste de la Península Ibérica*

ENTIDAD ORGANIZADORA: *Sociedad Española de Ciencias Forestales*

TIPO DE PARTICIPACIÓN: *Comunicación oral*

TÍTULO DEL CONGRESO: *VI Congreso Forestal Español*

ÁMBITO (Nacional/internacional): *Nacional*

Volumen: *6CFE01-018*

Editorial: *DIALNET*

Páginas (inicial y final): *1-13*

País de Publicación: *España*

Año de publicación: *2013*

ISSN/ISBN (si lo tiene)/Depósito Legal: *978-84-937964-9-5*

LUGAR DE CELEBRACIÓN: *Vitoria-Gasteiz, País Vasco (España)*

FECHA: *10-06-2013 a 14-06-2013*

7. AUTOR/ES: **Martínez-López V.**, Robledano-Aymerich F., De la Rúa-Tarín P., Zapata-Pérez VM., Zamora-Marín JM., Pérez-Navarro MA.

TÍTULO: *Analysis of the dispersal flow in Mediterranean fleshy-fruited shrubs through the study of kinship relations: Pistacia lentiscus L. (Anacardiaceae) as a model*

ENTIDAD ORGANIZADORA: *Campus Mare Nostrum*

TIPO DE PARTICIPACIÓN: *Póster*

TÍTULO DEL CONGRESO: *MED-SOUK: I Congreso Internacional de Jóvenes Investigadores del Mediterráneo*

ÁMBITO (Nacional/internacional): *Internacional*

LUGAR DE CELEBRACIÓN: *Murcia*

FECHA: *24 -10-2013 al 25-10-2013*

8. AUTOR/ES: **Martínez-López V.**, Robledano F., De la Rúa P., Zapata V., Jara L., García-Castellanos F.

TÍTULO: *Análisis de la diversidad genética de poblaciones de Pistacia lentiscus L. Anacardiaceae) en un paisaje mediterráneo semiárido fragmentado*

ENTIDAD ORGANIZADORA: *Sociedad Españolas de la Biología de Conservación de Plantas (Sebicop)*

TIPO DE PARTICIPACIÓN: *Comunicación oral*

TÍTULO DEL CONGRESO: *6º Congreso de Biología de Conservación de Plantas*

ÁMBITO (Nacional/internacional): *Nacional*

LUGAR DE CELEBRACIÓN: *Murcia*

FECHA: *15-10-2013 al 18-10-2013*

9. AUTOR/ES: Zapata V., Robledano F., Romero A., Belmonte F., **Martínez-López V.**, Martínez-Hernández C., Pérez M.A., Zamora J.M

TÍTULO: *Evolución florística en cultivos de secano abandonados en el sureste de la Península Ibérica*

ENTIDAD ORGANIZADORA: *Sociedad Españolas de la Biología de Conservación de Plantas (Sebicop)*

TIPO DE PARTICIPACIÓN: *Comunicación oral*

TÍTULO DEL CONGRESO: 6º Congreso de Biología de Conservación de Plantas
ÁMBITO (Nacional/internacional): Nacional
LUGAR DE CELEBRACIÓN: Murcia
FECHA: 15-10-2013 al 18-10-2013

10. *AUTOR/ES: Amat F., del Campo R., López-García D., Manzano S., **Martínez-López V.***
TÍTULO: Análisis numérico y taxonómico de los catálogos regionales de protección de flora amenazada. Aspectos cuantitativos y cualitativos que subyacen a las políticas regionales de conservación de la flora
ENTIDAD ORGANIZADORA: Sociedad Españolas de la Biología de Conservación de Plantas (Sebicop)
TIPO DE PARTICIPACIÓN: Póster
TÍTULO DEL CONGRESO: 6º Congreso de Biología de Conservación de Plantas
ÁMBITO (Nacional/internacional): Nacional
LUGAR DE CELEBRACIÓN: Murcia
FECHA: 15-10-2013 al 18-10-2013

11. *AUTOR/ES: Robledano-Aymerich F., Zapata-Pérez VM., **Martínez-López V.**, Martínez-Hernández C., Romero-Díaz A., Belmonte-Serrato F.*
TÍTULO: Diversidad litológica como condicionante de la diversidad ecológica en cultivos abandonados del Sureste Ibérico.
ENTIDAD ORGANIZADORA:
TIPO DE PARTICIPACIÓN: Póster
TÍTULO DEL CONGRESO: XXIII Congreso de Geógrafos Españoles
ÁMBITO (Nacional/internacional): Nacional
LUGAR DE CELEBRACIÓN: Palma de Mallorca
FECHA: 23/10/2013 al 24/10/2013

12. *AUTOR/ES: Pérez-Navarro MA., García-Castellanos FA., Zamora JM., **Martínez López V.**, Zapata VM., Robledano F.*
TÍTULO: Efectos del abandono agrícola sobre la biodiversidad de aves en áreas mediterráneas semiáridas
ENTIDAD ORGANIZADORA: Asociación de Jóvenes Investigadores de la Universidad de Murcia (AJIUM) y Facultad de Biología de la Universidad de Murcia
TIPO DE PARTICIPACIÓN: Comunicación oral
TÍTULO DEL CONGRESO: III Jornadas de Inicio a la Investigación de Estudiantes de la Facultad de Biología
ÁMBITO (Nacional/internacional): Nacional
DATOS DE LA PUBLICACIÓN:

Volumen:1

Editorial: Servicio de publicaciones de la Universidad de Murcia

Páginas (inicial y final):62-74

País de Publicación: España

Año de publicación: 2014

ISSN/ISBN (si lo tiene)/Depósito Legal: 978-84-606-6609-7

LUGAR DE CELEBRACIÓN: Murcia
FECHA: 28-03-2014 al 04-04-2014

13. *AUTOR/ES: **Martínez-López V.**, Farinós P., Zapata V., Soto A., Robledano F.*
TÍTULO: Bee-eaters: pests or allies of bee pollinators? Assessment framework and preliminary evaluation in the Iberian southeast (Spain)

ENTIDAD ORGANIZADORA: *Eurbee*
TIPO DE PARTICIPACIÓN: *póster*
TÍTULO DEL CONGRESO: *Sixth European Conference of Apidology*
ÁMBITO (Nacional/internacional): *Internacional*

Volumen: *1*
Editorial: *Servicio de publicaciones de la Universidad de Murcia*
Páginas (inicial y final): *183-184*
País de Publicación: *España*
Año de publicación: *2014*
ISSN/ISBN (si lo tiene)/Depósito Legal: *978-84-697-0855-2*

LUGAR DE CELEBRACIÓN: *Murcia*
FECHA: *2014*

14. AUTOR/ES: *Muñoz I., Hernández-Mármol D., Martínez-López V., García-Villena M., Jara L., Ornos C., De la Rúa P*
TÍTULO: *Intraspecific bumblebee diversity: a case study of *Bombus pascuorum* (Hymenoptera, Apidae) in the Iberian peninsula*
ENTIDAD ORGANIZADORA: *Eurbee*
TIPO DE PARTICIPACIÓN: *Póster*
TÍTULO DEL CONGRESO: *Sixth European Conference of Apidology*
ÁMBITO (Nacional/internacional): *Internacional*

Volumen: *1*
Editorial: *Servicio de publicaciones de la Universidad de Murcia*
Páginas (inicial y final): *169-170*
País de Publicación: *España*
Año de publicación: *2014*
ISSN/ISBN (si lo tiene)/Depósito Legal: *978-84-697-0855-2*

LUGAR DE CELEBRACIÓN: *Murcia*
FECHA: *2014*

15. AUTOR/ES: *Cejas D., Martínez-López V., Ornos C., De la Rúa P., Muñoz I.*
TÍTULO: *Genetic diversity of the endemic Iberian bumblebee *Bombus terrestris lusitanicus* (Hymenoptera, Apidae)*
ENTIDAD ORGANIZADORA: *Eurbee*
TIPO DE PARTICIPACIÓN: *Comunicación oral*
TÍTULO DEL CONGRESO: *Sixth European Conference of Apidology*
ÁMBITO (Nacional/internacional): *Internacional*

Volumen: *1*
Editorial: *Servicio de publicaciones de la Universidad de Murcia*
Páginas (inicial y final): *170*
País de Publicación: *España*
Año de publicación: *2014*
ISSN/ISBN (si lo tiene)/Depósito Legal: *978-84-697-0855-2*

LUGAR DE CELEBRACIÓN: *Murcia*
FECHA: *2014*

16. AUTOR/ES: **Martínez-López V.**, Zapata V., Smith-Ramírez C. & Robledano-Aymerich F.
TÍTULO: *Dispersal of fleshy-fruited trees and shrubs by birds in Mediterranean-type regions: a review*

ENTIDAD ORGANIZADORA: *FSD Symposium-Workshop*

TIPO DE PARTICIPACIÓN: *Póster*

TÍTULO DEL CONGRESO: *6th International Symposium- Workshop on Frugivory & Seed Dispersal*

ÁMBITO (Nacional/internacional): *Internacional*

LUGAR DE CELEBRACIÓN: *Sudáfrica*

FECHA: *2015*

17. AUTOR/ES: Zapata VM., **Martínez-López V.** & Robledano F.

TÍTULO: *Assessment of bird dispersal potential for the passive restoration of Mediterranean semiarid oldfields from forest source patches*

ENTIDAD ORGANIZADORA: *FSD Symposium-Workshop*

TIPO DE PARTICIPACIÓN: *Póster*

TÍTULO DEL CONGRESO: *6th International Symposium- Workshop on Frugivory & Seed Dispersal*

ÁMBITO (Nacional/internacional): *Internacional*

LUGAR DE CELEBRACIÓN: *Sudáfrica*

FECHA: *2015*

18. AUTOR/ES: Robledano-Aymerich F., Romero-Díaz A., Zapata V., Martínez-Hernández C., **Martínez-López V.**

TÍTULO: *Ecogeomorphological assesment of agricultural abandonment in semiarid Mediterranean areas: a basis for natural reconstruction*

ENTIDAD ORGANIZADORA: *Society for Conservation Biology Global y Society for Conservation Biology Europe Section*

TIPO DE PARTICIPACIÓN: *Comunicación oral*

TÍTULO DEL CONGRESO: *ICCB: 27th International Congress for Conservation Biology – 4th European Congress for Conservation Biology*

ÁMBITO (Nacional/internacional): *Internacional*

LUGAR DE CELEBRACIÓN: *Montpellier, France*

FECHA: *02-08-2016 al 06-08-2016*

19. AUTOR/ES: Zapata V., Robledano F., **Martínez-López V.**

TÍTULO: *Directrices para la conservación de la vegetación natural en paisaje mediterráneo semiáridos fragmentados*

ENTIDAD ORGANIZADORA: *Sociedad Españolas de la Biología de Conservación de Plantas (Sebicop)*

TIPO DE PARTICIPACIÓN: *Comunicación oral*

TÍTULO DEL CONGRESO: *VII Congreso de Biología de la Conservación de Plantas*

ÁMBITO (Nacional/internacional): *Nacional*

LUGAR DE CELEBRACIÓN: *Vitoria-Gasteiz, País Vasco*

FECHA: *30-09-2015 al 2-10-2015*

20. AUTOR/ES: **Martínez-López V.**, Zapata-Pérez V., Galián-Galvez D., Robledano-Aymerich F.
TÍTULO: *Estudio de la viabilidad de semillas del lentisco (Pistacia lentiscus L.) en el sureste ibérico*

ENTIDAD ORGANIZADORA: *Sociedad Españolas de la Biología de Conservación de Plantas (Sebicop)*

TIPO DE PARTICIPACIÓN: *Comunicación oral*

TÍTULO DEL CONGRESO: *VII Congreso de Biología de la Conservación de Plantas*

ÁMBITO (Nacional/internacional): Nacional
LUGAR DE CELEBRACIÓN: Vitoria-Gasteiz, País Vasco
FECHA: 30-09-2015 al 2-10-2015

21. AUTOR/ES: Robledano Aymerich F., Romero Díaz A., Belmonte Serrato F., Zapata Pérez VM.,
Martínez Hernández C., **Martínez López V.**

TÍTULO: *La renaturalización de cultivos abandonados como contribución a la infraestructura verde del Sureste ibérico*

ENTIDAD ORGANIZADORA: Asociación de Naturalistas del Sureste (Anse) y Universidad de Murcia

TIPO DE PARTICIPACIÓN: Comunicación oral

TÍTULO DEL CONGRESO: V CONGRESO DE LA NATURALEZA DE LA REGIÓN DE MURCIA

ÁMBITO (Nacional/internacional): Nacional

LUGAR DE CELEBRACIÓN: Murcia

FECHA: 25-11-2015 al 28-11-2015

22. AUTOR/ES: Jiménez-Franco MV., Robledano F., Hernández I., Zapata V., **Martínez- López V.**

TÍTULO: *Propuestas para a gestión de fincas agroforestales ubicadas en espacios Natura 2000: el convenio de colaboración Universidad de Murcia-Castillo de Chuecos*

ENTIDAD ORGANIZADORA: Asociación de Naturalistas del Sureste (Anse) y Universidad de Murcia

TIPO DE PARTICIPACIÓN: Póster

TÍTULO DEL CONGRESO: V CONGRESO DE LA NATURALEZA DE LA REGIÓN DE MURCIA

ÁMBITO (Nacional/internacional): Nacional

LUGAR DE CELEBRACIÓN: Murcia

FECHA: 25-11-2015 al 28-11-2015

23. AUTOR/ES: García-Castellanos FA, Robledano F., Zapata VM., **Martínez-López V.**, Barberá G.

TÍTULO: *Bebederos de aves: dinámica de uso y función en la dispersión ornitócora*

ENTIDAD ORGANIZADORA: Asociación de Naturalistas del Sureste (Anse) y Universidad de Murcia

TIPO DE PARTICIPACIÓN: Comunicación oral

TÍTULO DEL CONGRESO: V CONGRESO DE LA NATURALEZA DE LA REGIÓN DE MURCIA

ÁMBITO (Nacional/internacional): Nacional

LUGAR DE CELEBRACIÓN: Murcia

FECHA: 25-11-2015 al 28-11-2015

24. AUTOR/ES: **Martínez-López V.**, Zapata V., Robledano F., De la Rúa P.

TÍTULO: *Diversidad genética y estructuración de poblaciones de lentisco (*Pistacia lentiscus* L.) en la Cuenca Mediterránea*

ENTIDAD ORGANIZADORA: Sociedad Española de Biología Evolutiva (SESBE)

TIPO DE PARTICIPACIÓN: Comunicación oral

TÍTULO DEL CONGRESO: V CONGRESO DE LA SOCIEDAD ESPAÑOLA DE BIOLOGÍA EVOLUTIVA (SESBE)

ÁMBITO (Nacional/internacional): Nacional

DATOS DE LA PUBLICACIÓN:

Volumen: 1

Editorial: Azarbe, S.L.

Páginas (inicial y final): 30-31

País de Publicación: España

Año de publicación: 2016

ISSN/ISBN (si lo tiene)/Depósito Legal: 978-84-15162-58-2

LUGAR DE CELEBRACIÓN: Murcia

FECHA: 18-01-2016 al 21-01-2016

25. AUTOR/ES: Zapata V., Robledano F., Romero-Díaz, A., Belmonte F., Martínez-Hernández C., **Martínez-López, V.**

TÍTULO: *Evolución del paisaje en campos de cultivo abandonados bajo diferencias litológicas en condiciones climáticas semiáridas*

ENTIDAD ORGANIZADORA: *Associação Portuguesa de Ecologia da Paisagem (APEP), European Association for Landscape Ecology (IALE Europa) y Universidad de Extremadura*

TIPO DE PARTICIPACIÓN: *Comunicación oral*

TÍTULO DEL CONGRESO: *III Jornadas Ibéricas de Ecología del Paisaje*

ÁMBITO (Nacional/internacional): *Internacional*

LUGAR DE CELEBRACIÓN: *Cáceres (Extremadura). España*

FECHA: *05-05-2016 a 06-05-2016*

26. AUTOR/ES: Zapata V., Robledano F., **Martínez-López V.**, Cabezas JD.

TÍTULO: *Retos paisajísticos en la gestión de pinares de repoblación bajo condiciones climáticas semiáridas*

ENTIDAD ORGANIZADORA: *Associação Portuguesa de Ecologia da Paisagem (APEP), European Association for Landscape Ecology (IALE Europa) y Universidad de Extremadura*

TIPO DE PARTICIPACIÓN: *Comunicación oral*

TÍTULO DEL CONGRESO: *III Jornadas Ibéricas de Ecología del Paisaje*

ÁMBITO (Nacional/internacional): *Internacional*

LUGAR DE CELEBRACIÓN: *Cáceres (Extremadura). España*

FECHA: *05-05-2016 a 06-05-2016*

27. AUTOR/ES: **Martínez-López V.**, Carreño M. F., Zapata V., Chahbar N., Robledano F., De la Rúa P.

TÍTULO: *Habitat fragmentation and genetic diversity in western Mediterranean Pistacia lentiscus populations*

ENTIDAD ORGANIZADORA: *Asociación Española de Ecología Terrestre*

TIPO DE PARTICIPACIÓN: *Póster*

TÍTULO DEL CONGRESO: *XIV MEDECOS & XIII AEET meeting*

ÁMBITO (Nacional/internacional): *Internacional*

LUGAR DE CELEBRACIÓN: *Sevilla, España*

FECHA: *31-01-2017 a 04-02-2017*

28. AUTOR/ES: Zapata V., Robledano F., **Martínez-López V.**, Romero-Díaz A., Belmonte-Serrato F., Martínez-Hernández C.

TÍTULO: *Biodiversity responses to land abandonment over different lithologies in semiarid Mediterranean areas*

ENTIDAD ORGANIZADORA: *Asociación Española de Ecología Terrestre*

TIPO DE PARTICIPACIÓN: *Póster*

TÍTULO DEL CONGRESO: *XIV MEDECOS & XIII AEET meeting*

ÁMBITO (Nacional/internacional): *Internacional*

LUGAR DE CELEBRACIÓN: *Sevilla, España*

FECHA: *31-01-2017 a 04-02-2017*

ASISTENCIA A CONGRESOS (sin comunicación)

1. TÍTULO DEL CONGRESO: XI Congreso Ibérico de Mirmecología Taxomara 2016

AMBITO: Nacional

ENTIDAD ORGANIZADORA: Asociación Ibérica de Mirmecología (AIM), Asociación de Naturalistas del Sureste (ANSE) y Facultad de Biología de la Universidad de Murcia

LUGAR DE CELEBRACIÓN: Murcia

FECHA: 13-07-2016 a 15-07-2016

ESTANCIAS EN CENTROS EXTRANJEROS

1. TÍTULO DEL PROYECTO: Estancia predoctoral

CENTRO: Philipps-University of Marburg, Faculty of Biology

LOCALIDAD: Marburg

PAÍS: Alemania

DURACIÓN: 3 meses

FECHA INICIO: 01-02-2016

FECHA FIN: 30-04-2016

OBJETIVO, RELEVANCIA Y NOVEDAD DEL PROYECTO: En el proyecto se estudiaron las redes de dispersión de semillas en paisajes degradados. Las especies objetivo fueron los arbustos productores de fruto carnoso y las aves frugívoras. Se aplicaron técnicas novedosas para el estudio de la dispersión de semillas como el barcoding.

SI HA RECIBIDO AYUDA O FINANCIACIÓN SEÑALAR EL ORGANISMO Y ENTIDAD: Estancia financiada por Ministerio de Educación, Cultura y Deporte bajo el programa Ayudas complementarias para beneficiarios de ayudas (FPU): Estancias Breves y Traslados Temporales.

2. TÍTULO DEL PROYECTO: Estancia predoctoral

CENTRO: CIBIO/InBIO

LOCALIDAD: Porto

PAÍS: Portugal

DURACIÓN: 3 meses

FECHA INICIO: 01-04-2017

FECHA FIN: 30-06-2017

*OBJETIVO, RELEVANCIA Y NOVEDAD DEL PROYECTO: En el proyecto se estudió la diversidad genética del lentisco (*Pistacia lentiscus*) a partir del análisis de 1200 muestras procedentes de toda su área de distribución. El estudio permitió conocer los patrones genético-poblacionales de la especie.*

SI HA RECIBIDO AYUDA O FINANCIACIÓN SEÑALAR EL ORGANISMO Y ENTIDAD: Estancia financiada por Ministerio de Educación, Cultura y Deporte bajo el programa Ayudas complementarias para beneficiarios de ayudas (FPU): Estancias Breves y Traslados Temporales.

EXPERIENCIA DOCENTE

1. CURSO 2015-2016

CENTRO: Facultad de Veterinaria

DURACIÓN: Venia docendi Desde 01-09-2015 Hasta 31-07-2016

Asignatura: Biología general. Grado en Ciencia y Tecnología de los Alimentos. Departamento de Zoología y Antropología Física. Facultad de Veterinaria. Universidad de Murcia

Dedicación: 3 créditos ECTS

2. CURSO 2015-2016

CENTRO: Facultad de Biología

DURACIÓN: Venia docendi Desde 01-09-2015 Hasta 31-07-2016

Asignatura: Evaluación de impacto ambiental. Grado en Ciencia Ambientales. Departamento de Ecología e Hidrología. Facultad de Biología. Universidad de Murcia

Dedicación: 1,5 créditos ECTS

Asignatura: Evaluación de impacto. Grado en Biología. Departamento de Ecología e Hidrología. Facultad de Biología. Universidad de Murcia

Dedicación: 1,5 créditos ECTS

3. CURSO 2016-2017

CENTRO: Facultad de Biología

DURACIÓN: Venia docendi Desde 01-09-2016 Hasta 31-07-2017

Asignatura: Evaluación de impacto ambiental. Grado en Ciencia Ambientales. Departamento de Ecología e Hidrología. Facultad de Biología. Universidad de Murcia

Dedicación: 1,5 créditos ECTS

Asignatura: Evaluación de impacto. Grado en Biología. Departamento de Ecología e Hidrología. Facultad de Biología. Universidad de Murcia

Dedicación: 1,5 créditos ECTS

4. CURSO 2016-2017

CENTRO: Facultad de Veterinaria

DURACIÓN: Venia docendi Desde 01-09-2016 Hasta 31-07-2017

Asignatura: Biología general. Grado en Ciencia y Tecnología de los Alimentos. Departamento de Zoología y Antropología Física. Facultad de Veterinaria. Universidad de Murcia

Dedicación: 1,5 créditos ECTS

Asignatura: Biología general y molecular. Grado en Veterinaria. Departamento de Zoología y Antropología Física. Facultad de Veterinaria. Universidad de Murcia

Dedicación: 1,5 créditos ECTS

Trabajos Fin de Grado Dirigidos

Título: Restauración natural de áreas agroforestales semiáridas: papel de las interacciones animal- planta en la dispersión de semillas a larga distancia.

Estudiante: José Joaquín Martínez Gómez

Fecha: Febrero 2017

Grado: Grado en Ciencias Ambientales

Créditos:12 ECTS

Calificación: 9,1 (Sobresaliente)

Título: Papel de las aves migratorias en la dispersión de semillas a larga distancia.

Estudiante: Francisco Javier Muñoz Galvez

Fecha: Junio 2018

Grado: Grado en Ciencias Biología

Créditos:9 ECTS

Calificación: 9,2 (Sobresaliente)

PRÁCTICAS PROFESIONALES

DENOMINACIÓN PUESTO DE TRABAJO: Estudiante en prácticas

CENTRO: BIOCZYMA, CONSULTORA EN MEDIO AMBIENTE Y CALIDAD, S.L.

DURACIÓN: Desde 01-07-2010 Hasta 31-07-2010 (126 horas)

DENOMINACIÓN PUESTO DE TRABAJO: Estudiante en prácticas

CENTRO: BIOCZYMA, CONSULTORA EN MEDIO AMBIENTE Y CALIDAD, S.L.

DURACIÓN: Desde 01-07-2011 Hasta 31-07-2011 (100 horas)

OTROS MÉRITOS O ACLARACIONES QUE SE DESEE HACER CONSTAR

- *Revisor de artículos en revistas científicas: American Journal of Botany, Molecular Ecology, Ecosistemas*
- *Experiencia en organización de actividades de I+D+i*
 - *Organización de congresos, seminarios, jornadas, etc., científico-tecnológicos:*

Título: XXIV Semana de Biología

Tipo de actividad: Coordinador

Ámbito: Regional

Fecha: Febrero 2011

Título: V Simposio Nacional sobre Control de la Degradación y Uso Sostenible del Suelo

Tipo de actividad: Colaboración en la organización

Ámbito: Nacional

Fecha: Junio 2011

Título: XXV Semana de Biología

Tipo de actividad: Coordinador

Ámbito: Nacional

Fecha: Febrero 2012

Título: VII Olimpiada Española de Biología
Tipo de actividad: Colaboración en la organización *Ámbito: Nacional*
Fecha: Marzo 2012

Título: Congreso Eurbee (Sixth European Conference of Apidology)
Tipo de actividad: Comité organizador *Ámbito: Europeo*
Fecha: Septiembre 2014

Título: Seminarios de Biodiversidad y Gestión Ambiental
Tipo de actividad: Organizador *Ámbito: Programa de doctorado de Biodiversidad y Gestión ambiental. Facultad de Biología. Universidad de Murcia*
Fecha: Enero 2015 a Diciembre 2015

Título: V Congreso de la Sociedad Española de Biología Evolutiva (SESBE)
Tipo de actividad: Comité organizador *Ámbito: Nacional*
Fecha: Enero 2016

Título: IV Jornadas de Inicio a la Investigación en Biología
Tipo de actividad: Comité organizador y científico *Ámbito: Regional*
Fecha: Junio 2016

- *Participación como ponente en cursos y jornadas:*

- Participación en la organización y desarrollo de la actividad “Biodiversidad en la Región de Murcia: ¿Cómo conservarla?” en las Jornadas de Puertas Abiertas” organizadas por la Facultad de Biología de la Universidad de Murcia. Ediciones:

- *XXVI Semana de Biología. 25 de Febrero al 1 de Marzo de 2013. Dedicación de 15 horas*
- *XXVII Semana de Biología. 24 al 28 de Febrero de 2014. Dedicación de 15 horas*
- *XXVIII Semana de Biología. 23 al 27 de Febrero de 2015. Dedicación de 10 horas*
- *XXX Semana de Biología. 20-24 de Febrero de 2017. Dedicación de 20 horas*

- Participación como ponente en el curso oficial de la Universidad Internacional del Mar “ESCUELA DE VERANO FABIOUMU: UN PASEO POR LA BIOLOGÍA”, celebrado en Murcia. Ediciones 2017 y 2018. Dedicación de 50 horas (25 horas cada edición).

- *Actividades de divulgación científica:*

- *Organizador del taller “La importancia de los polinizadores” en “La Noche europea de l@s investigador@s” 2017. 29 de Septiembre. Murcia. España*

- *Actividades de representación estudiantil y del colectivo investigador:*

- Delegado de Facultad durante el curso 2010-2011. Facultad de Biología. Universidad de Murcia.

- Presidente de la Asociación de Jóvenes Investigadores de la Universidad de Murcia (05-03-2015 a 17-11-2016).